CONTENTS

PREFACE

Maize and onion are important agricultural crops in South Africa. The country is one of the biggest producers of onion seed in the world. In the 2004/2005 growing season 358 tons of onion seed were produced, of which 282 tons were for the export market. Maize is the most important grain crop in South Africa, being both the major animal feed grain and the staple food of the majority of the population. For the 2003/2004 marketing year maize was responsible for the second largest contribution to the gross value of agricultural production in the country. The South African maize industry is also the largest maize industry in Africa. Commercial farmers are cultivating nearly three million hectares of maize per year. In the past five years, South Africa produced between 7.2 and 10.1 million tons of maize per annum, with an average of 9.2 million tons. The main maize production areas in South Africa are the Free State, Northwest and Mpumalanga Provinces. These three provinces are responsible for 85% of the total maize produced in the country.

Numerous fungal diseases cause excessive damage to maize and onion in South Africa and efforts to control the quality of these crops concentrated on the detection and control of fungal pathogens. Consequently, little is known about plant pathogenic bacteria that may be present.

In 1981, leaf and seed stalk necrosis of onion, caused by *Erwinia herbicola* (syn. *Pantoea agglomerans*) was reported in the onion seed production areas. In 2000, leaf blight of onion, caused by *Xanthomonas axonopodis* pv. *allii* was observed in a few commercial fields of the Limpopo Province. These two diseases have not been observed again and no attempts have been made to screen the locally produced onion seed for the presence of *P. agglomerans* and *X. axonopodis* pv. *allii*.

In 1997, a disease similar to leaf and seed stalk necrosis was observed in onion fields in Georgia, USA. The disease, named center rot, has occurred in commercial fields in Georgia every year since 1997 and accounted for 100% loss in some fields. The causal agent of center rot is a gram-negative, facultatively anaerobic, seed-borne bacterium, identified as *Pantoea ananatis*. The seed associated with the first outbreak of center rot of onion in Georgia, USA, was produced in South Africa and it was suggested that the center rot

pathogen was possibly introduced on infested seed lots. *P. ananatis* is the causal agent of bacterial blight and dieback of *Eucalyptus* in South Africa, but center rot of onion has not been reported from this country.

The first goal of this study was to determine if pathogenic *P. ananatis* was present in South African onion seed and to compare such strains to those associated with center rot of onions in the USA. Nutrient agar is the common growth medium used to isolate *P. ananatis* from plant material and seed. Nutrient agar, however, is non-selective and many other organisms present as saprophytes or endophytes in plant material and in seed may hamper the detection of the target pathogen. In Chapter 2, a semi-selective medium, PA 20, is described, which suppresses growth of many saprophytic microorganisms and serves as a suitable medium for growth and enumeration of *P. ananatis*. The medium was specifically developed to detect this pathogen on onion seed. Chapter 3 describes the isolation and identification of *P. ananatis* from South African onion seed.

In 2004 and 2005, a new disease was observed in commercial maize fields in the Northwest and Mpumalanga Provinces. Diseased plants were scattered throughout the fields and 10-70% of the crop was affected. Gram-negative bacteria producing yellow colonies were consistently isolated from diseased tissues and these were tentatively identified as belonging to the genus *Pantoea.* Two *Pantoea* species have been reported to cause diseases on maize worldwide. *P. stewartii* subsp. *stewartii* causes Stewart's wilt in Europe, Asia and the Americas. *P. ananatis* was described as an agent of leaf spot on maize in Brazil.

The second goal of this study was to identify and characterise the causal agent of brown stalk rot of maize observed in South Africa. Chapter 4 describes the identification of the bacteria causing this new disease of maize.

The third goal of this study was to characterise a collection of *Pantoea* strains from onion and maize using a polyphasic approach based on analyses of carbon source utilisation, physiological characteristics, fluorescent amplified fragment length polymorphism (F-AFLP), repetitive sequence based PCR (rep-PCR) genomic fingerprinting, 16S rRNA gene sequence analysis and DNA-DNA hybridisations. The results are presented in Chapter 5.

CHAPTER 1

The Genus Pantoea in Plant Pathology; Literature Review

INTRODUCTION

The genus *Pantoea* was established in 1989 (Gavini et al., 1989) and it is classified in the family *Enterobacteriaceae*. *Pantoea* species are associated with plants as pathogens, saprophytes and epiphytes (Cottyn et al., 2001; Gavini et al., 1989; Gitaitis et al., 2000; Grimont and Grimont, 2005; Iimura and Hosono, 1996; Perombelon, 1981; Walcott et al., 2002). Strains of *P. ananatis* and *P. agglomerans* were reported to cause diseases in humans (De Beare et al., 2004; Gavini et al., 1989; Kratz et al., 2003; Lim et al., 2006; Maki et al., 1976).

Bacteria belonging to the genus (Grimont and Grimont, 2005) are gram-negative, non-capsulated, non-spore forming straight rods; most are motile and are peritrichously flagellated; are facultatively anaerobic, oxidase negative and catalase positive; colonies on nutrient agar are round, smooth, with entire margins; colonies might be yellow, cream/white or beige. There are seven described species within the genus *Pantoea* and these include: *P. agglomerans, P. ananatis, P. citrea, P. dispersa, P. punctata, P. terrea* and *P. stewartii,* containing two subspecies, *indologenes* and *stewartii* (Grimont and Grimont, 2005).

Bacterial wilt of maize was the first plant disease ever reported caused by a bacterium of the genus *Pantoea*. The disease is known as Stewart's wilt, to honour F. C. Stewart, who described the symptoms in 1897 on sweet corn in the USA (Stewart, 1897). At that time, the bacterium causing the disease was named *Bacillus stewartii.* It was also known as: *Bacterium stewartii, Erwinia stewartii, Pseudobacterium stewartii, Pseudomonas stewartii, Phytobacterium stewartii* and *Xanthomonas stewartii* (Brenner et al., 1984). The pathogen has been responsible for serious crop loses since its first discovery (Coplin et al., 2002). In 1993, the bacterium was transferred to the genus *Pantoea* as *Pantoea stewartii* subsp. *stewartii* (Mergaert et al., 1993).

Although the pink disease of pineapple was originally described in 1915 in Hawaii (Lyon et al., 1915), the pathogen responsible for this disease remained unknown until 1997. Cha et al. (1997) identified the bacterium causing the pink disease as *Pantoea citrea,* using molecular techniques. In 1928, Serrano (1928) reported brown rot of pineapple fruitlets, caused by *P. ananatis*, also in Hawaii. In the same year, Brown (1928) described a bacterial pocket disease of sugar beet, caused by *P. agglomerans*. In 1934, *P. agglomerans* was reported to induce galls on *Gypsophila* (Brown, 1934).

During the following 47 years, only one report was published, in 1954, on *P. ananatis* parasitic on uredia of cereal rust (Pon et al., 1954). Since 1981, however, bacterial species within the genus *Pantoea* have become increasingly important as plant pathogens worldwide. Seventeen new diseases caused by *P. ananatis, P. agglomerans, P. punctata* and *P. stewartii* subsp. *indologenes* have been described on a variety of crops (Table 1), including beetroot (Burr et al., 1991), cantaloupe (Bruton et al., 1991), cotton (Medrano and Bell, 2006), *Eucalyptus* (Coutinho et al., 2002), honeydew melon (Wells et al., 1987), maize (Paccola-Meirelles et al., 2001), mandarin orange (Kageyama et al., 1992), millet (Frederickson, 1997), onion (Gitaitis and Gay, 1997), pear (Lindow et al., 1998), rice (Azegami et al., 1983; Cother et al., 2004) and sudangrass (Azad et al., 2000). Disease symptoms are diverse and include galls, rots, wilt, leaf blights, necrosis and spots, dieback and stem necrosis (Grimont and Grimont, 2005). In the case of infection of honeydew melons, pineapple and cantaloupe fruit, the symptoms occur only after harvest (Bruton et al., 1986; Wells et al., 1987; Bruton et al., 1991, Cha et al., 1997).

Despite increasing frequency of plant diseases caused by *Pantoea* species, identification and characterisation of these bacteria were usually based on colony morphology, few physiological and biochemical tests and sometimes fatty acids analyses. Only *P. ananatis* strains causing dieback of *Eucalyptus* (Coutinho et al., 2002) and stem necrosis of rice (Cother et al., 2004) have been identified based on phenotypic and genotypic characteristics. Consequently, relatively little is known about isolation, identification, host specificity, genetic relatedness or epidemiology of these bacterial pathogens.

This review focuses on the detection, pathogenicity, ecology and taxonomy of the bacteria belonging to the genus *Pantoea.* The role of *Pantoea* species in plant pathology is

demonstrated and a list of *Pantoea* species causing diseases is presented. The techniques used in the diagnosis of plant diseases, and the methods for the identification of bacterial pathogens are discussed.

THE GENUS *PANTOEA*

In the past, all phytopathogenic bacteria in the family *Enterobacteriaceae* belonged to the genus *Erwinia*. The genus was proposed by Winslow et al. (1917) for all plant-associated gram-negative, non-spore forming, fermentative, rod-shaped bacteria (Beji et al., 1988). In the 1960's, Dye divided the genus *Erwinia* into four "natural" groups, founded on the type of disease symptoms produced on plants (Verdonck et al., 1987): amylovora (Dye, 1968) causing dry necrotic or wilt diseases; carotovora (Dye, 1969a) inducing soft rots; herbicola (Dye, 1969b) containing saprophytes and putative plant pathogens and atypical erwinias (Dye, 1969c).

The Herbicola group, of interest in this review, consisted of *Erwinia* strains that usually produced a yellow pigment on general growth media, including nutrient agar (Dye, 1969b; Beji et al., 1998). In 1974, Lelliot (1974) included four species in the Herbicola group. These were: *Erwinia uredovora* (Dye, 1963b), *E. milletiae* (Dye, 1969b), *E. stewartii* (Dye, 1963a) and *E. herbicola* (*E. herbicola* var. *herbicola* and *E. herbicola* var. *ananas*) (Dye, 1969b).

In 1971, Herbicola bacteria were involved in a nosocomial septicemia outbreak in the United States, when 40 of 378 patients died (Maki et al., 1976; Brenner et al., 1984). After comparing human and plant isolates, Ewing and Fife (1972) concluded that all strains belonged to a single species, which were named *Enterobacter agglomerans.* That conclusion was confirmed in 1986 by examining the DNA relatedness among 86 strains of *Enterobacter agglomerans,* isolated from humans, and the type strains of *Erwinia herbicola* and *E. milletiae* (Lind and Ursing, 1986). However, phytopathologists preferred to use the nomenclature of Dye while medical bacteriologists used the nomenclature of Edwig and Fife (Beji et al., 1988). This group of organisms was referred too in the literature as the "Erwinia herbicola-Enterobacter agglomerans complex" (Grimont and Grimont, 2005).

In 1984, Brenner et al. (1984) studied 124 strains isolated from plants and humans that belonged to the "Erwinia herbicola-Enterobacter agglomerans complex". The results of the

DNA-DNA hybridisations showed that 90 of these strains formed 13 hybridisation groups, numbered I-XIII. The authors demonstrated the immense genetic diversity of these bacteria and inadequacy of the nomenclature used at the time.

In 1987, Verdonck et al. (1987) performed the numerical analysis of phenotypic features of 529 enterobacterial strains, including those assigned to the "Erwinia herbicola-Enterobacter agglomerans complex" and other *Erwinia* species. The strains received as *Enterobacter agglomerans, Erwinia herbicola, E. milletiae, E. ananas, E. uredovora* and *E. stewartii* were dispersed into 23 phenotypic groups or phena. However, the type strains of *Enterobacter agglomerans, Erwinia herbicola and E. milletiae* grouped into a single phenon 8, the type strains of *E. ananas* and *E. uredovora* into phenon 12 and eight strains of *E. stewartii* into phenon 29.

In 1988, Beji et al. (1988) studied the DNA relatedness of the type strain *Enterobacter agglomerans*, ATCC 27155, to 54 strains from the "Erwinia herbicola-Enterobacter agglomerans complex". These 54 strains represented phenons 7B and 8 of Verdonck et al. (1987) and hybridisation groups V and XIII of Brenner et al. (1984). Seventy-three reference strains belonging to different species of the family *Enterobacteriaceae* were also included. "Erwinia herbicola-Enterobacter agglomerans complex" strains produced seven distinct protein profiles and showed 62-97% DNA binding to the *Enterobacter agglomerans* type strain, forming the hybridisation group 27155. The group included the type strains of *Enterobacter agglomerans, Erwinia herbicola* and *E. milletiae*. The authors concluded that these species names are subjective synonyms.

In 1989, based on the research of Beji et al. (1988), a new genus, *Pantoea,* was proposed (Gavini et al., 1989), with the type species, *Pantoea agglomerans*. The new species accommodated the type strains of *Enterobacter agglomerans*, *Erwinia herbicola* and *E. milletiae*, as well as the other strains belonging to electrophoretic protein profile groups I and III to VI of Beji et al. (1988) and DNA hybridisation group XIII of Brenner et al. (1984). At the same time, the second species, *P. dispersa*, was proposed, for the strains of the DNA hybridisation group III of Brenner et al. (1984) and phenon 10 of Verdonck et al (1987).

In 1992, bacterial strains were isolated from fruit and soil samples in Japan. The strains were classified as three new species within the genus *Pantoea*, namely *P. citrea*, *P. punctata* and

P. terrea (Kageyama at al., 1992). Grimont and Grimont (2005) suggested that additional taxonomic research was necessary to validate the placement of these three species within the genus *Pantoea*". The suggestion was based on the phylogenetic analysis of the 16S rRNA gene sequences and the *rpoB* gene sequences of all *Pantoea* species. *P. citrea, P. punctata* and *P. terrea* formed a separate cluster in phylogenetic trees that "joined the cluster containing other described *Pantoea* species at a lower level" (Grimont and Grimont, 2005).

In 1993, *Erwinia ananas* and *Erwinia uredovora* were united as a single species and transferred to the genus *Pantoea* as *P. ananatis* (Mergaert at al., 1993). *P. ananatis* comprised strains belonging to the hybridisation group VI of Brenner et al. (1984) and phenon 12 of Verdonck et al. (1987). The species *Pantoea stewartii* (Mergaert at al., 1993) contained two groups of strains. The first group, received as *Erwinia stewartii*, was originally isolated from maize or from corn flea beetles and formed phenon 29 of Verdonck et al. (1987). The DNA binding values among these strains were 93 to 99% and they were named hybridisation group 2715. The second group, DNA hybridisation group 2632, included strains received as *Erwinia herbicola* and *E. ananas* that were part of phenon 13 of Verdonck et al. (1987), and were very similar biochemically to *P. ananatis*. The DNA binding values among the strains of these two groups were between 60 and 83% and they were assigned to two subspecies of *P. stewartii*: The hybridisation group 2715 was named *P. stewartii* subsp. *stewartii* and the hybridisation group 2632, *P. stewartii* subsp. *indologenes.*

There are also four hybridisation groups from a study by Brenner et al. (1984); groups I, II, IV and V; which according to the latest edition of the Bergey's Manual of Systematic Bacteriology, belong to the genus *Pantoea* (Grimont and Grimont, 2005), but have not yet been described.

PANTOEA SPECIES AS PLANT PATHOGENS

Members of the genus *Pantoea* are mainly plant-pathogenic and plant-associated bacteria. A list of diseases caused by these pathogens is presented in Table 1.

Table 1. The diseases of plants caused by bacteria belonging to the genus *Pantoea*.

Host and Pathogen	Disease Name	References
Baby's breath (*Gypsophila paniculata*)		
P. agglomerans	Galls	Brown, 1934 Cooksey, 1986
Beetroot (*Beta vulgaris*)		
P. agglomerans	Root galls	Brown, 1928 Burr et al., 1991
Cotton (*Gossypium hirsutum*)		
P. agglomerans	Seed and boll rot	Medrano & Bell, 2006
Eucalyptus		
P. ananatis	Bacterial blight and dieback	Coutinho et al., 2002
Foxtail millet (*Setaria italica*)		
P. stewartii subsp. indologenes	Leaf spot	Mergaert et al., 1993
Maize (*Zea mays*)		
P. stewartii subsp. *stewartii*	Stewart's wilt	Stewart, 1897
P. ananatis	Leaf spot	Paccola-Meirelles et al., 2001
Mandarin orange (*Citrus nobilis*)		
P. citrea	Pink disease	Kageyama et al., 1992
P. punctata	Brown spot	Kageyama et al., 1992
Melon and cantaloupe (*Cucumis melo*)		
P. ananatis	Soft rot	Bruton et al., 1986 Wells et al., 1989
	Postharvest rot	Bruton et al., 1991
Onion (*Allium cepa*)		
P. agglomerans	Leaf and seed stalk necrosis	Hattingh & Walters, 1981
	Leaf blight and bulb rot	Edens et al., 2006
P. ananatis	Leaf blight, seed stalk rot, and bulb decay,	Gitaitis & Gay, 1997 Schwartz & Otto, 2000

Host and Pathogen	Disease Name	References
	Center rot	Walcott et al., 2002
Pear (*Pyrus communis*)		
P. agglomerans	Russetting of pear fruits	Lindow et al., 1998
Pearl millet (*Penissetum glaucum*, *P americanum*)		
P. agglomerans	Necrosis of the leaf tips and margins	Frederickson, 1997
P. stewartii subsp. indologenes	Leaf spot	Mergaert et al., 1993
Pineapple (*Ananas cosmosus*)		
P. ananatis	Bacterial fruitlet brown rot	Serrano, 1928
P. citrea	Pink disease	Lyon, 1915 Kageyama et al., 1992 Cha et al., 1997
Rice (*Oryza sativa*)		
P. ananatis	Palea browning Stem necrosis	Azegami et al., 1983 Cother et al., 2004
Rust on cereals		
P. ananatis	Parasite on uredia	Pon et al., 1954
Sudangrass (*Sorghum sudanense*)		
P. ananatis & *P. stewartii* subsp. *indologenes*	Leaf blotch	Azad et al., 2000
Sugarcane (*Saccharum* spp.)		
P. ananatis	Soft rot	Serrano, 1928

Many plant pathogenic *Pantoea* species are seed-borne and seed-transmitted, such as *P. ananatis* in onion (Walcott et al., 2002), sudangrass (Azad et al., 2000), rice (Tabei et al., 1988), buckwheat (Iimura and Hosono, 1996) and *P. agglomerans* in cotton (Medrano and Bell, 2006). *P. stewartii* subsp. *stewartii*, the causal agent of Stewart's wilt of maize, is also seed-borne and many countries have banned the importation of maize seed, unless they are certified free of *P. stewartii* (Coplin et al, 2002).

Pantoea stewartii subsp. *stewartii* infects its host plant systemically. It multiplies quickly and produces exopolysaccharide materials in the vascular system. Wilting of maize plants is caused by the blockage of vessels by exopolysaccharides (Grimont and Grimont, 2005; Pepper, 1967). The corn flea beetle (*Chaetocnema publicaria*) is a host of *P. stewartii* subsp. *stewartii* during winter. Throughout the growing season, the pathogen is spread from plant to plant and from field to field by the beetle. The epidemics of Stewart's wilt usually occur after mild winters that facilitate the survival of the insect vector (Grimont and Grimont, 2005; OEPP/EPPO, 1978). *P. ananatis,* causing center rot of onion, is transmitted via tobacco thrips, *Frankliniella fusca* (Gitaitis *et al.*, 2003; Wells *et al.*, 2002*). P. ananatis* has also been reported as a gut inhabitant of brown planthoppers, *Nilaparvata lugens* (Watanabe et al., 1996) and mulberry pyralids, *Glyphodes pyloalis* (Watanabe and Sato, 1999), but none of these insects are a vector of the pathogen.

The tumorigenic *P. agglomerans*, which induces galls on *Gypsophila* and beetroot and *P. citrea* causing pink disease of pineapple, are spread mechanically and through infected plant propagation material (Cooksey, 1986; Burr et al., 1991; Cha et al., 1997). *P. agglomerans* strains that produce plant hormones may also induce diseases. For example, *P. agglomerans* strains producing indole acetic acid and cytokinin (Guo et al., 2001) cause galls formation on *Gypsophila* (Manulis et al., 1998) and russeting of pear fruits (Lindow et al., 1998). Some strains of indoleacetic acid-producing *P. agglomerans* increase the severity of the olive knot disease, caused by *Pseudomonas savastanoi* pv. *savastanoi* (Marchi et al., 2006).

Some *P. agglomerans* and *P. ananatis* strains are ice nucleation active (Hirano and Upper, 2000). The ice nucleation active bacteria, when present on plant surfaces, increase the chances of frost injury at subzero temperatures (Hirano and Upper, 1995; Hirano and Upper, 2000; Lindow et al., 1982). *P. agglomerans*, found as an epiphyte on leaves of maize, was reported to enhance the frost injury in this host (Lindow et al., 1978). The ice nucleation active *P. ananatis* was isolated from the gemmisphere of tea, phyllosphere of many vegetables, the flowers of magnolia (Goto et al., 1988; Goto et al., 1993) as well as from wheat and barley (Newton and Hayward, 1986).

PANTOEA SPECIES AS BENEFICIAL MICROORGANISMS IN AGRICULTURE

Not all *Pantoea* strains are plant pathogens. Many strains have been used for biological control of other plant pathogens. In general, "an effective biological control agent has to be able to live and multiply in the same ecological niche as the pathogen" (Özactan and Bora, 2004; Pusey, 1997; Vanneste et al., 1999). There are two main mechanisms, by which biological control strains hamper the growth of pathogens.

The first mechanism is competition for sites, growth space and growth-limiting nutrients. *P. agglomerans* strains were reported to suppress the fire blight pathogen, *Erwinia amylovora*, in pear fruits and apple blossoms (Beer et al., 1984; Özactan and Bora, 2004). Application of naturally occurring Ice $^{-}$ strains of, among others, *P. agglomerans*, significantly reduced frost injury to sensitive crops by (Hirano and Upper, 2000; Lindow, 1982; Lindow et al., 1983; Wilson and Lindow, 1994). The post-harvest control of blue mould on pome fruits, caused by *Penicillum expansum* was achieved by application of epiphytic *P. agglomerans* and *P. ananatis* isolated from the fruits and leaf surfaces of apples and pears (Nunes et al., 2001; Torres et al., 2005).

The second biocontrol mechanism is production of bio-fungicides and bio-bactericides that inhibit the growth of pathogens. A strain of *P. dispersa* producing chitinase has been used as a biocontrol agent against fungal plant pathogens (Gohel et al., 2004). A strain of *P. agglomerans* is effective in controlling many banana pathogens (Gunasinghe et al., 2004). *P. agglomerans* strains control damping-off caused by *Phythium* species on canola, safflower, dry pea and sugar beet (Barding et al., 2003). A strain of a *Pantoea* species was reported to induce resistance in cucumber against *Pseudomonas syringae* pv. *lachrymans*, the agent of angular leaf spot (Hoon et al., 2006). A strain of *P. citrea* that contains the gene for albicdin detoxification has been reported to attenuate the pathogenicity of *Xanthomonas albilineans* to sugarcane (Zhang and Birch, 1997a). The gene is a promising candidate to transfer into sugarcane to confer a form of resistance against leaf scald (Zhang and Birch, 1997b).

Many researchers isolated and identified free-leaving nitrogen-fixing bacteria from leaves and stems of diverse plants (Hirano and Upper, 2000; Ladha et al., 1983; Ruinen, 1975; Sprent and Sprent, 1990). In 2004, a nitrogen-fixing *Pantoea* sp. was isolated from sugarcane in Cuba that may be valuable in agriculture (Loiret et al., 2004). The strain of *P.*

agglomerans, isolated from the rhizosphere of wheat in Morocco, had a positive effect on the aggregation and macroporosity of rhizosphere soil. The ultimate goal of the researchers is to use that exopolysaccharide-producing bacterium as inoculant in wheat fields to regulate water stress and, therefore, improve yields (Amellal et al., 1998).

ISOLATION OF *PANTOEA* SPECIES FROM PLANTS

Plant disease diagnosis and the identification of pathogen consist of several steps and these usually include: isolation of a pathogen from diseased plant tissues; obtaining a pure culture of a suspected pathogen; microscopic examination, for example to determine the Gram-stain reaction; serology, if a pathogen-specific antibodies are available; phenotypic tests including nutritional, physiological and biochemical characterisation and DNA-based identification (Alvarez, 2004; Goszczynska et al., 2000; Houpikian and Raoult, 2002).

Alvarez (2004) and Houpikian and Raoult (2002) wrote that although DNA-based techniques have become indispensable in the detection and identification of bacterial pathogens, they could not completely replace other, more traditional methods. For example, isolation of bacteria on agar media is crucial in plant pathology. A pure culture of a pathogen allows studying both known and emerging bacterial diseases. Purified bacterial strains can be characterised in detail and easily compared with similar strains isolated by other researchers. Isolation and obtaining a pure culture of bacteria remains "the ultimate goal of pathogen identification" (Houpikian and Raoult, 2002).

Majority of plant pathogenic bacteria, including *Pantoea* species, can be easily isolated from diseased tissues, especially from freshly collected samples (Coplin et al., 2002; Goszczynska et al., 2000). When a new disease is observed, isolations should be done from many plants, to make sure that the particular bacterium is associated with a disease (Alvarez, 2004). Azad et al. (2000), for instance, used 151 affected plants in their study while investigating a new leaf blotch disease of sudangrass, caused by two *Pantoea* species.

Media specifically selective for *Pantoea* strains are not yet available, but all media designed for the isolation *of Enterobacteriaceae* can be used for the isolation of *Pantoea* species and these include: MacConkey agar, Drigalski lactose agar, blood agar and Luria-Bertani (LB) agar (Grimont and Grimont, 2005). Although LB has been the most popular agar medium for

isolation of human pathogens, plant pathologists preferred nutrient agar (NA). Most of the plant pathogenic *Pantoea* species, in the first reports, were isolated on NA, including *P. agglomerans* from onion (Hattingh and Walters, 1981) and *P. ananatis* from *Eucalyptus* (Coutinho et al., 2002).

Isolation of *P. ananatis* from onion seed on NA is difficult due to abundant growth of saprophytes. Walcott et al. (2002) tried to solve this problem by using a polyclonal antiserum against *P. ananatis*. Seed extracts were incubated with immunomagnetic beads coated with the antiserum to immuno-bind the pathogen. Then the beads with bound *P. ananatis* were plated on NA. However, 64% of colonies recovered from seed lots by immuno-plating were not *P. ananatis*.

DETERMINATION OF PATHOGENICITY

Determination of pathogenicity and fulfilment of Koch's postulates is a crucial step in the identification of plant pathogenic bacteria. Pathogenicity tests are not standardised and are dependant on the host-pathogen combination (Goszczynska et al., 2000). Many authors used several different inoculation methods to confirm pathogenicity of *Pantoea* species associated with disease symptoms.

Coutinho et al. (2002) employed two inoculation techniques for *P. ananatis* causing bacterial blight and dieback of *Eucalyptus*. Three-month-old *Eucalyptus* plants were used in pathogenicity tests. A fine needle was dipped into a bacterial suspension and then gently inserted into the surface of young leaves and into the petioles of young leaves. All plants were incubated at temperatures between 20 and 23^{o}C and relative humidity between 80 and 90%.

Paccola-Meirelles et al. (2001) investigating leaf spot of maize, also used two inoculation methods, but tests were performed on 15-, 30-, and 45-day old plants. In the first method, a bacterial suspension was sprayed onto leaves after carefully wounding them with carborundum or puncturing them with a sterile needle. In the second method, the bacterial suspension was injected into leaves with a syringe and 26-gauge needle.

Azad et al. (2000) inoculated sudangrass using five methods: suspensions were sprayed onto leaf surfaces, infiltrated into leaf tissues with a syringe, injected into stems, mixed with an abrasive material and applied to leaves with a cloth and bacterial colonies were applied directly to wounds made in leaves and stems. Three plant growth conditions were tested for each inoculation method, which varied in temperature and relative humidity. All inoculation methods and growth conditions were favourable for development of symptoms. The infection was most rapid, however, when plants were inoculated by infiltration of bacterial suspensions into the tissues, and by direct application of bacterial colonies to needle induced wounds. Symptoms appeared 2-3 days after inoculation on such plants when they were kept in a growth chamber with a constant temperature of 32^{o}C. In contrast, in a greenhouse with a lower temperature (20^{o}C), symptoms were not visible until 17 days after inoculation. Cother et al. (2004) reported that stem necrosis developed in rice following inoculation of inflorescences and stems. No lesions were present on spray-inoculated leaves.

PHENOTYPIC IDENTIFICATION OF *PANTOEA* SPECIES

If a bacterium induces disease symptoms in pathogenicity tests, computerised commercial identification systems can be employed for identification. Examples include: metabolic tests, API 20E and API 50CHE (BioMérieux, La Balme les Grottes, Montalieu Vercieu, France); substrate utilisation, Biolog (Biolog, Inc., Hayward, CA) and Pheno 100 (BioMérieux) and analysis of fatty methyl esters (MIDI, Newark, DE). The obtained results are compared to that in the built-in database, and the name of the species name is given by the programme. Alvarez (2004) cautioned that the phenotypic identification systems are not always correct, as not all bacterial species are included in the databases. This is particularly true in the case of *Pantoea* species.

The phenotypic characteristics of named species and unnamed hybridisation groups of *Pantoea* are summarised in the latest edition of the Bergey's Manual of Systematic Bacteriology (Grimont and Grimont, 2005). The differentiation between *Pantoea* species based solely on phenotypic characteristics is sometimes difficult. For example, *P. agglomerans* and *P. dispersa* could be distinguished by only two tests, the ability to utilise malonate and erythitol (Gavini et al, 1989). *P. ananatis* and *P. stewartii* subsp. *indologenes*, two indole producing species of the genus, are only differentiated by the ability to produce acid from sorbitol and α-methyl-D-mannoside (Mergaert et al., 1993).

Tumorigenic *P. agglomerans* from *Gypsophila* and table beet (Burr et al., 1991; Cooksey et al., 1986), *P. ananatis* associated with a leaf spot disease of maize (Paccola-Meirelles et al., 2001), *P. ananatis* and *P. stewartii* causing leaf blotch on sudangrass (Azad et al., 2000) and *P. agglomerans* isolated from pearl millet (Frederickson, 1997), were identified solely by selected biochemical and physiological characteristics. Walcott et al. (2002) confirmed *P. ananatis* isolated from onion seed by the Hugh-Leifson assay, indole test and fatty acid methyl ester analysis.

DETECTION OF PLANT PATHOGENIC BACTERIA USING THE POLYMERASE CHAIN REACTION (PCR)

The first report about the polymerase chain reaction (PCR), titled "Enzymatic amplification of beta-globulin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia" was published by Saiki et al. (1985). Two years later, Mullis and Falona (1987) published the paper "Specific synthesis of DNA in vitro via a polymerase chain reaction". In 1988, Saiki et al. (1988) reported about the "Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase" allowing them to make unlimited copies of a fragment of DNA. The PCR technique revolutionised research in wide-ranging fields of the biological sciences (Babalola, 2003). The impact of PCR on biology could be compared with the unravelling the structure of DNA and decoding of the genetic code. In 1995, K. Mullis received the Nobel Price in chemistry for the discovery of PCR.

In the field of phytobacteriology, numerous research groups have reported the development of pathogen specific primers, allowing detection and identification of these pathogens in heterogeneous mixtures (Alvarez, 2004). Several primers pairs were developed for the specific detection and identification of *P. stewartii subsp. stewartii* in plant material (Blakemore et al., 1992; Blakemore et al., 1999; Coplin et al., 2002). The pathogen was easily detected in the tissue extracts from infected plants. The minimum number of *P. stewartii* cells needed for the detection was 200 CFU (Coplin et al., 2002).

The PCR could be inhibited by many compounds present in the plant and seed extracts (Alvarez, 2004; Schaad et al., 1995). Immunocapture or immunomagnetic separation (IMS) has been used to overcome this problem. The role of the antibody in the IMS-PCR is to

capture the pathogen from a plant extract containing mixture of bacteria and PCR-inhibitory substances. The IMS-PCR assay developed to detect *P. ananatis* in onion seed, demonstrated detection and recovery of 10^1 to 10^4 CFU/ml of spiked seed wash (Walcott et al., 2002). Schaad et al. (1995) developed the "Bio-PCR" to enhance the sensitivity of PCR reaction. Bio-PCR detects living cells of pathogens, those that could cause a disease, as bacterial colonies are picked up from agar plates preceding the PCR reaction. A plant or seed extract is plated on semi-selective media, plates are incubated for two days (longer incubation time is required for the slow-growing bacteria) and then the bacterial growth is removed from the agar plates and suspended in sterile distilled water. This bacterial suspension is used as a template in the PCR with primers targeting specific pathogen.

In the early 1990's, Higuchi et al. (1992) demonstrated the simultaneous amplification and detection of specific DNA sequences. The technique, named the "real-time PCR" (Higuchi et al., 1993), consists of "the amplification of DNA by PCR that is monitored using fluorescent technology, while the amplification is occurring" (Valasek and Repa, 2005). This method is: extremely sensitive; detects less than five copies of a target sequence; very quick, results are obtained within 30-50 min; performed in a close reaction environment so the chances for cross contaminations are minimised (Valasek and Repa, 2005). Many researchers pointed out that recent advances in the molecular-based diagnostic techniques, especially the real-time PCR, are invaluable in early and rapid detection of pathogens in propagation material, seed and diseased crops. Correct phytosanitary measures can be implemented at once, contributing to the crop biosecurity (Martin et al., 2000; Schaad et al., 2003; Strange and Scott, 2005).

DNA FINGERPRINTING-BASED METHODS USED FOR IDENTIFICATION AND TAXONOMY OF BACTERIA

The DNA-based, fingerprinting methods used for identification and taxonomy of bacteria can be divided into three groups. The first group is the restriction fragment analysis based on the digestion of the whole genome DNA with restriction enzymes. Examples include restriction fragment length polymorphisms (RFLP) and pulsed-field gel electrophoresis (PFGE). PCR-based typing techniques comprise the second group of fingerprinting methods with random amplified polymorphic DNA (RAPD) and repetitive sequence based PCR (Rep-PCR). Amplified fragment length polymorphism (AFLP) combines the PCR and the

digestion of DNA with restriction enzymes. All these techniques have been used by countless research groups for identification, differentiation and classification of almost every cultivable bacterial group.

The perfect genomic fingerprinting technique should be applicable to all bacterial strains to be studied, reproducible, highly discriminatory, easy to do and fast (Olive and Bean, 1999; Vandamme et al., 1996). Genomic fingerprints obtained by using these techniques are usually complex and computerised analysis is necessary to interpret the results and to compare a large number of strains (de Bruijn et al., 1996; Gürtler and Mayall, 2001; Olive and Bean, 1999; Rademaker and de Bruijn, 1997; Savelkoul et al., 1999). One of the best software available for such analysis is BioNumerics (Applied Maths, Kortrijk, Belgium). None of the fingerprinting methods is perfect, of course, but some are more suitable for specific bacterial taxa than others are.

Restriction fragment length polymorphism (RFLP). The restriction fragment analysis consists of four stages: extraction of the whole-genome DNA, digestion of DNA with restriction enzyme or enzymes, agarose gel electrophoresis of DNA fragments and interpretation of results (Vandamme et al., 1996). The selection of restriction enzymes and the digestion conditions has to be determined experimentally for the group of bacteria to be studied. Additionally, the obtained patterns of DNA fragments are usually complicated, difficult to compare and not always reliable (Vandamme et al., 1996). Despite these obvious drawbacks, RFLP was used successfully in 1989 in Australia, for identification of *Ralstonia solanacearum* race 2, the causal agent of Moko disease of banana (Hayward, 1996; Hyde et al., 1992).

Pulsed-field gel electrophoresis (PFGE) also known as the low-frequency restriction fragment analysis. PFGE is regarded as the most discriminatory fingerprinting method available (Struelens et al., 2001). The PFGE technique is an improved RFLP. There are many differences, contributing to the enhancement in reproducibility, reliability and discrimination power (Olive and Bean, 1999; Trenover et al., 1995; Vandamme et al., 1996). The DNA is not extracted from the cells by conventional techniques. Bacterial strains are cultivated on an appropriate agar medium or in a broth and mixed with molten agarose to form “agarose plugs”. Bacterial cells in agarose plugs are lysed and digested with restriction enzymes *in situ*. The restriction enzymes are cutting DNA infrequently (recognise specific

combination of six to eight bases). Consequently, the number of obtained DNA fragments is smaller than in conventional RFLP, but they are large (10 to 800 kb). Special electrophoretic techniques are used to separate large DNA fragments, known as pulsed-field gel electrophoresis (PFGE).

The PFGE procedure is simple to perform and the results are reproducible, but it is time consuming and therefore, not convenient for assessment of the large number of strains (Olive and Bean, 1999). Despite the time factor the PFGE remains the "gold standard" of DNA-based typing (Olive and Bean, 1999). Gürtler and Mayall (2001) were concerned about growing evidence regarding the negative effect of mobile genetic elements on the determination of bacterial relatedness by PFGE. Plasmids and transposons present in some strains, but absent in others of the same species, may contain restriction sites for infrequent cutting enzymes, for example, up to seven *SmaI* sites (Thal et al., 1997). PFGE patterns of such strains were different, but still the strains belonged to a single species.

PFGE was evaluated to confirm the identity of *P. stewartii* subsp. *stewartii* (Coplin et al., 2002). After DNA digestion with *Spe*I and *Xba*I restriction enzymes, *P. stewartii* strains could be easily distinguished from related *Erwinia* and *Pantoea* species and from each other. PFGE analysis of *P. stewartii* revealed many common bands among strains from different geographic regions. The genome of *P. stewartii* strains was highly conserved (similarity from 60 to 100%) based on these common fragments. On the other hand, there was sufficient divergence in the PFGE profiles to differentiate between *P. stewartii* strains from different geographical regions, showing that the technique also may be a useful tool for population genetics and epidemiological studies.

Random amplified polymorphic DNA (RAPD) assay. The RAPD fingerprinting is PCR-based. The technique was named by its developers as an arbitrary primed PCR (Welsh and McCleland, 1990; Williams et al., 1990). In RAPD, short (about 10 bases long), random primers are used to produce genomic fingerprints of bacterial strains. The primers that generate the best pattern for identification or discrimination of the studied bacteria have to be selected empirically (Olive and Bean, 1999). Trebaol et al. (2001) used 340 RAPD primers to investigate the genetic diversity in *Xanthomonas cynarae*, causing bacterial bract spot of artichoke. Among these 340 primers tested, only 40 produced reproducible and reliable fingerprints.

As the RAPD primers are arbitrary (not complimentary to any specific locus on the genome), the technique is very sensitive to changes in the reaction conditions, including the annealing temperature and reagents. That sensitivity makes RAPD banding patterns difficult to reproduce (Meunier et al., 1993; Olive and Bean, 1999; Welsh and McClelland, 1990). The RAPD technique is relatively easy to execute, quick and does not require expensive laboratory equipment. Any laboratory owning a thermocycler and an electrophoresis apparatus can do it. Hence, many studies contributed to standardisation of the procedure.

Vogel et al. (1999) performed RAPD typing of *Klebsiella pneumoniae*, *K. oxytoca, Serratia marcescens* and *Pseudomonas aeruginosa* isolates using standardised reagents. The use of Ready-To-Go RAPD Analysis beads resulted in reproducible and stable banding patterns with a high discriminatory capacity. A sequencing primer, M13 used in RAPD fingerprinting assays also allows for standardisation of the procedure (Olive and Bean, 1999; Vila et al., 1996). In 2005, Rosetti and Giraffa (2005) reported that the strains of dairy lactic acid bacteria are rapidly identified by M13-generated, RAPD-PCR databases.

A web-based database for the provisional identification of bacterial species using only genotypes was developed in Japan (Watanabe et al., 2002). The PCR reactions with DNA of all kinds of bacteria are performed using a set of four RAPD primers so all species can be compared on the same platform. The PCR products are separated, using a temperature-gradient gel electrophoresis and the images are processed to generate species-identification dots, named spiddos. The protocol was standardised to make the system reproducible and reliable.

Repetitive sequence based PCR (Rep-PCR). The rep-PCR genomic fingerprints are produced by performing PCR with primers complimentary to repetitive DNA elements present within bacterial genomes. The PCR amplifies genomic regions located between the repetitive sequences, generating reproducible patterns characteristics for examined bacterial strains (de Bruijn et al., 1996; Versalovic et al., 1991). There are several families of repetitive sequences (de Bruijn, 1992; Versalovic et al., 1991; Versalovic et al., 1994), but three of them have been used extensively to characterise diverse bacterial species. These are (de Bruijn et al., 1996; Olive and Bean, 1999): the 35-40 bp repetitive extragenic palindromic (REP) sequence (Stern et al., 1984); the 124-127 bp enterobacterial repetitive

intergenic consensus (ERIC) sequence (Hulton et al., 1991) and the 154 bp BOX element (Koeuth et al., 1995).

The discriminatory power of rep-PCR increases when strains are analysed with multiple sets of primers (Olive and Bean, 1999; Rademaker et al., 2000). Rep-PCR has only slightly less discriminatory power than PFGE (Barbier et al., 1996; Georghiou et al., 1995; Liu and Wu, 1997; Weigel et al., 2004), but unlike PFGE, is simple, quick and inexpensive. Olive and Bean (1999) wrote that rep-PCR "is becoming the most widely used method of DNA typing". Purification of DNA from bacterial cells prior to rep-PCR is not essential. Many researchers obtained identical patterns using broth cultures, single colonies, extract from lesions and purified DNA as templates for the reaction (de Bruijn et al., 1996).

Rep-PCR has been used to differentiate and identify, among many others, strains of *Escherichia coli* from water (dos Anjos Borges et al., 2003), soft-rot *Erwinia* from ornamental plants (Norman et al., 2003), *Serratia marcencens* causing the cucurbit yellow wine disease (Zhang et al., 2003) and *Rhizobium meliloti* (de Bruiin, 1992). A database of rep-PCR patterns of a large collection of *Xanthomonas* isolates coupled to a computer assisted phylogenetic analysis, has been used as a tool for strain diagnosis (de Bruijn et al., 1996; Rademaker and de Bruijn, 1997).

Amplified fragment length polymorphism (AFLP). In 1993, Zabeau and Vos, (1993), patented a new technique for DNA fingerprinting of plant genomes, the selective restriction fragment amplification. Vos et al. (1995) described the method in details and named it the amplified fragment length polymorphism (AFLP). AFLP was adapted and optimised for the analysis of bacterial genomes and for the identification of bacteria by Janssen et al. (1996).

The AFLP protocol is quite complicated and contains several stages (Janssen et al., 1996; Kassama et al., 2002; Olive and Bean, 1999; Savelkoul et al., 1999; Vandamme et al., 1996). Extraction and purification of DNA from pure bacterial cultures is essential, because the AFLP fingerprints produced by the direct use of DNA from e.g. soil are not instructive. The DNA (about 100 ng) is digested with two restriction enzymes to produce DNA fragments with two types of "sticky ends", corresponding to the restriction enzymes used. One enzyme is a frequent cutter, for example *Mse*I, recognising a 4 bp restriction site. The second enzyme recognises a 6 bp site (not so frequent cutter), *Eco*RI being the most popular. Short

nucleotides (adapters) are then ligated to the restriction fragments. These adapters contain a restriction site complementary to a "sticky end" and a sequence homologous to a primer for a subsequent PCR. Pre-amplification PCR is performed with primers complimentary to the sequences of an adapter and a restriction site. Pre-amplification PCR amplifies only restriction fragments that have an adapter ligated to both ends. Selective PCR amplification is done with selective primers that are complementary to the restriction sites and have one to three selective nucleotides at their 3'-ends. A selective primer complimentary to the *Mse*I with two selective nucleotides GG will amplify only the *Mse*I sites flanked by the CC nucleotides, thus reducing the complexity of DNA fingerprints. One of the selective primers, containing the normal frequency restriction site (*Eco*RI for example) is labelled with a fluorescent dye, to visualise obtained fingerprints on gels. The PCR products are separated by a polyacrylamide gel electrophoresis and visualised by using a laser light.

The discriminatory power of AFLP is equal to that of PFGE for most bacterial taxa (Jureen et al., 2004; Keto-Timonen et al., 2003; Lindstedt et al., 2000, Olive and Bean, 1999). Keto-Timonen et al. (2003) reported that AFLP was faster than and not as laborious as PFGE when applied to the discrimination of *Listeria* isolates. In some cases, for example in differentiation of *Streptococcus pyogenes* strains (Desai et al., 1998), and recognition of individual strains of *Xanthomonas axonopodis* (Janssen et al., 1996), AFLP was superior to PFGE (Savelkoul et al., 1999).

Countless researchers have used AFLP analysis in bacterial studies. The technique was applied to the identification and taxonomy of the soft rot bacteria *Erwinia carotovora* (syn. *Pectobacterium carotovorum* subsp. *carotovorum*) and *E. chrysanthemi* (syn. *Dickeya* spp) (Avrova et al., 2002). AFLP analysis of *Klebsiella pneumoniae*, K. *oxytoca* and other *Klebsiella* species allowed the discrimination of the species within the genus and recognition of epidemiologically non-related isolates (Jonas et al., 2004). Kassama et al. (2002) used AFLP for identification of bacterial isolates from urinary tract infections. The relatedness of 69 bacteria was determined by cluster analysis of the AFLP-generated fingerprints. The bacteria grouped into eight clusters, corresponding to eight bacterial species. Brady (2005) developed an AFLP-based typing system for the genus *Pantoea*. Seventy-nine strains included in the study, both reference strains of *Pantoea* species and suspected *Pantoea* from *Eucalyptus*, formed 15 distinct clusters in the dendrogram derived from the fingerprint data.

As the genus *Pantoea* contains seven species, the results indicated that some strains could belong to new, yet undescribed species.

AFLP and rep-PCR genomic fingerprinting techniques were compared to DNA-DNA hybridisation studies (Rademaker et al., 2000). AFLP and rep-PCR genomic fingerprints (rep-PCR with three different primers sets) were produced for 178 *Xanthomonas* strains, belonging to 20 defined DNA-DNA homology groups within *Xanthomonas*. The authors calculated similarity values obtained from rep-PCR and AFLP generated fingerprints and compared them with the DNA-DNA homology values. The similarity values were highly correlated, suggesting that genomic fingerprinting using AFLP and rep-PCR shows real genotypic and phylogenetic relationships among *Xanthomonas*. The study gave evidence that rep-PCR and AFLP could be used for identification of bacterial strains, studying the taxonomic diversity of bacterial groups and determination of phylogenetic structure of bacterial populations (Rademaker et al., 2000).

Other DNA-based typing methods. Apart from RFLP, PFGE, RAPD, rep-PCR and AFLP, the introduction of molecular biological techniques into the microbiology laboratory yielded a large variety of other DNA-based typing methods. Restriction fragment analysis of plasmids, PCR-based locus specific RFLP, ribotyping, cleavase fragment length polymorphism have all been used and evaluated as tools for taxonomic investigations (Gürter and Mayall, 2001; Houpikian and Raoult, 2004; Louws et al., 1999; Olive and Bean, 1999; Rademaker et al., 2000; Vandamme et al., 1996; Versalovic and Lupski 2002).

16S rRNA GENE SEQUENCE ANALYSIS

In the 1970's, Sanger, Gilbert and Maxam developed a technique for DNA sequencing (Broughton, 2003). Today, almost 40 years later, it is difficult to imagine biological sciences without DNA sequences. There are over 400 000 sequences of the 16S rRNA gene available in the GenBank, the largest database of nucleotide sequences (National Center for Biotechnology Information, U.S. National Institute of Health, Bethesda, MD). Since the landmark studies by Woese in 1970's (Woese et al., 1975) and 1980's (Woese et al., 1985; Woese, 1987), the 16S rRNA gene sequence has become an essential tool for bacterial classification and identification (Clarridge, 2004; Vandamme et al., 1996). Clarridge (2004)

compared the impact of the 16S rRNA gene sequence on bacterial taxonomy with that of the Gram-stain, developed in 1884 by C. Gram (Broughton, 2003).

The 16S rRNA gene supports protein synthesis in bacteria, a fundamental element of every cell function (Noller et al., 2005). The 16S rRNA gene (Clarridge, 2004) is present in all bacteria, the sequence is approximately 1,550 bp long, is the most conserved bacterial gene, contains both conserved and variable regions, variable regions are used for the comparative taxonomy and is easily amplified by PCR using universal primers (Weisburg et al., 1991). The gene could be amplified directly from diseased tissue, allowing characterisation and identification of bacteria that are difficult or impossible to culture (Avilla et al., 1998; Houpikian and Raoult, 2002; Relman, 1999)

Kwon et al. (1997) and Hauben et al. (1998) showed, using 16S rDNA sequences of plant associated strains, representing *Erwinia, Pantoea* and other *Enterobacteriaceae*, that three phylogenetic groups exist within *Pantoea*. *Pantoea* species group in a monophyletic unit that is closely related to the true *Erwinia* species. The results indicated that 16S rDNA sequences could be used to differentiate and identify bacteria of the family *Enterobacteriaceae*, including the genus *Pantoea*.

The 16S rDNA sequences have been used, together with phenotypic characterisation, in identification of *P. ananatis* causing stem necrosis of rice (Azad et al., 2000), *P. ananatis* and *P. agglomerans* isolated from human hosts (De Beare et al. 2004; Kratz et al., 2003), and *Pantoea* species from the environment (Loiret et al., 2004; Torres et al., 2005). Coutinho et al. (2002) showed that bacteria causing bacterial blight and dieback of *Eucalyptus* are *P. ananatis*, by, among other methods, the analysis of the 16S rRNA gene.

The 16S rDNA sequence is sometimes inadequate for the identification of closely related bacterial species because of: possible lateral transfers within the gene, the highly conserved nature of the gene and multiple copies of the 16S rRNA gene within a single cell (Broughton, 2003; Cillia et al., 1996; Keswani and Whitman, 2001). Alternatively, several other genes have been examined for their potential as phylogenetic markers. These include the *recA* gene involved in recombination and DNA repair (Waleron et al., 2002), the *groE*, which encodes stress proteins (Harada and Ishikawa, 1997), and the *gyrB* gene encoding the ATPase domain of DNA gyrase (Dauga, 2002).

MULTI-LOCUS SEQUENCE ANALYSIS (MLSA)

Analysis of a single gene sequence does not always lead to correct identification of a bacterial isolate. Van Berkum et al. (2003) pointed out that phylogeny of a gene is phylogeny of that gene, not of its host. The authors observed inconsistency in the 16S rRNA, ITS and 23S rRNA-derived phylogenetic relationships among bacterial strains of *Rhizobiaceae*. Fukushima et al. (2002) evaluated the *gyrB* and 16S rRNA sequences of *Salmonella, Shigella* and *Escherichia coli* for identification purposes. The classification of bacterial strains obtained by the 16S rRNA sequences analysis was different from that determined by the analysis of the gyrB gene sequences.

"The ad hoc committee for the re-evaluation of the species definition in bacteriology" proposed that not one, but at least five genes sequences of any given strain should be analysed, to obtain a phylogenetic data for species identification (Stackebrandt et al., 2002). Such genes should be: protein coding (housekeeping genes), located at diverse chromosomal loci, present in a single copy, not prone to recombination and widely distributed among bacterial taxa (Stackebrandt et al., 2002; Zeigler, 2003).

The multi-locus sequence analysis (MLSA), a "method for the genotyping characterisation of diverse groups of prokaryotes" (Gevers et al., 2005), stems from the multi-locus sequence typing (MLST), a "method for the genotyping characterisation of prokaryotes at the infraspecific level" (Gevers et al., 2005). MLST was developed to identify hypervirulent lineages of *Neisseria meningitides* (Maiden et al., 1998). This approach is used in clinical epidemiology for molecular typing of strains belonging to a single, described species (Gevers et al., 2005; Singh et al., 2006). The purpose of MLSA is to select the genes, which combined sequences, when analysed, could clearly divide the strains belonging to a genus into separate, previously described species. The division of strains into species obtained by MLSA should be congruent with other methods, specifically with the DNA-DNA hybridisation (Gevers et al., 2005).

Zeigler (2003) compared 49 sequences of complete bacterial genomes belonging to 23 described species to find the genes valuable for predicting relatedness of whole genomes in bacteria. He selected 32 protein-encoding genes applying the following criteria: genes were

present in all 49 genomes, only one copy of the gene was present in each of the genomes, the genes were "unique" (no close paralogues to confuse analysis), the gene sequence had to be long enough to include informative phylogenetic data and short enough for routine sequencing. The 16S rRNA gene was included as the most popular in phylogenetic studies. From the pool of 32 protein-coding genes, only eight showed an excellent ability for a species prediction. The best two genes for identification of diverse bacteria to a genus and species level were *rec*N, a recombination and repair protein-encoding gene and *dna*X, a gene encoding two subunits of DNA polymerase III. The 16S rRNA gene sequence had the poorest ability in assigning bacterial strains to the correct species.

Naser et al. (2005) examined the usefulness of RNA polymerase α subunit (*rpo*A) and phenylalanyl –tRNA synthase (*phe*S) gene sequences as a tool for identification of enterococci. Ninety-six representative strains comprising all currently described *Enterococcus* species were analysed. All species were clearly differentiated based on their *rpo*A and *phe*S sequences. The author concluded that these two genes could be successfully used for identification of *Enterococcus.* Richter et al. (2006) sequenced seven genes of various strains within *Borrelia burgdorferi sensu lato.* Only the strains for which DNA-DNA hybridisation values were available in the literature were selected. The delineation obtained with MLSA was fully congruent with that established by DNA-DNA reassociations. The results also revealed an existence of a novel species, *Borrelia spielmanii* sp. nov.

INTEGRATION OF VARIOUS METHODS FOR A POLYPHASIC IDENDIFICATION

Identification means assigning an unknown bacterial strain into one of the existing taxonomic groups (Vandamme et al., 1996). The basic unit of bacterial taxonomy is the species.

> The species is defined as "a group of strains with approximately 70% or greater DNA-DNA relatedness and with 5°C or less ΔT_m" (Wayne et al., 1987).

This definition is regarded the "golden rule" in delineation of bacterial species (Vandamme et al., 1996). However, the DNA-DNA hybridisation is difficult to perform, slow, reproducibility between laboratories is questionable, the data cannot be stored in databases

and each experiment requires the inclusion of sometimes several reference strains (Stackebrandt, 2002). Rosselló-Mora and Amann (2001) provided more universal and not as rigid definition:

> The species is "a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity in many independent characteristics, and is diagnosable by a discriminative phenotypic property" (Rosselló-Mora and Amann, 2001).

The definition calls for using a polyphasic approach, including both phenotypic and genotypic techniques, to identify a bacterial strain to the species level. "The ad hoc committee for the re-evaluation of the species definition in bacteriology" suggested the following components should be considered for species delineation (Stackebrandt et al., 2002): the 16S rRNA gene sequence (>1,300 bp), protein-coding gene sequences, genotyping using fingerprinting techniques targeting whole genomes (PFGE, RAPD, rep-PCR, AFLP), phenotypic characterisation using preferably commercial systems, MLSA and DNA-DNA reassociation. The polyphasic characterisation is compulsory for the description of novel species in the "International Journal of Systematic and Evolutionary Microbiology"(Kämpfer et al., 2003).

Xanthomonas arboricola pv. *fragariae*, a novel pathovar causing a new disease of strawberry, bacterial leaf blight, was described following polyphasic characterisation (Janse et al., 2001). The authors used a variety of phenotypic techniques, pathogenicity tests, AFLP analysis and DNA-DNA hybridisation to compare the leaf blight-causing strains with reference strains of other *Xanthomonas* species.

Coutinho et al. (2002) used a polyphasic approach to identify the causal agent of bacterial blight and dieback of *Eucalyptus.* Bacterial strains were characterised phenotypically by using the nutritional (Biolog) and physiological (API system) tests and fatty acid analysis. The 16S rDNA sequences were compared with those of other *Enterobacteriaceae* from the GenBank and phylogenetic analysis revealed that the isolates belong to the genus *Pantoea.* DNA-DNA hybridisation between the strains from *Eucalyptus* and the type strain of *P. ananatis* identified the bacterium causing blight and dieback of *Eucalyptus* as *P. ananatis*.

Polyphasic analysis has been used to identify previously undescribed pathogens of passion fruit. Goncalves and Rosato (2000) isolated *Xanthomonas campestris*-like bacteria from passion fruit plants and fruits. The genetic diversity of 55 strains was examined using RAPD, rep-PCR, RFLP of the 16S-23S rDNA intergenic spacer region, PFGE, AFLP and SDS-PAGE of whole cell proteins. *Xanthomonas* strains from passion fruit could not be classified by the analysis of these data. Only DNA-DNA hybridisation identified them as *X. axonopodis* pv. *passiflorae*.

CONCLUSIONS

A pure culture of the causal agent of the disease is essential for its identification. Isolation of *Pantoea ananatis* from onion seed on the general growth media such as nutrient agar, although possible, is difficult, due to abundant growth of other bacteria and fungi present in seed (Walcott et al., 2002). The development of an appropriate semi-selective medium would increase the efficiency of detection of *P. ananatis* in onion seed and other plant material.

Pantoea strains should be classified and identified based on genotypic and phenotypic characteristics, as identification based on phenotypic tests only does not always lead to clear results (Gavini et al., 1989, Mergaert et al., 1993).

PFGE is considered as the most-discriminatory DNA-based typing method (Struelens et al., 2001). Although it was used successfully to distinguish *P. stewartii* subsp. *stewartii* from related *Pantoea* species (Coplin et al., 2002), this technique is laborious and time consuming (Olive and Bean, 1999). RAPD fingerprints assays are quicker and easier to perform that PFGE. Unfortunately, the assay is difficult to standardise and the RAPD fingerprints are not always reproducible. Additionally, the RAPD primers that generate the best DNA patterns for identification and differentiation must be determined empirically for each taxon.

Rep-PCR and AFLP genomic fingerprinting are proven as reliable methods, with a high degree of reproducibility, for the identification and diagnosis of plant pathogenic bacteria. The discriminatory power of AFLP is equal to that of PFGE (Lindstedt at al. 2000) and the rep-PCR is only slightly less discriminatory than PFGE (Weigel et al., 2004). The genotypic and phylogenetic relationships among microorganisms obtained by the analysis of the AFLP and rep-PCR fingerprints were highly correlated to that obtained using the DNA-DNA

hybridisation studies (Rademaker et al., 2000). These two techniques are easy to perform and relatively inexpensive. However, the results are difficult to compare between laboratories, as the choice of primers, restriction enzymes and gel systems varies between laboratories.

Phylogenetic relationships between bacteria could be determined by comparing the sequences of the stable part of the genes. The gene most commonly used for taxonomic purposes is the 16S rRNA gene. Although this gene has the poorest ability to predict genome relatedness at the species level, compared to 32 protein-encoding genes studied by Zeigler (2003), the 16S rRNA gene sequence is available for a large number of strains of almost all bacterial species. The sequences are deposited in publicly accessible databases. The sequence of an unknown strain is easily compared with many previously deposited sequences, making identification quick and efficient.

MLSA of housekeeping genes has a superior potential for species discrimination than the 16S rDNA analysis (Zeigler, 2003). The analysis of several genes of diverse chromosomal loci has the potential to replace DNA-DNA hybridisation in the delineation of bacterial species (Gevers et al., 2005; Richter et al., 2006; Zeigler, 2003). MLSA is simple and portable between laboratories, but the cost of sequencing several gene fragments for each isolate makes it rather a distant option for the routine identification of bacteria.

One technique is not sufficient for identification of plant pathogenic bacteria especially those causing previously undescribed diseases of new hosts. The identification of such bacteria requires a polyphasic approach, employing several genotypic and phenotypic methods (Alvarez, 2004; Coutinho et al., 2002; Vandamme et al., 1996).

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CHAPTER 2

PA 20, a Semi-Selective Medium for Isolation and Enumeration of *Pantoea ananatis*

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Abstract

A semi-selective medium, PA 20, was developed for the isolation and enumeration of *Pantoea ananatis* from plant material, specifically from onion seed. The medium has a pH of 8.0 and contains $NH_4H_2PO_4$, K_2HPO_4, magnesium sulphate, NaCl, D (+) arabitol, crystal violet, bromothymol blue and thallium nitrate. All *P. ananatis* strains from a variety of hosts produced characteristic yellow colonies in 6-7 days at 25°C. Plating efficiencies on PA 20 in comparison to nutrient agar ranged from 92 to 112%. Recovery from naturally infested and artificially contaminated onion seed was high, with an almost total reduction of saprophytes.

Keywords: PA 20 medium; *Pantoea ananatis*, Seed; Selective isolation

1. Introduction

Pantoea ananatis is a pathogen causing diseases in a number of economically important plants including onion (Gitaitis and Gay, 1997; Schwartz and Otto, 2000), *Eucalyptus* (Coutinho et al., 2002), corn (Paccola-Meirelles et al., 2001), melons (Bruton et al., 1986; Wells et al., 1987), sudangrass (Azad et al., 2000), rice (Azegami et al., 1983) and pineapple (Serrano, 1928).

P. ananatis is both seed-borne and seed-transmitted in sudangrass (Azad et al., 2000), rice (Tabei et al., 1988) and onions (Walcott et al., 2002). The disease of onion, caused by *P. ananatis*, was named center rot. Onion seed associated with the first outbreak of center rot in Georgia, USA (Gitaitis and Gay, 1997), was produced in South Africa and it was suspected that the bacterium was introduced into that country on infected seed lots (Walcott et al., 2002). In South Africa, efforts to control the quality of commercially produced onion seed mainly focus on the detection of fungal pathogens, and little is known about potential bacterial pathogens associated with these seeds. Nutrient Agar (NA) and yeast extract dextrose calcium carbonate (YDC) (Wilson et al., 1967) are the common growth media used to isolate *P. ananatis* from plant material and seed (Azad et al., 2000; Coutinho et al., 2002; Walcott et al., 2002). These media, however, are non-selective and many other organisms present as saprophytes or endophytes in plant material and in seed may hamper the detection of the target pathogen. In this paper, we describe a semi-selective medium, PA 20, which suppresses growth of many saprophytic microorganisms and serves as a suitable medium for growth and enumeration of *P. ananatis*. The medium was specifically developed to detect this pathogen on onion seed.

2. Materials and Methods

2.1. Bacterial strains

Bacterial strains used in this study are listed in Table 1. Stock cultures of all isolates were maintained in milk-glycerol liquid medium (10% skim milk, 15% glycerol in distilled water) at –70°C. Stock strains were transferred onto NA (Difco) plates and incubated at 25°C to recover growing cultures. Cultures were routinely checked for purity and colony characteristics.

2.2. PA 20 development

Criteria for the semi-selective medium were: differentiation between *P. ananatis* and other bacteria by colony morphology and inhibition of fungal and bacterial contaminants commonly found on onion seed, without hampering the growth of *P. ananatis*.

Substrate utilisation profiles using the BIOLOG (BIOLOG, Inc., Hayward, CA) plates designed for gram negative bacteria revealed that *P. ananatis* strains utilised D (+) arabitol, while the majority of other bacteria listed in Table 1 did not (data not shown).

A variety of media, based on the utilisation of D (+) arabitol and modification of Medium C of Dye (Dye, 1968) were prepared. Different concentrations of NaCl: 0.5; 1.0; 2.0; 3.0 and 4.0%, and a range of pH from 7.0 until 10.0 were evaluated. The pH indicator bromothymol blue was added to monitor pH changes during bacterial growth. Crystal violet and thallium nitrate were added (Ishimaru and Klos, 1984) to suppress other bacteria and fungi (Norris et al., 1976; Srivastava et al., 1976). A loopful of bacterial suspensions of the strains listed in Table 1 was streaked on the media in triplicate and observed daily for bacterial growth.

The medium that satisfied the criteria specified above was named PA 20 and had the following composition per litre: NaCl, 20 g; K_2HPO_4, 1 g; $NH_4H_2PO_4$, 1 g; $MgSO_4$ x $7H_2O$, 0.2 g; bromothymol blue, 1 ml of 1.6% aq. solution; crystal violet, 2 ml of 0.075% aq. solution; agar, 15 g. The pH was adjusted to 8.0 with 1.0 N NaOH. After autoclaving and cooling to 50^o C, 3 g of D (+) arabitol dissolved in 5 ml water and 2 ml of 1% aq solution of thallium nitrate were added. Both solutions were filter sterilised. Plates were stored at room temperature for 24 hours before use.

2.3. Colony morphology and plating efficiency on PA 20

Sixteen *P. ananatis* strains were selected to determine colony morphology and plating efficiency on PA 20 (Table 2). The strains were grown on NA for 24-48 hours at 25^{o}C. Colonies were suspended in 9 ml quarter-strength, sterile Ringer (Oxoid) buffer (RB) to an absorbance density of 0.1 at 640 nm (UV-160A Shimazu spectrophotometer). Ten-fold serial dilutions were prepared in half-strength nutrient broth (Difco), and 0.1 ml of selected dilutions was spread-plated in triplicate on PA 20 and a non-selective NA (contained per litre: beef extract, 3.0 g; peptone, 5.0 g; agar, 15.0 g; pH 6.8 ± 0.2 at 25^{o}C). Plates were incubated at 25^{o}C. Colonies were assessed and counted after 7 days. Plating efficiencies

were determined by dividing the number of colonies growing on PA 20 by the number of colonies on NA, and multiplying the quotient by 100. The experiment was replicated twice.

2.4. PA 20 selectivity assessment

Selectivity was evaluated by assaying pathogen-free onion seed artificially infested with *P. ananatis* strain Pans 2002-2. One seed lot contained a large number of saprophytic bacteria (5.4 x 10^6 CFU/gram) while the second seed lot had a low population of saprophytes (2.2 x 10^3 CFU/gram). For each seed lot two sub-samples, 5 g each, were placed in separate sterile mortars and crashed with pestles. Crashed seeds were transferred to separate sterile Erlenmeyer's flasks containing 100 ml of RB. One of the sub-samples of each seed-lot was spiked with a suspension of *P. ananatis* to produce an estimated population of 10^3 CFU/ml. Flasks were incubated for 30 min at room temperature on a rotary shaker. Ten-fold serial dilutions were made in half-strength nutrient broth, and 0.1 ml of each dilution (spiked and non-spiked) was spread on the surface of PA 20 and NA. Colonies were counted after 7 days incubation at 25°C. Suspected *P. ananatis* (selection based on colony morphology), were purified and their identities confirmed by: oxidase test, indole production and Hugh-Leifson oxidation/fermentation (Walcott et al., 2002). Tests were replicated three times.

2.6. Isolations from naturally infested seed

Five onion seedlots obtained from seed companies and farmers were tested for the presence of *P. ananatis*. Seed extracts were prepared as described above. For each sample, ten-fold dilution series were made in half-strength nutrient broth, and 0.1 ml of each dilution and direct seed extract was plated in triplicate on NA and PA 20. Plates were incubated at 25°C and examined daily for the presence of characteristic *P. ananatis* colonies. Three suspected *P. ananatis* isolates were selected from each PA 20 plate (selection based on colony morphology) for further identification.

2.7. Identification of P. ananatis from naturally infested seed

Isolates were purified and their identities confirmed by pigment production on NA, motility, cell morphology, Gram stain, oxidase, catalase, indole production, Hugh-Leifson oxidation/fermentation, gas production from glucose and hydrogen sulphide from cysteine

(Mergaert et al., 1993). The tests were done according to methods described by Fahy and Hayward, 1983.

Pathogenicity was determined on onion (*Allium cepa,* cv. Granex 33) in greenhouse assays by using a stub inoculation test adapted from Fenwick and Guthrie, 1969. A sterile needle was dipped into the bacterial colony on NA (24-48 hours growth) and then the needle was inserted under the epidermis of a leaf. At least two leaves were inoculated per isolate. Inoculated plants were incubated in a greenhouse with 27°C/23°C day/night temperatures and observed daily for the development of symptoms.

3. Results and discussion

3.1. PA 20 development

D (+) arabitol as a single carbon source, the pH, NaCl concentration and presence of thallium nitrate all contributed to the PA 20 selectivity. *P. ananatis* grew on PA media at pH 7.0; 8.0 and 9.0, but some saprophytes grew as well, sometimes producing large, slimy colonies. The addition of thallium nitrate inhibited saprophytic bacteria at pH 9.0 but not at pH 7.0. However, the growth of *P. ananatis* on PA medium at pH 9.0 containing thallium nitrate was also hampered. Only at pH 8.0 with thallium nitrate did *P. ananatis* grow well and saprophytes listed in Table 1 were inhibited. The concentration of NaCl influenced the colony size and the time of growth to achieve a colony large enough for recognition and enumeration. When the concentration of NaCl was lower or higher than 2% colonies of *P. ananatis* were either too small for evaluation (<2%) or took 10 or more days to appear on the media (>2%). Thus the medium, named PA 20, containing thallium nitrate, 2% NaCl and a pH 8.0 was selected for further evaluation.

3.2. Colony morphology on PA 20 and plating efficiency

All strains of *P. ananatis* grew on PA 20 medium and colonies were visible after incubation for 6 to 7 days (Table 1). Colonies were yellow, 3-4 mm in diameter; shiny, drop-shaped with small, granular, darker yellow inclusions within. *P. ananatis* decreased the pH of the medium (degradation of D (+) arabitol to acid) and produced a diagnostic yellow zone around individual colonies (Fig. 1).

*Pseudomonas syringae, Pantoea agglomerans, Pectobacterium carotovorum*_subsp. *carotovorum, Xanthomonas campestris* and saprophytes isolated from onion seed did not grow on PA 20 medium. *Pantoea stewartii* subsp. *indologenes* produced small, 1-2 mm in diameter, yellow colonies with a dark green centre (Table 1).

Plating efficiencies of the 16 *P. ananatis* strains ranged from 92% to 112% with a mean of 99.5% on PA 20 medium compared to NA. Results are presented in Table 2.

3.3. PA 20 selectivity assessment

P. ananatis was not recovered from seed samples plated on NA or from non-amended seeds on a semi-selective PA 20. Plates of NA were overgrown by other bacteria and fungi. In contrast, on PA 20, saprophytic growth was reduced by 99-100% (Table 3, Fig. 2A and 2B) and *P. ananatis* was easily identified by characteristic, yellow, drop shaped colonies (Fig. 2B), that were all oxidase negative, indole positive and glucose fermentative. *P. ananatis* was recovered at the expected concentrations on PA 20 from both amended seed samples. The results are presented in Table 3.

3.4. Recovery from naturally infested seed

P. ananatis was isolated on PA 20 medium from two of the five onion seed samples obtained from seed companies and farmers. The selection of suspected *P. ananatis* on NA was difficult, because many saprophytes produced similar yellow colonies. The detection on PA 20 was not hampered, because other microorganisms present in onion seed either did not grow on the medium or had distinctly different colony morphology.

All suspected *P. ananatis* purified from PA 20 produced yellow colonies on NA, were gram-negative rods (1.5-2.0 µm length and 0.5-0.75 µm width), motile, oxidase negative, catalase and indole positive, and fermentatively utilised glucose. They did not produce gas from glucose and hydrogen sulphide from cysteine. The results of tests are characteristic for *P. ananatis* as described by Mergaert et al. (1993).

Pathogenicity of *P. ananatis* to the onion cultivars Granex 33 is shown in Table 1. All pathogenic isolates induced identical symptoms on onion leaves. Two to four days after inoculation water soaked spots appeared on leaves that expanded into longitudinal, bleached-green lesions with chlorotic margins.

In conclusion, PA 20 is a useful semi-selective medium for the isolation and enumeration of *P. ananatis* from onion seed. Experiments demonstrated the efficiency of the medium in recovering *P. ananatis* from seed containing large number of other microorganisms. The medium should not, however, be used as a single diagnostic tool. *P. ananatis* strains non-pathogenic to onion are indistinguishable from pathogens on PA 20 (Table 1, Table 2). Identity of the pathogen must be confirmed using selected phenotypic characteristics and by conducting pathogenicity tests described in this paper. *P. ananatis* from hosts other then onion also grew on PA 20, showing its potential as a general-purpose medium for isolation of these bacteria.

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Table 1. Bacterial strains [a] used in this study, their growth on PA 20 medium and their pathogenicity to onion (*Allium cepa*) cv. Granex 33.

Bacterium and strain number	Host	Growth on PA 20 [b]	Pathogenicity to onion in a stub-inoculation test [c]
P. ananatis			
LMG 2665^T	*Ananas cosmosus*	+	-
LMG 2676	*Puccinia graminis*	+	-
LMG 20103, 20104	*Eucalyptus*	+	-, -
ATCC 35400	*Cucumis melo*	+	-
ATCC BAA 515	*Allium cepa*	+	-
Blackshank 15, 24, 30	*Allium cepa*	+	+, -, -
Hort. Hill 7, 24, 31, 32			-, +, +, +
Pans 99-8, 99-23, 2002-2			-, -, +
BD 321, 322, 323,234,325,	*Allium cepa*	+	all -
326, 327, 328, 329			
BD 377, 381, 383, 386,	*Allium cepa*	+	all +
387, 388, 390, 392, 393			
P. agglomerans			
LMG 1286^T	knee laceration	-	-
SUH 2	*Allium cepa*	-	+
DAR 49828	*Allium cepa*	-	-
P. stewartii subsp. *indologenes*			
LMG 2632^T	*Setaria italica*	+ [d]	-
LMG 2671	*Ananas cosmosus*	+ [d]	-
Pseudomonas syringae	*Allium cepa*	-	not tested
Pectobacterium carotovorum subsp. *carotovorum*	*Solanum tuberosum*	-	not tested
Xanthomonas campestris pv. *allii*			
BD 142, 143, 211	*Allium cepa*	-	not tested
Saprophytes, 22 isolates	*Allium cepa* seed	-	all -

[a] LMG: BCCM/LMG Culture Collection, Universiteit Gent, Belgium; ATCC: American Type Culture Collection, Manassas, VA; DAR: Australian Collection of Plant Pathogenic Bacteria, Orange; BD: Plant Pathogenic and Plant Protecting Bacteria (PPPPB), ARC-PPRI, South Africa; Blackshank, Hort. Hill and Pans: R. Walcott, Department of Plant Pathology, University of Georgia, Athens; Saprophytes from onion seed: this study.

[b] Growth on PA 20 medium from suspension of cells plated for isolated colonies.

[c] A sterile needle was dipped into the bacterial colony on NA (24-48 hours growth) and then the needle was inserted under the epidermis of an onion leaf; results recorded after one week; pathogenic (+), non-pathogenic (-), not tested.

[d] Grew on the medium but colony morphology was different from *P. ananatis*.

Table 2. Plating efficiency of *P. ananatis* strains on PA 20 medium relative to NA medium.

Strain no	Number of CFU [a] on:		Plating efficiency (%) on PA 20[b]
	NA	PA 20	
Strains from onion			
ATCC BAA 515	24.6	22.8	92.6
BD 325	40.6	40.8	100.4
BD 327	13.3	13.2	100
BD 377	42	43	102
BD 387	29	28	96.6
BD 390	29	30	103.4
Blackshank 15	29.7	31	104.5
Hort. Hill 24	54.4	51.6	95
Hort. Hill 31	42.6	43.9	103
Pans 99-8	28	25.8	92
Pans 2002-2	44.6	50	112.1
		Average	100.1
Strains from other hosts			
ATCC 35400	27	24	92
LMG 2665^T	26.2	24.2	92.4
LMG 2676	23.2	25.4	109.5
LMG 20103	25.8	26	100
LMG 20104	33.8	33.6	100
		Average	98.8
TOTAL AVERAGE			99.5

[a] Strains were pre-cultured on NA medium 24-48 hours at 25°C, colonies removed with sterile cotton swab and suspended in SR to an absorbance density of 0.1 at 640 nm; tenfold dilution series were prepared in half-strength nutrient broth and 0.1 ml volumes were plated on test media and incubated at 25°C for 7 days. Figures in each row are from the same dilution.

[b] Plating efficiency = colony forming units on PA 20 medium/colony forming units on NA x 100. Figures were calculated from the mean colony numbers of minimum five plate counts per strain.

Table 3. Recovery of *P. ananatis* on a semi-selective medium PA 20 from two pathogen-free onion seed lots spiked with Pans 2002-2 to a final concentration ~103 CFU/ml. NA was used as a control, general growth medium. Characteristic yellow, drop shaped colonies on PA 20 (Fig. 1) were considered as *P. ananatis*.

Seed sample [a]	CFU/ml [b] recovered on			PA 20 evaluation
	NA medium	PA 20 medium		
	total CFU/ml	total CFU/ml	*P. ananatis* CFU/ml [c]	
not amended				% reduction saprophytes
A	105	0	0	100
B	270000	41	0	99
amended				% *P. ananatis* recovery
A	4220	4198	4197	100
B	275000	3130	3080	98

[a] A - seed with low (2.2 x 10^3 cfu/gram) population of saprophytes and B - seed with high (5.4 x 10^6 cfu/gram) population of saprophytes. Not amended seed - 5 gram seed crashed with mortar and pestle, 100 ml of quarter-strength Ringer (Oxoid) buffer; amended seed - as above plus a bacterial suspension of strain Pans 2002-2 to a final concentration ~10^3 CFU/ml were mixed and incubated for 30 min at room temperature on a rotary shaker.

[b] Values are the mean of three replications. Five ten-fold serial dilutions of each seed extracts were made in half-strength nutrient broth, 0.1 ml of each dilution and direct seed extract was plated on three plates of each of PA 20 and NA. Colonies were counted after 7 days incubation at 25°C.

[c] Identity of two suspected *P. ananatis* colonies from each PA 20 plate confirmed by physiological tests (oxidase negative, indole positive, glucose fermentative.

Fig. 1. Colony morphology of *P. ananatis* on PA 20 medium after 7 days of incubation at 25^{o}C. Colonies are yellow, 3-4 mm in diameter, shiny, drop shaped with small, granular, darker inclusions inside. Lighter zones around colonies on PA 20 are yellow and contrast with the dark blue of the medium.

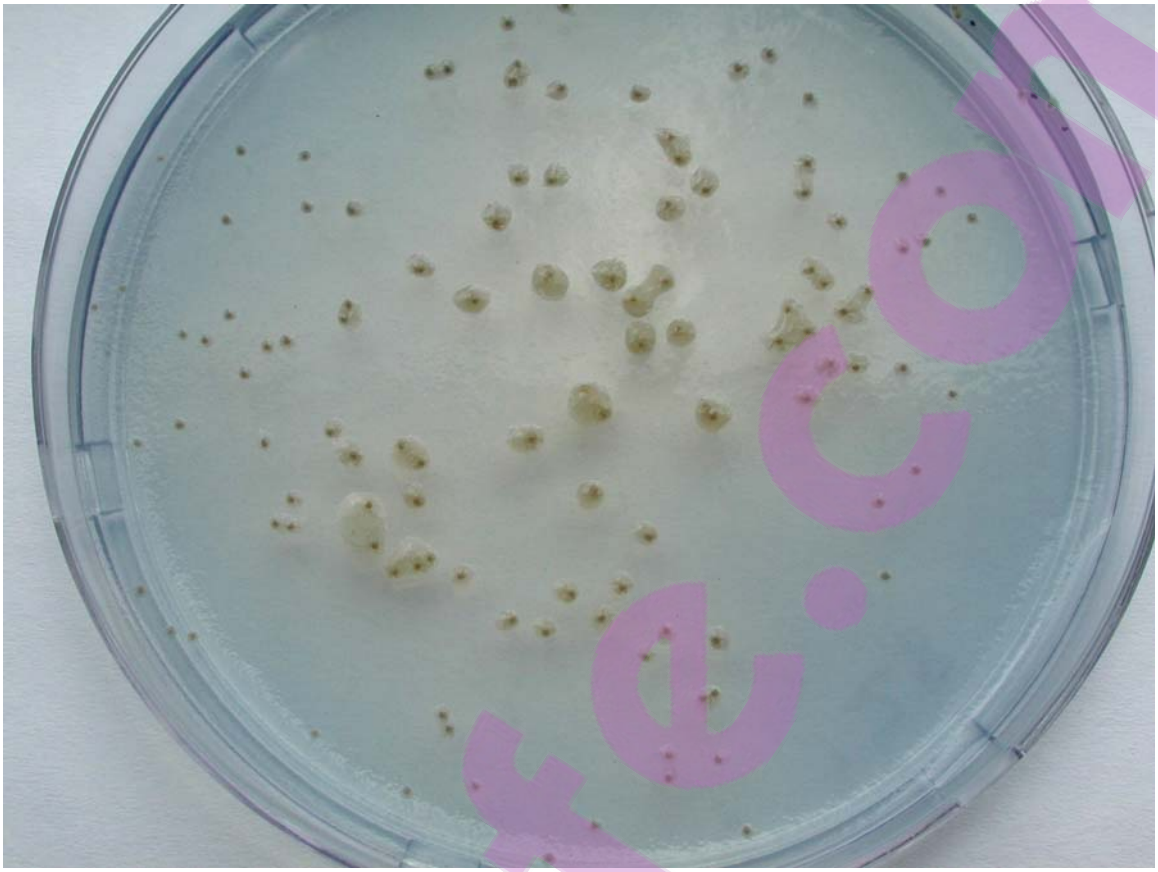

Fig. 1

Fig. 2. Comparison in selectivity between non-selective NA (left) and semi-selective medium PA 20 (right). Isolations were done from: A – *P. ananatis*-free onion seed; B – the same seed as in A spiked with *P. ananatis*. Note total suppression of non-target microflora on PA 20.

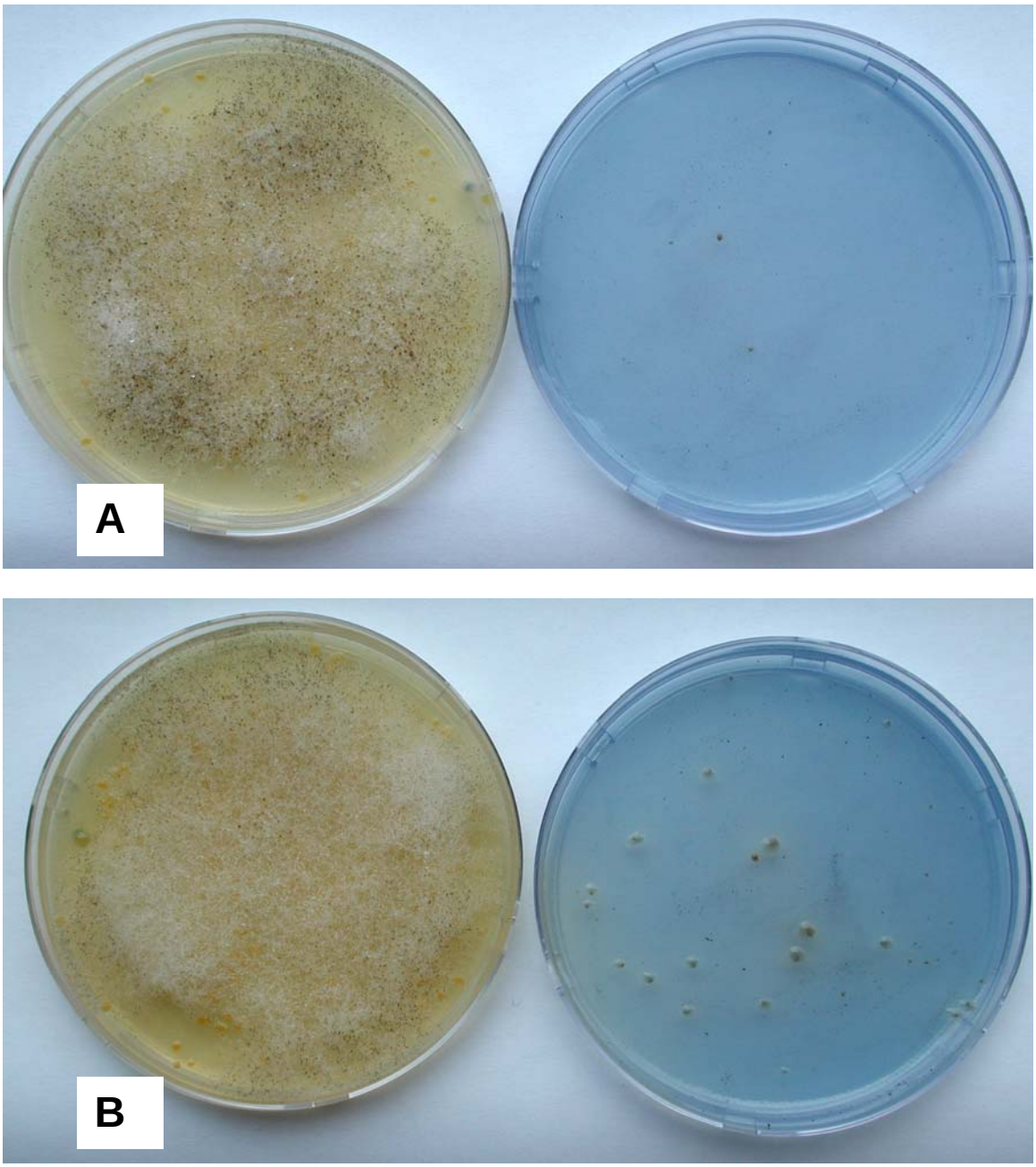

Fig. 2

CHAPTER 3

Isolation and Identification of P*antoea ananatis* from Onion Seed in South Africa

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Summary

Center rot of onion, caused by the gram-negative, facultatively anaerobic bacterium, *Pantoea ananatis*, was first described in Georgia, USA, in 1997. The disease was later reported in Colorado and Michigan, but not in any other area of the world. *P. ananatis* is seed-borne and seed-transmitted in onions. A similar disease, leaf and seed stalk necrosis of onion, was reported in South Africa in 1981, and it was suggested that *P. ananatis* was responsible for that disease and was introduced into the USA on infected seed lots from South Africa. However, a representative strain of the leaf and seed stalk necrosis pathogen, isolated in South Africa in 1981, was identified as *P. agglomerans* in this study. As center rot of onion has not been observed in South Africa, and *P. ananatis* was not isolated from diseased plants, the main objectives of this research were to determine if pathogenic *P. ananatis* is present in local onion seed and to compare the South African strains to center rot causing strains from the USA. Colonies resembling those of *P. ananatis* were isolated from four seed lots on a semi-selective medium, PA 20. Pathogenicity tests demonstrated that the South African strains could induce the same symptoms on onion as those caused by the American strains. Physiological and biochemical characterization using Biolog, API 20E and API 50 CHE systems, and analysis of the 16S rRNA gene sequences identified the strains from seed as *P. ananatis*. This is the first report on the presence of the center rot pathogen of onion, *P. ananatis*, in South African onion seed.

Introduction

Center rot disease of onion was first observed in Georgia, USA, on Sweet Vidalia onions in 1997 (Gitaitis and Gay, 1997) and later in Colorado (Schwartz and Otto, 2000a) and Michigan (Mark *et al.*, 2002). The disease has occurred in commercial fields in Georgia every year since 1997 and accounted for 100% loss in some fields (Walcott *et al.*, 2002). The causal agent of center rot is a Gram-negative, facultatively anaerobic bacterium, identified as *Pantoea ananatis* (Gitaitis and Gay, 1997). The disease affects the center leaves of onions, which become water-soaked, soft, and bleached white as the rot progresses. Advanced stages of the disease result in complete wilting and bleaching of all leaves (Mark *et al.*, 2002; Walcott *et al.*, 2002). Walcott *et al.* (2002), reported natural infestation and transmission of *P. ananatis* in onion seed and implied that seed was the primary source of inoculum. This pathogen is known to be seed-borne and seed-transmitted in rice (Tabei *et al.*, 1988), sudangrass (Azad *et al.*, 2000) and buckwheat (Iimura and Hosono, 1996).

In the 2004/2005 growing season 358 tons of onion seed were produced in South Africa, of which 282 tons were for the export market (Sansor Annual Report, 2005). Efforts to control the quality of commercially produced onion seed concentrated on the detection of fungal pathogens, and little is known about bacterial pathogens that may be present. *P. ananatis* is the causal agent of bacterial blight and dieback of *Eucalyptus* in South Africa (Coutinho *et al.*, 2002) but center rot of onion has not been reported. However, in 1981, *Erwinia herbicola* was reported to cause a similar disease, stalk and leaf necrosis of onion in South Africa (Hattingh and Walters, 1981). In 1989, Beji *et al*. (1988) proposed transferring *E. herbicola* (syn. *E. milletiae*) to the new genus *Pantoea* (Gavini *et al*., 1989), that included species formerly classified as *E. ananas*, *E. uredovora* and *E. stewartii* (Mergaert *et al.*, 1993). The seed associated with the first outbreak of center rot of onion in Georgia, USA, was produced in South Africa and Walcott *et al*. (2002) suggested that the center rot pathogen was possibly introduced on infested seed lots.

The goal of this study was to determine if pathogenic *P. ananatis* was present in South African onion seed and to compare such strains to those associated with center rot of onions in the USA.

Materials and methods

Media used for isolations

Two different culture media were used for isolations. The first was a general growth medium, nutrient agar (NA) (Difco, Sparks, MD) as recommended by Walcott *et al.* (2002). Another was a semi-selective medium PA 20 (Goszczynska *et al.*, 2006) developed specifically for isolation of *P. ananatis* from onion seed. PA 20 contained the following per liter: NaCl, 20 g; K_2HPO_4, 1 g; $NH_4H_2PO_4$, 1 g; $MgSO_4x7H_2O$, 0.2 g, bromothymol blue, 1 ml of 1.6% aq. solution; crystal violet, 2 ml of 0.075% aq. solution; agar, 15 g. The pH was adjusted to 8.0 with 1.0 N NaOH. After autoclaving and cooling to 50^o C, 3 g of D (+) arabitol dissolved in 5 ml water and 2 ml of 1% aq solution of thallium nitrate were added. Both solutions were filter sterilized prior to adding them to the medium.

Isolations from plants

Diseased onion fields in the Small Karoo area of the Western Cape Province were identified by the onion seed producers in June-July and November-January between 2001-2004. Disease symptoms on twenty-four experimental fields of *Allium cepa*, Granex type, were similar to leaf and stalk necrosis described by Hattingh and Walters (1981). Irregular oval spots, 4-10 cm long with a necrotic center and a dirty yellow or water-soaked margin lesions were found on onion leaves and seed stalks. Small water-soaked lesions were also present.

In June-July 2003, nineteen onion fields of commercial bulb producers in the Northern and Free State Provinces were inspected. Onions from these fields were healthy.

In April 2005, on one water-logged field in the Northern Province, where the cv. Granex 33 was grown, symptoms similar to that of center rot were observed. The youngest, center leaves of onions were chlorotic, becoming soft and wilted as the disease advanced. A light tan discoloration progressed into bulbs, resulting in the decay of the inner scales. The disease affected approximately 50% of the crop.

Ten to twenty diseased plants were collected from each field and isolations were done from all collected plants. Tissue from lesion margins of infected leaf, seed stalk or bulb was removed with a sterile scalpel and macerated in 1 ml of sterile quarter-strength Ringer buffer (Oxoid, Basingstoke, Hampshire, England) for 20 min at 25^{o}C. Plant extracts were streaked onto NA and PA 20. NA and PA 20 plates were evaluated after four and seven days of incubation at 25^{o}C, respectively. Suspected colonies were purified by streaking onto NA and

cultures stored in milk-glycerol liquid medium (10% skim milk, 15% glycerol in distilled water) at –20°C for further analysis.

Isolations from seed

Thirty-two seed samples were evaluated for the presence of *P. ananatis*: twenty-eight from *A. cepa*, two from *A. fistulosum* and two from *A. porri*. Fifteen *A. cepa* seed samples were commercial seed: six of cv. Granex 33, three of cv. Pegasus, two of cv. Pyramid, one of cv. Capricio and three of experimental onions (Granex type). The seed were produced in the Small Karoo area and harvested one or two years prior to testing. One sample, of an unknown cultivar, was from a farmer in the Northern Province, who produced the seed for his own use. The remaining twelve seed samples were harvested in January 2004 from umbels of experimental onion (Granex type) fields in the Small Karoo, in which leaf blight developed naturally. Twenty umbels were collected from each field and pooled into one sample. Seeds were extracted manually and stored in paper bags at 4°C until processing.

Isolations from seed were carried out as described previously (Goszczynska *et al.*, 2006). For each seed lot, two sub-samples, 5 g each, were crushed and placed into separate sterile Erlenmeyer's flasks containing 100 ml of a quarter-strength Ringer buffer. Flasks were incubated for 30 min at 25°C on a rotary shaker at 180 rpm/min. For each sample, four ten-fold serial dilutions were made in half strength nutrient broth. A volume of 0.1 ml of each dilution was plated onto three plates each of NA and PA 20. Plates were incubated at 25°C. Colonies were counted and evaluated after 7 days. Suspected colonies of the pathogen were purified by streaking onto NA and stored at –20°C in milk-glycerol liquid medium for further analysis.

Preliminary characterization of strains

From each seed lot, 20 yellow colonies from NA and 20 yellow colonies from PA 20 were purified and used in preliminary pathogenicity and biochemical tests. The strains from seed washings were characterized by colony morphology, pigment production on NA, motility, cell morphology, Gram stain, oxidase reaction, catalase reaction, indole production, Hugh-Leifson oxidation/fermentation test, gas production from glucose and hydrogen sulphide production from cysteine (Mergaert *et al.*, 1993), according to methods described by Fahy and Hayward (1983). Pathogenicity on onion was determined in green house assays by using a stub inoculation test (Goszczynska *et al.*, 2005). A sterile needle was dipped into the bacterial colony on NA (24-48 hours growth) and then the needle was inserted under the

epidermis of a leaf. At least two leaves were inoculated per isolate. Inoculated plants were incubated in a greenhouse with 27°C/23°C day/night temperatures and observed daily for the development of symptoms.

Bacterial strains

The following bacterial strains from onion were characterized in the study: three strains isolated from South African seed (Table 1), the strain reported to cause leaf and seed stalk necrosis of onion in 1981 and four *P. ananatis* strains associated with center rot in the USA (Table 2). Two *P. ananatis* strains causing blight of *Eucalyptus,* and the type strains of *P. ananatis* LMG 2665^T and *P. agglomerans* LMG 1286^T were used as reference strains (Table 2). Stock cultures of all strains were maintained in milk-glycerol liquid medium at –20°C. Stock strains were transferred onto NA plates and incubated at 25°C to recover growing cultures. Cultures were routinely checked for purity and colony characteristics on NA.

Pathogenicity tests

Spray inoculations were performed with strains listed in Table 2. Strains were grown on NA at 25°C for 24 hours. Bacterial suspensions were made in sterile distilled water to obtain approximately 10^7 colony forming units (CFU)/ml (as determined by dilution plating on NA). Six to eight week-old onion plants (*A. cepa* - cv. Granex 33 and cv. Pyramid; *A. fistulosum* - cv. White Welsh) and leek plants (*A. porri* - cv. Giant Italian) were spray inoculated to runoff with each bacterial suspension. Negative control plants were inoculated with sterile distilled water. Three plants were inoculated with each strain. Plants were incubated for 72 hours in a humidity chamber at 27°C and relative humidity of 95%. Later plants were maintained in a greenhouse with 27°C/23°C day/night temperatures and observed daily for three weeks for the development of symptoms.

The identities of the bacteria re-isolated from lesions were confirmed by colony morphology on NA and PA 20, Gram stain, oxidase reaction, Hugh-Leifson test, and indole production (Coutinho *et al.*, 2002; Walcott *et al.*, 2002).

Biochemical and physiological tests

Strains listed in Table 2 were characterized by sole carbon source utilization profiles, using Biolog GN Microplates together with Biolog MicroLog version 4.2 software (Biolog, Inc., Hayward, CA), according to the manufacturer's instructions. The strains were also tested with the API 20E and API 50CHE systems (BioMérieux, La Balme les Grottes,

Montalieu Vercieu, France) using the procedure recommended by the manufacturers. The results of the API 20E and API 50CHE tests were recorded after 24 and 48 hours of incubation at 30°C respectively. Profiles were identified using the APILAB V4.0 identification program (BioMérieux).

Amplification and sequencing of the 16S rRNA gene

Genomic DNA of strains was extracted by the cetyltrimethylammonium bromide (CTAB) method according to the DNA Miniprep protocol of Wilson (1989). Colonies from each strain were picked from the 48 hours NA plates and transferred into micro centrifuge tubes with sterile STE (10mM Tris-HCl, 1mM EDTA, 2M NaCl, pH 8.0) buffer. Cell lysis was obtained by incubation with sodium dodecyl sulfate and selective precipitation of cell debris and polysaccharides with CTAB/NaCl. DNA was extracted with phenol-chloroform-isoamyl alcohol, precipitated with isopropanol, washed with ethanol, air dried and dissolved in 100 µl of sterile water. Purified DNA was quantified using a Dyna Quant 200 fluorometer (Hoefer, San Francisco, CA) and Hoescht H 33258 intercalating dye (Polysciences, Warrington, PA). The DNA concentration was adjusted to 25-50 ng μl^{-1} with sterile water.

The 16S rRNA gene fragment was amplified using the universal primers fD1 (5'-AGA GTT TGA TCC TGG CTC AG-3') and rD1 (5'-AAG GAG GTG ATC CAG CCG CA-3') (Weisburg *et al.*, 1991). DNA was amplified in 50 µl reaction volumes containing polymerase chain reaction (PCR) buffer (10 mM Tris-HCl, pH 9.0 at 25°C; 50 mM KCl, 0.1% Triton X-100); 1.5 mM $MgCl_2$; 150 µM dNTPs; 1.0 µM each primer; Taq polymerase (Promega Corp., Madison, WI) 1 unit per reaction volume and 25-50 ng DNA template μl^{-1}. Amplifications were performed according to Weisburg *at al*. (1991) in a Hybaid Omni Gene thermocycler (Teddington, England).

The amplified PCR products were cut from the gel and purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany), and then cloned with the pGEM-T Easy Vector System II kit (Promega, Madison, WI) according to the manufacturer's instructions. Plasmids with inserts were purified using the QIAprep Spin kit (Qiagen). The sequencing was performed using the Dyenamic ET Dye terminator cycle sequencing kit for MegaBACE with dideoxynucletide chain-termination chemistry. Sequences were analyzed using a MegaBACE 500 Sequencer (Amersham Biosciences, Buckinghamshire, England) with MegaBACE 500 Sequence Analyzer (version 2.4) software.

The GenBank/EMBL databases were used for homology searches using the BLAST program (National Center for Biotechnology Information, U.S. National Institute of Health, Bethesda, MD).

Sequence analysis

The 16S rDNA nucleotide sequences obtained in this study were aligned with a selection of 16S rDNA sequences of *Pantoea* species from GenBank with the DNAMAN software (version 5.2.9, Lynnon Biosoft, Quebec, Canada), using an optimal alignment and a dynamic method according to Feng and Doolitlle (1987). Phylogenetic trees were constructed with the neighbor joining method (Saitou and Nei, 1987) and evolutionary distances calculated according to the method of Jukes and Cantor (1969) and Kimura (1980), using the DNAMAN software package. Bootstrap analysis of the data, based on 1000 permutations was used to assess the stability of relationships.

Results

Recovery from plants

P. ananatis was not isolated from diseased plants. The lesions on onions with symptoms similar to leaf and seed stalk necrosis were caused by *Pseudomonas syringae*, which was frequently isolated from these plants on a Milk-Tween medium (Goszczynska and Serfontein, 1998) and confirmed by pathogenicity tests and LOPAT characteristics (Lelliot *et al.*, 1966).

Yellow, mucoid colonies were consistently isolated on NA from diseased onions with center rot-like symptoms. Yellow bacteria were gram-negative rods, oxidase negative, catalase positive, fermentatively utilized glucose and produced gas from glucose. Five representative strains were identified as *Enterobacter cloacae* using the Biolog system, with a similarity index of 0.82-0.85. Five strains were positive in the following tests on the API 20E strips: β-galactosidase, arginine dihydrolase, lysine decarboxylase, utilization of citrate, tryptophane deaminase, production of acetoin, and acid production from glucose, mannitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. They were negative in the following tests: ornithine decarboxylase, H_2S production, urease, indole production, gelatinase, acid from inositol and sorbitol. The strains induced leaf rot on onion leaves in pathogenicity tests.

Rot of onions in South Africa and its causal agent *E. cloacae* were similar to that reported by Bishop (1990) and Schwartz and Otto (2000b) in the USA.

Recovery from seed

Yellow bacteria were recovered from most seed samples on NA. Preliminary identification using biochemical tests showed these strains were not *P. ananatis* (data not shown). All were negative in a stub inoculation pathogenicity test. On PA 20, *P. ananatis* was isolated from four of 28 *A. cepa* seed samples tested, but not from seed of *A. fistulosum* or *A. porri*. Characteristic yellow colonies, 3-4 mm in diameter, shiny, drop-shaped with small, granular, darker inclusions were visible on a PA 20 medium after 6-7 days of incubation at 25°C (Goszczynska *et al.*, 2006). The number of *P. ananatis*-like colonies recovered from the infested seed lots ranged from 2.2 x 10^3 CFU/gram to 5.0 x 10^6 CFU/gram of seed (Table 1). The strains from onion seed produced yellow colonies on NA, were gram-negative rods (1.5-2.0 µm length and 0.5-0.75 µm width), motile, oxidase negative and catalase positive. All strains fermentatively utilized glucose and produced indole from tryptophane. They did not produce gas from glucose nor hydrogen sulphide from cysteine.

One pathogenic strain per seed lot was selected for further characterization: BD 336 and PA 4 from two different experimental onion seed lots, harvested two years prior to testing and BD 390 from the seed produced by a farmer for his own use. None of the strains from the commercial seed cv. Granex 33 was pathogenic on onion cv. Granex 33 in a stub test and they were excluded from the study.

Pathogenicity test

All eight selected strains from onion were pathogenic to onion cv. Granex 33, but not to leek (Table 2). Strains BD 336, BD 310 and PA 4 did not produce disease symptoms on onion cv. Pyramid, all other strains did. Branching onion (*A. fistulosum*) cv. White Welsh was susceptible only to BD 287. All strains, including BD 287, induced identical symptoms on susceptible onion plants. Two to three days after inoculation, water-soaked spots appeared at the tips of leaves that expanded into longitudinal, bleached, green lesions with chlorotic margins. After two weeks, plants were completely wilted (Figure 1). Colonies recovered from onion plants with disease symptoms were yellow, gram-negative rods, oxidase negative and utilized glucose as a facultative anaerobe. All strains, except re-isolated BD 287 produced indole.

Two strains from *Eucalyptus*, LMG 20103 and LMG 20104, did not induce the disease on onions or leek. Control plants inoculated with water did not develop symptoms, nor were yellow bacteria isolated from leaves of these plants on NA and PA 20.

Physiological and biochemical characterization

The metabolic profiles of the eight strains from onion and two strains from *Eucalyptus* on Biolog GN microplates were most similar to the database profile of *P. agglomerans*, with a mean similarity index of 0.47. The type strains of *P. ananatis*, LMG 2665^T and *P. agglomerans*, LMG 1286^T were both identified as *P. agglomerans* by Biolog with a similarity index 0.51 (*P. ananatis* was not in the Biolog database). There was consistency in utilization of 66 substrates but some strains varied in the utilization of glycogen, adonitol, turanose, α-hydroxybutyric acid, bromosuccinic acid, succinamic acid, D-serine and uridine. The type strain of *P. agglomerans* and BD 287 gave nearly identical reactions and differed from others by not utilizing 11 substrates: Tween 40, Tween 80, gentiobiose, lactulose, D-melibiose, D-raffinose, D-sorbitol, citric acid, formic acid, D-glucosamic acid and quinic acid. Strain BD 287 did not utilize D-arabitol and psicose and LMG 1286^T did not utilize α-lactose and acetic acid.

Physiological and biochemical profiles obtained by using the API 20E and API 50CHE systems revealed that South African *P. ananatis* had similar biochemical characteristics and resembled the American and *Eucalyptus* strains and the type strain of *P. ananatis* (Table 3). In the API 20E tests, LMG 2665^T, BD 301, BD 309 and BD 315 were identical. African BD 390 and PA 4 differed by giving a positive reaction in the API 20E inositol test. Strain BD 310 did not produce β-galactosidase. The three profiles were identified by the APILAB program as *Pantoea* spp. 2. South African BD 336 differed from the others by not utilizing citrate and not producing acid from rhamnose and sucrose. However, in the API 50CHE acid from sucrose was produced after 48 hours. The profile was not recognized by the program. Unlike *P.ananatis* from onion and LMG 2665^T, two strains from *Eucalyptus* liquefied gelatin (Coutinho *et al*.,2002).

P. agglomerans LMG 1286^T and BD 287 differed from the other strains by the inability to utilize citrate, produce indole from tryptophan and acid from sorbitol.

There was consistency in the ability of the twelve strains to produce acid from 35 substrates (15 positives and 20 negatives) on the API 50CHE strips (Table 3). The type strain of *P.*

agglomerans and BD 287 differed from the other strains by not producing acid from glycerol, inositol, D-sorbitol, D-raffinose and D-arabitol.

16S rDNA sequence analysis

In this study, almost complete 16S rDNA sequences (~1500 bp) were determined for the four South African and four American strains. The sequences were deposited into the GenBank database. Accession numbers are shown in Table 2.

A BLAST search of the EMBL/GenBank database conducted with the sequences revealed a high degree of sequence identity (≥ 98%) with previously determined sequences of bacteria belonging to the genus *Pantoea*. The seven indole-producing strains had the highest homology to the 16S rDNA of *P. ananatis* strains LMG 20103 (accession number AF364847) and LMG 20106 (AF364844), ranging from 98.9% for BD 309 and BD 336 to 99.7% for BD 301 and BD 315. The strain BD 287 (Hattingh and Walters, 1981) had the highest homology (99.7%) to the 16S rDNA sequence of *P. agglomerans* strain LMG 2565 (Z96082).

Figure 2 shows the phylogenetic relationship derived from a neighbor-joining analysis of the pairwise comparison among the 16S rDNA sequences of eight strains from this study with nine sequences of four described species of the genus *Pantoea*. *Klebsiella pneumoniae* DSM 30104^T (X87276) was used as an outgroup taxon. Phylogenetic trees constructed by two different methods, with Jukes-Cantor and Kimura algorithms, were identical (not shown). Five strains, three American (BD 301, BD 310, BD 315) and two South African (BD 390, PA 4), formed a cluster with *P. ananatis* (syn. *E. uredovora)* at 72% confidence value from bootstrap analysis. The strain BD 287 grouped closely (77% confidence value) with *P. agglomerans* (syn. *E. herbicola*, *E. milletiae*). The sequences of the South African BD 336 and BD 309 from the USA clustered with *P. agglomerans* on phylogenetic trees. Some strains of *P. ananatis* from rice studied by Cother *et al*. (2004) also grouped with *P. agglomerans* on phylogenetic trees of the 16S rRNA gene sequences.

Discussion

Walcott *et al*. (2002) suggested that the center rot pathogen, *P. ananatis,* was introduced to the USA on infested onion seed lots produced in South Africa. The suggestion was based on the description of a similar disease of onion, stalk and leaf necrosis, reported in South Africa

in 1981(Hattingh and Walters, 1981). The causal agent of leaf and seed stalk necrosis, although pathogenic to onion, is not *P. ananatis*. Nutritional and genotypic characteristics of a representative strain, BD 287, closely resembled those of the *P. agglomerans* type strain, LMG 1286^T (Table 3, Figure 2). Our results are supported by those of Verdonck *et al.* (1987) and Beji *et al.* (1988) who also identified BD 287 as *P. agglomerans* in their taxonomic investigations.

In this study, *P. ananatis* pathogenic to onion was detected in three South African onion seed lots. Three strains isolated from onion seed lots induced the same symptoms on onions as those caused by strains isolated from diseased plants in the USA (Figure 1). There were, however, differences in susceptibility among onions cultivars (Table 2), providing an opportunity for the selection of tolerant material. Interestedly, *P. ananatis* causing leaf blight of *Eucalyptus* did not induce the disease on onions in pathogenicity tests done in this study, suggesting that *Eucalyptus* strains could be host specific (Azad et al., 2000).

When comparing the South African strains, the type strain *P. ananatis* and the American strains associated with center rot, by means of the Biolog system, we observed similar results in the utilization of 95 different carbon sources. With API 20E and API 50CHE strips, the profiles for the majority of onion strains were also similar to each other and to the reference strains of *P. ananatis*. Three strains (BD 336, BD 309 and BD 310) varied slightly in a few biochemical characteristics (Table 3). The production of acetoin and acid from L-rhamnose and methyl-α-D mannopyranoside differed among the 18 strains of *P. ananatis* studied by Mergaert *et al.*, 1993, while β-galactosidase and citrate utilization were reported to be positive for all strains. *P. ananatis* from *Eucalyptus* (this study, Coutinho *et al.*, 2002) and rice (Cother *et al.*, 2004), also showed a few biochemical differences from those characteristic for the species.

The genotypic characterization of the three strains from onion seed confirmed that South African strains are *P. ananatis*. Although the seed strain BD 336, and the American BD 309 clustered with *P. agglomerans* on phylogenetic trees (Figure 2), their 16S rDNA sequences were 98.8% homologous to the *P. ananatis* type strain. Additionally, all seed strains and strains from the USA were indole positive and their Biolog and API profiles were more similar to those of *P. ananatis* from *Eucalyptus*, LMG 2665^T, and those reported for 18 strains of *P. ananatis* examined by Mergaert *et al.* (1993). We concluded that the strains isolated from onion seed belong to this species.

Our results showed that *P. ananatis* pathogenic to onion is present in South African onion seed. This, however, does not support the claim that the pathogen was introduced to the USA

on infected seed lots from South Africa in 1997 (Walcott *et al*., 2002). *P. ananatis* was detected in the USA on Vidalia onions several years prior to the 1997 epidemic (Mark *et al.*, 2002). *P. ananatis* is widely distributed throughout Georgia on many types of weeds as epiphytes (Gitaitis *et al.*, 2000) and it has been reported to cause diseases on muskmelon (Bruton *et al.*, 1986), honeydew melon (Wells *et al.*,1987) and Sudangrass (Azad *et al.*, 2000) in the USA. The center rot pathogen is transmitted via tobacco thrips, *Frankliniella fusca* (Wells *et al.*, 2002; Gitaitis *et al.*, 2003). Although *P. ananatis* from sudangrass did not cause extensive disease in other plant species studied by Azad *et al*. (2000), the possibility that *P. ananatis* was transferred from another host to onions by thrips cannot be excluded.

P. ananatis was not isolated from diseased onion plants in this study. Leaf and seed stalk blights were caused by *P. syringae*, which was also present in the majority of surveyed onion seed lots (data not shown). Center rot-like symptoms observed on the water logged field in 2005 were caused by *E. cloacae*, an opportunistic pathogen and a common component of the micro flora in water, soil and on plant surfaces (Bishop, 1990; Schwartz and Otto, 2000b). Our survey of onion fields was limited to three provinces in South Africa, and we cannot conclude that the disease was not present in other areas of the country.

P. ananatis was found in three of twenty-eight locally produced *A. cepa* seed lots, and is a potential source of inoculum for the development of center rot. The diseases on sudangrass (Azad et al., 2000), *Eucalyptus* (Coutinho et al., 2002) and onions (Walcott et al., 2002) were prevalent during spring and summer, when temperatures and relative humidity were high. Onion is a winter crop in South Africa, and the arid environmental conditions could be not conductive for the disease development (Webster et al., 1983). A rapid screening technique to detect this bacterium in seed has to be evaluated, and commercially important seeds should be tested to determine their levels of infestation.

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Table 1. Recovery of *P. ananatis* from naturally infested *A. cepa* seed on PA 20 medium [a] and its pathogenicity to onion (cv. Granex 33) in a stub inoculation pathogenicity test [b].

Seed lot	Description Cultivar	*P. ananatis* CFU/gram of seed [a]	Pathogenicity to onion 20 strains per seed lot	Strains selected for characterization
1	Commercial, Granex 33	9.4×10^3	0 / 20	-
2	Experimental, Granex type	2.4×10^6	15 / 20	BD 336
3	Experimental, Granex type	2.2×10^3	9 / 20	PA 4
4	Farmer – own use, unknown	5.0×10^6	20 / 20	BD 390

[a] Characteristic yellow colonies, 3-4 mm in diameter, shiny, drop-shaped with small, granular, darker inclusions on PA 20 medium were regarded as *P. ananatis*. Colonies were counted after 7 days of incubation. Figures represent the mean of three replicates.

[b] Results were recorded after 4 days. Positive reaction: water soaked spots that expanded into longitudinal, bleached-green lesions with chlorotic margins (Goszczynska *et al*., 2006).

Table 2. Bacterial strains characterized in this study and their pathogenicity to three onion cultivars.

Strain [a]	Origin	16S rDNA GenBank accession number [c]	Pathogenicity to onion cultivars in a greenhouse test		
			A. cepa		*A. fistulosum*
			cv. Granex 33	cv. Pyramid	cv. White Welsh
	USA [b]				
BD 301	Blackshank 15, diseased onion	AY579209	+	+	-
BD 309	Horticulture Hill 24, diseased onion	AY579210	+	+	-
BD 310	Horticulture Hill 31, diseased onion	AY579211	+	-	-
BD 315	Pans 2002-2, diseased onion	AY579212	+	+	-
	South Africa				
BD 336	Onion seed	AY530794	+	-	-
BD 390	Onion seed	AY530795	+	+	-
PA 4	Onion seed	AY530796	+	-	-
BD 287 [b]	SUH 2, leaf & stalk necrosis	AY530797	+	+	+
	Reference strains				
LMG 20103	*P. ananatis*, Eucalyptus	AF364844	-	-	-
LMG 20104	*P. ananatis*, Eucalyptus	AF364845	-	-	-
LMG 1286 T	*P. agglomerans*, type strain	AJ233423	nd	nd	nd
LMG 2665 T	*P. ananatis*, type strain	Z96081	nd	nd	nd

[a] BD and PA strains: accession numbers of the Plant Pathogenic and Plant Protecting Bacteria (PPPPB) culture collection, ARC-PPRI, Pretoria, South Africa. American strains deposited by R. Walcott, Department of Plant Pathology, University of Georgia, Athens, USA. LMG strains: accession numbers of the BCCM/LMG Culture Collection, University Gent, Belgium.

[b] Isolated in 1981, Hattingh and Walters (1981).

[c] 16S rDNA sequences of reference strains obtained from the GenBank database.

nd – not done

Table 3. Physiological and biochemical characteristics of the American and South African onion strains and reference strains of *P. ananatis* and *P. agglomerans* using the API 20E and API 50CHE systems.

Characteristic [a]	*P. ananatis*		American strains				South African strains			*P. agglomerans*	
	LMG2665^T	LMG20103 LMG20104	BD 301	BD 309	BD 310	BD 315	BD336	BD390	PA4	LMG1286^T	BD 287
API 20E											
ß-galactosidase	+	+	+	+	-	+	+	+	+	+	+
Simmons citrate	+	+	+	+	+	+	-	+	+	-	-
Production of											
Indole	+	+	+	+	+	+	+	+	+	-	-
Acetoin	+	+	+	+	+	+	-	+	+	+	+
Gelatinase	-	+	-	-	-	-	-	-	-	-	-
API 50CHE											
Acid production											
Glycerol	+	+	+	+	+	+	+	+	+	-	-
L-rhamnose	+	+	+	+	+	+	-	+	+	+	+
Inositol	+	+	+	+	+	+	+	+	+	-	-
D-sorbitol	+	+	+	+	+	+	+	+	+	-	-
Methyl-α-D-mannopyranoside	+	-	+	-	+	+	-	+	+	-	-
Amygdalin	-	+	-	+	-	+	+	+	+	-	-

Characteristic [a]	*P. ananatis*		American strains				South African strains			*P. agglomerans*	
	LMG2665^T	LMG20103 LMG20104	BD 301	BD 309	BD 310	BD 315	BD336	BD390	PA4	LMG1286^T	BD 287
D-cellobiose	+	+	+	+	+	+	+	+	+	-	+
D-lactose	+	+	+	+	+	+	+	+	+	-	+
D-melibiose	+	+	+	+	+	+	+	+	+	-	+
D-raffinose	+	+	+	+	+	+	+	+	+	-	-
D-fucose	-	-	-	-	-	-	-	-	-	-	+
D-arabitol	+	+	+	+	+	+	+	+	+	-	-
Potassium gluconate	-	-	-	-	±	-	±	-	-	-	±
Aesculin hydrolysis	+	+	+	-	+	-	+	+	-	+	+

[a] API 20E tests data was recorded after 24 hours, API 50CHE data (acid production) after 48 hours. Incubation temperature was at 30^{o}C. Symbol (+) indicates positive reaction; symbol (-) negative reaction; symbol (±) uncertain.

All strains produced acid from: L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, N-acetyloglucosamine, gentibiose, arbutin, salicylin, D-maltose, sucrose and D-trehalose.

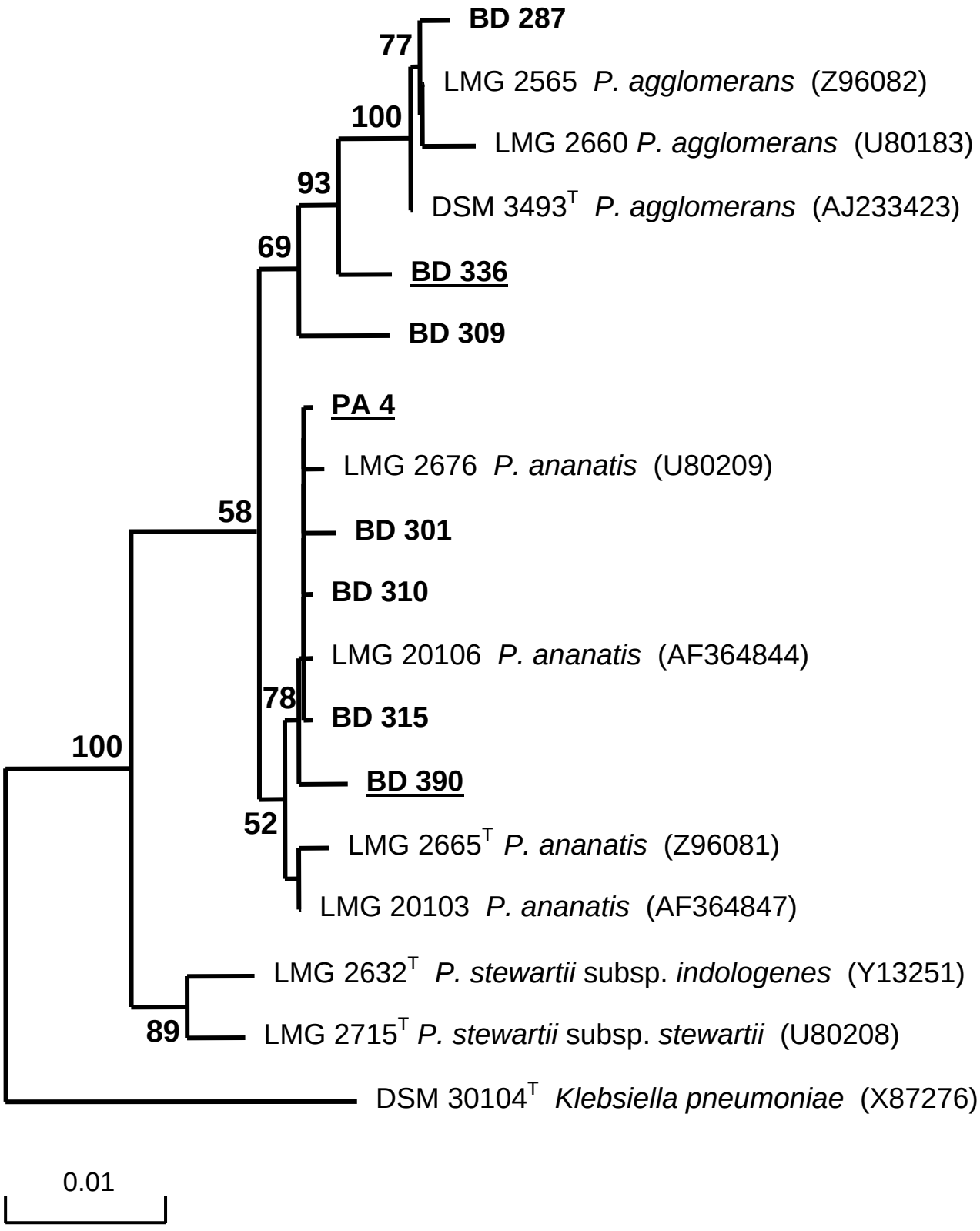
All strains were negative in the following tests: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S, urease, tryptophane desaminase and acid production from: erythitol, D-arabinose, L-xylose, D-adonitol, methyl-B-D-xylopyranoside, L-sorbose,

dulcitol, methyl-α-D-glucopyranoside, inulin, D-melezitose, starch, glycogen, xylitol, D-turanose, D-lyxose, D-tagatose, L-fucose, L-arabitol, potassium 2-ketogluconate and potassium 5-ketogluconate.

Fig. 1. Symptoms on onion induced by the strains associated with center rot in the USA and by the South African strains isolated from onion seed.

Fig. 1

Fig. 2. Phylogenetic tree showing the relationship among selected partial 16S rDNA sequences from *Pantoea* species, including onion strains (in bold; South African strains from seed are underlined), based on pairwise comparisons using the Jukes-Cantor parameter. The sequence of *Klebsiella pneumoniae* was used as an outgroup taxon. The numbers at the nodes indicate the levels of bootstrap support based on data for 1.000 replicates; only values that are greater then 50% are shown. Accession numbers (in brackets) and the sequences of *P. ananatis*, *P. agglomerans*, *P. stewartii* and *K. pneumoniae* were obtained from the GenGank/EMBL databases.


BD 287
77
LMG 2565 P. agglomerans (Z96082)
100
LMG 2660 P. agglomerans (U80183)
93
DSM 3493T P. agglomerans (AJ233423)
69
BD 336
BD 309
PA 4
LMG 2676 P. ananatis (U80209)
58
BD 301
BD 310
LMG 20106 P. ananatis (AF364844)
78
BD 315
100
BD 390
52
LMG 2665T P. ananatis (Z96081)
LMG 20103 P. ananatis (AF364847)
LMG 2632T P. stewartii subsp. indologenes (Y13251)
89
LMG 2715T P. stewartii subsp. stewartii (U80208)
DSM 30104T Klebsiella pneumoniae (X87276)
0.01

CHAPTER 4

Isolation and Identification of the Causal Agent of Brown Stalk Rot, a New Disease of Maize in South Africa

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ABSTRACT

During 2004-2005, an unreported disease of maize (*Zea mays*) was observed on commercial fields in the Northwest and Mpumalanga Provinces of South Africa. Infected plants were stunted, with a vertical crack at the fist internode. Inside the stem, a dark brown, narrow lesion was present along the crack. Internal browning inside the stem extended upwards, reaching the top internode in some plants. Seed cobs were underdeveloped. Diseased plants were scattered in the fields and 10-70% of the crop was affected. Gram-negative, facultatively anaerobic bacteria were consistently isolated from diseased tissues. Pathogenicity tests established that representative strains induced disease symptoms similar to those observed on maize plants in the field. Physiological and biochemical characterization using the API 20E and API 50CHE systems and 16S rRNA gene sequence analyses showed that the strains belonged to the genus *Pantoea*. The results of these tests also separated the strains into two groups. The first group, giving a positive reaction in the indole test, was similar to *P. ananatis*. The second group of strains was indole negative and resembled *P. agglomerans*. The F-AFLP genomic fingerprints generated by the indole positive strains and *P. ananatis* reference strains were similar and clustered together in the dendrogram, confirming that the indole positive bacteria causing brown stalk rot on maize were *P. ananatis*. The F-AFLP fingerprints produced by the indole negative strains were distinctly different from those generated by *P. ananatis, P. agglomerans, P. dispersa, P. citrea, P. stewartii* subsp. *stewartii* and *P. stewartii* subsp. *indologenes*. The results indicated that indole negative bacteria causing brown stalk rot on maize might belong to a previously undescribed species of the genus *Pantoea*. This is the first report of a new disease on maize, brown stalk rot, caused by two bacterial species, *P. ananatis* and an undescribed *Pantoea* sp.

Additional keywords: corn, detection

Maize (*Zea mays*) is the most important grain crop in South Africa, being both the major animal feed grain and the staple food of the majority of the population. For the 2003/2004 marketing year maize was responsible for the second largest contribution to the gross value of agricultural production in the country. The South African maize industry is also the largest maize industry in Africa (39). Commercial farmers cultivate nearly three million hectares of maize per year. In the past five years, South Africa produced between 7.2 and 10.1 million tons of maize per annum, with an average of 9.2 million tons. The main maize production areas in South Africa are the Free State, Northwest and Mpumalanga Provinces. These three provinces are responsible for 85% of the total maize produced in the country (7, 39).

Numerous fungal diseases cause excessive damage to maize in South Africa (12), especially *Puccinia* common rust (13), *Cercospora* gray leaf spot (20), *Aureobasidium* leaf spot (18), *Fusarium* (14, 15, 16) and *Stenocarpella* (13) stem, cob and root rots. By contrast, only two bacterial diseases have been reported on maize in the country, leaf streak (11) caused by *Xanthomonas campestris* pv. *zeae* and bacterial stalk rot (35), caused by *Dickeya zeae*.

In January 2004, a new disease was observed in a single breeder's field in the Mpumalanga Province on an inbred female line. In December 2004 and January/February 2005 the same symptoms were also found in commercial maize fields in the Northwest and Mpumalanga Provinces. The disease was prevalent on the inbreed female and single cross female lines and a commercial hybrid SR 52. Affected plants were stunted and a vertical crack at the first internode was always present (Fig. 1A). In most diseased plants, the crack extended into the second or sometimes the third internode. When the crack was split open, a dark brown, narrow lesion was visible along the crack (Fig.1B). Internal browning inside the stem extended upwards, reaching the top internode in some plants (Fig. 1C). Seed cobs were underdeveloped. On one field, the leaf margins were soft, bleached-green and curled inwards (Fig. 1D). Diseased plants were scattered throughout the fields and 10-70% of the crop was affected.

When examined under a microscope, cut edges of symptomatic tissues consistently exhibited bacterial streaming. Gram negative bacteria producing yellow colonies were consistently isolated from diseased tissues and these were tentatively identified as belonging to the genus *Pantoea* (32). Two bacterial species of *Pantoea* have been reported to cause diseases on maize and other *Poaceae* hosts. *P. stewartii* subsp. *stewartii* causes Stewart's wilt in Europe, Asia and the Americas, but not in South Africa (8, 33). *P. ananatis* was described as an agent of leaf spot on maize in Brazil (34), stem necrosis of rice in Australia

(9) and glume and grain discoloration of rice in Japan (4). *P. ananatis* and *P. stewartii* subsp. *indologenes* were reported to cause leaf blotch on sudangrass in California (3).

The aim of this study was to identify and characterize the causal agent of brown stalk rot of maize observed in South Africa.

MATERIALS AND METHODS

Isolation of the causal agent from plants. Maize breeders and commercial farmers reported ten diseased maize fields throughout Mpumalanga and Northwest Provinces. Typical brown stalk rot symptoms were observed in these fields, on which the inbreed female, the single cross female lines and the hybrid SR 52 were cultivated. One hundred diseased plants with brown stalk rot symptoms, approximately ten plants per field, were collected in January 2004, December 2004 and February 2005.

Isolations were done from all collected plants, from at least one stalk lesion per plant and from the necrotic stripes on leaves observed in one field. Small sections of stalk or leaf tissue with symptoms were cut aseptically from the margins of lesions and macerated in 1 ml of sterile distilled water for 20 min at 25°C. Plant extracts were streaked onto nutrient agar (Difco, Sparks, MD), tryptone glucose extract agar (TGA) (Difco) and King's B (30) media. Plates were incubated at 25°C and examined after 3 to 5 days for bacterial growth. On nutrient agar, bacterial colonies were very small, approximately 1 mm in diameter. On King's B, bacterial growth was very slimy and it was difficult to distinguish single colonies. On TGA, yellow, circular, well-separated colonies, 3-4 mm in diameter, were visible 2 to 3 days after plating. After the initial isolations from approximately 20 plants, subsequent isolations were made on TGA plates only. Plates were incubated at 25°C and examined after 3 to 5 days for bacterial growth. Suspected colonies were purified by streaking onto TGA and cultures stored in milk-glycerol liquid medium (10% skim milk, 15% glycerol in distilled water) at –20°C for further analysis. The same procedure was used to re-isolate bacteria from inoculated plants.

Preliminary identification of bacteria. Ten to twenty yellow bacterial colonies per field isolated on TGA medium were purified and used in preliminary identification tests. The strains were characterized by colony morphology on TGA, motility, cell morphology, Gram stain, catalase and oxidase reaction, Hugh-Leifson oxidation/fermentation test, production of

indole, gas from glucose and hydrogen sulphide from cysteine (32), according to methods described by Fahy and Hayward (17).

Bacterial strains. Bacterial strains from maize characterized in this study are listed in Table 1. Type strains of *P. ananatis*, *P. agglomerans*, *P. dispersa*, *P. citrea*, *P. stewartii* subsp. *indologenes* and *P. stewartii* subsp. *stewartii* and seven *P. ananatis* strains isolated from sudangrass, onion and *Eucalyptus* were used as reference strains (Table 1). *P. stewartii* subsp. *stewartii* is a quarantine organism in South Africa and only DNA was used in this study. Stock cultures of all strains were maintained in milk-glycerol liquid medium at –20°C. Stock strains were transferred onto TGA plates and incubated at 25°C to recover growing cultures. Cultures were routinely checked for purity and colony characteristics on TGA.

Pathogenicity tests. Pathogenicity on maize was determined in greenhouse assays on maize cultivar SR 52, shown to be susceptible in the field. Thirteen strains from maize, two *P. ananatis* from *Eucalyptus*, five from onion and one from sudangrass were used in pathogenicity tests (Table 1). Each bacterial strain was grown on TGA plates for 24-48 hours at 25°C, and each strain was suspended in sterile distilled water to obtain approximately 10^7 CFU/ml. The concentrations were confirmed by dilution plating on TGA plates. Maize plants were inoculated immediately after preparation of the bacterial suspensions. In the first experiment, maize plants were four weeks old and in the second experiment, plants were nine weeks old. Four-week-old plants were inoculated using two methods: (a) suspensions of single strains were infiltrated into leaf tissues with a syringe and (b) suspensions of single strains were injected into stems below the first leaf whorl. Inoculated plants were placed in plastic bags and maintained in a greenhouse with natural light and 28/23°C day/night temperature. After seven days the plastic bags were removed and plants were kept in the same greenhouse. Relative humidity (RH) fluctuated between 50 and 70%. Nine-week-old plants were inoculated by injecting a few drops of bacterial suspensions into the first internode of the stem. Stem-inoculated plants were maintained in a greenhouse with natural light and at temperatures and RH described above. At least two plants were inoculated with each bacterial strain per inoculation method. Each experiment continued for ten weeks. Control plants in all experiments were inoculated with sterile distilled water. Pathogenicity tests were performed twice.

Bacteria re-isolated from symptomatic tissues were confirmed by colony morphology on TGA, Gram stain, indole production, and Huigh-Leifson and oxidase tests (10, 22).

Biochemical and physiological tests. Biochemical and physiological characteristics of the strains listed in Table 1 were examined with the API 20E and API 50CHE systems (BioMérieux, La Balme les Grottes, Montalieu Vercieu, France), according to the procedure recommended by the manufacturers. The results of the API 20E and API 50CHE tests were recorded after 24 and 48 hours of incubation at 30°C, respectively. Profiles were identified using the APILAB V4.0 identification program (BioMérieux). The data for the *P. stewartii* subsp. *stewartii* strain LMG 2715^T was obtained from the paper by Mergaert et al. (32).

The cluster analysis was performed with the combined API 20E (21 characters) and API 50CHE (49 characters) data, using the Bionumerics software (version 4.5, Applied Maths, Kortrijk, Belgium). Similarity matrixes were constructed with the Dice coefficient and cluster analyses were performed by the unweighted pair group method (UPGMA). The data was presented as an UPGMA dendrogram.

DNA extraction. Genomic DNA of bacterial strains (Table 1) was extracted by using the GenElute Bacterial Genomic DNA Kit (Sigma, Steinheim, Germany), according to the manufacturer's instructions. Purified DNA was quantified using a Dyna Quant 200 fluorometer (Hoefer, San Francisco, CA) and Hoescht H 33258 intercalating dye (Polysciences, Warrington, PA). The DNA concentration was adjusted to 25-50 ng µl $^{-1}$ with sterile water. The DNA was stored at –20°C until further analysis.

Fluorescent amplified fragment length polymorphism analysis. All strains listed in Table 1 were examined by the fluorescent amplified fragment length polymorphism (F-AFLP)-based system for the identification of plant-associated species from the genus *Pantoea* (6).

Genomic DNA, 50-100 ng from each isolate, was digested with *Eco*RI and *Mse*I (Roche, Mannheim, Germany) restriction enzymes, and then ligated to the respective adaptors for 2 hours at 20°C. Ligation reactions were diluted 1:10 in nuclease-free water for pre-amplification with Eco-00 (5'-GAC TGC GTA CCA ATT C-3') and Mse-00 (5'-GAT GAG TCC TGA CTA A-3') primers (Inquaba, Pretoria, South Africa). Each 25 µl pre-amplification reaction contained PCR buffer (10 mM Tris-HCl, pH 9.0 at 25°C; 50 mM KCl, 0.1% Triton X-100); 1.5 mM $MgCl_2$, 250 µM of each dNTPs; 100 pmol of each primer; 1 U Taq polymerase (Qiagen, Hilden, Germany) and 2 µl diluted ligation reaction. Amplification

conditions were as follows: initial denaturation at 94°C for 3 min; 20 cycles at 94°C for 30 s, 56°C for 1 min and 72°C for 1 min; and final elongation at 72°C for 5 min. Each pre-amplification reaction was diluted 1:50 in nuclease-free water prior to selective amplification with Mse-CG (5'-GAT GAG TCC TGA CTA ACG-3') and fluorescently labeled Eco-G (5'-GAC TGC GTA CCA ATT CG-3') primers (Inquaba). A volume of selective PCR reactions was 20 µl, and each contained: PCR buffer; 1.5 mM $MgCl_2$; 250 µM of each dNTPs; 0.5 pmol Eco-G primer; 2.4 pmol Mse-CG primer; 1 U Taq polymerase and 5 µl diluted pre-amplification reaction. The selective PCR amplifications included the following steps: denaturation at 94°C for 5 min; 9 cycles of denaturation at 94°C for 30 s, initial annealing at 65°C for 30s (annealing temperature was reduced by 1°C per cycle until 56°C was reached) and elongation at 72°C for 1 min; 23 cycles at 94°C for 30 s, 56°C for 30 s and 72°C for 1 min, followed by 5 min extension at 72°C. Amplifications were performed at least twice for each strain in the Hybaid Omni Gene thermocycler (Teddington, England).

The selective amplification reactions (1 µl) were mixed with an equal volume of formamide loading buffer (95% formamide, 20 mM EDTA, bromophenol blue), heated for 3 min at 90°C and then chilled on ice for 10 min. A volume of 0.5-0.8 µl of each mixture was loaded onto the gel. The AFLP products were separated in 8% Long Ranger (LI-COR Biosciences, Lincoln, NE) denaturing gels on a LI-COR IR automated sequencer. Electrophoresis was carried out for 4 hours at 1500 V in 0.8 x TBE buffer. Images were imported into BioNumerics software. Gels were standardized with a 50-700 bp sizing standard (LI-COR) and the bands between 50 and 700 bp were analyzed (6). Similarity was calculated using the Dice correlation coefficient and cluster analysis was performed by the UPGMA method. The data was presented as an UPGMA dendrogram.

Amplification and sequencing of the 16S rRNA gene. The 16S rRNA gene fragment of thirteen strains from maize (Table 1) was amplified using the universal primers fD1 (5'-AGA GTT TGA TCC TGG CTC AG-3') and rD1 (5'-AAG GAG GTG ATC CAG CCG CA-3') (41). DNA was amplified in 50 µl reaction volumes containing PCR buffer (10 mM Tris-HCl, pH 9.0 at 25°C; 50 mM KCl, 0.1% Triton X-100); 1.5 mM $MgCl_2$; 150 µM dNTPs; 1.0 µM each primer; Taq polymerase (Promega Corp., Madison, WI) 1 unit per reaction volume and 25-50 ng DNA template μl^{-1}. Amplifications were performed according to Weisburg *at al*. (41) in a Hybaid Omni Gene thermocycler.

The amplified PCR products were cut from the gel and purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany), and then cloned with the pGEM-T Easy Vector System II kit (Promega, Madison, WI) according to the manufacturer's instructions. Plasmids with inserts were purified using the QIAprep Spin kit (Qiagen). The sequencing was performed using the Dyenamic ET Dye terminator cycle sequencing kit for MegaBACE with dideoxynucletide chain-termination chemistry. Sequences were analyzed using a MegaBACE 500 Sequencer (Amersham Biosciences, Buckinghamshire, England) with MegaBACE 500 Sequence Analyzer (version 2.4) software.

The GenBank/EMBL databases were used for homology searches using the BLAST program (National Center for Biotechnology Information, U.S. National Institute of Health, Bethesda, MD).

Sequence analysis. The 16S rDNA nucleotide sequences obtained in this study were aligned with a selection of 16S rDNA sequences of *Pantoea* species from GenBank with the MAFFT software, ver. 5.743 (28). Phylogenetic trees were constructed with the neighbor joining method (36) and evolutionary distances calculated according to the method of Kimura (29), using the MEGA software package, version 3.1, Kumar, Tamura, Nei, 2004 (31). Bootstrap analysis of the data, based on 1000 permutations was used to assess the stability of relationships.

RESULTS

Isolation of the causal agent. Yellow bacterial colonies were consistently isolated from all diseased tissues on TGA medium. Colonies were circular, 2-3 mm in diameter, raised, regular and shiny. One hundred and thirty-seven colonies were purified on TGA and used in

preliminary identification tests. All strains were gram negative rods, motile, oxidase negative and catalase positive. They did not produce H_2S from cysteine and gas from glucose. All strains fermentatively utilized glucose. The majority of isolates produced indole, with the exception of all strains isolated from a single field of cv. SR 52 maize in the Northwest Province.

Pathogenicity test. When four-week-old seedlings were inoculated, no lesions or other disease symptoms developed within ten weeks on any of the maize inoculated with any of the bacterial cultures.

When bacterial suspensions were injected into the stems of nine-week-old plants, all thirteen strains from maize produced brown stalk rot symptoms within six to seven weeks (Table 1). Four to six days after inoculation, small, 2-4 mm, light brown or dark green lesions developed around the inoculation point. Drops of yellow liquid slowly oozed from the lesion for two to three days. After 6 to 7 weeks, a single, long, vertical crack appeared on the injected internode. A dark brown, narrow lesion was present in an internal stem tissue along the crack. The internal browning within the stem was also observed in upper internodes. One strain from maize, BD 561, also induced the symptoms on leaves, similar to those observed in the field.

P. ananatis from *Eucalyptus*, onion and sudangrass did not cause brown stalk rot on maize. However, four strains, LMG 20103 and LMG 20104 from *Eucalyptus* and BD 315 and PA 4 from onion produced the symptoms on maize leaves, when nine-week-old plants were stem-inoculated. Four to five weeks after injection, yellow lesions appeared on young leaves. Within a week, lesions spread along leaf veins and turned bleached-green. Tissues inside the stem appeared to be healthy.

Colonies recovered from plants with disease symptoms were yellow, gram negative rods, oxidase negative and utilized glucose as a facultative anaerobe. All strains, except re-isolated BD 500, BD 502 and BD 639 (isolated from one field in the Northwest Province) produced indole. Control plants inoculated with water did not develop symptoms, nor were yellow bacteria isolated from leaves and stems of these plants on TGA.

Physiological and biochemical characterization. The results of physiological and biochemical tests using the API 20E and API 50CHE systems separated thirteen strains from maize into two groups (Fig. 2). Ten indole positive strains had similar biochemical characteristics and resembled the type strain of *P. ananatis* LMG 2665^T and *P. ananatis*

reference strains from *Eucalyptus*, onion and sudangrass (Table 2). Unlike other *P. ananatis* used in the study and 18 strains of *P. ananatis* studied by Mergaert et al. (32), three strains from maize produced acid from D-lyxose. The profiles of all these strains were identified by APILAB program as *Pantoea* spp. 2.

Three indole negative strains from maize, BD 500, BD 502 and BD 639, were similar to *P. agglomerans* LMG 1286^T, and formed a separate group on the dendrogram (Fig. 2). The ability to produce acid from inositol, D-melibiose, starch, glycogen, D-fucose and D-arabitol, distinguished three indole negative isolates from LMG 1286^T (Table 2). None of the 21 *P. agglomerans* strains studied by Beji et al. (5), 16 by Gavini et al. (19) and 70 strains studied by Verdonck et al. (40), produced acid from glycogen or D-fucose, but some did from starch, D-melibiose and D-arabitol. The profiles of LMG 1286^T, BD 500, BD 502 and BD 639 were identified as *Pantoea* spp. 3.

The type strains of *P. citrea, P. dispersa, P. stewartii* subsp. *indologenes* and *P. stewartii* subsp. *stewartii* produced different profiles and did not cluster with maize strains on the UPGMA dendrogram constructed with the results of the API 20E and API 50CHE tests (Fig. 2).

F-AFLP analysis. Strains from maize, *P. ananatis* reference strains and five type strains of other *Pantoea* species generated complex DNA fingerprints from extracted genomic DNA (Fig. 3). The selective primers Eco-G/Mse-CG (6) yielded well-defined DNA fingerprints, with an average of 80 bands per isolate. Amplified DNA fragments ranged in size from approximately 50 bp to slightly greater than 700 bp and revealed a high degree of genetic diversity among the strains. Although significant differences were observed between the major groups, within each group of strains the fingerprints correlated.

Ten indole positive strains from maize generated fingerprints that were similar to *P. ananatis* and they were contained in a cluster with *P. ananatis* type strain, LMG 2665^T, and *P. ananatis* reference strains (Fig. 3). The similarity values among these strains were between 73 and 96%.

F-AFLP fingerprints generated by the three indole negative strains from maize were almost identical (88 to 94% similarity), but clearly different from that produced by the indole positive isolates and the type strains of *P. ananatis*, *P. agglomerans*, *P. citrea*, *P. dispersa*, *P. stewartii* subsp. *stewartii* and *P. stewartii* subsp. *indologenes.*

16S rDNA sequence analysis. The 16S rDNA sequences of thirteen strains from maize obtained in this study were deposited in the GenBank database. Accession numbers are listed in Table 1.

BLAST searches with the 1.5 kb sequences showed that 16S rDNA of ten indole positive strains had the highest homology (99.3 to 99.9%) to the 16S rDNA of *P. ananatis* strains LMG 20103 (accession number AF364847), LMG 20106 (AF364844) and PA 4 (AY530796). The 16S rDNA sequence of the indole negative strains had the highest homology, 99.6%, to *P. agglomerans* DSM 3493^T (AJ233423).

Figure 4 shows the phylogenetic relationship derived from a neighbor-joining analysis of the pairwise comparison among the 16S rDNA sequences of thirteen strains from this study with fifteen sequences of five described species of the genus *Pantoea*. *Enterobacter cloacae* ATCC 13047^T (AJ251469) *Klebsiella pneumoniae* DSM 30104^T (X87276) and *Escherichia coli* ATCC 11775^T (X80725) were used as the outgroup taxons. Phylogenetic evaluation confirmed the division of strains from maize into two groups at 99% confidence value from bootstrap analysis. The tree demonstrated clearly that the sequences of all indole positive isolates from maize clustered with *P. ananatis*. Three indole negative strains grouped closely with *P. agglomerans*. However, the three sequences formed a subcluster in the tree, separate from *P. agglomerans*, at a high, 91% confidence value.

DISCUSSION

In this study, we describe a new disease on maize, brown stalk rot, caused by two species of bacteria belonging to the genus *Pantoea*, *P. ananatis* and what appears to be a previously undescribed *Pantoea* sp. Koch's postulates were confirmed using thirteen strains, which were subjected to further taxonomic investigations.

Nutritional and biochemical characterization using the commercial systems API 20E and API 50CHE, showed that the ten strains from maize had similar biochemical characteristics and resembled the type strain of *P. ananatis* and *P. ananatis* reference strains (Fig 2, Table 2). Although all indole positive, some strains varied slightly in a few biochemical tests from that characteristic for the species (32), such as producing acid from D-lyxose but not from D-sorbitol or arbutin. Several *P. ananatis* from *Eucalyptus* (10), rice (9) and onion (22) also showed a few unusual biochemical properties. The genotypic characterization confirmed that the ten strains were *P. ananatis*. The 16S rDNA sequences of all indole positive isolates were 99.3 to 99.9% homologous to that of *P. ananatis* and all clustered with *P. ananatis* in a

neighbor-joining phylogenetic tree (Fig. 4). The AFLP genomic fingerprinting technique was demonstrated as a rapid, discriminatory method not only to determine the taxonomic diversity of bacteria (37), but also to identify them to the species level (2, 24, 25, 27). The F-AFLP fingerprints produced by the ten indole positive strains using selective primers Eco-G/Mse-CG (6) strongly resembled that yielded by *P. ananatis* (Fig. 3). We conclude that the indole positive bacteria associated with brown stalk rot of maize in South Africa are *P. ananatis*.

Nutritional and physiological characteristics of the three indole negative strains, BD 500, BD 502 and BD 639 were similar to *P. agglomerans* LMG 1286^T (Fig. 2, Table 2) and their 16S rDNA sequences were 99.6% homologous to the *P. agglomerans* type strain. However, the F-AFLP fingerprints generated by these three strains, which were almost identical to each other, were distinctly different from that of *P. agglomerans* and other *Pantoea* species used in the study. BD 500, 502 and BD 639 could also be differentiated from LMG 1286^T by giving positive reaction in the tryptophane deaminase test and not producing acid from amygdalin on the API 20E strips (Table 2). Therefore, the taxonomic status of these three strains, that were isolated from only one field of maize with brown stalk rot (Table 1), needs to be further investigated.

Two different bacterial species of the genus *Pantoea* were reported to cause similar disease symptoms on other crops. *P. ananatis* and *P. agglomerans* were isolated from onion (21, 23) and proved to induce indistinguishable symptoms on this host in pathogenicity tests (22). *P. punctata* and *P. citrea* were both isolated from mandarin orange (26). *P. ananatis* and *P. stewartii* subsp. *indologenes* were causing leaf blotch of sudangrass (3) and rot of pineapple (32, 38). *P. ananatis* and *Pantoea* spp. isolated from diseased maize produced identical brown stalk rot symptoms on maize SR 52 when the pathogens were injected into the stems of nine-weeks-old plants. However, the disease did not develop following leaf inoculation. Only one strain, *P. ananatis* BD 561, produced the leaf stripes observed in a field, but failed to induce stalk rot when the leaf was inoculated. The results suggested that brown rot develops in stalks if the pathogen enters into plants through the stem. The most damaging insect pests of maize in South Africa are several species of stalk borers (1). The larvae enter the stem by boring a hole, usually some distance above the ground and bores upward in the stem. Stalk borer was present on several maize fields with brown stalk rot and *P. ananatis* was occasionally isolated from the larvae in this study (data not shown). It is possible that stalk borers play a role in the spreading of the brown stalk rot pathogens.

The sudden appearance of brown stalk rot prompted investigations into potential sources of inoculum. A strain associated with the leaf blotch disease of sudangrass in the USA (3) was non pathogenic on maize, but two strains from onion generated leaf symptoms on this plant. Center rot disease of onion has not been found in South Africa on plants and the pathogen was only isolated from onion seed (22), eliminating that host as a source of disease on maize. Although two strains of *P. ananatis* from *Eucalyptus* used in the study did not induce stalk rot on maize, they produced lesions on leaves in pathogenicity tests when injected into the stem. Bacterial blight and dieback of *Eucalyptus* was first observed in South Africa in 1998 (10), six years before the appearance of brown stalk rot on maize. The possibility that *P. ananatis* was transmitted into maize from *Eucalyptus* by an unknown vector cannot be excluded.

The impact of brown stalk rot on maize yield and quality has not been measured. Disease was most severe on inbreed and single cross female lines. This is of considerable concern, as the lines represent some of the crucial breeding stock on which the maize industry is based. More research is required to understand the epidemiology of this new disease, and to develop the management strategies to reduce its expansion.

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Table 1. Strains of *Pantoea* characterized in this study.

Strain [a]	Host and origin MP-Mpumalanga Province NW-Northwest Province	16S rDNA (GenBank accession number) [b]	Pathogenicity test on maize [c]
Strains isolated from maize with brown stalk rot symptoms			
BD 435	Maize, stalk, MP, field 1	AY898642	Brown stalk rot
BD 442	Maize, stalk, MP, field 2	AY898643	Brown stalk rot
BD 500	Maize, stalk, NW, field 3	DQ849042	Brown stalk rot
BD 502	Maize, stalk, NW, field 3	DQ849043	Brown stalk rot
BD 639	Maize, stalk, NW, field 3	DQ512489	Brown stalk rot
BD 543	Maize, stalk, NW, field 4	DQ133545	Brown stalk rot
BD 561	Maize, leaf, NW, field 4	DQ133546	Brown stalk rot Leaf stripe
BD 577	Maize, stalk, MP, field 5	DQ133547	Brown stalk rot
BD 588	Maize, stalk, MP, field 6	DQ133548	Brown stalk rot
BD 602	Maize, stalk, MP, field 7	DQ195522	Brown stalk rot
BD 622	Maize, stalk, MP, field 8	DQ195523	Brown stalk rot
BD 640	Maize, stalk, MP, field 9	DQ195524	Brown stalk rot
BD 647	Maize, stalk, MP, field 10	DQ195525	Brown stalk rot
P. ananatis reference strains from other hosts			
BD 301	Onion, USA	AY579209	No symptoms
BD 315	Onion, USA	AY579212	Leaf stripe
BD 333	Onion, SA	DQ512490	No symptoms
BD 336	Onion, SA	AY530794	No symptoms
PA 4	Onion, SA	AY530796	Leaf stripe
0197-28	Sudangrass, USA	-	No symptoms
LMG 20103	Eucalyptus, SA	AF364847	Leaf stripe
LMG 20104	Eucalyptus, SA	AF364844	Leaf stripe
Type strains			
LMG 2665^T	*P. ananatis*	Z96081	-
LMG 1286^T	*P. agglomerans*	AJ233423	-
LMG 2603^T	*P. dispersa*	DQ504305	-
LMG 2632^T	*P. stewartii* subsp.	Y13251	-

Strain [a]	Host and origin MP-Mpumalanga Province NW-Northwest Province	16S rDNA (GenBank accession number) [b]	Pathogenicity test on maize [c]
	indologenes		
LMG 2715^T	*P. stewartii* subsp. *stewartii*	U80208	-
LMG 22049^T	*P. citrea*	-	-

[a] BD and PA strains: accession numbers of the Plant Pathogenic and Plant Protecting Bacteria (PPPPB) culture collection, ARC-PPRI, Pretoria, South Africa; BD 301 and BD 315 were deposited by R. Walcott, Department of Plant Pathology, University of Georgia, Athens. LMG strains: accession numbers of the BCCM/LMG Culture Collection, University Gent, Belgium. Strain from sudangrass: D.A. Cooksey, Department of Plant Pathology, University of California, Riverside. * *P. stewartii* subsp. *stewartii* is a quarantine organism in South Africa, and only DNA was used in this study.

[b] 16S rDNA sequences of strains from maize were obtained in this study; accession numbers and sequences of other strains were obtained from the GenBank databases.

[c] Symptoms induced on the stem-inoculated maize SR 52 within six to seven weeks, when bacterial suspensions were injected into the stems of nine-week-old plants.

- not done

Table 2. Physiological and biochemical characteristics of the strains isolated from maize with brown stalk rot, and reference strains of *P. ananatis* and *P. agglomerans* using the API 20E and API 50CHE systems.

Characteristic [a]	*P. ananatis* LMG 2665^T Type strain	*P. ananatis* from onion, *Eucalyptus* and sudangrass	BD435,442,543,561, 577,588,602,622,640, 647 from maize	*P. agglomerans* LMG 1286^T Type strain	BD 500, 502, 639 from maize
API 20E					
Citrate utilization	+	d	+	-	-
Tryptophane deaminase	-	-	-	-	+
Indole	+	+	+	-	-
Gelatinase	-	d	d	-	+
Acid from					
Inositol	+	d	d	-	+
D-sorbitol	+	+	d	-	-
L-rhamnose	+	d	+	+	+
D-sucrose	+	d	+	+	+
Amygdalin	+	+	d	+	-
API 50CHE					
Acid from					
Glycerol	+	+	+	±	±
L-rhamnose	+	d	+	+	+
Inositol	+	+	+	-	+

Characteristic [a]	*P. ananatis* LMG 2665^T Type strain	*P. ananatis* from onion, *Eucalyptus* and sudangrass	BD435,442,543,561, 577,588,602,622,640, 647 from maize	*P. agglomerans* LMG 1286^T Type strain	BD 500, 502, 639 from maize
D-sorbitol	+	+	d	-	-
Methyl-α-D-mannopyranoside	+	d	d	-	-
Amygdalin	-	d	d	-	-
Arbutin	+	+	d	+	d
D-cellobiose	+	+	+	-	d
D-maltose	+	d	d	+	+
D-lactose	+	+	+	-	-
D-melibiose	+	+	+	-	+
Sucrose	+	d	+	+	+
D-raffinose	+	d	+	-	-
Starch	-	-	-	-	d
Glycogen	-	-	-	-	d
Gentibiose	+	+	d	±	+
D-lyxose	-	-	d	-	-
D-fucose	-	-	-	-	±
D-arabitol	+	+	+	-	+
Potassium gluconate	-	d	d	-	-

Characteristic [a]	*P. ananatis* LMG 2665^T Type strain	*P. ananatis* from onion, *Eucalyptus* and sudangrass	BD435,442,543,561, 577,588,602,622,640, 647 from maize	*P. agglomerans* LMG 1286^T Type strain	BD 500, 502, 639 from maize
Aesculin hydrolysis	+	d	d	+	d

[a] API 20E test data was recorded after 24 hours, API 50CHE test data was recorded after 48 hours. Incubation temperature was at 30°C. Symbol (+) indicates positive reaction; symbol (-) negative reaction; symbol (±) weak positive reaction; symbol (d) reaction differs.

All strains gave positive reactions in the following tests: API 20E – ß-galactosidase, acetoin and acid from D-glucose, D-mannitol, D-melibiose, L-arabinose; API 50CHE – acid production from: L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, N-acetyloglucosamine, salicylin and D-trehalose.

All strains were negative in the following tests: API 20E – arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S, urease; API 50 CHE – acid production from: erythitol, D-arabinose, L-xylose, D-adonitol, methyl-ß-D-xylopyranoside, L-sorbose, dulcitol, methyl-α-D-glucopyranoside, inulin, D-melezitose, xylitol, D-turanose, D-tagatose, L-fucose, L-arabitol, potassium 2-ketogluconate and potassium 5-ketogluconate.

Fig. 1. Brown stalk rot symptoms on maize observed in a field. A, vertical crack on the first internode of the stem; B, long, brown lesion along the crack; C, internal browning inside the stem; D, curling of the leaf edges.

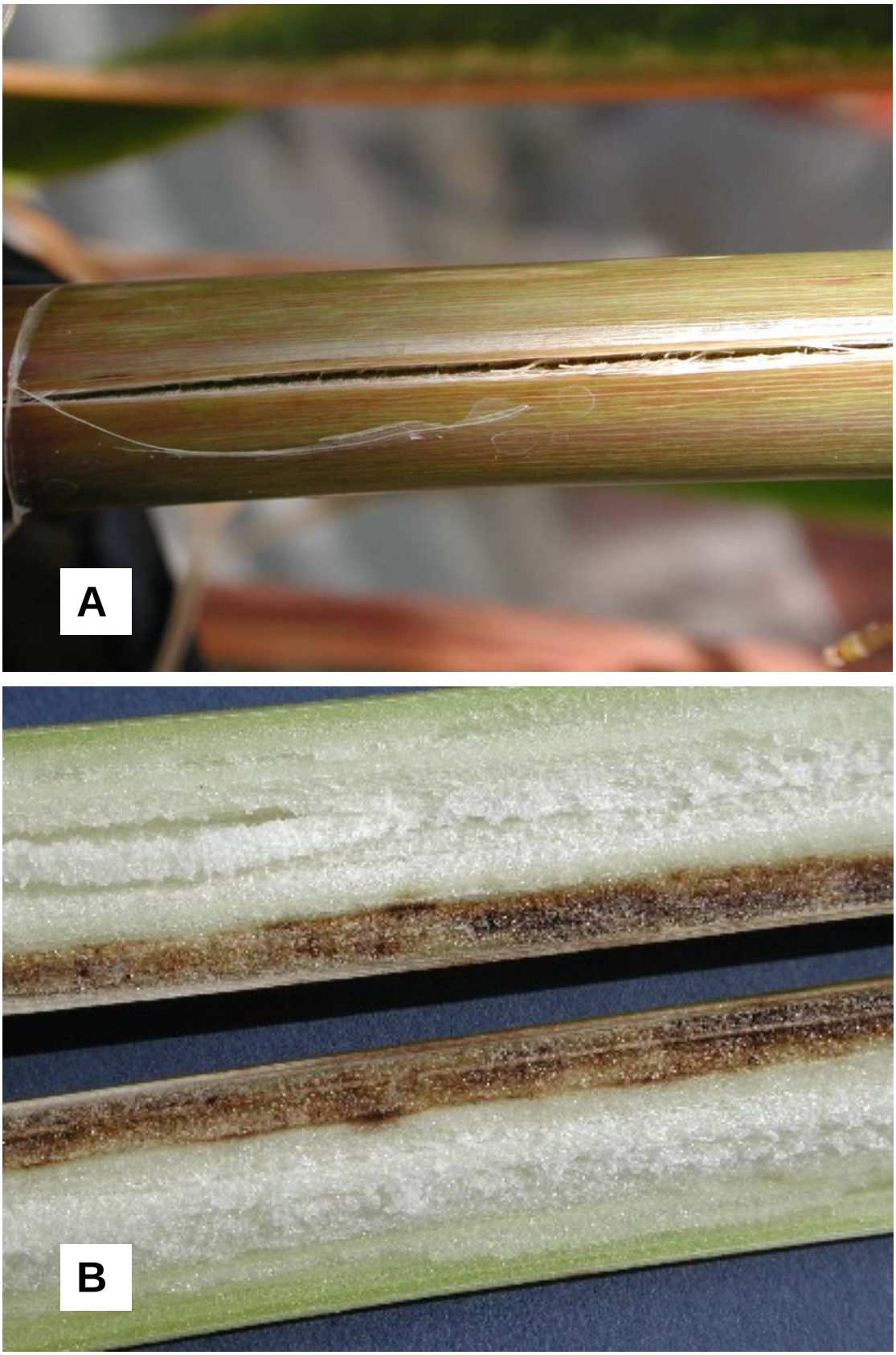


Fig. 1

Fig. 1

Fig. 2. A dendrogram based on the results of the API 20E and API 50CHE systems (BioMérieux, La Balme les Grottes, Montalieu Vercieu, France) tests, showing relationships among bacterial strains isolated from maize with brown stalk rot, *P. ananatis* reference strains, *P. agglomerans*, *P. citrea*, *P. dispersa*, *P. stewartii* subsp. *stewartii* and *P. stewartii* subsp. *indologenes.* Similarity matrixes were constructed using the Dice coefficient and cluster analyses were performed by the unweighted pair group method with the Bionumerics program (version 4.5, Applied Maths, Kortrijk, Belgium). Strains from maize are in bold (indole negative strains are underlined). The data for *the P. stewartii* subsp. *stewartii* LMG 2715^{T} was obtained from the paper by Mergaert et al. (32).

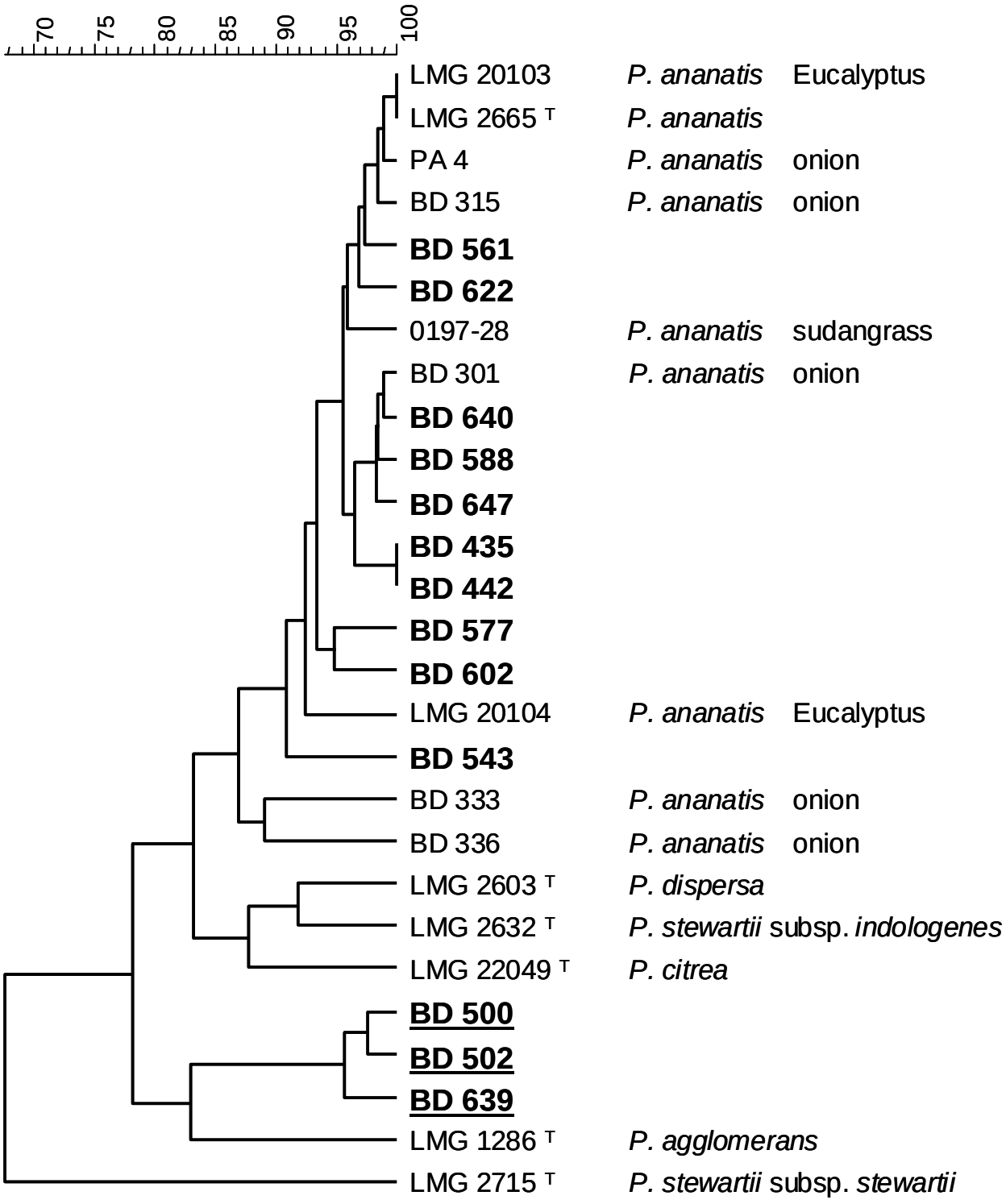

70
75
80
85
90
95
100
LMG 20103 P. ananatis Eucalyptus
LMG 2665 T P. ananatis
PA 4 P. ananatis onion
BD 315 P. ananatis onion
BD 561
BD 622
0197-28 P. ananatis sudangrass
BD 301 P. ananatis onion
BD 640
BD 588
BD 647
BD 435
BD 442
BD 577
BD 602
LMG 20104 P. ananatis Eucalyptus
BD 543
BD 333 P. ananatis onion
BD 336 P. ananatis onion
LMG 2603 T P. dispersa
LMG 2632 T P. stewartii subsp. indologenes
LMG 22049 T P. citrea
BD 500
BD 502
BD 639
LMG 1286 T P. agglomerans
LMG 2715 T P. stewartii subsp. stewartii

Fig. 3. A dendrogram showing similarity among the fluorescent amplified fragment length polymorphism (F-AFLP) DNA fingerprints of bacterial strains isolated from maize with brown stalk rot, *P. ananatis* reference strains, *P. agglomerans*, *P. citrea*, *P. dispersa*, *P. stewartii* subsp. *stewartii* and *P. stewartii* subsp. *indologenes.* The fingerprints were generated using the selective primers Eco-G/Mse-CG (6). Similarity matrixes were constructed using the Dice coefficient and cluster analyses were performed by the unweighted pair group method with the Bionumerics program (version 4.5, Applied Maths, Kortrijk, Belgium). Strains from maize are in bold (indole negative strains are underlined).

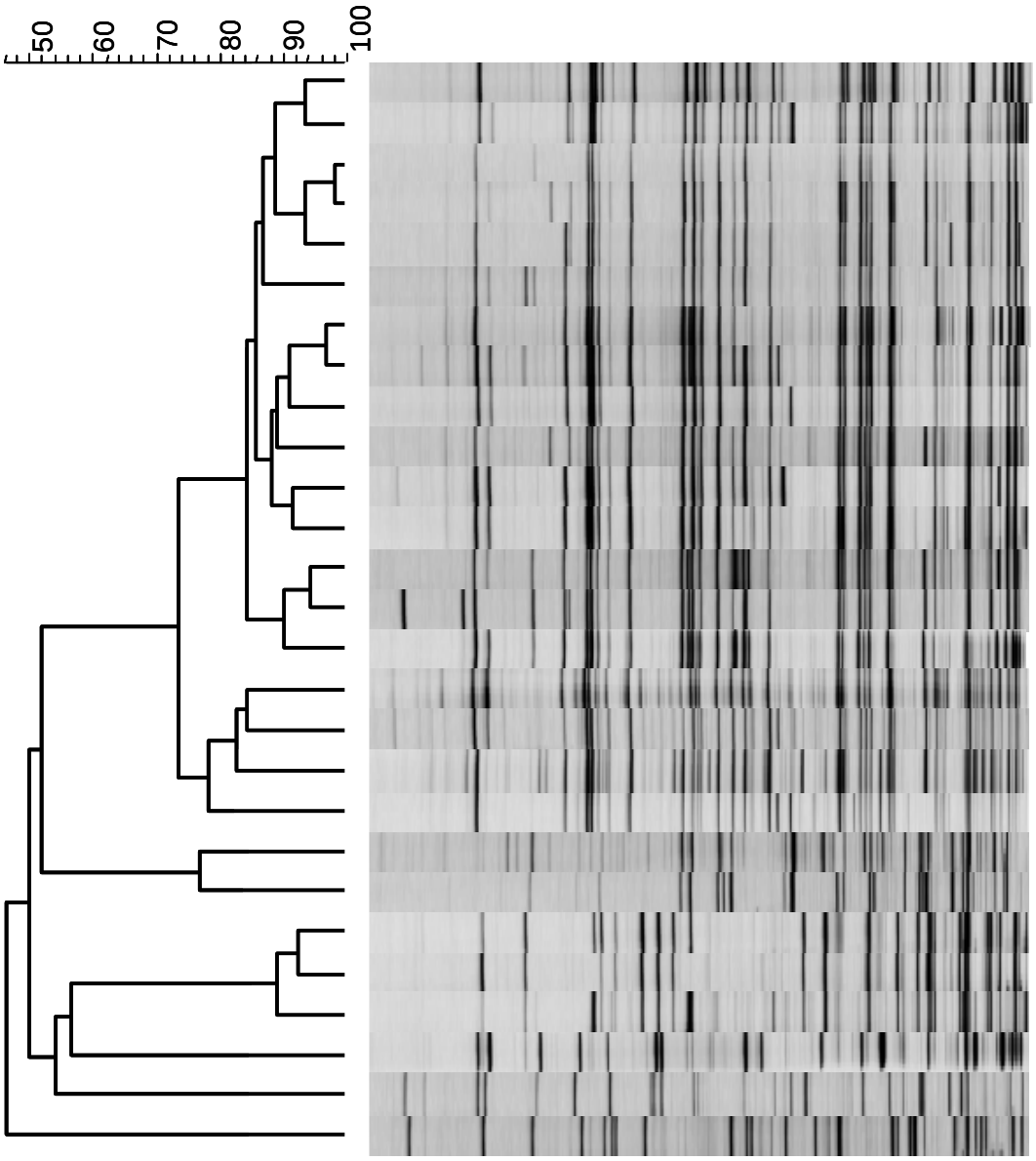
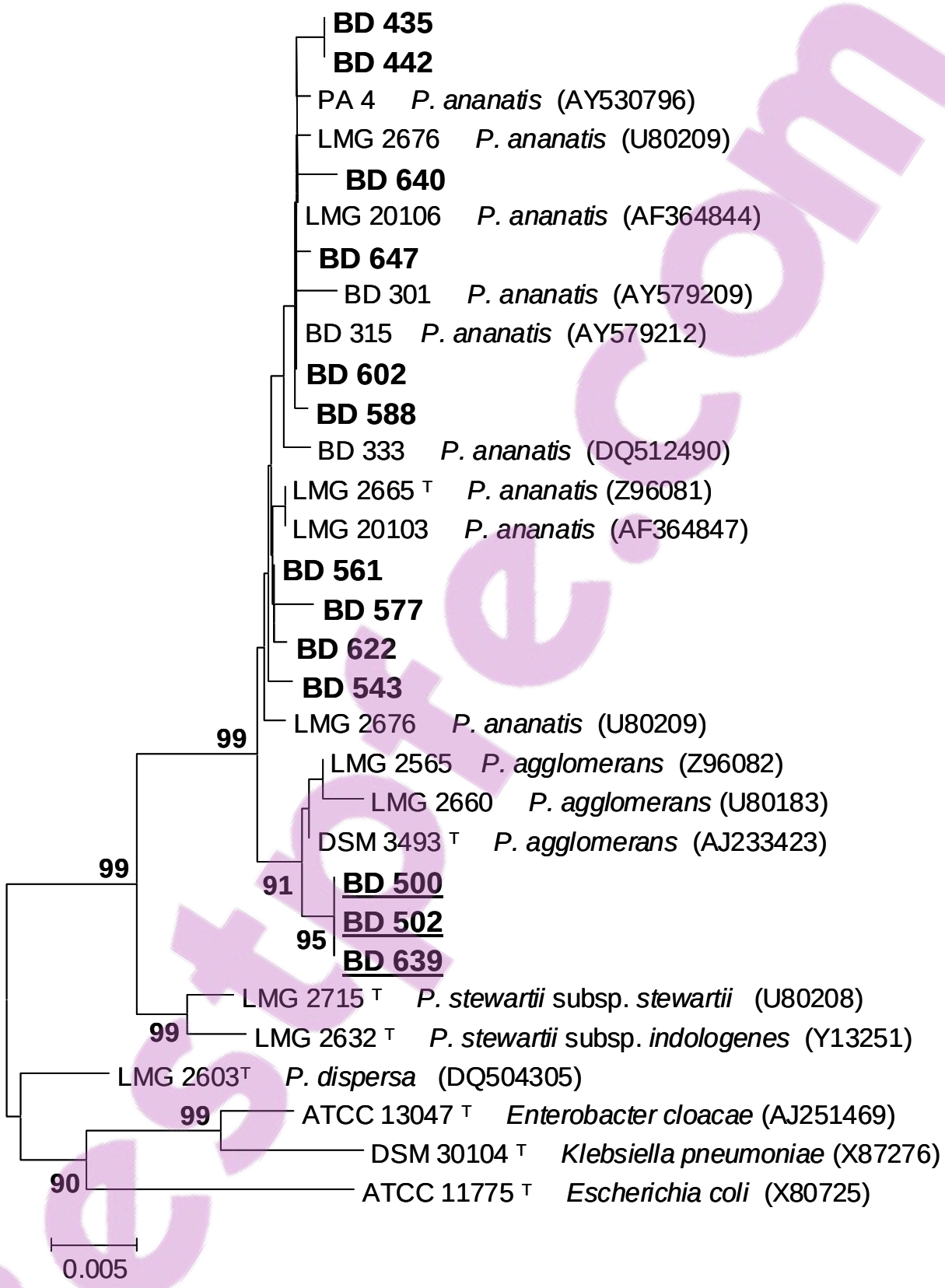

50
60
70
80
90
100
BD 640
BD 647
BD 315 P. ananatis onion
BD 435
BD 543
BD 301 P. ananatis onion
BD 561
BD 588
BD 622
BD 442
BD 577
BD 602
BD 336 P. ananatis onion
PA 4 P. ananatis onion
BD 333 P. ananatis onion
LMG 20104 P. ananatis Eucalyptus
LMG 2665 T P. ananatis
0197-28 P. ananatis sudangrass
LMG 20103 P. ananatis Eucalyptus
LMG 2632 T P. stewartii subsp. indologenes
LMG 2715 T P. stewartii subsp. stewartii
BD 500
BD 639
BD 502
LMG 1286 T P. agglomerans
LMG 2603 T P. dispersa
LMG 22049 T P. citrea

Fig. 4. Phylogenetic tree showing the relationship among selected partial 16S rDNA sequences from *Pantoea* species and strains isolated from maize with brown stalk rot (in bold, indole negative strains are underlined). Phylogenetic tree was constructed with the neighbor-joining method (39) and evolutionary distances calculated according to the method of Kimura (32), using the MEGA software package, version 3.1; Kumar, Tamura, Nei, 2004 (34). The sequences of *Enterobacter cloacae, Klebsiella pneumoniae* and *Escherichia coli* were used as outgroup taxons. The numbers at the nodes indicate the levels of bootstrap support based on data for 1.000 replicates, only values greater than 90 % are shown. Accession numbers (in brackets) and the sequences of *P. ananatis, P. agglomerans, P. dispersa, P. stewartii, E. cloacae, K. pneumoniae* and *E. coli* were obtained from the GenBank/EMBL databases. Bar represents genetic distance.


BD 435
BD 442
PA 4 P. ananatis (AY530796)
LMG 2676 P. ananatis (U80209)
BD 640
LMG 20106 P. ananatis (AF364844)
BD 647
BD 301 P. ananatis (AY579209)
BD 315 P. ananatis (AY579212)
BD 602
BD 588
BD 333 P. ananatis (DQ512490)
LMG 2665 T P. ananatis (Z96081)
LMG 20103 P. ananatis (AF364847)
BD 561
BD 577
BD 622
BD 543
LMG 2676 P. ananatis (U80209)
99
LMG 2565 P. agglomerans (Z96082)
LMG 2660 P. agglomerans (U80183)
DSM 3493 T P. agglomerans (AJ233423)
99
91
BD 500
BD 502
95
BD 639
LMG 2715 T P. stewartii subsp. stewartii (U80208)
99
LMG 2632 T P. stewartii subsp. indologenes (Y13251)
LMG 2603T P. dispersa (DQ504305)
99
ATCC 13047 T Enterobacter cloacae (AJ251469)
DSM 30104 T Klebsiella pneumoniae (X87276)
90
ATCC 11775 T Escherichia coli (X80725)
0.005

CHAPTER 5

Polyphasic Characterisation of *Pantoea* Strains from Onion and Maize, and the Description of *Pantoea allii* sp. nov.

Abstract

Bacteria from the genus *Pantoea* have become increasingly important plant pathogens around the world. In South Africa, they cause diseases of two economically important crops, *Eucalyptus* and maize. The strains inducing center rot of onion have only been found in onion seed in this country, and have not yet been associated with any disease outbreak. The identity and taxonomic position of *Pantoea* isolates from onion and maize has not been fully determined. Forty-seven strains were subjected to a polyphasic study that included phenotypic characterisation, analysis of the F-AFLP patterns, rep-PCR genomic fingerprinting, 16S rDNA gene sequences and DNA-DNA hybridisation. The results revealed that the strains belong to three different species within the genus *Pantoea*. Majority of strains were identified as *P. ananatis*. Indole-negative strains from maize were identified as *P. vagens*. Some strains from onion, previously classified as *P. ananatis*, were shown to form a novel species. The name *Pantoea allii* sp. nov. is proposed for these strains (type strain BD 390^T) and the description of the species is presented.

INTRODUCTION

Bacteria belonging to the genus *Pantoea* are common on plant surfaces, in seeds, soil, and water and have been isolated from humans and animals (Gavini et al., 1989; Mergaert et al., 1993). Many microbiologists have studied the taxonomy of the genus. The studies included the numerical analysis of phenotypic characteristics (Verdonck et al., 1987), 16S rRNA gene sequence analysis (Hauben et al., 1998), fatty acid and protein profile analyses, DNA-DNA hybridisation (Brenner et al., 1984; Beji et al., 1988; Gavini et al., 1989, Mergaert et al., 1993) and AFLP fingerprints analysis (Brady, 2005). There are seven described species within the genus *Pantoea*: *P. agglomerans, P. ananatis, P. citrea, P. dispersa, P. punctata, P. terrea*, and *P. stewartii* containing two subspecies, *indologenes* and *stewartii.* There are also several hybridisation groups from a study by Brenner et al. (1984), and a protein profile group of Beji et al. (1988), that according to the latest edition of the Bergey's manual of systematic bacteriology belong to the genus *Pantoea* (Grimont and Grimont, 2005).

Plant pathogenic *Pantoea* species cause diseases on diverse crops, such as pineapple (Serrano, 1928; Kageyama et al., 1992), melons (Bruton et al., 1986; Wells et al., 1987), cantaloupe (Bruton et al., 1991) sudangrass (Azad et al., 2000), *Eucalyptus* (Coutinho et al., 2002), rice (Azegami et al., 1983; Cother et al., 2004), mandarin orange (Kageyama et al., 1992), sugarcane (Serrano, 1928), beets (Brown, 1928), *Gypsophila* (Brown, 1934), onion (Hattingh and Walters, 1981; Gitaitis and Gay, 1997; Schwartz and Otto, 2000; Goszczynska et al., 2006) and maize (Elliott, 1941; Paccola-Meirelles et al., 2001; Goszczynska et al., 2007). Disease symptoms are diverse and include galls, rots, wilt, leaf blights, necrosis and spots, dieback and stem necrosis (Grimont and Grimont, 2005).
However, not all *Pantoea* strains are plant pathogens, and some have been used for biological control of *Erwinia amylovora* (Beer et al., 1984) and *Xanthomonas albilineans* (Zhang and Birch, 1997). Some strains of *P. agglomerans* (Nunes et al., 2001) and *P. ananatis* (Torres et al., 2005) are effective for the biological control of post harvest pome fruit diseases caused by fungi.

Diseases of onion are characterised by leaf blight, central leaf rot, seed stalk necrosis and rot, and bulb decay and these symptoms can lead to economically significant loses (Hattingh and Walters, 1981; Walcott et al., 2002). These diseases are caused by *P. ananatis* (Gitaitis and Gay, 1997) and *P. agglomerans* (Hattingh and Walters, 1981). The strains of *P. ananatis*

inducing center rot of onion have only been found in onion seed in South Africa, and have not yet been associated with any disease outbreak (Goszczynska et al., 2006).

Brown stalk rot of maize was first observed in South Africa in 2004 and was caused by *P. ananatis* and an unknown *Pantoea* sp. similar phenotypically to *P. agglomerans* (Goszczynska et al., 2007). Affected plants were stunted, with a vertical crack at the first internode, surrounded by a dark brown, narrow lesion. Internal browning inside the stem extended upwards, reaching the top internode in some plants. Seed cobs were underdeveloped. Diseased plants were scattered over the fields and 10-70% of the crop was affected (Goszczynska et al., 2007).

The diseases of onion and maize caused by *Pantoea* species were recorded for the first time during the last decade. Their taxonomic position has not been fully clarified. Bacteria isolated from onion in the USA and South Africa was considered to be *P. ananatis*. This classification was based only on biochemical and physiological characteristics (Gitaitis and Gay, 1997; Schwartz and Otto, 2000; Walcott et al., 2002) and the analysis of the 16S rDNA sequences (Goszczynska et al., 2006). Such methods are often insufficient for species delineation according to the recommendation of Wayne et al. (1987) and Stackebrandt et al. (2002). *Pantoea* strains isolated from maize with brown stalk rot symptoms in South Africa were additionally examined by using fluorescent amplified fragment length polymorphism analysis (F-AFLP) (Goszczynska et al., 2007). The majority of strains isolated from diseased maize were *P. ananatis*. Several isolates, although similar to *P. agglomerans* on the basis on biochemical tests and 16S rDNA sequences, produced F-AFLP fingerprints that were distinctly different from that generated by *P. ananatis, P. agglomerans, P. dispersa, P. citrea, P. stewartii* subsp. *stewartii* and *P. stewartii* subsp. *indologenes*. The results indicated that these bacteria belong to a previously undescribed species of the genus *Pantoea* and their taxonomic status needed to be further investigated. Bacteria recently isolated from *Eucalyptus* in Uganda were proposed to form a new species within a genus *Pantoea*, *P. vagens* (unpublished results(Goszczynska et al., 2007).). The type strain of *P. vagens*, BCC 105^{T} was included in this study.

The present research was initiated to characterise a collection of *Pantoea* strains from onion and maize by a polyphasic approach based on analyses of carbon source utilisation,

physiological characteristics, 16S rRNA gene sequence analysis, DNA-DNA hybridisation and F-AFLP. Based on the results obtained, the new species *Pantoea allii* is proposed.

MATERIALS AND METHODS

Bacterial strains. Sixty-seven bacterial strains used in the study are listed in Table 1. Twenty-four strains were originally isolated from onion plants and seed in the USA and South Africa. Twenty-three strains were isolated from maize with brown stalk rot in South Africa. Twenty *Pantoea* strains, including the type strains of *P. ananatis*, *P. agglomerans, P. citrea, P. dispersa, P. vagens, P. stewartii* subsp. *indologenes* and *P. stewartii subsp. stewartii* were used as reference strains. The strains were routinely cultured on tryptone glucose extract agar (TGA) (Difco, Sparks, MD), at 26°C and preserved in milk glycerol liquid medium (10% skim milk, 15% glycerol in distilled water) at –20°C. All cultures were routinely checked for purity and colony characteristics on TGA.

Pathogenicity tests. Pathogenicity on onion cv. Granex 33 was determined in green house assays by using a stub inoculation test (Goszczynska et al., 2006). A sterile needle was dipped into the bacterial colony on TGA (24-48 hours growth) and then the needle was inserted under the epidermis of a leaf. At least two leaves were inoculated per isolate. Inoculated plants were incubated in a greenhouse with 27°C/23°C day/night temperatures and observed daily for the development of symptoms.

Pathogenicity on maize was determined in greenhouse assays on a susceptible maize cultivar SR 52 (Goszczynska et al., 2007). Nine-week-old plants were inoculated by injecting a few drops of bacterial suspensions into the first internode of the stem. Maize plants were maintained in a greenhouse with natural light and at temperatures and RH described above. At least two plants were inoculated with each bacterial strain per inoculation method. This experiment continued for ten weeks.
Three control plants in all experiments were inoculated with sterile distilled water.

Phenotypic methods

Morphological and physiological characteristics. Strains from onion and maize were characterised by colony morphology, pigment production on TGA, motility, cell

morphology, Gram-stain, oxidase reaction, catalase reaction, indole production, Hugh-Leifson oxidation/fermentation test, gas production from glucose, hydrogen sulphide production from cysteine, growth at different temperatures (4, 30, 37, 40 and 44°C), and tolerance to NaCl (Mergaert *et al.*, 1993). The tests were performed according to methods described by Fahy and Hayward (1983).

Utilisation of carbon sources. All strains listed in Table 1 were characterised phenotypically by the Biolog GN2 microplate system (Biolog, Inc., Hayward, CA), according to the manufacturer's instructions. The inoculated microplates were incubated at 30°C for 24 hours. Plates were then scored visually for carbon source utilisation patterns. The data was entered into the Bionumerics software (version 4.5, Applied Maths, Kortrijk, Belgium). Similarity matrixes were constructed using the Pearson coefficient. Groups of strains were defined by the unweighted pair group method (UPGMA) using the same software.

Biochemical tests. Biochemical characteristics of the strains listed in Table 1 were examined with the API 20E system (BioMérieux, La Balme les Grottes, Montalieu Vercieu, France), according to the procedure recommended by the manufacturers. Selected strains were also tested with the API 50CHE system. The results of the API 20E and API 50CHE tests were recorded after 24 and 48 hours of incubation at 30°C respectively.

Two cluster analyses were performed using the Bionumerics software. The first analysis was done with the API 20E data (21 characters) for all strains listed in Table 1 and the second analysis with the API 50CHE (49 characters) data for selected strains. Similarity matrixes were constructed with the Dice coefficient and cluster analyses were performed by the UPGMA method. The data was presented as UPGMA dendrograms.

Molecular methods

DNA extraction. Genomic DNA of bacterial strains (Table 1) was extracted by using the GenElute Bacterial Genomic DNA Kit (Sigma, Steinheim, Germany), according to the manufacturer's instructions. Purified DNA was quantified using a Dyna Quant 200 fluorometer (Hoefer, San Francisco, CA) and Hoescht H 33258 intercalating dye (Polysciences, Warrington, PA). The DNA was stored at –20^{o}C until further analysis.

Fluorescent amplified fragment length polymorphism (F-AFLP). All strains listed in Table 1 were examined by the fluorescent amplified fragment length polymorphism (F-AFLP)-based system for the identification of plant-associated species from the genus *Pantoea*, as described by Brady et al. (2006).

Genomic DNA, 50-100 ng from each isolate, was digested with *Eco*RI and *Mse*I (Roche, Mannheim, Germany) restriction enzymes, and then ligated to the respective adaptors. Pre-amplification PCR reactions were done with Eco-00 (5'-GAC TGC GTA CCA ATT C-3') and Mse-00 (5'-GAT GAG TCC TGA CTA A-3') primers. Selective PCR amplifications were performed with Mse-CG (5'-GAT GAG TCC TGA CTA ACG-3') and fluorescently labelled Eco-G (5'-GAC TGC GTA CCA ATT CG-3') primers. Amplifications were done at least twice for each strain in the Hybaid Omni Gene thermocycler (Teddington, England) according to the Brady (2005) specifications.

The selective amplification reactions (1 µl) were mixed with an equal volume of formamide loading buffer (95% formamide, 20 mM EDTA, bromophenol blue), heated for 3 min at 90^{o}C and then chilled on ice for 10 min. A volume of 0.5-0.8 µl of each mixture was loaded onto the gel. The AFLP products were separated in 8% Long Ranger (LI-COR Biosciences, Lincoln, NE) denaturing gels on a LI-COR IR automated sequencer. Electrophoresis was carried out for 4 hours at 1500 V in 0.8 x TBE buffer. Images were imported into BioNumerics software. Gels were standardized with a 50-700 bp sizing standard (LI-COR) and the bands between 50 and 700 bp were analysed (Brady, 2005).

Similarity of the AFLP fingerprints was calculated using the Dice correlation coefficient and the cluster analysis was performed using the unweighted pair group method (UPGMA). The results were presented as an UPGMA dendrogram.

Repetitive sequence based PCR genomic fingerprinting. Repetitive sequence based polymerase chain reaction (rep-PCR) genomic fingerprints were obtained for all strains listed in Table 1. PCR was performed in 10 µl volumes containing PCR buffer (10 mM Tris HCl, pH 9.0 at 25°C; 50 mM KCl; 0.1% Triton X-100); 3.5 mM $MgCl_2$; 150 µM dNTPs; 1.0 µM primer, 2.5% DMSO; Taq polymerase (Promega, Madison, WI) 0.15 units per reaction volume and 25-50 ng template µl $^{-1}$. Primers used in the PCR corresponded to the prokaryotic enterobacterial repetitive interogenic consensus (ERIC2) and the BOX1A subunit of the BOX element (Rademaker and de Bruijn, 1997). PCR reaction volumes were overlaid with 10 µl sterile mineral oil and PCR amplifications performed according to Rademaker and de Bruijn (1997) in a Hybaid Omni Gene thermocycler.

The rep-PCR products were separated in 1.5% agarose in 1 x TBE buffer at 80 V. Gels were stained in ethidium bromide (10 mg ml $^{-1}$) for 10 min and destained for 15 min in water. Images were imported into BioNumerics software. The similarity between strains was calculated using the Pearson's correlation coefficient applied to the entire densitometric curves of the gel tracks. Gels were standardized with DNA molecular weight marker VI (Roche, Steinheim, Germany). All PCR reactions were repeated at least twice. Cluster analysis was performed by the UPGMA method and the data presented as an UPGMA dendrogram.

16S rRNA gene sequence analysis. The 16S rRNA gene fragments of nine strains from onion and twelve strains from maize were amplified in previous studies (Goszczynska et al., 2006 and 2007). The GenBank accession numbers (National Center for Biotechnology Information, U.S. National Institute of Health, Bethesda, MD) are shown in Fig. 4. Ilse Cleenwerck (Ghent University, Belgium) provided the 16S rDNA sequences of *P. citrea, P. punctata* and *P. terrea*. Carrie Brady (University of Pretoria) supplied the sequence of *P. vagens* BD 105^T.

The 16S rDNA nucleotide sequences of strains from maize and onion were aligned with a selection of 16S rDNA sequences of *Pantoea* species from GenBank with the MAFFT software, ver. 5.743 (Katoh et al., 2002). Phylogenetic trees were constructed with the neighbor joining method (Saitou and Nei, 1987) and evolutionary distances calculated

according to the method of Kimura (1980), using the MEGA software package, version 3.1, Kumar, Tamura, Nei, 2004 (Kumar et al., 2004). Bootstrap analysis of the data, based on 1000 permutations was used to assess the stability of relationships.

DNA-DNA hybridisation. The DNA was extracted from the five strains from onion (BD 309, BD 310, BD 377, BD 390, PA 4) three strains from maize (BD 435, BD 442, BD 639), BCC 105 from *Eucalyptus*, and the type strains of *P. ananatis* LMG 2665^T, *P. agglomerans* LMG 1286^T, *P. stewartii* subsp. *stewartii* LMG 2715^T, *P. dispersa* LMG 2603^T and *P. vagens* BCC 105^T, using the method described by Wilson (1989) and modified by Cleenwerck et al (2002).

DNA-DNA hybridisations were performed with photo-biotin-labelled probes in microplate wells (Ezaki et al., 1989; Goris et al., 1998) as described by Cleenwerk et al. (2002). Fluorescent measurements were done with a HTS7000 BioAssay Reader (Perkin-Elmer Cetus, Norwalk, CT). The hybridisation temperature was 45^{o}C. Reciprocal experiments were performed for every pair of strains, and the means from reciprocal tests were presented.

RESULTS

Pathogenicity tests. All *Pantoea* strains isolated from onion induced identical symptoms on onion leaves cv. Granex 33 in pathogenicity tests. Two to four days after inoculation water soaked spots appeared on leaves that expanded into longitudinal, bleached-green lesions with chlorotic margins (Fig. 1).

All *Pantoea* from maize produced brown stalk rot symptoms within six to seven weeks (Fig. 2). Four to six days after inoculation, small, 2-4 mm, light brown or dark green lesions developed around the inoculation point. Additionally, drops of yellow liquid slowly oozed from the lesion for two to three days. After 6 to 7 weeks, a single, long, vertical crack appeared on the injected internode. A dark brown, narrow lesion was present in the internal stem tissue along the crack. The internal browning within the stem was also observed in upper internodes.

Morphological and physiological characteristics. The strains from onion and maize produced yellow colonies on TGA, were gram-negative rods (1.5-2.0 µm length and 0.5-0.75

µm width), motile, oxidase negative and catalase positive. All strains fermentatively utilised glucose. They did not produce gas from glucose nor hydrogen sulphide from cysteine. Majority of strains produced indole from tryptophane, with the exception of *Pantoea* sp. from maize. Cells grew on nutrient agar containing up to 6% NaCl.

Pantoea strains should be classified and identified mainly based on genotypic characteristics, as identification based on phenotypic tests does not always lead to clear results (Gavini et al., 1989, Mergaert et al., 1993). Thus, the results of molecular characterisation of the strains from onion and maize are presented first, followed by the phenotypic description.

Molecular characterisation

F-AFLP analysis. *Pantoea* strains used in the study generated complex DNA fingerprints from extracted genomic DNA (Fig. 3). The selective primers Eco-G/Mse-CG (Brady, 2005) yielded well-defined DNA fingerprints, with an average of 80 bands per isolate. Amplified DNA fragments ranged in size from approximately 50 bp to slightly greater than 700 bp. A total of 101 fragments were generated and all these fragments were polymorphic. Sixty-seven *Pantoea* strains formed seven clusters. Majority of strains from onion and maize clustered with *P. ananatis* type strain LMG 2665^T and *P. ananatis* reference strains. The similarity values among these strains were between 73 and 98%.

The strains from maize, classified as *Pantoea* sp. in a previous study (Goszczynska et al., 2006), formed a cluster with *P. vagens* BCC 105^T. The F-AFLP fingerprints obtained for these strains showed 87 to 94% similarity.

Five strains from onion, indistinguishable from *P. ananatis* by biochemical and morphological characteristics, generated F-AFLP patterns that were clearly different from those produced by other isolates used in the study, including the type strains of *P. ananatis*, *P. agglomerans*, *P. citrea*, *P. dispersa*, *P. vagens, P. stewartii* subsp. *stewartii* and *P. stewartii* subsp. *indologenes.* Fingerprints of BD 304, BD 309, BD 377, BD 380 and BD 390 were 70 to 92% similar to each other.

Rep-PCR genomic fingerprinting. The rep-PCR fragments amplified with the BOX1A and ERIC2 primers, ranged in size from approximately 230 bp to greater than 3 kb. The banding

pattern revealed a high degree of genetic diversity among the strains. Although significant differences were observed between the major groups, within each group of strains fingerprints were similar (Fig. 4). The groups of strains obtained by the analysis of the rep-PCR data were identical to that obtained by using the F-AFLP fingerprints.

The DNA fingerprint similarity among most of isolates from onion, maize, and *P. ananatis* reference strains ranged from 50% to 99% and all these strains clustered together on the rep-PCR dendrogram. The indole-negative strains from maize (Goszczynska et al., 2007) produced characteristic and unique rep-PCR fingerprints. BD 502 and BD 639 formed a separate cluster on the dendrogram with the type strain of *P. vagens*.

A group of five strains from onion (BD 304, BD 309, BD 377, BD 380 and BD 390), generated rep-PCR fingerprints that were similar to each other (50 to 80%), but distinctly different from other strains. The same five isolates also grouped together on the F-AFLP dendrogram.

16S rRNA gene sequence analysis. Figure 5 shows the phylogenetic relationship derived from a neighbor-joining analysis of the pairwise comparison among the 16S rDNA sequences of twelve strains from maize and nine strains from onion. The 16S rDNA sequences of fourteen *Pantoea* reference strains, including the type strains of seven described species within the genus were included in the analysis. The multiple sequence alignment of thirty-five 16S rDNA sequences used in the study is presented in Appendix A.

Phylogenetic evaluation confirmed the division of strains from maize and onion into three groups. Nine maize and five onion strains, that produced genomic fingerprints similar to *P. ananatis*, clustered with the 16S rDNA sequences of *P. ananatis*. Two indole negative isolates from maize grouped closely, at 62% confidence value, with *P. vagens* BCC 105^{T}. Although the sequences of three onion isolates, BD 309, BD 377 and BD 390 were more than 99% similar to that of *P. ananatis,* they formed a separate cluster in the tree, at a high, 81% confidence value.

DNA-DNA hybridisation. Between two and four strains from each F-AFLP and rep-PCR cluster were selected for the DNA-DNA hybridisation. The results of DNA-DNA hybridisations of all examined strains are shown in Table 2.

DNA-DNA binding values obtained for four indole positive strains, BD 310 and PA 4 from onion and BD 435 and BD 442 from maize and *P. ananatis* LMG 2665^T, varied between 90 and 100%. Since 70% DNA binding value was recommended as the minimum level of genotypic relatedness within a species (Wayne et al., 1987), these four isolates were identified as *P. ananatis*.

Strain BD 639 showed 89% DNA relatedness to BCC 105^T. These two strains displayed an intermediate level of DNA similarity, 63 to 65%, to the type strain of *P. agglomerans*, and low levels to the known *Pantoea* species. Therefore, BD 639, and two indole negative strains, BD 500 and BD 502 (Goszczynska et al., 2007) that grouped with BCC 105^T in the F-AFLP, rep-PCR and 16S rDNA dendrograms, were identified as *P. vagens*.
DNA-DNA hybridisation data revealed that three strains from onion, BD 309, BD 377 and BD 390 displayed a high level of DNA relatedness, 90 to 100%, and low levels of relatedness to the known *Pantoea* species and *P. vagens*. The name *Pantoea allii* sp. nov. is proposed for this taxon.

Phenotypic characteristics

Utilisation of carbon sources. On the UPGMA dendrogram of the Biolog data (Fig. 6), the strains used in the study formed four clusters. The biggest group consisted of 54 strains and included all strains identified as *P. ananatis* by molecular fingerprinting, *P. stewartii* subsp. *indologenes* and *P. allii* sp. nov. There was consistency in utilisation of 69 substrates on the GN2 plates among these strains, 41 positive and 28 negative reactions. No single test was able to differentiate between *P. ananatis* and *P. allii*. *P. stewartii* subsp. *indologenes*, however, could be distinguished from these two other species by its inability to utilise L-rhamnose, L-histidine and D,L,α-glycerol phosphate.

The *P. vagens* strains from maize and the *P. vagens* type strain BCC 105^T clustered with *P. agglomerans*. The isolates within a cluster gave 41 positive and 27 negative reactions in Biolog GN2 plates. *P. vagens* differed from *P. agglomerans* by its ability to utilise nine substrates: D-melibiose, turanose, L-ornithine, D-serine, L-threonine and four acids, formic, α-hydroxybutyric, α-ketoglutaric and succinamic.

API 20E. The results of physiological and biochemical tests using the API 20E system separated 67 strains (Table 1) into six groups (Fig. 7). All *P. ananatis* and *P. allii* fell within a single cluster. These two species could not be distinguished from each other based on API 20E tests. Five *P. ananatis* strains isolated from onion seed did not produce acid from sucrose, unlike the other *P. ananatis* used in the study and 18 strains of *P. ananatis* studied by Mergaert et al. (1993).

Three strains of *P. vagens* formed a separate group in the dendrogram (Fig. 7). They gave positive reactions in the following tests: β-galactosidase, acetoin, gelatinase and produced acid from D-glucose, D-mannitol, inositol, L-rhamnose, D-sucrose, D-melibiose, and L-arabinose. They did not produce acid from D-sorbitol and amygdalin, indole from tryptophane, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate, H_2S and urease. Although in another study (Goszczynska et al., 2007) three strains from maize differed from other *Pantoea* species by giving a positive reaction in tryptophane deaminase test, the type strain of *P. vagens*, BCC 105^T, was negative for this test. In contrast to other indole negative *Pantoea* species, *P. vagens* did not produce acid from amygdalin, and could be distinguished by that characteristic.

API 50CHE. Thirty-three strains were examined by the analysis of anaerobic acid production from 48 substrates and the ability to hydrolyse esculin in the API 50CHE strips.

Contrary to Biolog and API 20E, *P. allii* could be differentiated from *P. ananatis* and other *Pantoea* species by the API 50CHE tests (Fig. 7). Five *P. allii* strains produced acid from at least one of the following compounds; D-adonitol, methyl-β-D-xylopyranoside, L-sorbose, inulin and xylitol, while all other *Pantoea* isolates did not. Production of acid from D-adonitol, methyl-ß-D-xylopyranoside, L-sorbose and inulin, were not reported for members of the genus (Gavini et al., 1989; Mergaert et al., 1993).

Biochemical characteristics of *P. vagens* were additionally defined by the API 50CHE. The isolates produced acid from D-fucose, unlike other strains used in the study.

DISCUSSION

Diseases caused by bacteria belonging to the genus *Pantoea* are emerging diseases in South Africa, and may have an increasing economic impact in the future. The causal agents, isolated from onion (Hattingh and Walters, 1981), *Eucalyptus* (Coutinho et al., 2002), onion seed (Goszczynska et al., 2006) and most recently from maize (Goszczynska et al., 2007), have been described as *P. agglomerans, P. ananatis* and *Pantoea* sp. closely related to *P. agglomerans*, respectively. The species allocation, with the exception *P. ananatis* from *Eucalyptus*, was based on a biochemical and physiological characterisation, analysis of the 16S rDNA sequences and, in the case of maize pathogens, F-AFLP genomic fingerprinting. Such a taxonomic system is insufficient for species delineation according to the recommendation of Wayne et al. (1987) and Stackebrandt et al. (2002). This study was performed to determine the accurate taxonomic position of bacteria from onion and maize in the genus *Pantoea*. To achieve this, a collection of strains from South African maize and strains pathogenic to onion isolated in South Africa and the USA were examined using a variety of methods, recommended for the taxonomic description of bacterial species (Stackebrandt et al., 2002).

Phenotypic characterisation confirmed that strains from maize and onion belong to the genus *Pantoea* and indicated, as expected, that at least two bacterial species were present. The 16S rDNA sequence analysis, however, revealed that three species could be present, as some strains from onion, thought to be *P. ananatis*, formed a separate cluster in the 16S rDNA tree (Fig. 5). However, 16S rDNA sequence analysis is known to be of limited value in terms of taxonomic resolution at the species level for members of *Enterobacteriaceae,* due to possible lateral transfers within the gene (Rayssiguier et al., 1989) and multiple copies of the 16S rRNA gene (Cillia et al., 1996).

Taxonomic relationships at the species level were assessed by rep-PCR and F-AFLP genomic fingerprints analysis and DNA-DNA hybridisation. All indole positive *Pantoea* from maize and the majority of strains from onion generated genomic fingerprints that were similar to that produced by the reference strains of *P. ananatis* (Fig. 3, Fig. 4). Four strains of that group, two from onion and two from maize, showed 90-100% DNA binding to *P. ananatis* type strain LMG 2665^{T} (Table 2). As strains with similar genomic fingerprints are

genomically closely related (Gavini et al., 1989; Rademaker et al., 2000), all strains within *P. ananatis* rep-PCR and F-AFLP clusters are thus identified as *P. ananatis*.

The indole negative strains from maize, although similar to *P. agglomerans* by phenotypic tests and the 16S rDNA sequences, produced genomic fingerprints that clustered with *P. vagens* BCC 105^T (Fig. 3, Fig. 4). BD 639 showed a high level, 89%, of DNA relatedness to BCC 105^T. Although DNA biding ratios of these two strains to *P. agglomerans* were between 63 and 65%, they should not be classified as a subspecies of *P. agglomerans*. The fingerprints of *P. vagens* were distinctly different from that of *P. agglomerans*, further supporting the establishment of a separate species to contain them.

The strains BD 390 and BD 309 were identified in a previous study as *P. ananatis* (Goszczynska et al., 2006). However, the genomic fingerprints generated by BD 309, BD 390 and three other isolates from onion, did not resemble that produced by *P. ananatis*, and formed a separate cluster in the F-AFLP and rep-PCR dendrograms (Fig. 3, Fig. 4). The DNA-DNA hybridisation values among three strains of this cluster were between 90 and 100%, confirming that they belong to a single species. The most closely related type strain was *P. ananatis* with 44 to 57% DNA reassociation, below the 70% threshold allowed for species delineation (Wayne et al., 1987). The name *Pantoea allii* sp. nov. is proposed for this species.

Table 3 gives phenotypic characteristics useful in the differentiation of the species of the genus *Pantoea*. *P. vagens* could not be easily differentiated from *P. agglomerans* and *P. allii* from *P. ananatis* by an exclusive biochemical or physiological tests. A similar problem was reported by Gavini et al. (1989) with *P. agglomerans* versus *P. dispersa* and by Mergaert et al. (1993) concerning *P. ananatis* versus *P. stewartii* subsp. *indologenes*.
Pantoea strains should be classified and identified mainly based on genotypic characteristics, as identification based on phenotypic tests does not always lead to clear results (Gavini et al., 1989, Mergaert et al., 1993).

Description of *Pantoea allii* sp. nov.

Pantoea allii [al'li.i. N.L. adj. *allii* from *Allium*, the genus of onion (*Allium cepa* L.).

The description below is based on the data obtained for the five strains, BD 304, BD 309, BD 377, BD 380 and BD 390, isolated from onion plants with centre rot symptoms in the USA and from onion seed in South Africa. In pathogenicity tests, strains induce symptoms identical to those caused by *P. ananatis* on onion plants.

Gram-negative, non-capsulated, non-spore forming straight rods, motile. Colonies on nutrient agar and TGA are yellow, smooth, round, and convex with entire margins. They are facultatively anaerobic, oxidase negative and catalase positive. Cells tolerate up to 6% NaCl and grow at 30°C, 37°C and 40°C, but not at 4°C and 44°C.

P. allii does not produce arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S, urease, tryptophane deaminase and gas from glucose. Citrate is utilised. Indole, acetoin, and β-galactosidase are produced.

P. allii strains produce acid from at least one of the following carbon sources: D-adonitol, methyl-β-D-xylopyranoside, L-sorbose, inulin and xylitol, while *P. ananatis* do not produce acid from this compounds. Other biochemical characteristics of *P. allii* at 30°C are shown in Table 3.

The following carbon sources are utilised at 30°C (as assessed by Biolog GN microplates), by 100% of strains: dextrin, tween 40, tween 80, N-acetyl-D-glucosamine, L-arabinose, D-arabitol, D-cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, inositol, α-D-lactose, lactulose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, D-raffinose, L-rhamnose, sucrose, D-trehalose, pyruvic acid methyl ester, succinic acid mono-methyl ester, acetic acid, cis-aconitic acid, citric acid, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, D,L-lactic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, D-alanine, L-alanine, L-alanyl-glutamic acid, L-histidine, L-proline, L-serine, urocanic acid, inosine, thymidine, glycerol, D,L,α-glycerol phosphate, α-D-glucose-1-phosphate and D-glucose-6-phosphate. The following carbon substrates are not utilised: α-cyclodextrin, N-acetyl-D-galactosamine, erythitol, L-fucose, turanose, xylitol, D-galactonic acid lactone, β-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, sebacic acid, L-alaninamide, hydroxy-L-proline, L-leucine, L-phenylalanine, L-pyroglutamic acid, L-threonine, D,L-carnitine, γ-aminobutyric acid, phenylethylamine, putrescine, 2-

aminoethanol and 2,3-butanediol. *P. allii* varies in the utilisation of the following carbon sources in the Biolog GN plates: glycogen, adonitol, maltose, D-psicose, D-sorbitol, formic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, succinamic acid, glucuronamide, L-ornithine and D-serine.

The proposed type strain is BD 390, isolated from onion seed in South Africa.

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Table 1. *Pantoea* strains used in this study.

Strain	Other strain designations	Species (as received)	Host	Location	Source
0197-28		*P. ananatis*	*Sorghum sudanense*	USA, California	D. Azad
BCC 105^{T}		*Pantoea vagens.*	*Eucalyptus*	Uganda	T. A. Coutinho
BD 250	98-1	*P. ananatis*	*Allium cepa*	USA	R. Gitaitis
BD 287	SUH 2	*P. agglomerans*	*Allium cepa*	South Africa	PPPPB
BD 295	ATCC BAA 517	*P. ananatis*	*Allium cepa*	USA, Colorado	ATCC
BD 296	ATCC BAA 516	*P. ananatis*	*Allium cepa*	USA, Colorado	ATCC
BD 301	Blackshank 15	*P. ananatis*	*Allium cepa*	USA, Georgia	R. Walcott
BD 304	Blackshank 24	*P. ananatis*	*Allium cepa*	USA, Georgia	R. Walcott
BD 305	Blackshank 30	*P. ananatis*	*Allium cepa*	USA, Georgia	R. Walcott
BD 309	Hort. Hill 24	*P. ananatis*	*Allium cepa*	USA, Georgia	R. Walcott
BD 310	Hort. Hill 31	*P. ananatis*	*Allium cepa*	USA, Georgia	R. Walcott
BD 311	Hort. Hill 32	*P. ananatis*	*Allium cepa*	USA, Georgia	R. Walcott
BD 312	Pans	*P. ananatis*	*Allium cepa*	USA, Georgia	R. Walcott
BD 315	Pans 2002-2	*P. ananatis*	*Allium cepa*	USA, Georgia	R. Walcott
BD 317	Pans	*P. ananatis*	*Allium cepa*	USA, Georgia	R. Walcott
BD 325		*P. ananatis*	*Allium cepa*	South Africa	R. Walcott
BD 326		*P. ananatis*	*Allium cepa*	South Africa	PPPPB
BD 331		*P. ananatis*	*Allium cepa*	South Africa	PPPPB

Strain	Other strain designations	Species (as received)	Host	Location	Source
BD 333		*P. ananatis*	*Allium cepa*	South Africa	PPPPB
BD 334		*P. ananatis*	*Allium cepa*	South Africa	PPPPB
BD 335		*P. ananatis*	*Allium cepa*	South Africa	PPPPB
BD 336		*P. ananatis*	*Allium cepa*	South Africa	PPPPB
BD 377		*P. ananatis*	*Allium cepa*	South Africa	PPPPB
BD 380		*P. ananatis*	*Allium cepa*	South Africa	PPPPB
BD 390		*P. ananatis*	*Allium cepa*	South Africa	PPPPB
BD 435		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 442		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 478		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 494		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 515		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 527		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 541		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 543		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 551		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 556		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 561		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 577		*P. ananatis*	*Zea mays*	South Africa	PPPPB

Strain	Other strain designations	Species (as received)	Host	Location	Source
BD 588		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 596		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 602		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 614		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 620		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 622		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 629		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 639		*Pantoea* sp.	*Zea mays*	South Africa	PPPPB
BD 640		*P. ananatis*	*Zea mays*	South Africa	PPPPB
BD 647		*P. ananatis*	*Zea mays*	South Africa	PPPPB
CTB 1061		*P. ananatis*	*Oryza sativa*	Japan	CTB
CTB 1135		*P. ananatis*	*Oryza sativa*	Japan	CTB
DAR 49828		*P. agglomerans*	*Pyrrus communis*	Australia	ACPPB
DAR 72041		*P. agglomerans*	*Allium cepa*	Australia	ACPPB
LMG 1286T		*P. agglomerans*	Human	Zimbabwe	BCCM/LMG
LMG 20103		*P. ananatis*	*Eucalyptus*	South Africa	BCCM/LMG
LMG 20104		*P. ananatis*	*Eucalyptus*	South Africa	BCCM/LMG
LMG 22049T		*P. citrea*	Mandarin orange	Japan	BCCM/LMG
LMG 2565		*P. agglomerans*	Cereals	Canada	BCCM/LMG

Strain	Other strain designations	Species (as received)	Host	Location	Source
LMG 2603T		*P. dispersa*	Soil	Japan	BCCM/LMG
LMG 2632		*P. stewartii* subsp. *indologenes*	*Setaria italica*	India	BCCM/LMG
LMG 2665T		*P. ananatis*	*Ananas cosmosus*	Hawaii	BCCM/LMG
LMG 2671		*P. stewartii* subsp. *indologenes*	*Ananas cosmosus*	Hawaii	BCCM/LMG
LMG 2676		*P. ananatis*	*Puccinia graminis*	USA	BCCM/LMG
LMG 2713		*P. stewartii* subsp. *stewartii*	*Zea mays*	USA	BCCM/LMG
LMG 2715T		*P. stewartii* subsp. *stewartii*	*Zea mays*	USA	BCCM/LMG
LMG 2749		*P. dispersa*	Human		BCCM/LMG
PA 3		*P. ananatis*	*Allium cepa*	South Africa	PPPPB
PA 4		*P. ananatis*	*Allium cepa*	South Africa	PPPPB

BCCM/LMG Culture Collection: Universiteit Gent, Belgium; ATCC: American Type Culture Collection, Manassas, VA; ACPPB: Australian Collection of Plant Pathogenic Bacteria, Orange; PPPPB: Plant Pathogenic and Plant Protecting Bacteria, ARC-PPRI, South Africa; R. Walcott: Department of Plant Pathology, University of Georgia, Athens; T. A. Coutinho: Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; R. Gitaitis: Department of Plant Pathology, University of Georgia; CTB: Centre Technique du Bois, Paris, France.

Table 2. DNA binding values of examined *Pantoea* strains. The tests were performed at the University of Ghent, Belgium.

	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13
	P. ananatis													
1	LMG 2665^T	100												
2	BD 310	90	100											
3	BD 442	93	100	100										
4	BD 435	90	87	91	100									
5	PA 4	92	97	99	92	100								
	***P. allii* sp. nov.**													
6	BD 377	44					100							
7	BD 390T	55					99	100						
8	BD 309	57					90	99	100					
	P. agglomerans													
9	LMG 1286^T	21						26		100				
	P. vagens													
10	BCC 105^T	20						24		65	100			
11	BD 639									63	89	100		
	P. stewartii* subsp. *stewartii													
12	LMG 2715^T	20						18		6	9		100	
	P. dispersa													
13	LMG 2603^T	20						22		24	19		22	100

Table 3. Differential characteristics of *Pantoea allii* and some biochemically similar species within the genus *Pantoea*.

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**
API 20E tests						
Citrate utilisation	+	+	+	-	-	-
Tryptophane deaminase	-	-	-	d	-	-
Indole	+	+	+	-	-	-
Gelatinase	+	d	-	+	d	-
Acid from:						
Inositol	d	d	-	+	d	-
D-sorbitol	+	d	-	-	-	-
L-rhamnose	+	d	d	+	+	d
D-sucrose	+	d	+	+	+	+
Amygdalin	+	+	+	-	+	d
API 50CHE tests						
Esculin hydrolysis	+	d	d	d	+	-
Acid from:						
D-adonitol	d	-	-	-	-	-
Methyl-β-D-xylopyranoside	d	-	-	-	-	-
L-sorbose	d	-	-	-	-	-
Inositol	+	+	+	+	d	+
D-sorbitol	+	d	-	-	-	-
Amygdalin	+	d	-	-	-	-
D-lactose	+	+	+	-	-	-
Inulin	d	-	-	-	-	-
Starch	-	-	-	d	-	-
Glycogen	-	-	-	d	-	-
Xylitol	+	-	-	-	-	-
D-fucose	-	-	-	+	-	-

+ , positive reaction for at least 90% of the strains; – ,negative reaction for at least 90 % of the strains; d, 11-89% strains positive. 1, *Pantoea allii*; 2, *P. ananatis*; 3, *P. stewartii* subsp. *indologenes*; 4, *P. vagens*; 5, *P. agglomerans*; 6, *P. dispersa*

Data for *P. ananatis* and *P. stewartii* subsp. *indologenes* are from this study and Mergaert et al. (1993); for *P. agglomerans* and *P. dispersa* are from this study and Gavini et al. (1989).

Fig. 1. The symptoms induced by *Pantoea ananatis* (A) and *P. allii* (B) strains isolated from onion on onion leaves in pathogenicity tests. Two to four days after inoculation water soaked spots appeared on leaves that expanded into longitudinal, bleached-green lesions with chlorotic margins.

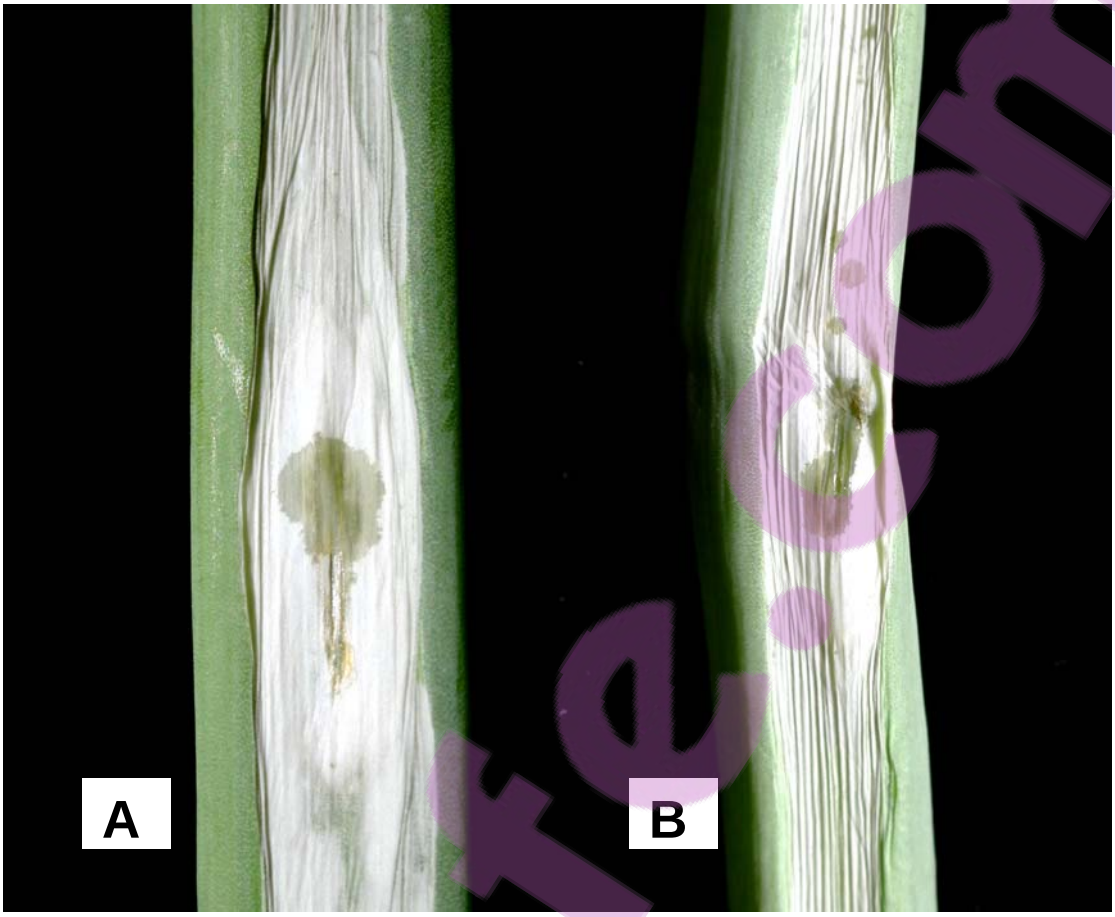


Fig. 1

Fig. 2. The symptoms produced by *P. ananatis* isolated from maize with brown stalk rot on maize SR 52. A, Four to six days after inoculation, small, 2-4 mm, light brown or dark green lesions developed around the inoculation point. Additionally, drops of yellow liquid slowly oozed from the lesion for two to three days. B, after 6 to 7 weeks, a single, long, vertical crack appeared on the injected internode. C, A dark brown, narrow lesion was present in an internal stem tissue along the crack. D, Browning of the internal tissue in an upper internode. The symptoms induced by *P. vagens* were indistinguishable from those induced by *P. ananatis*.

Fig. 2

Fig. 2

Fig. 3. A dendrogram showing the relationships between *Pantoea* strains used in the study based on F-AFLP fingerprints. *P. allii* strains are highlighted in bold. Reference strains, including the type strains of *Pantoea* species, are in italics.

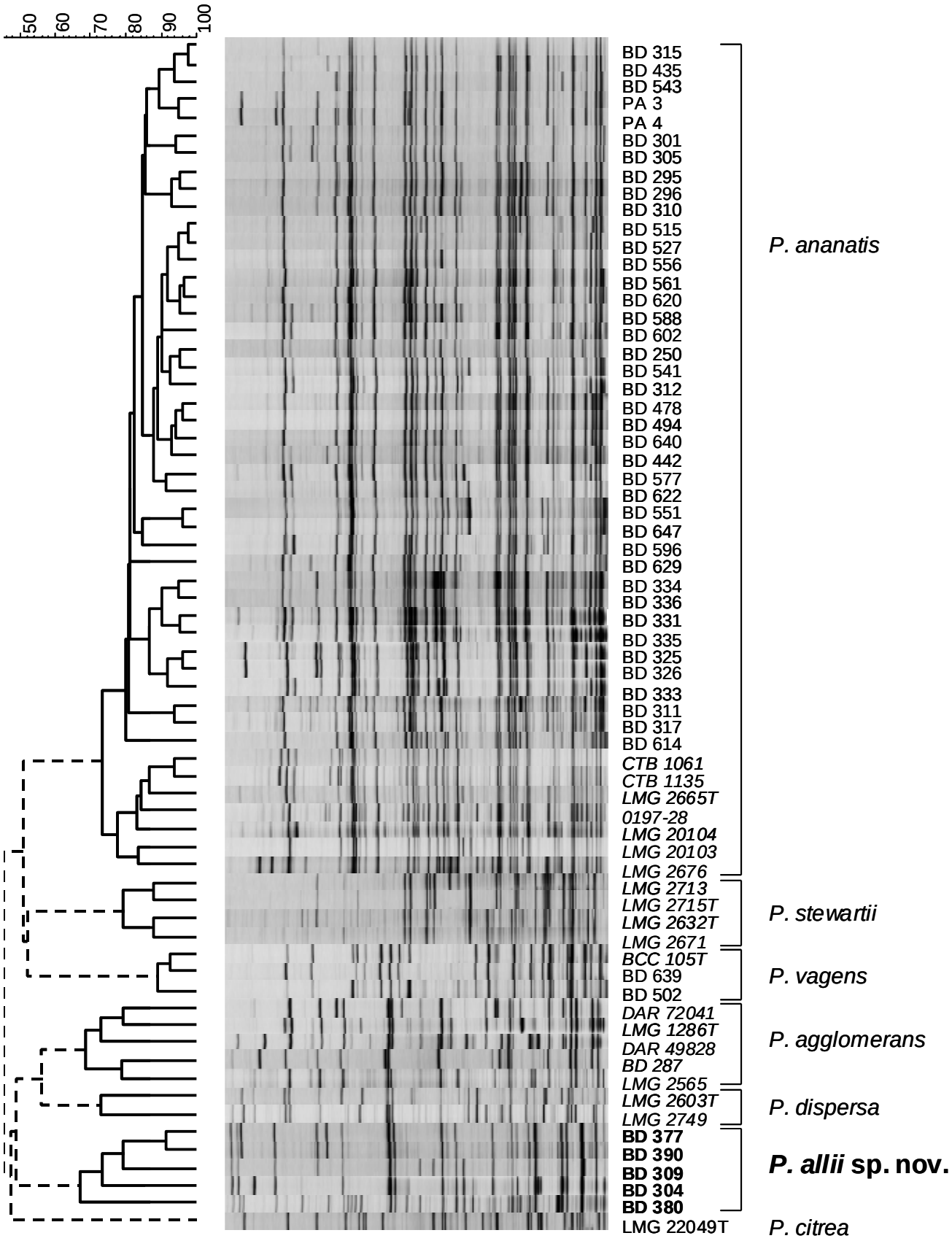

F-AFLP
50
60
70
80
90
100
BD 315
BD 435
BD 543
PA 3
PA 4
BD 301
BD 305
BD 295
BD 296
BD 310
BD 515
BD 527
BD 556
BD 561
BD 620
BD 588
BD 602
BD 250
BD 541
BD 312
BD 478
BD 494
BD 640
BD 442
BD 577
BD 622
BD 551
BD 647
BD 596
BD 629
BD 334
BD 336
BD 331
BD 335
BD 325
BD 326
BD 333
BD 311
BD 317
BD 614
CTB 1061
CTB 1135
LMG 2665T
0197-28
LMG 20104
LMG 20103
LMG 2676
LMG 2713
LMG 2715T
LMG 2632T
LMG 2671
BCC 105T
BD 639
BD 502
DAR 72041
LMG 1286T
DAR 49828
BD 287
LMG 2565
LMG 2603T
LMG 2749
BD 377
BD 390
BD 309
BD 304
BD 380
LMG 22049T
P. ananatis
P. stewartii
P. vagens
P. agglomerans
P. dispersa
P. allii sp. nov.
P. citrea

Fig. 4. A dendrogram showing the relationships between the *Pantoea* strains used in the study based on the rep-PCR fingerprints. ***P. allii*** are highlighted in bold. Reference strains, including the type strains of *Pantoea* species, are in italics.

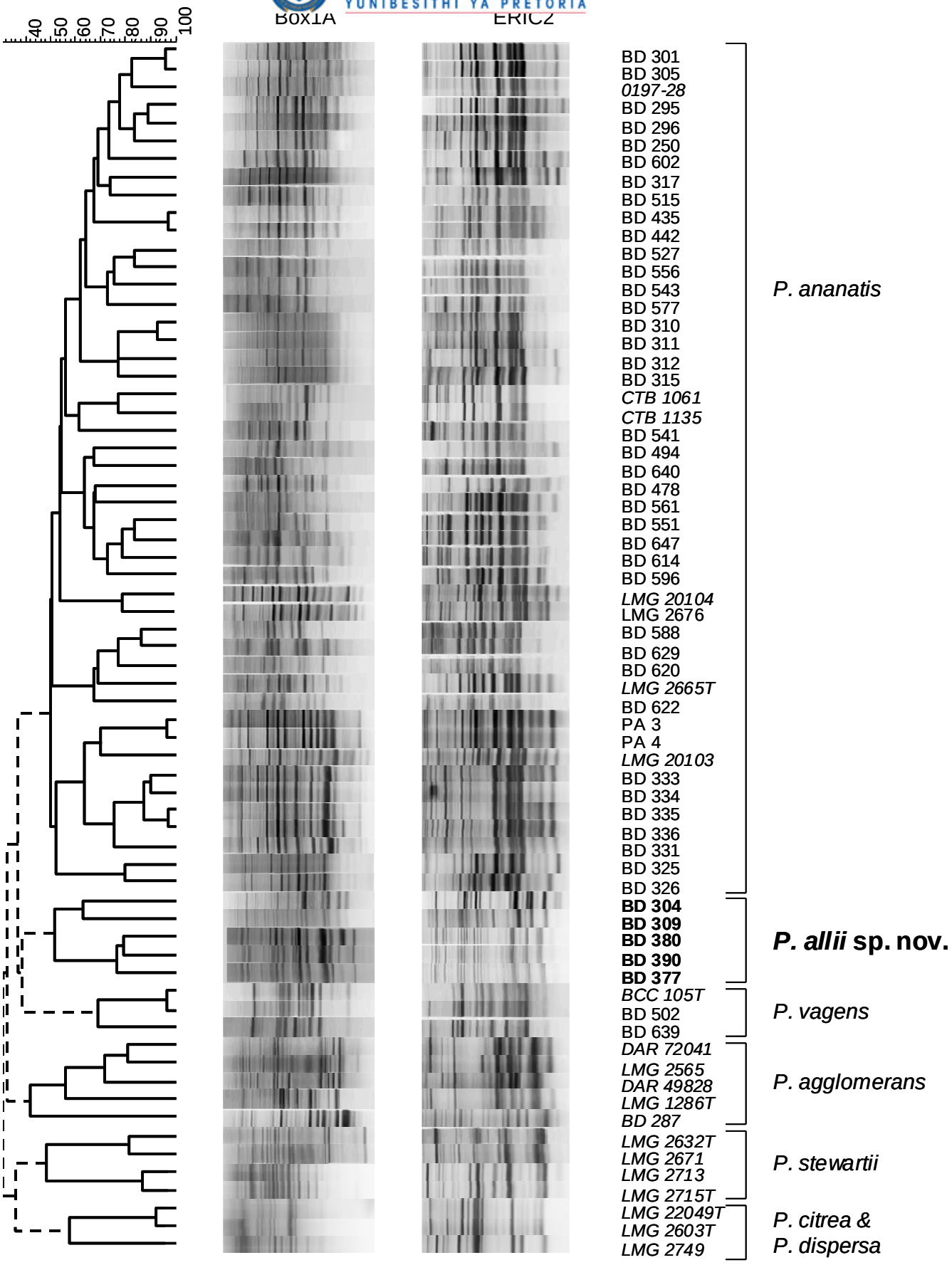

40
50
60
70
80
90
100
B0x1A
ERIC2
BD 301
BD 305
0197-28
BD 295
BD 296
BD 250
BD 602
BD 317
BD 515
BD 435
BD 442
BD 527
BD 556
BD 543
BD 577
BD 310
BD 311
BD 312
BD 315
CTB 1061
CTB 1135
BD 541
BD 494
BD 640
BD 478
BD 561
BD 551
BD 647
BD 614
BD 596
LMG 20104
LMG 2676
BD 588
BD 629
BD 620
LMG 2665T
BD 622
PA 3
PA 4
LMG 20103
BD 333
BD 334
BD 335
BD 336
BD 331
BD 325
BD 326
BD 304
BD 309
BD 380
BD 390
BD 377
BCC 105T
BD 502
BD 639
DAR 72041
LMG 2565
DAR 49828
LMG 1286T
BD 287
LMG 2632T
LMG 2671
LMG 2713
LMG 2715T
LMG 22049T
LMG 2603T
LMG 2749
P. ananatis
P. allii sp. nov.
P. vagens
P. agglomerans
P. stewartii
P. citrea &
P. dispersa

Fig. 5. Neighbour-joining tree reflecting the phylogenetic position of *Pantoea* strains from maize and onion based on 16S rDNA sequences. Numbers at branching points indicate bootstrap values derived from 1000 samples. The bar represents genetic distance. *P. allii* strains are highlighted in bold. Reference strains, including the type strains of *Pantoea* species, are in italics.

The 16S rDNA accession numbers of maize and onion strains:

Maize: BD 602, DQ195522; BD 647, DQ195525; BD 435, AY89864; BD 442, AY898643; BD 640, DQ195524; BD 588, DQ133548BD 561, DQ133546; BD 622, DQ195523; BD 577, DQ133547; BD 639, DQ512489; BD 500, DQ849042; BD 502, DQ849043.Onion: BD 309, AY579210; BD 390, AY530795; BD 377, DQ512491; BD 310, AY579211; BD 301, AY579209; BD 315, AY579212; PA 4, AY530796; BD 336, AY530794; BD 287, AY530797.

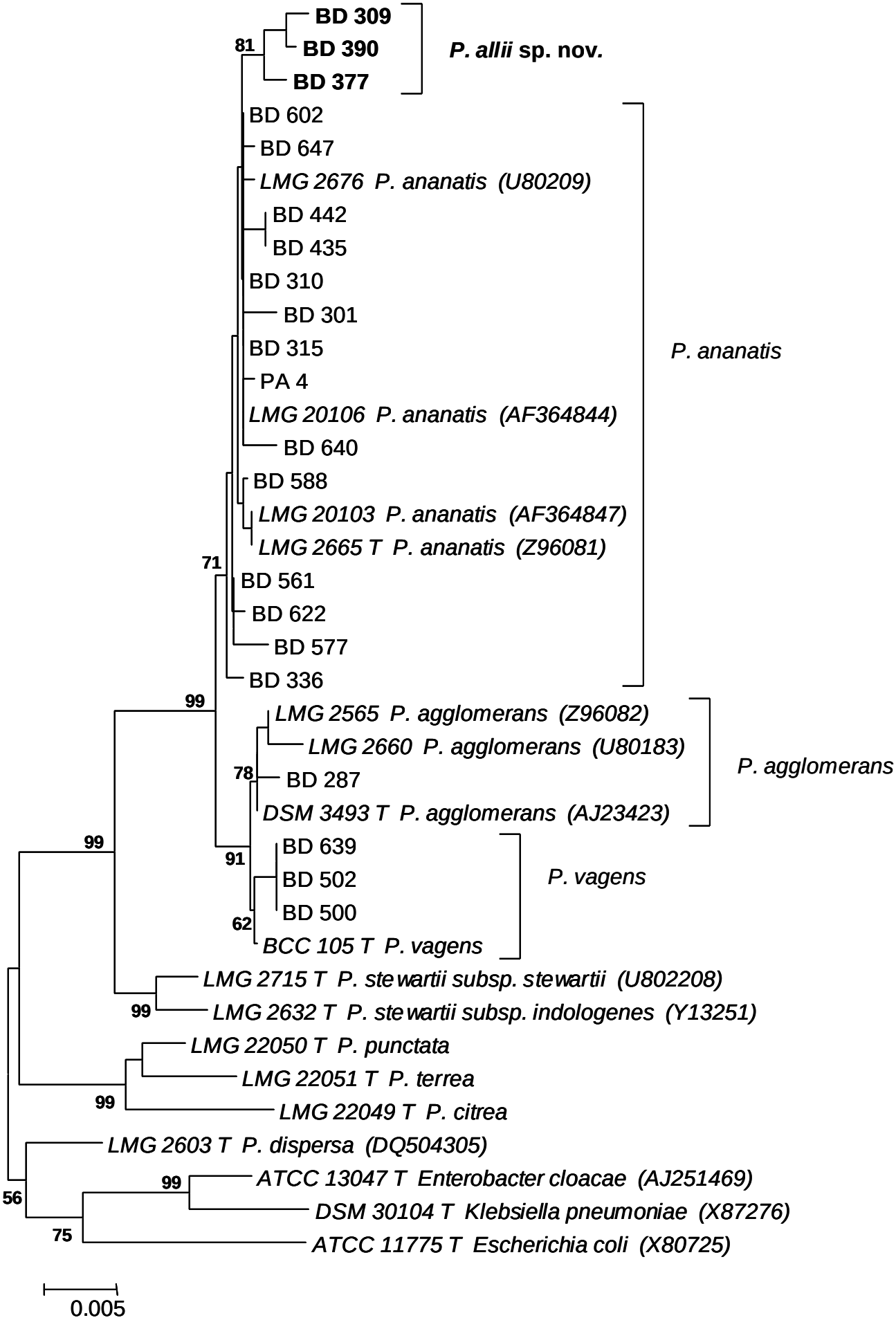

BD 309
BD 390
BD 377
P. allii sp. nov.
BD 602
BD 647
LMG 2676 P. ananatis (U80209)
BD 442
BD 435
BD 310
BD 301
BD 315
PA 4
LMG 20106 P. ananatis (AF364844)
BD 640
BD 588
LMG 20103 P. ananatis (AF364847)
LMG 2665 T P. ananatis (Z96081)
BD 561
BD 622
BD 577
BD 336
P. ananatis
LMG 2565 P. agglomerans (Z96082)
LMG 2660 P. agglomerans (U80183)
BD 287
DSM 3493 T P. agglomerans (AJ23423)
P. agglomerans
BD 639
BD 502
BD 500
BCC 105 T P. vagens
P. vagens
LMG 2715 T P. stewartii subsp. stewartii (U802208)
LMG 2632 T P. stewartii subsp. indologenes (Y13251)
LMG 22050 T P. punctata
LMG 22051 T P. terrea
LMG 22049 T P. citrea
LMG 2603 T P. dispersa (DQ504305)
ATCC 13047 T Enterobacter cloacae (AJ251469)
DSM 30104 T Klebsiella pneumoniae (X87276)
ATCC 11775 T Escherichia coli (X80725)
81
71
99
78
99
91
62
99
99
99
56
75
0.005

Fig. 6. Relationships among 67 *Pantoea* strains, including strains isolated from onion and maize, based on Biolog GN microplate substrate utilisation patterns. *P. allii* strains are highlighted in bold. Reference strains, including the type strains of *Pantoea* species, are in italics.

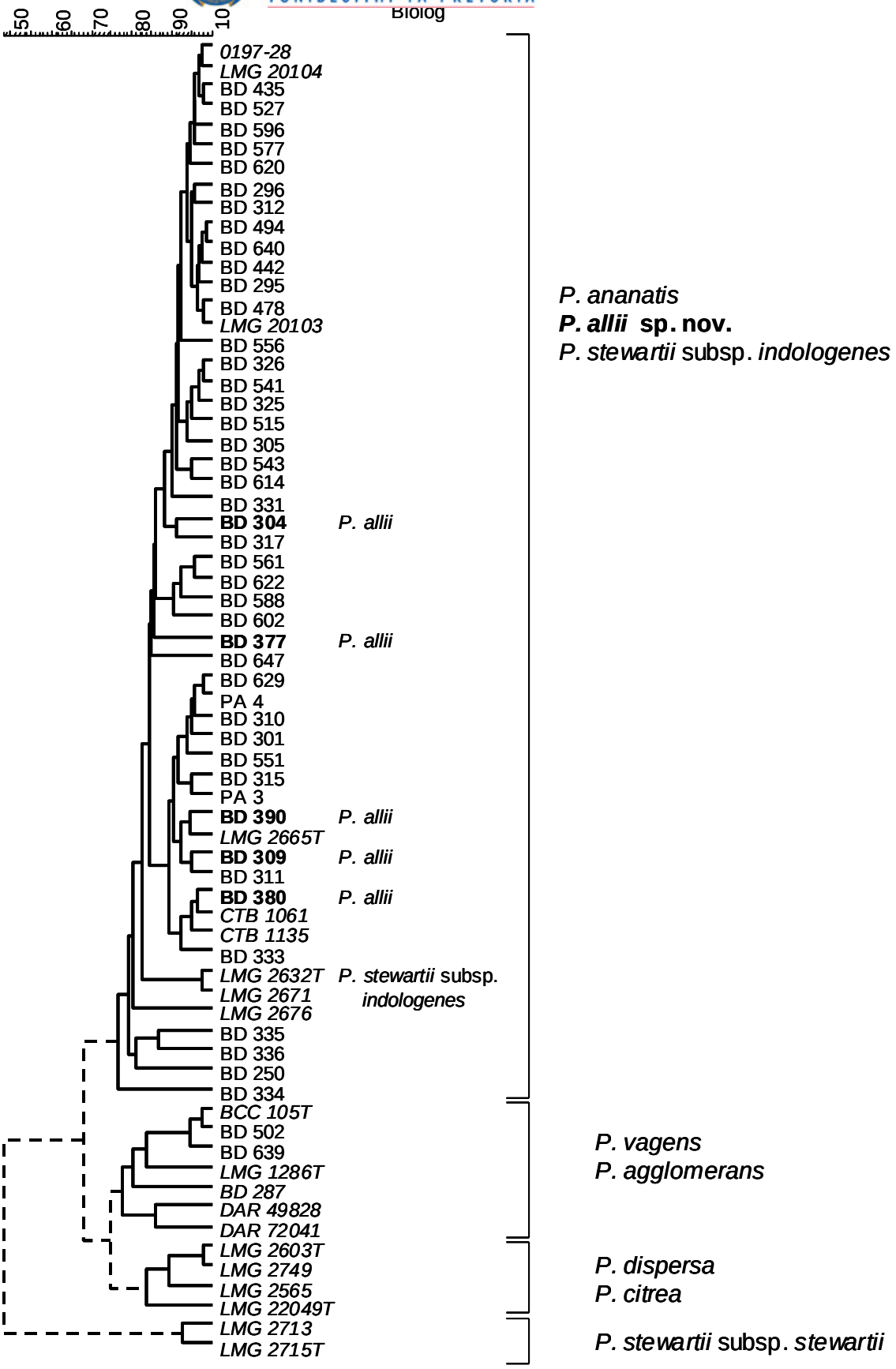

Biolog
50
60
70
80
90
100
0197-28
LMG 20104
BD 435
BD 527
BD 596
BD 577
BD 620
BD 296
BD 312
BD 494
BD 640
BD 442
BD 295
BD 478
LMG 20103
BD 556
BD 326
BD 541
BD 325
BD 515
BD 305
BD 543
BD 614
BD 331
BD 304
P. allii
BD 317
BD 561
BD 622
BD 588
BD 602
BD 377
P. allii
BD 647
BD 629
PA 4
BD 310
BD 301
BD 551
BD 315
PA 3
BD 390
P. allii
LMG 2665T
BD 309
P. allii
BD 311
BD 380
P. allii
CTB 1061
CTB 1135
BD 333
LMG 2632T
P. stewartii subsp.
LMG 2671
indologenes
LMG 2676
BD 335
BD 336
BD 250
BD 334
BCC 105T
BD 502
BD 639
LMG 1286T
BD 287
DAR 49828
DAR 72041
LMG 2603T
LMG 2749
LMG 2565
LMG 22049T
LMG 2713
LMG 2715T
P. ananatis
P. allii sp. nov.
P. stewartii subsp. indologenes
P. vagens
P. agglomerans
P. dispersa
P. citrea
P. stewartii subsp. stewartii

Fig. 7. Relationships among 67 *Pantoea* strains, including strains isolated from onion and maize, based on API 20E tests. ***P. allii*** strains are highlighted in bold. Reference strains, including the type strains of *Pantoea* species, are in italics. A black rectangle indicates a positive reaction in the test while a white rectangle indicates a negative reaction in the test.

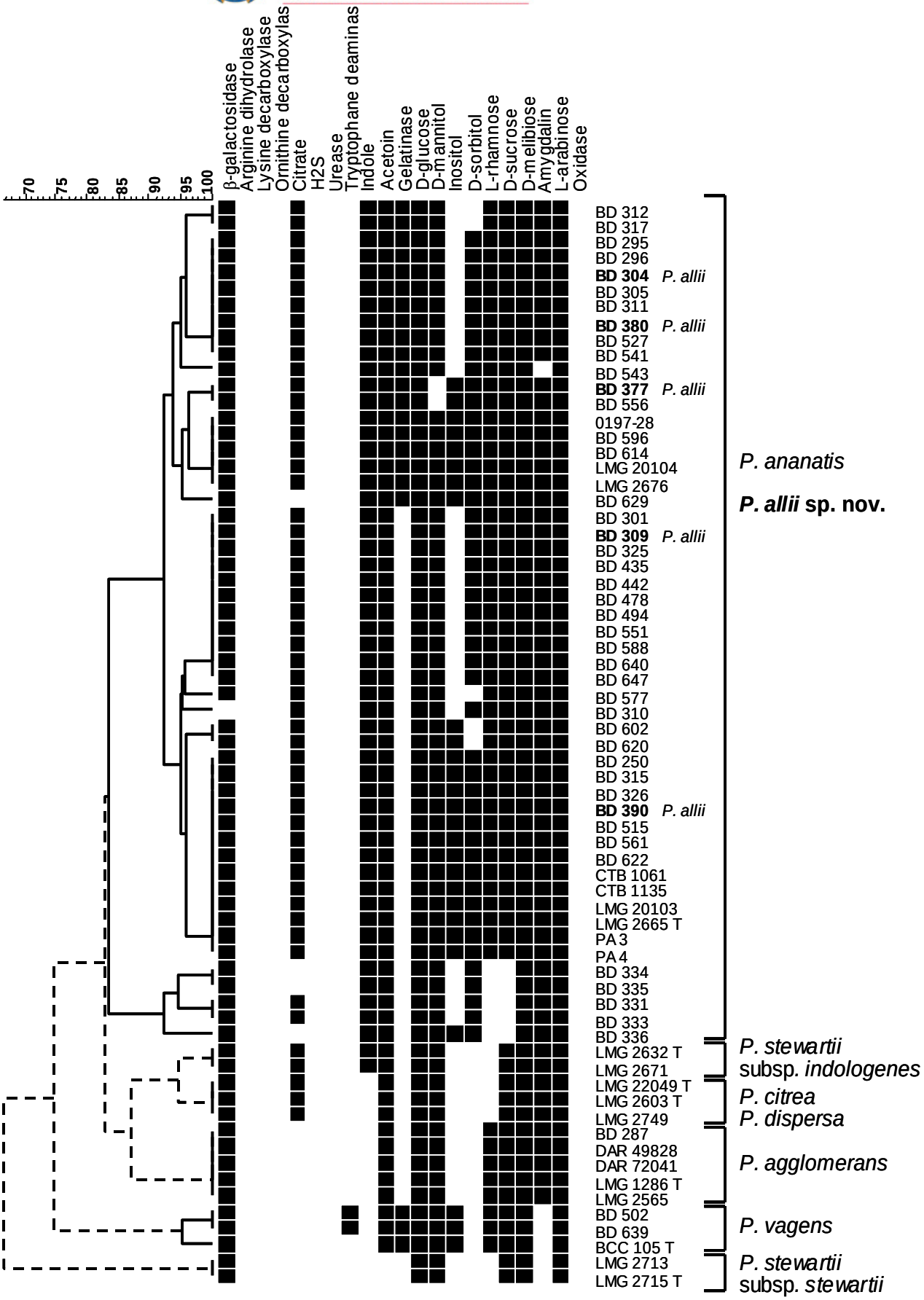
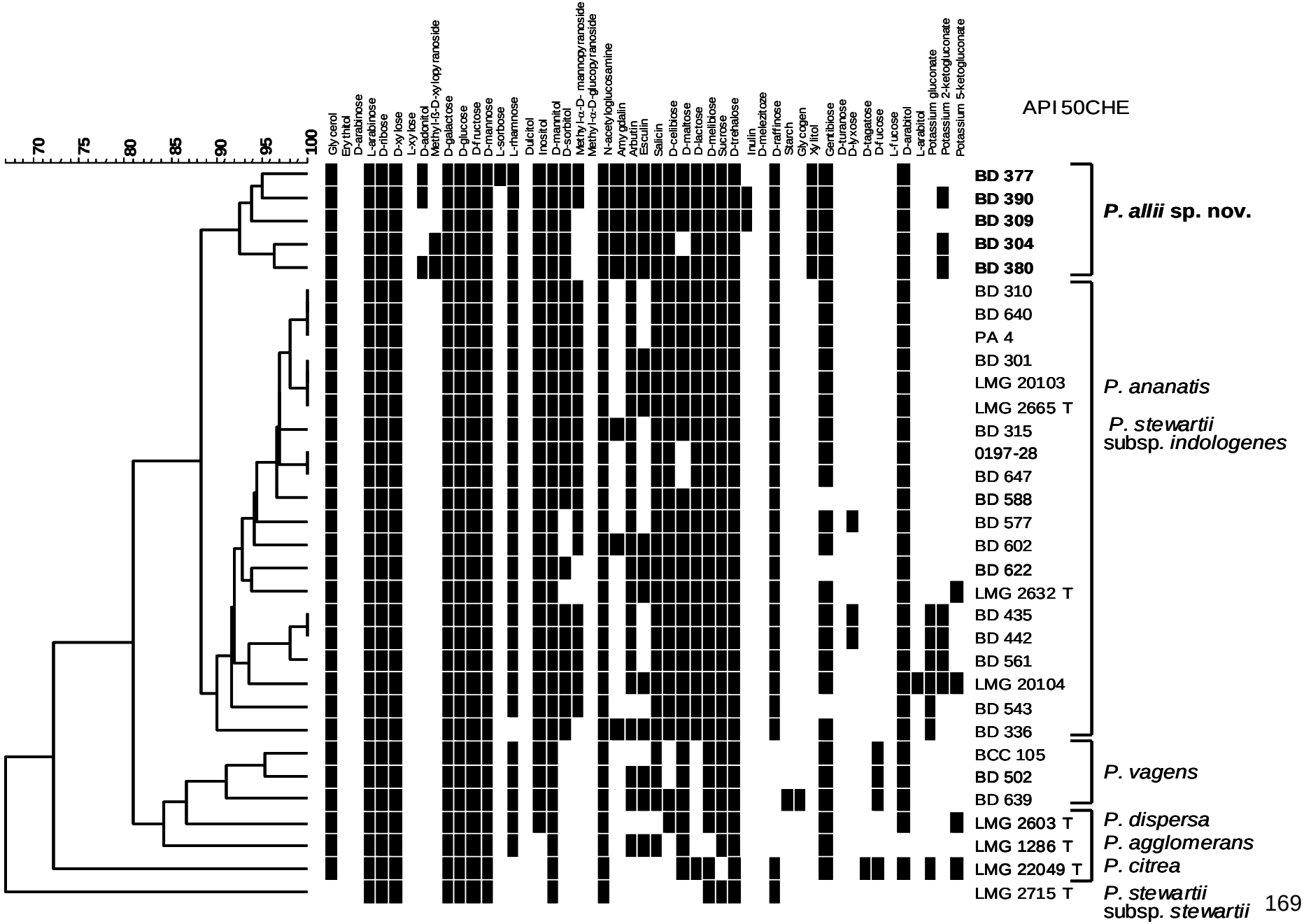

70
75
80
85
90
95
100
β-galactosidase
Arginine dihydrolase
Lysine decarboxylase
Ornithine decarboxylase
Citrate
H2S
Urease
Tryptophane deaminase
Indole
Acetoin
Gelatinase
D-glucose
D-mannitol
Inositol
D-sorbitol
L-rhamnose
D-sucrose
D-melibiose
Amygdalin
L-arabinose
Oxidase
BD 312
BD 317
BD 295
BD 296
BD 304 P. allii
BD 305
BD 311
BD 380 P. allii
BD 527
BD 541
BD 543
BD 377 P. allii
BD 556
0197-28
BD 596
BD 614
LMG 20104
LMG 2676
BD 629
BD 301
BD 309 P. allii
BD 325
BD 435
BD 442
BD 478
BD 494
BD 551
BD 588
BD 640
BD 647
BD 577
BD 310
BD 602
BD 620
BD 250
BD 315
BD 326
BD 390 P. allii
BD 515
BD 561
BD 622
CTB 1061
CTB 1135
LMG 20103
LMG 2665 T
PA 3
PA 4
BD 334
BD 335
BD 331
BD 333
BD 336
LMG 2632 T
LMG 2671
LMG 22049 T
LMG 2603 T
LMG 2749
BD 287
DAR 49828
DAR 72041
LMG 1286 T
LMG 2565
BD 502
BD 639
BCC 105 T
LMG 2713
LMG 2715 T
P. ananatis
P. allii sp. nov.
P. stewartii subsp. indologenes
P. citrea
P. dispersa
P. agglomerans
P. vagens
P. stewartii subsp. stewartii

Fig. 8. Relationships among 33 selected *Pantoea* strains, based on API 50CHE tests. *P. allii* strains are highlighted in bold. Reference strains, including the type strains of *Pantoea* species, are in italics. A black rectangle indicates a positive reaction in the test while a white rectangle indicates a negative reaction in the test.


API 50CHE
70
75
80
85
90
95
100
Gly cerol
Ery thitol
D-arabinose
L-arabinose
D-ribose
D-xy lose
L-xy lose
D-adonitol
Methyl-ß-D-xy lopy ranoside
D-galactose
D-glucose
D-fructose
D-mannose
L-sorbose
L-rhamnose
Dulcitol
Inositol
D-mannitol
D-sorbitol
Methyl-α-D- mannopy ranoside
Methyl-α-D-glucopy ranoside
N-acety lglucosamine
Amy gdalin
Arbutin
Esculin
Salicin
D-celibiose
D-maltose
D-lactose
D-melibiose
Sucrose
D-trehalose
Inulin
D-melezitoze
D-raffinose
Starch
Gly cogen
Xy litol
Gentibiose
D-turanose
D-lyxose
D-tagatose
D-fucose
L-fucose
D-arabitol
L-arabitol
Potassium gluconate
Potassium 2-ketogluconate
Potassium 5-ketogluconate
BD 377
BD 390
BD 309
BD 304
BD 380
BD 310
BD 640
PA 4
BD 301
LMG 20103
LMG 2665 T
BD 315
0197-28
BD 647
BD 588
BD 577
BD 602
BD 622
LMG 2632 T
BD 435
BD 442
BD 561
LMG 20104
BD 543
BD 336
BCC 105
BD 502
BD 639
LMG 2603 T
LMG 1286 T
LMG 22049 T
LMG 2715 T
P. allii sp. nov.
P. ananatis
P. stewartii subsp. indologenes
P. vagens
P. dispersa
P. agglomerans
P. citrea
P. stewartii subsp. stewartii

APPENDIX A: Multiple Sequence Alignment, 16S rDNA sequences of *P. allii, P. ananatis, P. agglomerans, P. vagens, P. stewartii* subsp. *stewartii, P. stewartii* subsp. *indologenes, P. punctata, P. terrea, P. citrea, P. dispersa, Enterobacter cloacae, Klebsiella pneumoniae* and *Escherichia coli***.**Number of sequences: 38

Maximum length: 1560

Strains:

P. allii: BD 309, BD 390, BD 377

P. ananatis: BD 301, BD 315, LMG 20106, BD 442, BD 435, BD 310, LMG 2676, BD 647, PA 4, BD 602, BD 640, BD 588, LMG 20103, LMG 2665T, BD 561, BD 622, BD 577, BD 336

P. agglomerans: LMG 2565, LMG 2660, BB 287, DSM 3493 T (= LMG 1286 T)

P. vagens: BCC 105 T, BD 500, BD 502, BD 639

P. stewartii subsp. *stewartii*: LMG 2715 T; *P. stewartii* subsp. *indologenes*: LMG 2632 T; *P. punctata*: LMG 22050 T; *P. terrea*: LMG 22051 T; *P. citrea*: LMG 22049 T; *P. dispersa* LMG 2603 T

Enterobacter cloacae: ATCC 13047 T*; Klebsiella pneumoniae*: DSM 30104 T*; Escherichia coli* ATCC 11775 T

The differences in nucleotides between *P. allii* and *P. ananatis* are marked in green: BD 309 nucleotide numbering, position 378-179.

The differences in nucleotides between *P. vagens* and *P. agglomerans* are marked in blue: BCC 105 nucleotide numbering, position 375-376.

Upper line in the alignment:

symbol " - " indicates sites excluded from the construction of phylogenetic tree (Fig. 3).

Symbol " * "indicates sites included in the construction of phylogenetic tree (Fig. 3).

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                ------------------------------------------------------------
BD 309          ............................................................  0
BD 390          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
BD 377          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
BD 602          .agagtttgatcctggctcagattgaacg.ccggcggcaggcctaacacat.gcaagtcg  57
BD 647          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
LMG 2676        ........................................aacacat.gcaagtcg  15
BD 442          ............................................................  0
BD 435          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
BD 310          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
BD 301          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
BD 315          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
PA 4            .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacattgcaagtcg  58
LMG 20106       ...agtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  55
BD 640          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
BD 588          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
LMG 20103       .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
LMG 2665 T      ..................agtgaacg.ctggcggcagccctaacacat.gcaagtcg  37
BD 561          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
BD 622          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
BD 577          .agagtttgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
BD 336          ................tcaagatgaacg..ctgcggcaggcctaacacat.gcaagtcg  40
LMG 2565        ..................attgaacg.ctggcggcaggcctaacacat.gcaagtcg  37
LMG 2660        ........................................aacacat.gcaagtcg  15
BD 287          .agagtttgatcctggctcagattgaacgnctggcggcaggcctaacacat.gcaagtcg  58
LMG 1286 T      ....................................ggcctaacacat.gcaagtct  20
BD 639          ............................................................  0
BD 502          ............................................................  0
BD 500          ............................................................  0
BCC 105 T       ............................................................  0
LMG 2715 T      ........................................aacacat.gcaagtcg  15
LMG 2632 T      tagagtntgatcctggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  58
LMG 22050 T     ..................attgaacg.ctggcggcaggcctaacacat.gcaagtcg  37
LMG 22051 T     ..................attgaacg.ctggcggcaggcctaacacat.gcaagtcg  37
LMG 22049 T     ..................attgaacg.ctggcggcaggcctaacacat.gcaagtcg  37
LMG 2603 T      ............................................................  0
ATCC 13047 T    ....................tgaacg.ctggcggcaggcctaacacat.gcaagtcg  35
DSM 30104 T     .agagtttgatnntggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  57
ATCC 11775 T    ...agtttgatcatggctcagattgaacg.ctggcggcaggcctaacacat.gcaagtcg  55
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BD 309          .............GGGAGCTTGCTCCTCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     46
BD 390          gacggtagcacagaG.AGCTTGCTCt.CGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    114
BD 377          gacggtagcacagaG.AGCTTGCTCt.CGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    114
BD 602          gacggtagcacagaG.AGCTTGCTC.TCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    114
BD 647          gacggtagcacagaG.AGCTTGCTC.TCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    114
LMG 2676        gacggtagcacagaG.AGCTTGCTCt.CGtGTGACGAGTGGCGGACGGGTGAGTAATGT.     72
BD 442          ...ggtagcacagaG.AGCTTGCTC.TCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     54
BD 435          gacggtagcacagaG.AGCTTGCTC.TCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    114
BD 310          gacggtagcacagaGGAGCTTGCTCCTCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    116
BD 301          gacggtagcacagaGGAGCTTGCTCCTCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    116
BD 315          gacggtagcacagaGGAGCTTGCTCCTCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    116
PA 4            gacggtagcacagaG.AGCTTGCTCt.CGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    115
LMG 20106       gacggtagcacagGGGAGCTTGCTCCTCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    114
BD 640          gacggtagcacagaG.AGCTTGCTCt.CGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    114
BD 588          gacggtagcacagaGGAGCTTGCTCCTCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    116
LMG 20103       gacggtagcacagaG.AGCTTGCTCt.CGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    114
LMG 2665 T      ggcggtagcacagaG.AGCTTcCTC.TCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     94
BD 561          gacggtagcacagaGGAGCTTGCTCCTCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    116
BD 622          gacggtagcacagaG.AGCTTGCTCt.CGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    114
BD 577          gacggtagcacagaG.AGCTTGCTCt.CGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    114
BD 336          gacg.tagcacagaG.AGCTTGCTnt.CGGGTGACGA.TtGCGGACGGGTGAGTAATGTt    96
LMG 2565        gacggtancacagaG.AGnTTGtTCt.nGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     94
LMG 2660        gacggtagcacagaGGAGCTTGCTCtctGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     74
BD 287          gacggtagcacagaG.AGCTTGCTCt.tGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    115
LMG 1286 T      gacggtagcacagaGGAGCTTGCTCCTtGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     79
BD 639          ........................CTtGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     34
BD 502          ........................CTtGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     34
BD 500          ......................CCTtGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     35
BCC 105 T       ............................GGGTGACGAGTGGCGGACGGGTGAGTAATGT.     31
LMG 2715 T      gacggtagcacagaGGAGCTTGCTC.TCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     73
LMG 2632 T      gacggtagcacagaGGAGCTTGCTCCTCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.    117
LMG 22050 T     aacggtagcacagGGGAGCTTGCTCCcCGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     96
LMG 22051 T     aacggtagcacagaGGAGCTTGCTCCTtGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     96
LMG 22049 T     aacggtagcacagaGGAGCTTGCTCCTtGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     96
LMG 2603 T      ........................................GGACGGGTGAGTAATGT.     17
ATCC 13047 T    aacggtagcacagaG.AGCTTGCTCt.CGGGTGACGAGTGGCGGACGGGTGAGTAATGT.     92
DSM 30104 T     agcggtagcacagaG.AGCTTGCTCt.CGGGTGACGAGcGGCGGACGGGTGAGTAATGT.    114
ATCC 11775 T    aacggtaacaggaaGcAGCTTGCTgCTttGcTGACGAGTGGCGGACGGGTGAGTAATGT.    114
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BD 309          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    106
BD 390          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    174
BD 377          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    174
BD 602          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    174
BD 647          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    174
LMG 2676        CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    132
BD 442          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    114
BD 435          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    174
BD 310          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    176
BD 301          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    176
BD 315          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    176
PA 4            CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    175
LMG 20106       CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    174
BD 640          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    174
BD 588          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    176
LMG 20103       CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    174
LMG 2665 T      CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    154
BD 561          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    176
BD 622          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    174
BD 577          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    174
BD 336          CTGGGnATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    156
LMG 2565        CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    154
LMG 2660        CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    134
BD 287          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    175
LMG 1286 T      CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC    139
BD 639          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC     94
BD 502          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC     94
BD 500          CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC     95
BCC 105 T       CTGGGGATCTGCCCGATAGAGGGGGATAACCACTGGAAACGGTGGCTAATACCGCATAAC     91
LMG 2715 T      CTGGGaAaCTGCCCGATgGAGGGGGATAACtACTGGAAACGGTaGCTAATACCGCATAAC    133
LMG 2632 T      nTGGGaAaCTGCCCGATgGAGGGGGATAACtACTGGAAACGGTaGCTAATACCGCATAAC    177
LMG 22050 T     CTGGGaAaCTGCCtGATgGAGGGGGATAACtACTGGAAACGGTaGCTAATACCGCATAAt    156
LMG 22051 T     CTGGGaAaCTGCCtGATgGAGGGGGATAACtACTGGAAACGGTaGCTAATACCGCATAAn    156
LMG 22049 T     CTGGGaAaCTGCCtGATgGnGGGGGATAACtACTGGAAACGGTaGCTAATACCGCATAAC    156
LMG 2603 T      CTGGGaAaCTGCCCGATgGAGGGGGATAACtACTGGAAACGGTaGCTAATACCGCATAAC     77
ATCC 13047 T    CTGGGaAaCTGCCtGATgGAGGGGGATAACtACTGGAAACGGTaGCTAATACCGCATAAn    152
DSM 30104 T     CTGGGaAaCTGCCtGATgGAGGGGGATAACtACTGGAAACGGTaGCTAATACCGCATAAC    174
ATCC 11775 T    CTGGGaAaCTGCCtGATgGAGGGGGATAACtACTGGAAACGGTaGCTAATACCGCATAAC    174
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BD 309          GTCGCAAGACCAAAGAGGGGGACCTTAGGGCCTCTCACTATCGGATGAACCCAGATGGGA   166
BD 390          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   234
BD 377          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   234
BD 602          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   234
BD 647          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   234
LMG 2676        GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   192
BD 442          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   174
BD 435          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   234
BD 310          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   236
BD 301          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   236
BD 315          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   236
PA 4            GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   235
LMG 20106       GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   234
BD 640          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   234
BD 588          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   236
LMG 20103       GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   234
LMG 2665 T      GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   214
BD 561          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   236
BD 622          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   234
BD 577          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   234
BD 336          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   216
LMG 2565        GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   214
LMG 2660        GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   194
BD 287          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   235
LMG 1286 T      GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   199
BD 639          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   154
BD 502          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   154
BD 500          GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   155
BCC 105 T       GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTCACTATCGGATGAACCCAGATGGGA   151
LMG 2715 T      GTCGCAAGACCAAAGtGGGGGACCTccGGGCCTCaCACCcATCGGATGtgCCCAGATGGGA   193
LMG 2632 T      GTCGCAAGACCAAAGtGGGGGACCTTcGGGCCTCaCACCcATCGGATGtgCCCAGATGGGA   237
LMG 22050 T     GTCGCAAGACCAAAGcGGGGGACCTTcGGGCCTCgCACCcATCGGATGtgCCCAGATGGGA   216
LMG 22051 T     GTCGCAAGACCAAAGtGGGGGACCTTcGGGCCTCaCACCcATCGGATGtgCCCAGATGGGA   216
LMG 22049 T     GTCGCAAGACCAAAGtGGGGGACCTTcGGGCCTCaCACCcATCGGATGtgCCCAGATGGGA   216
LMG 2603 T      GTCGCnAGACCAAAGtGGGGGACCTTcGGGCCTCaCACCcATCGGATGtgCCCAGATGGGA   137
ATCC 13047 T    GTCGCAAGACCAAAGAGGGGGACCTTcGGGCCTCTtgCcATCaGATGtgCCCAGATGGGA   212
DSM 30104 T     GTCGCAAGACCAAAGtGGGGGACCTTcGGGCCTCatgCcATCaGATGtgCCCAGATGGGA   234
ATCC 11775 T    GTCGCAAGcaCAAAGAGGGGGACCTTAGGGCCTCTtgCcATCGGATGtgCCCAGATGGGA   234
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BD 309          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    226
BD 390          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
BD 377          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
BD 602          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
BD 647          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
LMG 2676        TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    252
BD 442          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    234
BD 435          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
BD 310          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    296
BD 301          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    296
BD 315          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    296
PA 4            TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGtTCCCTAGCTGGTCTGAGAGGA    295
LMG 20106       TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
BD 640          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
BD 588          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    296
LMG 20103       TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
LMG 2665 T      TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    274
BD 561          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    296
BD 622          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
BD 577          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
BD 336          TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    276
LMG 2565        TTAGCTAGTAGGCGGGGTAAtGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    274
LMG 2660        TTAGCTAGTAGGCGGGGTAAtGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGgGGA    254
BD 287          TTAGCTAGTAGGCGGGGTAAtGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    295
LMG 1286 T      TTAGCTAGTAGGCGGGGTAAtGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    259
BD 639          TTAGCTAGTAGGCGGGGTAAtGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    214
BD 502          TTAGCTAGTAGGCGGGGTAAtGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    214
BD 500          TTAGCTAGTAGGCGGGGTAAtGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    215
BCC 105 T       TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    211
LMG 2715 T      TTAGCTAGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    253
LMG 2632 T      TTAGCTtGTAGGCGGGGTAACGGCCCACCTAGGCGACGATCCCTAGCTGGTnTGAGAGGA    297
LMG 22050 T     TTAGCTAGTAGGtGGGGTAAtGGCtCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    276
LMG 22051 T     TTAGCTAGTAGGtGGGGTAAtGGCtCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    276
LMG 22049 T     TTAGCTAGTAGGtGGGGTAACGGCtCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    276
LMG 2603 T      TTAGCTAGTAGGtGGGGTAAtGGCtCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    197
ATCC 13047 T    TTAGCTAGTAGGtGGGGTAACGGCtCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    272
DSM 30104 T     TTAGCTAGTAGGtGGGGTAACGGCtCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
ATCC 11775 T    TTAGCTAGTAGGtGGGGTAACGGCtCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGA    294
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BD 309            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGAGA    286
BD 390            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    354
BD 377            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACcCCTACGGGAGGCAGCAGTGGgGA    354
BD 602            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    354
BD 647            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    354
LMG 2676          TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    312
BD 442            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    294
BD 435            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    354
BD 310            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    356
BD 301            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    356
BD 315            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    356
PA 4              TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    355
LMG 20106         TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    354
BD 640            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    354
BD 588            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    356
LMG 20103         TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    354
LMG 2665 T        TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    334
BD 561            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    356
BD 622            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    354
BD 577            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    354
BD 336            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    336
LMG 2565          TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    334
LMG 2660          TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTC.TACGGGAGGCAGCAGTGGgGA    313
BD 287            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    355
LMG 1286 T        TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    319
BD 639            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    274
BD 502            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    274
BD 500            TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    275
BCC 105 T         TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    271
LMG 2715 T        TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    313
LMG 2632 T        TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    357
LMG 22050 T       TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    336
LMG 22051 T       TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    336
LMG 22049 T       TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    336
LMG 2603 T        TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    257
ATCC 13047 T      TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    332
DSM 30104 T       TGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    354
ATCC 11775 T      TGACCAGCaACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGgGA    354
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BD 309            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    345
BD 390            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    413
BD 377            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    413
BD 602            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    413
BD 647            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    413
LMG 2676          ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    371
BD 442            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    353
BD 435            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    413
BD 310            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    415
BD 301            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAgGAAGGCCTTC    415
BD 315            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    415
PA 4              ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    414
LMG 20106         ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    413
BD 640            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    413
BD 588            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    415
LMG 20103         ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    413
LMG 2665 T        ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    393
BD 561            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    415
BD 622            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    413
BD 577            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCcTC    413
BD 336            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    395
LMG 2565          ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    393
LMG 2660          ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    372
BD 287            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGaCGTGTATGAAGAAGGCCTTC    415
LMG 1286 T        ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    378
BD 639            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    333
BD 502            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    333
BD 500            ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    334
BCC 105 T         ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    330
LMG 2715 T        ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    372
LMG 2632 T        ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    416
LMG 22050 T       ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    395
LMG 22051 T       ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    395
LMG 22049 T       ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    395
LMG 2603 T        ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    316
ATCC 13047 T      ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTATGAAGAAGGCCTTC    391
DSM 30104 T       ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG.CGTGTgTGAAGAAGGCCTTC    413
ATCC 11775 T      ATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCnG.CGTGTATGAAGAAGGCCTTC    413
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BD 309 T          GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCATTGTGGTTAATAACCGCAGTGATTGA    405
BD 390            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCATTGTGGTTAATAACCGCAGTGATTGA    473
BD 377            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCATTGTGGTTAATAACCGCAtTGATTGA    473
BD 602            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCAtTGATTGA    473
BD 647            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCAtTGATTGA    473
LMG 2676          GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCAtTGATTGA    431
BD 442            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCAtTGATTGA    413
BD 435            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCAtTGATTGA    473
BD 310            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCAtTGATTGA    475
BD 301            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCAtTGATTGA    475
BD 315            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCnCAtTGATTGA    475
PA 4              GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCAtTGATTGA    474
LMG 20106         GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCAtTGATTGA    473
BD 640            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCAtTGAcTGA    473
BD 588            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCAtTGATTGA    475
LMG 20103         GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCgtcGATTGA    473
LMG 2665 T        GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTnTGGTTAATAACCnngtcGATTGA    453
BD 561            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCgtcGATTGA    475
BD 622            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCttgtcGATTGA    473
BD 577            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCgtcGATTGA    473
BD 336            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTaaGGcTAATAACCttgtcGATTGA    455
LMG 2565          GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgacGgGGTTAATAACCctgtcGATTGA    453
LMG 2660          GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgacGgG.TTAATAACCctgtcGATTGA    431
BD 287            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGgGGTTAATAACCctgtcGATTGA    475
LMG 1286 T        GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGgGGTTAATAACCttgtcGATTGA    438
BD 639            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCggTGcGGTTAATAACCGCgccGATTGA    393
BD 502            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCggTGcGGTTAATAACCGCgccGATTGA    393
BD 500            GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCggTGcGGTTAATAACCGCgccGATTGA    394
BCC 105 T         GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCgaTGTGGTTAATAACCGCgtcGATTGA    390
LMG 2715 T        GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGtggTGaGGTTAATAACCtCAtcaATTGA    432
LMG 2632 T        GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGtggTGaGGTTAATAACCtCAtcaATTGA    476
LMG 22050 T       GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCATTGcGGTTAATAACCGCAGTGATTGA    455
LMG 22051 T       GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCAgTcgtGTTAATAgCacgncTGATTGA    455
LMG 22049 T       GGGTTGTAAAGTACTTTCAGtcaGGAGGAAGGgtgTagtcTTAATAcggctAtgcATTGA    455
LMG 2603 T        GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCggTGaGGTTAATAACCtngccGATTGA    376
ATCC 13047 T      GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGtgTTGTGGTTAATAACCGCAGcaATTGA    451
DSM 30104 T       GGGTTGTAAAGcACTTTCAGCGGGGAGGAAGGCgaTGaGGTTAATAACCtCAtcGATTGA    473
ATCC 11775 T      GGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGgAgTaaaGTTAATAcCtttgcTcATTGA    473
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BD 309          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    463
BD 390          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    531
BD 377          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    531
BD 602          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    531
BD 647          CGTTACCC.GCAGAAGAgGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    531
LMG 2676        CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    489
BD 442          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    471
BD 435          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    531
BD 310          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGnCAGCAGCCGCGGnTAATACGGAG    534
BD 301          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    533
BD 315          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    533
PA 4            CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    532
LMG 20106       CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    531
BD 640          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    531
BD 588          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    533
LMG 20103       CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    531
LMG 2665 T      CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    511
BD 561          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    533
BD 622          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    531
BD 577          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    531
BD 336          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    513
LMG 2565        CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    511
LMG 2660        CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    489
BD 287          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGnCGCGG.TAATACGGAG    533
LMG 1286 T      CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    496
BD 639          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    451
BD 502          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    451
BD 500          CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    452
BCC 105 T       CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    448
LMG 2715 T      CaTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    490
LMG 2632 T      CGTTACnC.GCAnAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    534
LMG 22050 T     CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    513
LMG 22051 T     CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    513
LMG 22049 T     CGTTACtg.aCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    513
LMG 2603 T      CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    434
ATCC 13047 T    CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    509
DSM 30104 T     CGTTACCCtGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    532
ATCC 11775 T    CGTTACCC.GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG.TAATACGGAG    531
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                **********************************************************
BD 309          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    523
BD 390          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    591
BD 377          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    591
BD 602          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    591
BD 647          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    591
LMG 2676        GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    549
BD 442          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    531
BD 435          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    591
BD 310          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAaCGCACGCAGGCGGTCTGTTAAGTCA    594
BD 301          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    593
BD 315          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    593
PA 4            GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    592
LMG 20106       GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    591
BD 640          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    591
BD 588          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    593
LMG 20103       GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    591
LMG 2665 T      GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    571
BD 561          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    593
BD 622          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    591
BD 577          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    591
BD 336          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    573
LMG 2565        GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    571
LMG 2660        GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    549
BD 287          GGTGCAAGCGTnAATCGGAATTACTGGGCGTAAcGCGCACGCAGGCGGTCTGTTAAGTCA    593
LMG 1286 T      GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    556
BD 639          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    511
BD 502          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    511
BD 500          GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    512
BCC 105 T       GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    508
LMG 2715 T      GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    550
LMG 2632 T      GGTGCAAGCGTTAATCGGAATTACnGGGCGTAAAnCGCACGCAGGCGGTCTGTTAAGTCA    594
LMG 22050 T     GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTcAAGTCg    573
LMG 22051 T     GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTcAAGTCg    573
LMG 22049 T     GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTcAAGTCg    573
LMG 2603 T      GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTTAAGTCA    494
ATCC 13047 T    GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTcAAGTCg    569
DSM 30104 T     GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTcAAGTCg    592
ATCC 11775 T    GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTtTGTTAAGTCA    591
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BD 309          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    582
BD 390          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    650
BD 377          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTcGAAACTGGCAGGCTTGAGTCT    650
BD 602          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    650
BD 647          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    650
LMG 2676        GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    608
BD 442          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    590
BD 435          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    650
BD 310          GATGnnnAAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    654
BD 301          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    652
BD 315          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    652
PA 4            GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    651
LMG 20106       GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    650
BD 640          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCgGGCTTGAGTCT    650
BD 588          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    652
LMG 20103       GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    650
LMG 2665 T      GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    630
BD 561          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    652
BD 622          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    650
BD 577          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    650
BD 336          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    632
LMG 2565        nATGTG.AAAnCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    630
LMG 2660        GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    608
BD 287          GATGTG.AAAnCCCnnGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    652
LMG 1286 T      GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    615
BD 639          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    570
BD 502          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    570
BD 500          GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    571
BCC 105 T       GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    567
LMG 2715 T      GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    609
LMG 2632 T      GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAnTCT    653
LMG 22050 T     GATGTG.AAATCCCCGGGCTcAACCcGGGAACTGCATTcGAAACTGGCAGaCTaGAGTCT    632
LMG 22051 T     GATGTG.AAATCCCCGGGCTcAACCcGGGAACTGCATTcGAAACTGGCAGaCTaGAGTCT    632
LMG 22049 T     GATGTG.AAATCCCCGGGCTcAACCcGGGAACTGCATTcGAAACTGGCAGGCTaGAGTCT    632
LMG 2603 T      GATGTG.AAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCT    553
ATCC 13047 T    GATGTG.AAATCCCCGGGCTcAACCTGGGAACTGCATTcGAAACTGGCAGGCTgGAGTCT    628
DSM 30104 T     GATGTG.AAATCCCCGGGCTcAACCTGGGAACTGCATTcGAAACTGGCAGGCTaGAGTCT    651
ATCC 11775 T    GATGTG.AAATCCCCGGGCTcAACCTGGGAACTGCATcTGAtACTGGCAaGCTTGAGTCT    650
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BD 309            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    641
BD 390            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    709
BD 377            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    709
BD 602            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    709
BD 647            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    709
LMG 2676          CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    667
BD 442            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    649
BD 435            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    709
BD 310            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    713
BD 301            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    711
BD 315            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    711
PA 4              CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    710
LMG 20106         CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    709
BD 640            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    709
BD 588            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    711
LMG 20103         CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    709
LMG 2665 T        CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    689
BD 561            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    711
BD 622            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    709
BD 577            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    709
BD 336            CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATn    691
LMG 2565          tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    689
LMG 2660          tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    667
BD 287            tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAAaTGCGnnnnGATCTGGAGGAATA    712
LMG 1286 T        tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    674
BD 639            tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    629
BD 502            tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    629
BD 500            tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    630
BCC 105 T         CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    626
LMG 2715 T        CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    668
LMG 2632 T        CGTAGAGGGGGGTAGAATTCCAnGTGTAGCGGTGAAA.TGCGTAnAGATCTGGAGGAATA    712
LMG 22050 T       tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    691
LMG 22051 T       tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    691
LMG 22049 T       tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    691
LMG 2603 T        CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    612
ATCC 13047 T      tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    687
DSM 30104 T       tGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    710
ATCC 11775 T      CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA.TGCGTAGAGATCTGGAGGAATA    709
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            ***********-**************************************************
BD 309      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  700
BD 390      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  768
BD 377      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  768
BD 602      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  768
BD 647      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  768
LMG 2676    CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  726
BD 442      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  708
BD 435      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  768
BD 310      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  772
BD 301      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  770
BD 315      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  770
PA 4        CCGGTGGCGAAGaGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  770
LMG 20106   CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  768
BD 640      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  768
BD 588      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  770
LMG 20103   CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  768
LMG 2665 T  CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  748
BD 561      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  770
BD 622      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  768
BD 577      CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  768
BD 336      nCnGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  750
LMG 2565    CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  748
LMG 2660    CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  726
BD 287      CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  771
LMG 1286 T  CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  733
BD 639      CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  688
BD 502      CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  688
BD 500      CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  689
BCC 105 T   CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  685
LMG 2715 T  CCGGTGGCGAAG.GCGGtCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  727
LMG 2632 T  CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  771
LMG 22050 T CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  750
LMG 22051 T CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  750
LMG 22049 T CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  750
LMG 2603 T  CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  671
ATCC 13047 T CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  746
DSM 30104 T CCGGTGGCGAAG.GCGGCCCCCTGGACaAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  769
ATCC 11775 T CCGGTGGCGAAG.GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA  768
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                ************************************************************
BD 309          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    760
BD 390          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    828
BD 377          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    828
BD 602          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    828
BD 647          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    828
LMG 2676        GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    786
BD 442          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGgGGTTGT    768
BD 435          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGgGGTTGT    828
BD 310          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTnGACTTGGAGGTTGT    832
BD 301          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    830
BD 315          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    830
PA 4            GCAAACAGGATTAGATACCCTGGTAnTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    830
LMG 20106       GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    828
BD 640          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    828
BD 588          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    830
LMG 20103       GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    828
LMG 2665 T      GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    808
BD 561          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    830
BD 622          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    828
BD 577          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    828
BD 336          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    810
LMG 2565        GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    808
LMG 2660        GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    786
BD 287          GCAAACAGGATTAGATACCCTGGTAnTCCgCGCCGTAAACGATGTCGACTTGGAGGTTGT    831
LMG 1286 T      GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    793
BD 639          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    748
BD 502          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    748
BD 500          GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    749
BCC 105 T       GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    745
LMG 2715 T      GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    787
LMG 2632 T      GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    831
LMG 22050 T     GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGcTGT    810
LMG 22051 T     GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGcTGT    810
LMG 22049 T     GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    810
LMG 2603 T      GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    731
ATCC 13047 T    GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGAtTTGGAGGTTGT    806
DSM 30104 T     GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGAtTTGGAGGTTGT    829
ATCC 11775 T    GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT    828
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                ************************************************************
BD 309          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    820
BD 390          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    888
BD 377          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    888
BD 602          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    888
BD 647          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    888
LMG 2676        TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    846
BD 442          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    828
BD 435          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    888
BD 310          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    892
BD 301          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    890
BD 315          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    890
PA 4            TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    890
LMG 20106       TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    888
BD 640          TCCCTTGAaGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    888
BD 588          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    890
LMG 20103       TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    888
LMG 2665 T      TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    868
BD 561          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    890
BD 622          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    888
BD 577          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCcTTAAGTCGACCGCCTGGGGAGTACGGCC    888
BD 336          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    870
LMG 2565        TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    868
LMG 2660        TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    846
BD 287          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGnGGAGTACGGCC    891
LMG 1286 T      TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    853
BD 639          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    808
BD 502          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    808
BD 500          TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    809
BCC 105 T       TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    805
LMG 2715 T      TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    847
LMG 2632 T      TCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    891
LMG 22050 T     gCCCTTGAGGcGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    870
LMG 22051 T     TCCCnTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    870
LMG 22049 T     gCCCTTGAGGnGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    870
LMG 2603 T      gCCCTTGAGGnGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    791
ATCC 13047 T    gCCCTTGAGGcGTGGCTTCCGGAGCTAACGCGTTAAaTCGACCGCCTGGGGAGTACGGCC    866
DSM 30104 T     gCCCTTGAGGcGTGGCTTCCGGAGCTAACGCGTTAAaTCGACCGCCTGGGGAGTACGGCC    889
ATCC 11775 T    gCCCTTGAGGcGTGGCTTCCGGAnnTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC    888
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                ************************************************************
BD 309          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    880
BD 390          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    948
BD 377          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    948
BD 602          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    948
BD 647          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    948
LMG 2676        GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATaTGGTTT    906
BD 442          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    888
BD 435          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    948
BD 310          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    952
BD 301          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGcGGAGCATGTGGTTT    950
BD 315          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    950
PA 4            GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    950
LMG 20106       GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    948
BD 640          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    948
BD 588          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    950
LMG 20103       GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    948
LMG 2665 T      GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    928
BD 561          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    950
BD 622          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    948
BD 577          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    948
BD 336          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    930
LMG 2565        GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    928
LMG 2660        GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    906
BD 287          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    951
LMG 1286 T      GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    913
BD 639          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    868
BD 502          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    868
BD 500          GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    869
BCC 105 T       GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    865
LMG 2715 T      GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    907
LMG 2632 T      GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    951
LMG 22050 T     GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    930
LMG 22051 T     GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    930
LMG 22049 T     GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    930
LMG 2603 T      GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    851
ATCC 13047 T    GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    926
DSM 30104 T     GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT    949
ATCC 11775 T    GCAAGGTTAAAACTCAAATGAATTGACGGGGGCC.GCACAAGCGGTGGAGCATGTGGTTT    947
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BD 309          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCACGGAATTCGGCAGAG    938
BD 390          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG   1006
BD 377          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG   1006
BD 602          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCACGGAATTtGGCAGAG   1006
BD 647          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCACGGAATTtGGCAGAG   1006
LMG 2676        AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG    964
BD 442          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAATTtaGCAGAG    946
BD 435          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCACGGAATTtGGCAGAG   1006
BD 310          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTGgACATCCAgaGAAcTtaGCAGAG   1011
BD 301          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG   1008
BD 315          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG   1008
PA 4            AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG   1008
LMG 20106       AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG   1006
BD 640          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG   1006
BD 588          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG   1008
LMG 20103       AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG   1006
LMG 2665 T      AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtGGCAGAG    986
BD 561          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCACGGAATTCGGCAGAG   1008
BD 622          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG   1006
BD 577          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG   1006
BD 336          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCACGGAATTCGGCAGAG    988
LMG 2565        AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCACGGAATTtGGCAGAG    986
LMG 2660        AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCACGGAATTtGGCAGAG    964
BD 287          AATTCGATGCAACGCGAAGAAgCCTTACCTACTCTTG.ACATCCACGGAATTtGGCAGAG   1010
LMG 1286 T      AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCACGGAATTtGGCAGAG    971
BD 639          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAnnGAAnTnnGCAGAG    926
BD 502          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAnnGAAnTnGGCAGAG    926
BD 500          AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCACnGAAnTnnGCAGAG    927
BCC 105 T       AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG    923
LMG 2715 T      AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgcGAAcTtGGCAGAG    965
LMG 2632 T      AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgcGAAcTttnCAGAG   1009
LMG 22050 T     AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG    988
LMG 22051 T     AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTttcCAGAG    988
LMG 22049 T     AATTCGATGCAACGCGAAGAA.CCTTACCTACTCTTG.ACATCCAgaGAAcTtaGCAGAG    988
LMG 2603 T      AATTCGATGCAACGCGAAGAA.CCTTACCTggcCTTG.ACATCCAgaGAAcTtaGCAGAG    909
ATCC 13047 T    AATTCGATGCAACGCGAAGAA.CCTTACCTggTCTTG.ACATCCACaGAAcTttcCAGAG    984
DSM 30104 T     AATTCGATGCAACGCGAAGAA.CCTTACCTggTCTTG.ACATCCACaGAAcTttcCAGAG   1007
ATCC 11775 T    AATTCGATGCAACGCGAAGAA.CCTTACCTggTCTTG.ACATCCACGGAAgTtttCAGAG   1005
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BD 309          ATGCCTTAGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT    998
BD 390          ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1066
BD 377          ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1066
BD 602          ATGCCTTAGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1066
BD 647          ATGCCTTAGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1066
LMG 2676        ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1024
BD 442          ATGCgTTgGTGCCTTCGGGAACCcTGAGACAGGTGCTGCATGGCTGTtGTCAGCTCGTGT   1006
BD 435          ATGCgTTAGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTtGTCAGCTCGTGT   1066
BD 310          ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1071
BD 301          ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1068
BD 315          ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1068
PA 4            ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1068
LMG 20106       ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1066
BD 640          ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1066
BD 588          ATGCtTTgGTGCCTTCGGGAgCtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1068
LMG 20103       ATGCtTTgGTGCCTTCGGGAgCtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1066
LMG 2665 T      ATGCtTTgGTGCCTTCGGGAgCCcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1046
BD 561          ATGCCTTAGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1068
BD 622          ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1066
BD 577          ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1066
BD 336          ATGCCTTAGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1048
LMG 2565        ATGCCTTAGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1046
LMG 2660        ATGCCTTAGTGCCTTCGGGgACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1024
BD 287          ATGCCTTAGTGCCTTCGGGAACCGTGAnACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1070
LMG 1286 T      ATGCCTTAGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1031
BD 639          ATGCnTTnGTGCCTTCGGGAACnnTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT    986
BD 502          ATGCCTTnGTGCCTTCGGGAACnnTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT    986
BD 500          ATGCnTTnGTGCCTTCGGGAACnnTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT    987
BCC 105 T       ATGCtTTgGTGCCTTCGGGAACCcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT    983
LMG 2715 T      ATGCCTTgGTGCCTTCGGGAACgcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1025
LMG 2632 T      ATGgaTTgGTGCCTTCGGGAACgcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1069
LMG 22050 T     ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1048
LMG 22051 T     ATGgaTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1048
LMG 22049 T     ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1048
LMG 2603 T      ATGCtTTgGTGCCTTCGGGAACtcTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT    969
ATCC 13047 T    ATGgaTTgGTGCCTTCGGGAACtGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1044
DSM 30104 T     ATGgaTTgGTGCCTTCGGGAACtGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1067
ATCC 11775 T    ATGagaatGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT   1065
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BD 309          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1058
BD 390          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1126
BD 377          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1126
BD 602          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1126
BD 647          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1126
LMG 2676        TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1084
BD 442          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1066
BD 435          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1126
BD 310          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1131
BD 301          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1128
BD 315          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1128
PA 4            TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1128
LMG 20106       TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1126
BD 640          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1126
BD 588          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1128
LMG 20103       TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1126
LMG 2665 T      TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1106
BD 561          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1128
BD 622          TGTGAAATGTTGGGTTAAGTCCtGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1126
BD 577          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1126
BD 336          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1108
LMG 2565        TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1106
LMG 2660        TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1084
BD 287          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1130
LMG 1286 T      TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1091
BD 639          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1046
BD 502          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1046
BD 500          TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1047
BCC 105 T       TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1043
LMG 2715 T      TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1085
LMG 2632 T      TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGAT   1129
LMG 22050 T     TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCcTATCCTTTGTTGCCAGCGAT   1108
LMG 22051 T     TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCcTATCCTTTGTTGCCAGCGAT   1108
LMG 22049 T     TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCcTATCCTTTGTTGCCAGCGAT   1108
LMG 2603 T      TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGgn   1029
ATCC 13047 T    TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGgT   1104
DSM 30104 T     TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGgT   1127
ATCC 11775 T    TGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGgT   1125
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BD 309          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1118
BD 390          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1186
BD 377          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1186
BD 602          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1186
BD 647          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1186
LMG 2676        TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1144
BD 442          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1126
BD 435          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1186
BD 310          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1191
BD 301          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1188
BD 315          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1188
PA 4            TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1188
LMG 20106       TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1186
BD 640          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1186
BD 588          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1188
LMG 20103       TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1186
LMG 2665 T      TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1166
BD 561          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1188
BD 622          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1186
BD 577          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1186
BD 336          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1168
LMG 2565        TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1166
LMG 2660        TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1144
BD 287          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1190
LMG 1286 T      TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1151
BD 639          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1106
BD 502          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1106
BD 500          TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1107
BCC 105 T       TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1103
LMG 2715 T      TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1145
LMG 2632 T      TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1189
LMG 22050 T     TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1168
LMG 22051 T     TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1168
LMG 22049 T     TCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1168
LMG 2603 T      TCGGcCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTC   1089
ATCC 13047 T    cCGGcCGGGAACTCAAAGGAGACTGCCaGTGATAAACtGGAGGAAGGTGGGGATGACGTC   1164
DSM 30104 T     TaGGcCGGGAACTCAAAGGAGACTGCCaGTGATAAACtGGAGGAAGGTGGGGATGACGTC   1187
ATCC 11775 T    cCGGcCGGGAACTCAAAGGAGACTGCCaGTGATAAACtGGAGGAAGGTGGGGATGACGTC   1185
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BD 309          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1178
BD 390          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1246
BD 377          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1246
BD 602          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1246
BD 647          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1246
LMG 2676        AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1204
BD 442          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1186
BD 435          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1246
BD 310          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1251
BD 301          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1248
BD 315          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1248
PA 4            AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1248
LMG 20106       AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1246
BD 640          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1246
BD 588          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1248
LMG 20103       AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1246
LMG 2665 T      AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1226
BD 561          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1248
BD 622          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1246
BD 577          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1246
BD 336          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1228
LMG 2565        AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1226
LMG 2660        AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1204
BD 287          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1250
LMG 1286 T      AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1211
BD 639          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1166
BD 502          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1166
BD 500          AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1167
BCC 105 T       AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1163
LMG 2715 T      AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1205
LMG 2632 T      AAGTCATCATGGCCCTTACcAGTAnGGCTACACACnTGCTACAATGGCGCATACAAnGAG   1249
LMG 22050 T     AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1228
LMG 22051 T     AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGtATACAAAGAG   1228
LMG 22049 T     AAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1228
LMG 2603 T      AAGTCATCATGGCCCTTACGgccAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1149
ATCC 13047 T    AAGTCATCATGGCCCTTACGAccAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1224
DSM 30104 T     AAGTCATCATGGCCCTTACGAccAGGGCTACACACGTGCTACAATGGCatATACAAAGAG   1247
ATCC 11775 T    AAGTCATCATGGCCCTTACGAccAGGGCTACACACGTGCTACAATGGCGCATACAAAGAG   1245
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BD 309          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1238
BD 390          AAGCGACCTCGCGAGAGCAAGCGGACCTCAcAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1306
BD 377          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1306
BD 602          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1306
BD 647          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1306
LMG 2676        AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1264
BD 442          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1246
BD 435          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1306
BD 310          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1311
BD 301          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1308
BD 315          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1308
PA 4            AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1308
LMG 20106       AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1306
BD 640          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1306
BD 588          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1308
LMG 20103       AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1306
LMG 2665 T      AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1286
BD 561          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1308
BD 622          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1306
BD 577          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1306
BD 336          AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1288
LMG 2565        AAGCGACCTCGCGAGAGCAAGCGGACCTCAcAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1286
LMG 2660        AAGCaACCTCGCGAGAGCAAGCGGACCTCAcAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1264
BD 287          AAGCGACCTCGCGAGAGCAAGCGGACCTCAcAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1310
LMG 1286 T      AAGCGACCTCGCGAGAGCAAGCGGACCTCAcAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1271
BD 639          AAGCGACCTCGCGAGAGCAAGCGGACCTCAcAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1226
BD 502          AAGCGACCTCGCGAGAGCAAGCGGACCTCAcAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1226
BD 500          AAGCGACCTCGCGAGAGCAAGCGGACCTCAcAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1227
BCC 105 T       AAGCGACCTCGCGAGAGCAAGCGGACCTCAcAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1223
LMG 2715 T      AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1265
LMG 2632 T      AAGCGACCTCGCGAGAnCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATCGGAGTCT   1309
LMG 22050 T     AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATtGGAGTCT   1288
LMG 22051 T     AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTaCGTCGTAGTCCGGATtGGAGTCT   1288
LMG 22049 T     AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATtGGAGTCT   1288
LMG 2603 T      AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATtGGAGTCT   1209
ATCC 13047 T    AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATtGGAGTCT   1284
DSM 30104 T     AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTatGTCGTAGTCCGGATtGGAGTCT   1307
ATCC 11775 T    AAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATtGGAGTCT   1305
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BD 309            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1297
BD 390            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATCtGTGGATCAGAATGCCACGGTGA   1366
BD 377            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1365
BD 602            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1365
BD 647            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1365
LMG 2676          GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1323
BD 442            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1305
BD 435            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1365
BD 310            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGnCACGGTGA   1370
BD 301            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATt.GTGGATCAGAATGCCACGGTGA   1367
BD 315            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGnCACGGTGA   1367
PA 4              GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1367
LMG 20106         GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1365
BD 640            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1365
BD 588            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1367
LMG 20103         GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1365
LMG 2665 T        GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1345
BD 561            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1367
BD 622            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1365
BD 577            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTgATC.GTGGATCAGAATGCCACGGTGA   1365
BD 336            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1347
LMG 2565          GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1345
LMG 2660          GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1323
BD 287            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1369
LMG 1286 T        GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1330
BD 639            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1285
BD 502            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1285
BD 500            GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1286
BCC 105 T         GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1282
LMG 2715 T        GCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1324
LMG 2632 T        GCAACTCnACTCCGTGAAGTCGGAATCGCTAGTAATC.GTGGATCnnAATGCCACnGTGA   1368
LMG 22050 T       GCAACTCGACTCCaTGAAGTCGGAATCGCTAGTAATC.GTaGATCAGAATGCtACGGTGA   1347
LMG 22051 T       GCAACTCGACTCCaTGAAGTCGGAATCGCTAGTAATC.GTaGATCAGAATGCtACGGTGA   1347
LMG 22049 T       GCAACTCGACTCCaTGAAGTCGGAATCGCTAGTAATC.GTaGATCAGAATGCtACGGTGA   1347
LMG 2603 T        GCAACTCGACTCCaTGAAGTCGGAATCGCTAGTAATC.GTaGATCAGAATGCtACGGTGA   1268
ATCC 13047 T      GCAACTCGACTCCaTGAAGTCGGAATCGCTAGTAATC.GTaGATCAGAATGCtACGGTGA   1343
DSM 30104 T       GCAACTCGACTCCaTGAAGTCGGAATCGCTAGTAATC.GTaGATCAGAATGCtACGGTGA   1366
ATCC 11775 T      GCAACTCGACTCCaTGAAGTCGGAATCGCTAGTAATC.GTGGATCAGAATGCCACGGTGA   1364
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BD 309          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1357
BD 390          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1426
BD 377          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1425
BD 602          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1425
BD 647          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1425
LMG 2676        ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1383
BD 442          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1365
BD 435          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1425
BD 310          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1430
BD 301          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1427
BD 315          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1427
PA 4            ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1427
LMG 20106       ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1425
BD 640          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1425
BD 588          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1427
LMG 20103       ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1425
LMG 2665 T      ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1405
BD 561          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1427
BD 622          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1425
BD 577          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1425
BD 336          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1407
LMG 2565        ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAnAA   1405
LMG 2660        ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1383
BD 287          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1429
LMG 1286 T      ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1390
BD 639          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1345
BD 502          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1345
BD 500          ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1346
BCC 105 T       ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGG.................   1325
LMG 2715 T      ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1384
LMG 2632 T      ATnCGTTCCCGGGCCTTGTACACACnGCCCGTCACnCCATGGGAGTGGGTTGCAnAAGAA   1428
LMG 22050 T     ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1407
LMG 22051 T     ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1407
LMG 22049 T     ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1407
LMG 2603 T      ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1328
ATCC 13047 T    ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1403
DSM 30104 T     ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1426
ATCC 11775 T    ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA   1424
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BD 309          GTAGGTAGCTTAACCTCCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1417
BD 390          GTAGGTAGCTTAACCTCCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1486
BD 377          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1485
BD 602          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1485
BD 647          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1485
LMG 2676        GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1443
BD 442          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATcCATGACTGGGGTGAAG   1425
BD 435          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATcCATGACTGGGGTGAAG   1485
BD 310          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1490
BD 301          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1487
BD 315          GTAGGTAGCTTAACCTtCGGGgGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1487
PA 4            GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1487
LMG 20106       GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1485
BD 640          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTcGTGATTCATGACTGGGGTGAAG   1485
BD 588          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1487
LMG 20103       GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1485
LMG 2665 T      GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1465
BD 561          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1487
BD 622          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1485
BD 577          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1485
BD 336          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1467
LMG 2565        GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCAC.........................   1440
LMG 2660        GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1443
BD 287          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1489
LMG 1286 T      GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1450
BD 639          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1405
BD 502          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1405
BD 500          GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGG.....   1401
BCC 105 T       ............................................................   1325
LMG 2715 T      GTAGGTAGCTTAACCctCGGGAGGGCGCTTACtACTTTGTGATTCATGACTGGGGTGAAG   1444
LMG 2632 T      GTnnGTAGCTTAACCaCCGGGAGGGCGCTTACCAnTTTGTGATTCnnGACTGGGGTnAAG   1488
LMG 22050 T     GTAGaTAGCTTAACCTtCGGGAGGGCGtTTACCACTTTGTGATTCATGACTGGGGTGAAG   1467
LMG 22051 T     GTAGaTAGCTTAACCTtCGGGAGGGCGtTTACCACTTTGTGATTCATGACTGGGGTGAAG   1467
LMG 22049 T     GTAGaTAGCTTAACCTtCGGGAGGGCGtTTACCACTTTGTGATTCATGACTGGGGTGAAG   1467
LMG 2603 T      GTAGGTAGCTTAACCTtCG..........................................   1347
ATCC 13047 T    GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1463
DSM 30104 T     GTAGGTAGCTTAACCTtCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAG   1486
ATCC 11775 T    GTAGGTAGCTTAAC.TtCGGGAGGGCG.................................   1450
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BD 309          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1464
BD 390          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1533
BD 377          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1532
BD 602          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1532
BD 647          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1532
LMG 2676        TCGTAACAAGGT................................        1455
BD 442          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1472
BD 435          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1532
BD 310          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1537
BD 301          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1534
BD 315          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTTa    1535
PA 4            TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1534
LMG 20106       TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1532
BD 640          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1532
BD 588          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1534
LMG 20103       TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1532
LMG 2665 T      TCGTAACAAGGTAACCGcAGGGGAACCTGC.................    1495
BD 561          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1534
BD 622          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1532
BD 577          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1532
BD 336          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1514
LMG 2565        ..............................................      1440
LMG 2660        TCGTAACAAGGT................................        1455
BD 287          TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTT.    1536
LMG 1286 T      TCGTAACAAGGTAACCGTAGGGG.....................        1473
BD 639          TCGTAACAAGGTAACCGTAGGGGAACC.................        1432
BD 502          TCGT........................................        1409
BD 500          ..............................................      1401
BCC 105 T       ..............................................      1325
LMG 2715 T      TCGTAACAAGGT................................        1456
LMG 2632 T      TCGTnnCAAGG.................................        1499
LMG 22050 T     TCGTAACAAGGTAACCGTAGGGGAACC.................        1494
LMG 22051 T     TCGTAACAAGGTAACCGTAGGGGAACC.................        1494
LMG 22049 T     TCGTAACAAGGTAACCGTAGGGGAACC.................        1494
LMG 2603 T      ..............................................      1347
ATCC 13047 T    TCGTAACAAGGTAACCGTAGGGGAACCTGCGGCTGGATCACCTCCTTg    1511
DSM 30104 T     TCGTAACAAGGTAACCGTAGGGGAACCTGCGGtTGGATCACCTCCTTt    1534
ATCC 11775 T    ..............................................      1450
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