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# To split or not to split: an opinion on dividing the genus *Burkholderia*

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**Abstract** The genus *Burkholderia* is a large group of species of bacteria that inhabit a wide range of environments. We previously recommended, based on multilocus sequence analysis, that the genus be separated into two distinct groups—one that consists predominantly of human, plant, and animal pathogens, including several opportunistic pathogens, and a second, much larger group of species comprising plant-associated beneficial and environmental species that are primarily known not to be pathogenic. This second group of species is found mainly in soils, frequently in association with plants as plant growth-promoting bacteria. They also possess genetic traits that bestow them with an added potential for agriculture and soil restoration, such as nitrogen fixation, phosphate solubilization, iron sequestration, and xenobiotic degradation, and they are not pathogenic. In this review, we present an update of current information on this second group of *Burkholderia* species, with the goal of focusing attention on their use in agriculture and environmental remediation. We describe their distribution in the environment, their taxonomy and genetic

features, and their relationship with plants as either associative nitrogen-fixers or legume-nodulating/nitrogen-fixing bacteria. We also propose that a concerted and coordinated effort be made by researchers on *Burkholderia* to determine if a definitive taxonomic split of this very large genus is justified, especially now as we describe here for the first time intermediate groups based upon their 16S rRNA sequences. We need to learn more about the plant-associated *Burkholderia* strains regarding their potential for pathogenicity, especially in those strains intermediate between the two groups, and to discover whether gene exchange occurs between the symbiotic and pathogenic *Burkholderia* species. The latter studies will require both field and laboratory analyses of gene loss and gain.

**Keywords** *Burkholderia* · Nitrogen fixation · Nodulation · Bioremediation · Plant growth-promoting bacteria

## Introduction

*Burkholderia* is a bacterial genus that contains a large and ever increasing number of species, with the current count being around 100. It belongs to the class  $\beta$ -proteobacteria, within the family *Burkholderiaceae*, along with *Cupriavidus*, *Lautropia*, *Limnobacter*, *Pandoraea*, *Paucimonas*, *Polynucleobacter*, *Ralstonia*, and *Thermotrix*. The *B. cepacia* complex (Bcc), which consists of 20 species [Electronic Supplementary Material (ESM) Table 1], has been the major focus of most of the research on *Burkholderia* and is probably the best known group within this genus. The Bcc is found in soil, the rhizosphere, and clinical environments, but it is the ability of the members of this group to act as opportunistic pathogens, especially in cystic fibrosis patients, which has resulted in the Bcc being not only well studied, but also a source of major concern (Mahenthiralingam et al. 2008). The other medically

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important cluster is the *B. pseudomallei* group, which consists of four species: *B. pseudomallei*, *B. mallei*, *B. thailandensis*, and *B. oklahomensis*. Of these, *B. pseudomallei* is the causative agent of melioidosis (Cheng and Currie 2005), and *B. mallei* causes glanders, a disease of equines (Nierman et al. 2004). Other *Burkholderia* species are plant pathogens and responsible for diseases such as wilts, rots, blights, or cankers. Many of the phytopathogenic species were originally classified as *Pseudomonas* (e.g., *P. andropogonis*, *P. gladioli*, *P. cepacia*, *P. glumae*, and *P. plantarii*), but following a polyphasic taxonomic investigation, they were transferred to the genus *Burkholderia* (Yabuuchi et al. 1992; Urakami et al. 1994; Coenye et al. 2001).

The purpose of this review is not to discuss plant and mammalian pathogens or the opportunistic pathogens, as numerous reviews have been written about the Bcc, the *B. pseudomallei* cluster, and the plant pathogenic *Burkholderia* species (Sprague and Neubauer 2004; Coenye and Vandamme 2007; Gonzalez et al. 2007). In a recent study, we demonstrated that *Burkholderia* could be split into two phylogenetic groups, indicating that this genus consists of distinct taxonomic lineages (Estrada-de los Santos et al. 2013). Gyaneshwar et al. (2011) proposed that the plant-beneficial–environmental (PBE) group be collectively categorized as the genus *Caballeronia*, and Sawana et al. (2014) recently described this same group as *Paraburkholderia*, but these authors did not adhere to the criteria required for a valid description of a new genus (see section *Burkholderia* taxonomy update).

In this review, we summarize the latest information about the plant-associated beneficial and environmental bacterial species, i.e., the PBE cluster (Suarez-Moreno et al. 2012), which are not allied to the pathogenic clade. We provide an overview of their taxonomy, distribution in the environment, and interaction with plants as nitrogen (N)-fixing and/or legume-nodulating bacteria and also discuss the various traits that these bacteria use to promote plant growth. Special emphasis will be placed on the PBE *Burkholderia* species that possess these traits and their potential use in agriculture.

### ***Burkholderia* taxonomy update**

Over the last 20 years, information on the genus *Burkholderia* has expanded. It began with the description of *Burkholderia* gen. nov. in 1992 and the transfer of seven species (*B. cepacia*, *B. caryophylli*, *B. gladioli*, *B. mallei*, *B. pseudomallei*, *B. pickettii*, and *B. solanacearum*) from the genus *Pseudomonas* homology rRNA group II into the new genus *Burkholderia* (Yabuuchi et al. 1992). Since then and particularly over the last decade, a steadily increasing number of new species have been described. There are currently 99 named *Burkholderia* species, although not all are validated in the International Journal of Systematic and Evolutionary Microbiology

(IJSEM), which is the official journal of record of bacterial names of the International Committee on Systematics of Prokaryotes of the International Union of Microbiological Societies (ESM Table 1). The discovery of so many different *Burkholderia* species is in part due to a reclassification of a number of already known species (*B. phenazinium*, *B. pyrrocinia*, *B. glathei*, among others) (Viillard et al. 1998), but mostly due to the exploration of new environments and subsequent discovery of new species (*B. tropica*, *B. unamae*, *B. caballeronis*, *B. aspalathi*, *B. dipogonis* and *B. cordobensis*, and many more) (ESM Table 1).

In our first publication on *Burkholderia* in 2001 (Estrada-de los Santos et al. 2001) and in subsequent publications (Caballero-Mellado et al. 2004; Reis et al. 2004; Perin et al. 2006; Suarez-Moreno et al. 2012), we have noted that the genus can be classified into two large groups, namely, Group A and Group B, based on 16S rRNA gene sequencing data. A similar outcome occurred using multilocus sequence analysis (MLSA) of 55 type strains and 26 reference *Burkholderia* strains. Group A consists of strains equivalent to the PBE cluster of Suarez-Moreno et al. (2012), whereas Group B is composed of plant, human, and animal pathogens, as well as opportunistic pathogens (Estrada-de los Santos et al. 2013). Subsequent to these studies, Sawana et al. (2014) performed a phylogenetic analysis of 21 conserved proteins and the 16S rRNA gene sequence from 45 *Burkholderia* species (26 species of *Burkholderia*, 18 strains of *Burkholderia* spp., and one Candidatus *Burkholderia*), with the results also providing evidence for the existence of two major clades that were basically the same as those described in Estrada-de los Santos et al. (2013). Sawana et al. (2014) split the species from Group A from *Burkholderia* to form a new genus, which was named *Paraburkholderia*. In yet another study, Zuleta et al. (2014) phylogenetically analyzed 545 housekeeping genes from 15 *Burkholderia* species, and their results also supported the existence of two distinct groups. We had earlier suggested the name *Caballeronia* as a new name for the genus encompassing the PBE *Burkholderia* species (Gyaneshwar et al. 2011), but proposing a new legitimate genus name that can be validated by the scientific community requires additional studies (see later sections).

For this review, we have updated the phylogenetic tree of *Burkholderia* taking into account one or more (up to 5) 16S rRNA gene sequences from each *Burkholderia* species to obtain even stronger support for the presence of two or more clades. Using a single gene, such as the 16S rRNA gene, for taxonomic analyses is thought by some researchers to be disadvantageous and/or even produce irrelevant results (Yarza et al. 2014). Nevertheless, this gene is still the only widely used taxonomic marker for which sufficient information is available and which is commonly accessible in databases (Yarza et al. 2014).

We have therefore performed an updated phylogenetic analysis with maximum likelihood, similar to that previously described (Estrada-de los Santos et al. 2013). The new analysis shows that since our 2013 publication, Group A has expanded significantly and now includes many new *Burkholderia* species, several of which have only been described within the last few years (Fig. 1). By contrast, Group B has remained essentially the same in the context that the number of described species has hardly changed. In our analysis, *B. andropogonis* was found not to be closely related to any former or current group. Moreover, there are two Transition Groups (1 and 2) among the *Burkholderia* clusters (Fig. 1). We determined the intra-similarity for each group using MEGA v6 (Tamura et al. 2013) and found that for Group A, the similarity was 97.6 % and that for each of the other groups, it was >98.0 % (Table 1). In comparison, the overall inter-group similarity was found to be 95.9 % for Group A and Group B, 96.3 % for Group A and Transition Group 1, 96.2 % for Group A and Transition Group 2, and 95 % for Group A and *B. andropogonis* (Table 1). Interestingly, the inter-similarity among Group B, *B. andropogonis*, and the two Transition Groups was found to be >97 %. The 95.9 % inter-group similarity between Groups A and B is above the cut-off value of 95 % for establishing the separateness of two genera (Tindall et al. 2010). Yarza et al. (2014) recently analyzed the 16S rRNA gene sequences from both bacteria and archaea and proposed rational taxonomic boundaries for taxonomic ranks above the genus level. These authors reported that a sequence identity of  $\leq 94.5$  % between two 16S rRNA gene sequences provided strong evidence for distinct genera. However, they also mentioned that this threshold represents a minimum value and that when supported by other evidence, higher than threshold sequence identities can be considered. To paraphrase Yarza et al. (2014). "... the 94.5 % threshold for genera does not preclude the formation of genera that have sequence identities of 96 % if it is supported by other phenotypic, genetic, or environmental data". This is the case for the PBE Group A of *Burkholderia*, with 95.9 % 16S rRNA gene sequence identity to Group B. Moreover, most Group A species are symbionts or saprophytes and not pathogens or parasites. Group A bacteria also exhibit traits that are applicable to soil remediation and restoration. The relationship between Group A and the Transition Groups is >96.0 % (Table 1), and species in all of these clusters are found in soil, water, and/or rhizosphere or are associated with plants or fungi.

Although the analysis of 16S rRNA gene sequences is a very helpful approach when the aim is to describe bacteria, the resolving power of this technique may be limited, as seen for the Bcc (Vandamme and Dawyndt 2011). Newer alternatives have been developed for the description of novel microorganisms, such as whole genome sequence comparison (Tindall et al. 2010), but because the sequences of many PBE *Burkholderia* genomes are not available for comparative

analysis (ESM Table 2), we appeal to the international community working on this genus to start an initiative based on MLSA, which is a highly accepted approach for describing novel species in *Burkholderia* (Vandamme and Peeters 2014). Such an analysis will definitely improve our understanding of the phylogenetic relationship among *Burkholderia* species and will set the stage for splitting the genus.

Curiously, two *Burkholderia* species described by different authors in the same year and isolated from completely different locations have the same name, *B. humi*. One species (Rs7<sup>T</sup>), which is closely related to *B. tropica*, was isolated from peat soil in Russia (Srinivasan et al. 2013), whereas the second species (RA1-5<sup>T</sup>), which is closely related to *B. terrestris*, was isolated from rhizospheric soil in the Netherlands (Dalmastrri et al. 1999; Vandamme et al. 2013). The latter species was published in IJSEM and therefore, should keep the name *B. humi*. The *Burkholderia* described by Srinivasan et al. (2013) should be renamed to avoid confusion.

### Distribution of *Burkholderia* species in the environment

Members of the genus *Burkholderia* are found almost everywhere in the environment, but mainly as an important component of the soil microbial community (Dalmastrri et al. 1999). Suarez-Moreno et al. (2012) summarized all information available on the distribution of the *Burkholderia* species up to 2012. In the years following the publication of their review, many more *Burkholderia* species have been described. Our updated 2015 list of *Burkholderia* species, which is shown in ESM Table 1, contains a description of the environmental source of each isolated species. Few efforts have been made to study the ecology and distribution of these species, which is especially unfortunate considering that many of them had originally been described from only a single strain. Roselló-Mora and Amann (2001) point out that a single-strain species description (SSSD) ignores the diversity of strains within a species and, consequently, the resulting description is incomplete. A large number of *Burkholderia* species consist of SSSDs (e.g., *B. aspalathi*, *B. australis*, *B. dabaoshenensis*, *B. denitrificans*, *B. eburnea*, *B. endofungorum*, *B. ferrariae*, *B. ginsengisoli*, *B. grimmiae*, *B. humi* Rs7, *B. insulsa*, *B. jiangsuensis*, *B. kururiensis*, *B. megalochromosomata*, *B. monticola*, *B. oxyphila*, *B. phenoliruptrix*, *B. phymatum*, *B. rhizoxinica*, *B. rinojensis*, *B. sacchari*, *B. sediminicola*, *B. soli*, *B. terrae*, *B. terrestris*, *B. terricola*, *B. tuberum*, and *B. zhejiangensis*). Although some species consisting of two or more strains have been described (ESM Table 1), information about the ecology of these bacterial species is limited. Fortunately, recently multiple strains have been isolated for *B. phenoliruptrix*, *B. phymatum*, *B. kururiensis*, and *B. tuberum* species (Estrada-de los Santos et al. 2001; Chen et al. 2005a, b; Caballero-Mellado et al.

2007; Elliott et al. 2007a, b; Anandham et al. 2009; Bontemps et al. 2010; Liu et al. 2012; Mishra et al. 2012; Beukes et al. 2013; Gehlot et al. 2013; Zuleta et al. 2014), and additional information should be forthcoming.

The ability of the genus *Burkholderia* to thrive in totally different environments is remarkable and comparable to that of other versatile genera, such as *Pseudomonas* and the enterobacterial group. Two possible explanations of this diversity are (1) the likelihood of horizontal gene transfer among *Burkholderia* (Blaha et al. 2006) and (2) the prevalence of insertion sequences in *Burkholderia* genomes that modulate gene expression (Lessie et al. 1996; Miché et al. 2001), although the latter feature has been analyzed almost exclusively in the pathogenic *Burkholderia* species. For example, in the *B. pseudomallei* group, the results of a subtractive hybridization analysis indicate that genomic islands are key determinants of genome plasticity in *B. pseudomallei* and *B. thailandensis* (Brown and Beacham 2000). In preliminary analyses we have noted that numerous insertion sequences are also present in the genomes of the symbiotic species (manuscript in preparation).

### Nitrogen fixation and legume nodulation in *Burkholderia* species

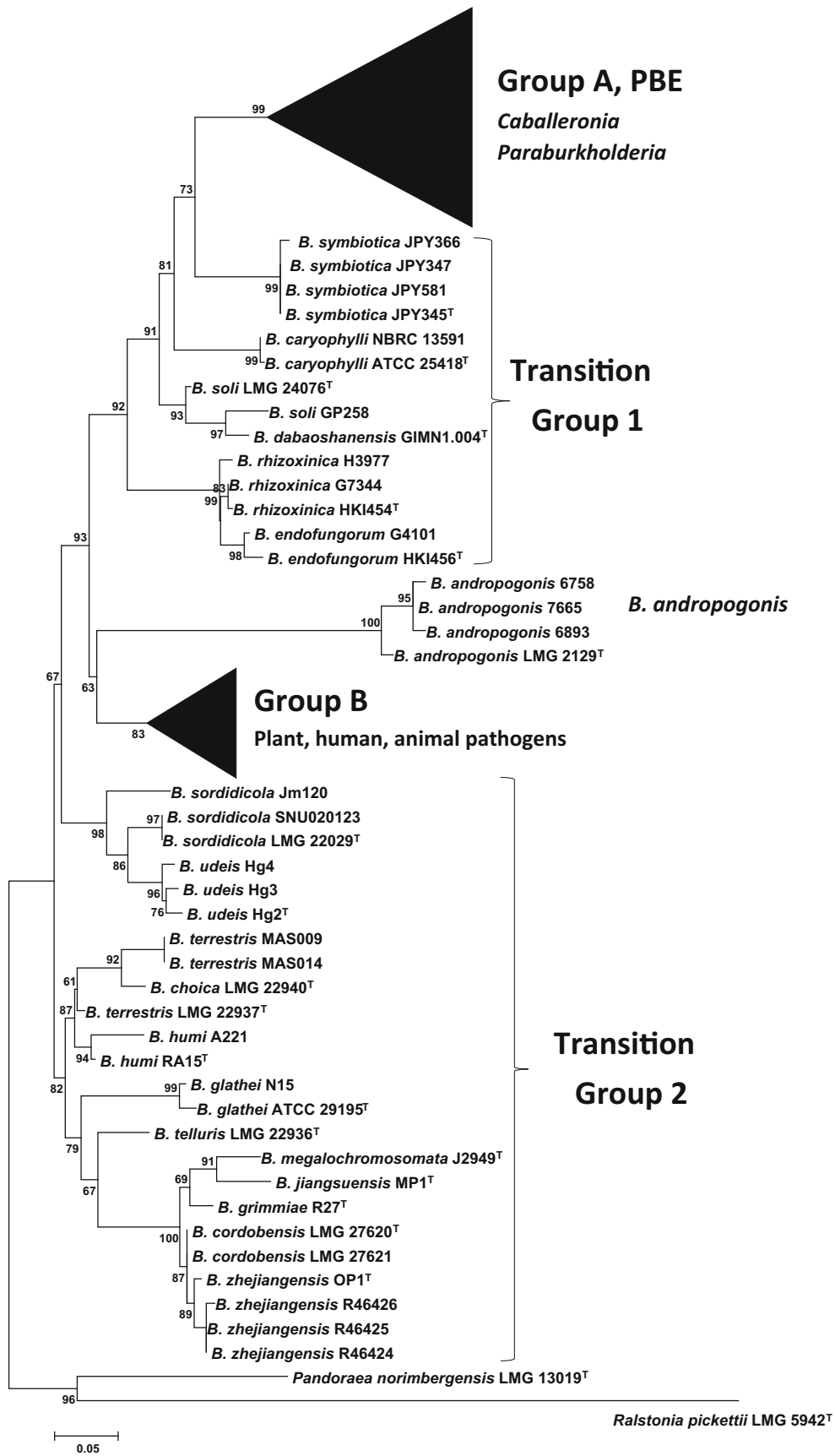
The first evidence of dinitrogen ( $N_2$ ) fixation by a member of genus *Burkholderia* was found in *B. vietnamiensis*, a species isolated from the *Oryza sativa* L. soil rhizosphere in Vietnam (Gillis et al. 1995). However, some time passed before it was realized that the genus *Burkholderia* is actually rich in diazotrophic species (Estrada-de los Santos et al. 2001). *Burkholderia* species that have been shown to be free-living  $N_2$ -fixers are *B. caballeronis*, *B. caryophylli*, *B. contaminans*, *B. ferrariae*, *B. fungorum*, *B. heleia*, *B. kururiensis*, *B. lata*, *B. nodosa*, *B. phymatum*, *B. silvatlantica*, *B. terrae*, *B. tuberum*, *B. tropica*, *B. unamae*, *B. xenovorans*, and *B. vietnamiensis* (ESM Table 1). Other putative  $N$ -fixing *Burkholderia* species (*B. australis*, *B. acidipaludis*, and *B. bannensis*) have been reported, but actual diazotrophy has not been authenticated. Nitrogen fixation in *Burkholderia* has not been solely limited to newly described new species, but some previously described species have also been shown to fix nitrogen, such as *B. caryophylli* (Glagoleva et al. 1996), *B. kururiensis* (Estrada-de los Santos et al. 2001), and *B. ferrariae* (Martínez-Aguilar et al. 2008; Estrada-de los Santos et al. 2013). The presence of *nifH*, the first structural gene encoding the enzyme nitrogenase, has been detected and sequenced from *B. caryophylli* (Chen et al. 2003; Martínez-Aguilar et al. 2008) and the ability to fix nitrogen, as determined by acetylene reduction activity, was reported earlier for this species than it was for *B. vietnamiensis*, which was subsequently named *Pseudomonas caryophylli* (Postgate 1982; Haahtela et al. 1983). The NCBI database contains a partial

**Fig. 1** Phylogenetic relationships among *Burkholderia* species based on 16S rRNA gene sequences, determined using maximum likelihood analysis. *Bar* Number of expected substitutions per site under the GTR + G model. The 16S rRNA gene sequences were taken from their original publication or from the Taxonomy Browser. *PBE* Plant-associated beneficial and environmental species. *Caballeronia* and *Paraburkholderia* were previously proposed to define *Burkholderia* group A as a new genus. A list of *Burkholderia* species from Group A and Group B is given in ESM Table 3

*nifH* sequence from *B. fungorum* strain S4 2R (Accession number AM110722), but this strain's true identity is unknown because a 16S rRNA sequence is not available and no other attempt to classify it has been pursued. Moreover, some strains of *B. xenovorans*, which fix nitrogen, were first identified as *B. fungorum* (NCBI Taxonomy Browser). Consequently, whether *B. fungorum* fixes nitrogen remains an enigma. Also, the NCBI database contains records of numerous *nifH* sequences in *Burkholderia*, but a sole sequence from the *nif* operon is not sufficient evidence to confirm  $N$ -fixing ability. At least nine *nif* gene sequences must be present for nitrogen fixation to occur in certain bacteria (Wang et al. 2013).

A remarkable discovery was that pertaining to the capacity of some  $\beta$ -proteobacteria species to nodulate legumes. Moulin et al. (2001) showed that two *Burkholderia* strains which had been apparently isolated from *Aspalathus* and *Machaerium* nodules (both sub-family Papilionoideae) were able to form nodules on the promiscuous legume siratro [*Macropodium atropurpureum* (DC) Urb], but the nodules were ineffective. These two strains were later described as *B. tuberum* and *B. phymatum* (Vandamme et al. 2002), and Elliott et al. (2007a, b) later demonstrated their ability to nodulate and fix nitrogen, but with legumes different to their originally described hosts: *Cyclopia* spp. and siratro in the case of *B. tuberum* STM678<sup>T</sup> and *Mimosa* spp. in the case of *B. phymatum* STM815<sup>T</sup>. Chen et al. (2005a, b) provided the first conclusive evidence for symbiotic nitrogen fixation and nodulation by *Burkholderia* using high-resolution microscopy and green fluorescent protein tagging of strains. A full description of the history of nodulation in beta-rhizobia is reported in Gyaneshwar et al. (2011) and Suarez-Moreno et al. (2012). However, new findings on nodulation by *Burkholderia* have emerged since then, and these are described in the following sections of this review.

Although most earlier studies focused on *Burkholderia* symbionts of *Mimosa* spp. either in their native (mainly) neotropical range or as pan-tropical invasives (Gyaneshwar et al. 2011), recently many more *Burkholderia* strains, potentially new species, have been isolated from nodules on papilionoid legumes from South Africa (Beukes et al. 2013). Most of these isolates were from *Cyclopia* and related genera in the tribes Podalyriaceae or Hypocalypteae; they induced nodules on cowpea and/or siratro, and had *nod* genes similar to those of *B. tuberum* STM678<sup>T</sup>, which has previously been shown to



**Table 1** Similarity percentage among *Burkholderia* groups based on the analysis of 16S rRNA gene sequence

Species	(1)	(2)	(3)	(4)	(5)	Inter-similarity
<i>Burkholderia</i> Group A (1)						97.6
<i>Burkholderia</i> Group B (2)	95.9					99.4
Transition Group 1 (3)	96.3	97.8				98.2
Transition Group 2 (4)	96.2	97.4	97.3			98.5
<i>B. andropogonis</i> (5)	95.0	97.4	96.6	97.0		99.7
Out group (6)	93.5	94.2	94.0	94.3	93.5	–

The groups are based on the phylogenetic tree reported in Fig. 1. The analysis was performed with MEGA v6 (Tamura et al. 2013)

nodulate *Cyclopia* spp. (Elliott et al. 2007a). Similar data were obtained with a wider range of legumes from the Cape Core subregion of South Africa by Lemaire et al. (2015). i.e., *Burkholderia* strains related to *B. phytofirmans*, *B. sprentiae*, *B. tuberum*, *B. rhynchosiae*, and some unnamed *Burkholderia* spp., but all possessed symbiosis genes related to *B. tuberum* STM678<sup>T</sup>. These were isolated from various species in the tribes Podalyriaceae, Indigoferaceae, Phaseoleaceae and Crotalariae, including species of *Bolusafraga*, *Crotalaria*, *Indigofera*, *Podalyria*, *Rafnia*, *Virgilia*, *Amphithalea*, and *Aspalathus*. Recently, based on Beukes et al. (2013), a novel *Burkholderia* species was identified as *B. kirstenboschensis* (Steenkamp et al. 2015), and at least three more species are being characterized as new species (S. Venter, personal communication). These, together with *B. dilworthii*, *B. rhynchosiae*, and *B. sprentiae*, and probably also *B. dipogonis* (Liu et al. 2014; Sheu et al. 2015). illustrate the plethora of recent new descriptions of *Burkholderia* species that nodulate South African native legumes in their native range (and/or as introduced plants in Australasia) and confirm the Fynbos biome/Cape Core subregion as a major center of diversity for beta-rhizobia (Gyaneshwar et al. 2011; Howieson et al. 2013).

With regard to the other major center of beta-rhizobial diversity, South America, and its enormous variety of *Mimosa* species that are nodulated by *Burkholderia* (Gyaneshwar et al. 2011), a recent study on related legume genera in the same group as *Mimosa*, i.e., the Piptadenia group in tribe Mimosaceae (sub-family Mimosoideae), showed that these were mainly nodulated by nine different *Burkholderia* species, of which three are likely to be new species and one that was identified as *B. phenoliruptrix* (Bournaud et al. 2013). *Burkholderia phenoliruptrix* was previously found to be a symbiont of *Mimosa flocculosa* Bukart (Chen et al. 2005a; Cunha et al. 2012). Therefore, it appears that in South America nodulation by *Burkholderia* is mainly confined to *Mimosa* spp. and related neotropical genera in the sub-family Mimosoideae, whereas the South African burkholderias only nodulate legumes in the sub-family Papilionoideae. However, although these two geographically distant groups of symbiotic burkholderias are

distinct in terms of the member species of their respective host range (owing to their very different plasmid-borne *nod* genes), there are exceptions; for example, *B. phymatum* and *B. tuberum* can both nodulate *siratro* and common bean (*Phaseolus vulgaris* L.) (Elliott et al. 2007a; Gyaneshwar et al. 2011; Angus et al. 2013). Moreover, *B. tuberum* is also an exception to the apparent geographical segregation of the symbiotic species, as it is present as a symbiotic nodulator of native and/or endemic legumes on both continents—i.e., it nodulates *Cyclopia* and other papilionoid legumes in South Africa and is a major component of the *Mimosa*-nodulating population in South America (Bontemps et al. 2010; Mishra et al. 2012). Consequently, *B. tuberum* has been proposed to have two biovars in terms of host range, geographical distribution, and *nod* gene phylogeny: biovar mimosae (*Mimosa* symbionts) and biovar papilionoideae (*Cyclopia* and other papilionoideae symbionts) (Mishra et al. 2012).

Mexico represents an interesting case in terms of symbiotic burkholderias, as a recent survey of the symbionts of the highly diverse *Mimosa* spp. native to Mexico (and which are taxonomically distant from their Brazilian cousins), showed that the native and endemic species from some central states were not nodulated by *Burkholderia* but by *Rhizobium* and *Ensifer* (Bontemps et al. 2016). The authors of this study attributed this difference to the separate evolution of these two groups of *Mimosa* spp. for >30 million years in very different soils, i.e., the Brazilian spp. are highly endemic to very acidic soils which support a diverse population of acid-tolerant burkholderias, whereas the Mexican spp. are endemic to mainly neutral-alkaline soils which support a wider range of potential symbionts. The exceptions to the apparent absence of *Burkholderia* symbionts in Mexican *Mimosa* spp. are a closely related group of *B. tuberum*-like burkholderias that were isolated from the widespread neotropical species, *Mimosa somnians* and *M. skinneri*, in Jalisco. These were genetically almost identical to a strain (CCGE1002) which was isolated from nodules on *M. occidentalis* in the adjacent state of Nayarit, and which according to EzTaxon was also identified as *B. tuberum* (98.7 %) (Fig. 1) (Bontemps et al. 2016). We believe that more work needs to be done regarding the isolation of *Burkholderia* from legume nodules in Mexico. For example, we have described *B. caballeronis*, which was isolated from the tomato rhizosphere, and surprisingly it nodulates *Phaseolus vulgaris* L. (Martinez-Aguilar et al. 2013). Nodulating bacteria are normally isolated from legume nodules or rhizospheres. We are currently assessing *B. caballeronis* on different legume species, including *Mimosa* spp., to determine its host range.

The symbiotic N-fixing *Burkholderia* described to date are: *B. caballeronis*, *B. caribensis*, *B. diazotrophica*, *B. dilworthii*, *B. dipogonis*, *B. kirstenboschensis*, *B. mimosarum*, *B. nodosa*, *B. phenoliruptrix*, *B. phymatum*, *B. rhynchosiae*, *B. sabiae*, *B. sprentiae*, *B. symbiotica*, and *B. tuberum* (see references in



ESM Table 1). Ferreira et al. (2012) reported the isolation of three *B. fungorum* strains from nodules of *Macroptilium atropurpureum* (DC.) Urb that nodulate common beans but which lack the ability to fix nitrogen. All nodulating *Burkholderia* species are located in the PBE group, with the exception of the symbiont of the Brazilian endemic legume *Mimosa cordistipula*, namely, *B. symbiotica* (Sheu et al. 2012), which sits in the Transition Group 1 along with *B. endofungorum*, *B. rhizoxinica*, and *B. caryophylli*, among others, which were previously placed in the Group B (Fig. 1). This is not surprising because *Burkholderia* is a genus with a continuously growing number of species, and it is just a matter of time until more nodulating species are discovered outside the PBE Group A.

### ***Burkholderia* Group A species: virulent or not?**

That the N-fixing species *B. vietnamiensis*, a member of the Bcc, and other Group B *Burkholderia* species are either dedicated or opportunistic pathogens has led to concerns about their use in agriculture. The biotechnological use of Bcc species was restricted in 2003 by the U.S. Environmental Protection Agency (EPA). Nevertheless, the transmissibility and clinical impacts of the Bcc differ widely from one species to another, thus opening the door to discussions on the existing restriction measures. Indeed, suggestions have been put forth that the ban should be lifted on some strains belonging to distinct Bcc species (Chiarini et al. 2006; Li et al. 2013).

As mentioned earlier, the PBE clade defined by Suarez-Moreno et al. (2012) falls into a clade separate from the pathogenic species. When PCR-amplified *cblA* and *esmR* transmissibility-factor encoding-genes from *B. cenocepacia* strain J2315<sup>T</sup> were used to probe plant-associated diazotrophic (Perin et al. 2006) or non-diazotrophic *Burkholderia* species in the PBE cluster (Castro-González et al. 2011), the results of both PCR and Southern hybridization studies were negative (Perin et al. 2006). Similarly, Angus et al. (2014) analyzed the genomes of several *Burkholderia* species using functional and genomic methods to determine whether virulence determinants could be found in Group A species. Their genomics analysis showed that many of the Group A strains lack the Type 3 secretion system, especially T3SS-3, which is responsible for *B. pseudomallei* virulence in mammalian hosts. Although some Group A strains have a T3SS, such as *B. phytofirmans* and *B. phenoliruptrix* Br3459, these secretion systems lack the genes that are required for cell invasion in *B. pseudomallei* BsaN (Chen et al. 2014). Many of the Group A strains also lack a canonical Type 4 secretion system. In addition to the genomic data, several of the Group A strains were tested on *Caenorhabditis elegans* and on HeLa cells; in both systems, the Group A strains tested did not cause mortality or lysis as did treatment with *Pseudomonas aeruginosa* (A.A. Angus and A.M. Hirsch, unpublished data) or

*Burkholderia thailandensis* E264 (Angus et al. 2014). This same study reported that the tested Group A strains also demonstrated greater susceptibility to commonly used antibiotics than did the pathogenic strains tested, which included *B. thailandensis* E264, *B. vietnamiensis* G4, and *B. gladioli* BSR3.

### **Potential use of *Burkholderia* in agro-biotechnology**

The Bcc has been used to control plant pests, promote plant growth, produce important industrial compounds, and degrade toxic molecules (Jaeger et al. 1999; Van et al. 2000; Hussain et al. 2007; Li et al. 2013). However, due to their opportunistic pathogenic behavior and the spread of Bcc into diverse environments, many of which function as a natural reservoir, these bacteria have been banned for agricultural use in the USA. Nonetheless, the beneficial behavior of *Burkholderia* is not limited to just the Bcc. Indeed, many species from the PBE group have interesting features with potential applications in agro-biotechnology.

### **Bioremediation**

Modern industrial activity has led to an accumulation of artificially synthesized pollutants, many of which damage the environment. Alternatives considered for soil remediation/decontamination are involve both plants (phytoremediation) and microorganisms (rhizoremediation), and which taken together is referred to as bioremediation. *Burkholderia* has a potential role in rhizoremediation because several species metabolize toxic compounds. For example, different strains of the plant-associated diazotroph *B. unamae* use phenol and benzene as their sole carbon sources (Caballero-Mellado et al. 2007). Also, *B. kururiensis*, a trichloroethylene-metabolizing, 2,4,6-trichlorophenol degrader, and a plant-associated, N-fixing species, breaks down phenol, benzene, and toluene (Zhang et al. 2000; Caballero-Mellado et al. 2007; Gómez-De Jesús et al. 2009). A *B. tropica* strain isolated from the Santa Alejandrina marsh in Veracruz, Mexico, degrades benzene, toluene, and xylene (De Los Cobos-Vasconcelos et al. 2006). In addition, *B. xenovorans* strain LB400<sup>T</sup> is one of the most potent aerobic polychlorobiphenyl (PCB)-degrading microorganisms studied to date (Seeger et al. 1999). This species was tested for PCB degradation in the rhizosphere of *Panicum virgatum* L. and was found to be responsible for the removal of 47.3 % of the PCB pollutants present (Liang et al. 2014). Recently, *Burkholderia* sp. VUN10013 was found to be able to degrade phenanthrene and anthracene, the latter being elevated in acidic soils (Somtrakoon et al. 2008a, b). Interestingly, when the 16S rRNA sequence (AF068011) from strain VUN10013 was compared to sequences in the NCBI database, the best hit was *B. phytofirmans* with 99 % similarity. *Burkholderia phytofirmans* PsJN<sup>T</sup> is a plant growth-promoting bacterium with high aminocyclopropane-1-carboxylate (ACC)-

deaminase (AcdS) activity (Sessitsch et al. 2005). It also has the capacity to degrade thiocyanate, a common contaminant in effluents from gold mine tailings (Vu et al. 2013). Another environmental *Burkholderia* species involved in pollutant degradation is *B. sartisoli*. This species was isolated from a polycyclic aromatic hydrocarbon-contaminated soil in New Zealand (Vanlaere et al. 2008) and grew on naphthalene and phenanthrene (Laurie and Lloyd-Jones 1999). *Burkholderia phenoliruptrix* type strain AC1100 was isolated after successive plating from a chemostat inoculated with waste contaminated with 2,4,5-trichloroethylene acid (2,4,5-T), a potent herbicide (Kellogg et al. 1981; Coenye et al. 2004). This bacterium also degrades 2,3,4,6-tetrachlorophenol and pentachlorophenol (Karns et al. 1983). Strain AC1100<sup>T</sup> can remove >99 % of 2,4,5-T present at 1 mg g<sup>-1</sup> of soil within 1 week and >90 % from a heavily contaminated soil containing 20 mg g<sup>-1</sup> of soil within 6 weeks (Kilbane et al. 1983). Moreover, the elimination of 2,4,5-T by strain AC1100<sup>T</sup> supports the growth of plants inoculated by this strain in soil containing low concentrations of this contaminant. The same effect was observed on the germination and seedling vigor of *Solanum lycopersicum* L. grown in soil contaminated with 2,4,5-T after inoculation with *B. phenoliruptrix* AC1100<sup>T</sup> (Gangadhara and Kunhi 2000). *Burkholderia terricola* and *B. hospita* were isolated as transconjugants that acquired the catabolic plasmids pJP4 or pEMT1, both encoding enzymes for the degradation of 2,4-dichlorophenoxyacetic acid, in an agricultural soil inoculated with a *Pseudomonas putida* UWC3 donor strain harboring either one or the other plasmid.

Pérez-Pantoja et al. (2012) analyzed several *Burkholderia* genomes for aromatic compound biodegradation and reported that the *Burkholderia* species studied contained the pathways for protocatechuate *ortho* ring-cleavage, catechol *ortho* ring-cleavage, homogentisate ring-cleavage, and phenylacetyl-CoA ring-cleavage. Many of these species belong to phylogenetic Group A. A number of *Burkholderia* strains are involved in biodegradation processes (Mueller et al. 1997; Coenye and Vandamme 2003), but many have not been assigned to any new or already described *Burkholderia* species. In summary, the ability of the PBE *Burkholderia* species to degrade toxic compounds is either more common than originally thought, or it has just simply been overlooked until now.

### Plant growth promotion abilities

Many *Burkholderia* species are known for their ability to promote plant growth. The mechanisms involved in plant promotion include indole acetic acid (IAA) production, siderophore synthesis, nitrogen fixation, phosphate solubilization, ACC-deaminase activity, and induction of systemic resistance, among others (see ESM Table 1 for more examples and references). AcdS degrades ACC, the ethylene precursor, which is an inhibitor of plant growth. AcdS is found in a diversity of

*Burkholderia* strains from both phylogenetic groups (Castro-González et al. 2011).

A number of *Burkholderia* species have been reported to produce IAA, as measured by the Salkowski test, with or without the addition of tryptophan (Castro-González et al. 2011; de Oliveira-Langatti et al. 2014; Naveed et al. 2014). One publication describes the identification of IAA not only by the Salkowski test but also by chromatography (Singh et al. 2013), although it should be noted that the species in this particular study was *B. cepacia* RRE25. Regardless, the exact mechanism(s) used by the Group A *Burkholderia* for IAA production has not been elucidated, and whether or not any other phytohormones are produced by the beta-rhizobial strains has not to our knowledge been reported as yet.

In summary, although the plant-promoting activity exhibited by *Burkholderia* is promising, the presence of human pathogens and opportunistic pathogens in this genus together with some very effective plant growth-promoting rhizobacteria, such as *B. vietnamiensis* (Van et al. 2000), has so far limited its application in agriculture.

### Concluding remarks

The number of species within the genus *Burkholderia* is steadily increasing, with many species having been described within the last 10 years and with numerous attempts having been made to consolidate the various species into different phylogenetic groups and ultimately to describe new genera. Although the description of a new taxonomic lineage must be thoroughly comprehensive, there is a lingering reluctance to split the genus *Burkholderia*. However, the current approach of basing the separation of a genus on phenotypic features, especially when the phenotypic traits are highly inconsistent, which is always the case whenever large populations are studied (see Xu et al. 1995; Yao et al. 2002; Vinuesa et al. 2005), is problematic. Ackermann (2015) mentioned that the expectation has been that all individuals in a clonal population will express the same phenotype. However, in some situations only a minority of individuals in a clonal population will express a given phenotype, while others will benefit from it without contributing. Therefore, phenotype cannot always be a conclusive factor in determining taxonomic lineage.

The potential use in agriculture of many *Burkholderia* species from the environmental, plant-associated, and non-pathogenic clade is definitely one reason, among others, why it is desirable to split the PBE cluster from the pathogen-containing Group B clade and describe it as a new genus with a less controversial name which does not contain the word “*Burkholderia*”, as does “*Paraburkholderia*”. Moreover, although many *Burkholderia* species, such as *B. graminis*, have been proposed as species which should be placed in genus *Paraburkholderia*, based on Fig. 1 and on

other unpublished data *B. graminis* is nested well within the PBE clade with other *Burkholderia* species that have also been properly validated. Although we believe strongly that the A group should be separated from the B group and renamed, we propose that *B. graminis* and all the other PBE clade members remain in the genus *Burkholderia* until more robust evidence is provided beyond what has been published to date. Therefore, our review is a plea for a concerted international effort to study the entire genus and determine whether MLSA or other strategies are better for separating *Burkholderia* into two or more genera. For example, experiments whereby symbiotic genes are transferred into Group B bacteria and virulence genes moved into Group A strains might address these concerns, but to our knowledge, such experiments have not been pursued. In our previous publication (Angus et al. 2014), we described the development of functional assays to test whether select PBE *Burkholderia* strains inhibited nematode worm and HeLa survival. Additional assays need to be developed to test the efficacy and safety of these PBE strains. Nevertheless, although many *Rhizobium* and closely related species are tarred with the opportunistic or serious pathogen (citrus greening disease) brush, the *Rhizobiaceae* are still widely used as inoculants. “The bottom line is that different clusters of genes and different G+C content of genomes correlate with either the symbiotic or parasitic lifestyle in the *Rhizobiaceae*” (Angus et al. 2014). The same is true for the *Burkholderiaceae*. A multi-faceted scientific effort that encompasses many disciplines and focuses on the PBE *Burkholderiaceae* is needed to understand fully the differences between the A and B groups.

We view as achievable the goal of using PGPR *Burkholderia* strains, particularly those from the PBE group, for bioremediation, biofertilizer production, and protection from plant pests, with the ultimate aims to eliminate our dependence on and use of chemical fertilizers, herbicides, and pesticides and to help us attain truly sustainable agriculture. We realize that the quest to separate the PBE *Burkholderia* from the Group B species cannot be performed by a few laboratories—it will “take a village”. Demonstrating how internationally relevant PBE *Burkholderia* species are is shown by the fact that South African forage legumes, which are nodulated by Group A *Burkholderia* (J. Howieson, personal communication), thrive in the acid, infertile, and arid soils of Western Australia and have already been planted in experimental plots in farmers’ fields. Farmers in Western Australia can no longer use *Rhizobium* inoculants and their hosts because the Mediterranean forage crops used for grazing are no longer productive due to the change in Western Australia’s climate. The time is right to direct research efforts towards the Group A *Burkholderia* so that they can be utilized for agriculture. Splitting the genus is the first stop towards achieving this goal.

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## References

- Ackermann M (2015) A functional perspective on phenotypic heterogeneity in microorganisms. *Nat Rev Microbiol* 13:497–508
- Anandham R, Gandhi PI, Kwon SW, Sa TM, Kim YK, Jee HJ (2009) Mixotrophic metabolism in *Burkholderia kururiensis* subsp. thiooxydans subsp. nov., a facultative chemolithoautotrophic thio-sulfate oxidizing bacterium isolated from rhizosphere soil and proposal for classification of the type strain of *Burkholderia kururiensis* as *Burkholderia kururiensis* subsp. *kururiensis* subsp. nov. *Arch Microbiol* 191:885–894
- Angus AA, Lee AS, Lum MR, Shehaye M, Hessabi R, Fujishige NA, Yerrapragada S, Kano S, Song N, Yang P, Estrada-de los Santos P, de Faria SM, Dakora FD, Weinstock G, Hirsch AM (2013) Nodulation and effective nitrogen fixation of *Macroptilium atropurpureum* (siratiro) by *Burkholderia tuberum*, a beta-proteobacterium, are influenced by environmental factors. *Plant Soil* 362:543–562
- Angus AA, Agapakis CM, Fong S, Yerrapragada S, Estrada-de los Santos P, Yang P, Song N, Kano S, Caballero-Mellado J, de Faria SM, Dakora FD, Weinstock G, Hirsch AM (2014) Plant-associated symbiotic *Burkholderia* species lack hallmark strategies required in mammalian pathogenesis. *PLoS ONE* 9:e83779
- Beukes CW, Venter SN, Law IJ, Phalane FL, Steenkamp ET (2013) South African papilionoid legumes are nodulated by diverse *Burkholderia* with unique nodulation and nitrogen-fixation loci. *PLoS ONE* 8: e68406
- Blaah D, Pringent-Combaret C, Mirza MS, Moëgne-Loccoz Y (2006) Phylogeny of the 1-aminocyclopropane-1-carboxylic acid deaminase-encoding gene *acdS* in phytobeneficial and pathogenic *Proteobacteria* and relation with strain biogeography. *FEMS Microbiol Ecol* 56:455–470
- Bontemps C, Elliott GN, Simon MF, Dos Reis Junior FB, Gross E, Lawton R, Neto NE, Loureiro MF, de Faria SM, Sprent JI, James EK, Young JPW (2010) *Burkholderia* species are ancient symbionts of legumes. *Mol Ecol* 19:44–52
- Bontemps C, Rogel MA, Wiechmann A, Mussebekova A, Moody S, Simon MF, Moulin L, Elliott GN, Lacercat-Didier L, Dasilva C, Grether R, Camargo-Ricalde SL, Chen W, Sprent JI, Martinez-Romero E, Young JPW, James EK (2016) Endemic *Mimosa* species from Mexico prefer alphaproteobacterial rhizobial symbionts. *New Phytol* 209(1):319–333. doi:10.1111/nph.13573
- Boumaud C, de Faria SM, Ferreira dos Santos JM, Tisseyre P, Silva M, Chaintreuil C, Gross E, James EK, Prin Y, Moulin L (2013) *Burkholderia* species are the most common and preferred nodulating symbionts of the Piptadenia group (Tribe Mimoseae). *PLoS ONE* 8: e63478
- Brown NF, Beacham IR (2000) Cloning and analysis of genomic differences unique to *Burkholderia pseudomallei* by comparison with *Burkholderia thailandensis*. *J Med Microbiol* 49:993–1001
- Caballero-Mellado J, Martínez-Aguilar L, Paredes-Valdez G, Estrada-de los Santos P (2004) *Burkholderia unamae* sp. nov., an N<sub>2</sub>-fixing rhizospheric and endophytic species. *Int J Syst Evol Microbiol* 54: 1165–1172

- Caballero-Mellado J, Onofre-Lemus J, Estrada-de los Santos P, Martínez-Aguilar L (2007) The tomato rhizosphere, an environment rich in nitrogen-fixing *Burkholderia* species with capabilities of interest for agriculture and bioremediation. *Appl Environ Microbiol* 73:5308–5319
- Castro-González R, Martínez-Aguilar L, Ramírez-Trujillo A, Estrada-de los Santos P, Caballero-Mellado J (2011) High diversity of culturable *Burkholderia* species associated with sugarcane. *Plant Soil* 345:155–169
- Chen W-M, Moulin L, Bontemps C, Vandamme P, Béna G, Boivin-Masson C (2003) Legume symbiotic nitrogen fixation by  $\beta$ -Proteobacteria is widespread in nature. *J Bacteriol* 185:7266–7272
- Chen WM, de Faria SM, Stralioetto R, Pitard RM, Simoes-Araujo JL, Chou JH, Barrios E, Prescott AR, Elliott GN, Sprent JI, Young JPW, James EK (2005a) Proof that *Burkholderia* strains form effective symbioses with legumes: a study of novel *Mimosa*-nodulating strains from South America. *Appl Environ Microbiol* 71:7461–7471
- Chen WM, James EU, Chou JH, Sheu SY, Yang SZ, Sprent JI (2005b)  $\beta$ -Rhizobia from *Mimosa pigra*, a newly discovered invasive plant in Taiwan. *New Phytol* 168:661–675
- Chen Y, Schröder I, French CT, Jaroszewicz A, Yee XJ, Teh BE, Toesca IJ, Miller JF, Gan YH (2014) Characterization and analysis of the *Burkholderia pseudomallei* BsaN virulence regulon. *BMC Microbiol* 14:206
- Cheng AC, Currie BJ (2005) Melioidosis: epidemiology, pathophysiology, and management. *Clin Microbiol Rev* 18:383–416
- Chiarini L, Bevivino A, Dalmastrì C, Tabacchioni S, Visca P (2006) *Burkholderia cepacia* complex species: health hazards and biotechnological potential. *Trends Microbiol* 14:277–286
- Coenye T, Vandamme P (2003) Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environ Microbiol* 5:719–729
- Coenye T, Vandamme P (2007) *Burkholderia*: molecular microbiology and genomics. Horizon Scientific Press, Norfolk
- Coenye T, Laevens S, Gillis M, Vandamme P (2001) Genotypic and chemotaxonomic evidence for the reclassification of *Pseudomonas woodsii* (Smith 1911) Stevens 1925 as *Burkholderia andropogonis* (Smith 1911) Gillis et al. 1995. *Int J Syst Evol Microbiol* 51:183–185
- Coenye T, Henry D, Speert DP, Vandamme P (2004) *Burkholderia phenoliruptrix* sp. nov., to accommodate the 2, 4, 5-trichlorophenoxyacetic acid and halophenol-degrading strain AC1100. *Syst Appl Microbiol* 27:623–627
- Cunha CO, Zuleta LFG, De Almeida LGP, Ciapina LP, Borges WL, Pitard RM, Baldani JI, Stralioetto R, De Faria SM, Hungria M, Cavada BS, Mercante FM, De Vasconcelos ATR (2012) Complete genome sequence of *Burkholderia phenoliruptrix* BR3459a (CLA1), a heat-tolerant, nitrogen-fixing symbiont of *Mimosa fluculosa*. *J Bacteriol* 194:6675–6676
- Dalmastrì C, Chiarini L, Cantale C, Bevivino A, Tabacchioni S (1999) Soil type and maize cultivar affect the genetic diversity of maize root-associated *Burkholderia cepacia* populations. *Microb Ecol* 38:273–284
- De Los Cobos-Vasconcelos D, Santoyo-Tepole F, Juárez-Ramírez C, Ruiz-Ordaz N, Galindez-Mayer C (2006) Cometabolic degradation of chlorophenols by a strain of *Burkholderia* in fed-batch culture. *Enzymol Microb Technol* 40:57–60
- de Oliveira-Langatti SM, Marra LM, Soares BL, Bomfeti CA, da Silva K, Ferreira PAA, de Souza Moreira FM (2014) Bacteria isolated from soils of the western Amazon and from rehabilitated bauxite-mining areas have potential as plant growth promoters. *World J Microbiol Biotechnol* 30:1239–1250
- Elliott GN, Chen W-M, Bontemps C, Chou J-H, Young JPW, Sprent JI, James EK (2007a) Nodulation of *Cyclopia* spp. (Leguminosae, Papilionoideae) by *Burkholderia tuberum*. *Ann Bot* 100:1403–1411
- Elliott GN, Chen WM, Chou JH, Wang HC, Sheu SY, Perin L, Reis VM, Moulin L, Simon MF, Bontemps C, Sutherland JM, Bessi R, de Faria SM, Trinick MJ, Prescott AR, Sprent JI, James EK (2007b) *Burkholderia phymatum* is a highly effective nitrogen-fixing symbiont of *Mimosa* spp. and fixes nitrogen *ex planta*. *New Phytol* 173:168–180
- Estrada-de los Santos P, Bustillos-Cristales RO, Caballero-Mellado J (2001) *Burkholderia*, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. *Appl Environ Microbiol* 67:2790–2798
- Estrada-de los Santos P, Vinuesa P, Martínez-Aguilar L, Hirsch AM, Caballero-Mellado J (2013) Phylogenetic analysis of *Burkholderia* species by multilocus sequence analysis. *Curr Microbiol* 67:51–60
- Ferreira PAA, Bomfeti CA, Soares BL, de Souza Moreira FM (2012) Efficient nitrogen-fixing *Rhizobium* strains isolated from Amazonian soils are highly tolerant to acidity and aluminium. *World J Microbiol Biotechnol* 28:1947–1959
- Gangadhara K, Kunhi A (2000) Protection of tomato seed germination from the inhibitory effect of 2,4,5-trichlorophenoxyacetic acid by inoculation of soil with *Burkholderia cepacia* AC1100. *J Agric Food Chem* 48:4314–4319
- Gehlot HS, Tak N, Kaushik M, Mitra S, Chen WM, Poseleit N, Panwar D, Poonar N, Parihar R, Tak A, Sankhla IS, Ojha A, Rao SR, Simon MF, dos Reis Junior FB, Perigolo N, Tripathi AK, Sprent JI, Young JPW, Gyaneshwar P (2013) An invasive *Mimosa* in India does not adopt the symbionts of its native relatives. *Ann Bot* 112:1–18
- Gillis M, Tran VV, Bardin R, Goor M, Hebbar P, Willems A, Segers P, Kersters K, Heulin T, Fenandez MP (1995) Polyphasic taxonomy in the genus *Burkholderia* leading to an emended description of the genus and proposition of *Burkholderia vietnamiensis* sp. nov. for  $N_2$ -fixing isolates from rice in Vietnam. *Int J Syst Bacteriol* 45:274–289
- Glagoleva O, Kovalskaya N, Umarov M (1996) Endosymbiosis formation between nitrogen-fixing bacteria *Pseudomonas caryophylli* and rape root cells. *Edocyt Cell Res* 11:147–158
- Gómez-De Jesús A, Romano-Baez F, Leyva-Amezcuca L, Juárez-Ramírez C, Ruiz-Ordaz N, Galindez-Mayer J (2009) Biodegradation of 2,4,6-trichlorophenol in a packed-bed biofilm reactor equipped with an internal net draft tube riser for aeration and liquid circulation. *J Hazard Mater* 161:1140–1149
- Gonzalez CF, Venturi V, Engledow AS (2007) The phytopathogenic *Burkholderia*. In: Coenye T, Mahenthalingam E (eds) *Molecular microbiology and genomics*. Caister Academic Press, Norfolk, pp 153–176
- Gyaneshwar P, Hirsch AM, Moulin L, Chen WM, Elliott GN, Bontemps C, Estrada-de los Santos P, Gross E, dos Reis FB, Sprent JI, Young JPW, James EK (2011) Legume-nodulating betaproteobacteria: diversity, host range, and future prospects. *Mol Plant Microb Int* 24:1276–1288
- Hahtela K, Helander I, Nurmiaho-Lassila EL, Sundman V (1983) Morphological and physiological characteristics and lipopolysaccharide composition of  $N_2$ -fixing ( $C_2H_2$ -reducing) root-associated *Pseudomonas* sp. *Can J Microbiol* 29:874–880
- Howieson JG, De Meyer SE, Vivas-Marfisi A, Ratnayake S, Ardley JK, Yates RJ (2013) Novel *Burkholderia* bacteria isolated from *Lebeckia ambigua*—a perennial suffrutescent legume of the fynbos. *Soil Biol Biochem* 60:55–64
- Hussain S, Arshad M, Saleem M, Khalid A (2007) Biodegradation of  $\alpha$ - and  $\beta$ -endosulfan by soil bacteria. *Biodegradation* 18:731–740
- Jaeger K, Dijkstra B, Reetz M (1999) Bacterial biocatalysts: molecular biology, three-dimensional structures, and biotechnological applications of lipases. *Annu Rev Microbiol* 53:315–351
- Karns J, Kilbane J, Dutttagupta S, Chakrabarty A (1983) Metabolism of halophenols by 2,4,5-trichlorophenoxyacetic acid-degrading *Pseudomonas cepacia*. *Appl Environ Microbiol* 46:1176–1181

- Kellogg S, Chatterjee DK, Chakrabarty AM (1981) Plasmid-assisted molecular breeding: new technique for enhanced biodegradation of persistent toxic chemicals. *Science* 214:1133–1135
- Kilbane J, Chatterjee D, Chakrabarty A (1983) Detoxification of 2,4,5-trichlorophenoxyacetic acid from contaminated soil by *Pseudomonas cepacia*. *Appl Environ Microbiol* 45:1697–1700
- Laurie AD, Lloyd-Jones G (1999) The *phn* Genes of *Burkholderia* sp. strain RP007 constitute a divergent gene cluster for polycyclic aromatic hydrocarbon catabolism. *J Bacteriol* 181:531–540
- Lemaire B, Dlodlo O, Chimphango S, Stirton C, Schrire B, Boatwright JS, Honnay O, Smets E, Sprent J, James EK, Muasya AM (2015) Symbiotic diversity, specificity and distribution of rhizobia in native legumes of the Core Cape Subregion (South Africa). *FEMS Microbiol Ecol* 9:1–17
- Lessie TG, Hendrickson W, Manning BD, Devereux R (1996) Genomic complexity and plasticity of *Burkholderia cepacia*. *FEMS Microbiol Lett* 144:117–128
- Li G-X, Wu X-Q, Ye J-R (2013) Biosafety and colonization of *Burkholderia multivorans* WS-FJ9 and its growth-promoting effects on poplars. *Appl Microbiol Biotechnol* 97:10489–10498
- Liang Y, Meggo R, Hu D, Schnoor JL, Mattes TE (2014) Enhanced polychlorinated biphenyl removal in a switchgrass rhizosphere by bioaugmentation with *Burkholderia xenovorans* LB400. *Ecol Eng* 71:215–222
- Liu WYY, Ridgway HJ, James TK, James EK, Chen WM, Sprent JI, Young JPW, Andrews M (2014) *Burkholderia* sp. induces functional nodules on the South African invasive legume *Dipogon lignosus* (Phaseoleae) in New Zealand soils. *Microb Ecol* 68:542–555
- Liu XY, Wei S, Wang F, James EK, Guo XY, Zagar C, Xia LG, Dong X, Wang YP (2012) *Burkholderia* and *Cupriavidus* spp. are the preferred symbionts of *Mimosa* spp. in Southern China. *FEMS Microb Ecol* 80:417–426
- Mahenthalingam E, Baldwin A, Dowson CG (2008) *Burkholderia cepacia* complex bacteria: opportunistic pathogens with important natural biology. *J Appl Microbiol* 104:1539–1551
- Martínez-Aguilar L, Díaz R, Peña-Cabriaes JJ, Estrada-de los Santos P, Dunn MF, Caballero-Mellado J (2008) Multichromosomal genome structure and confirmation of diazotrophy in novel plant-associated *Burkholderia* species. *Appl Environ Microbiol* 74:4574–4579
- Martínez-Aguilar L, Salazar-Salazar C, Díaz-Mendez R, Caballero-Mellado J, Hirsch AM, Vasquez-Murrieta MS, Estrada-de los Santos P (2013) *Burkholderia caballeronis* sp. nov., a nitrogen fixing species isolated from tomato (*Lycopersicon esculentum*) with the ability to effectively nodulate *Phaseolus vulgaris*. *Antonie van Leeuwenhoek* 104:1063–1071
- Miché L, Faure D, Blot M, Cabanne-Giuli E, Balandreau J (2001) Detection and activity of insertion sequences in environmental strains of *Burkholderia*. *Environ Microbiol* 3:766–773
- Mishra RP, Tisseyre P, Melkonian R, Chaintreuil C, Miche L, Klonowska A, Gonzalez S, Bena G, Laguerre G, Moulin L (2012) Genetic diversity of *Mimosa pudica* rhizobial symbionts in soils of French Guiana: investigating the origin and diversity of *Burkholderia phymatum* and other beta-rhizobia. *FEMS Microbiol Ecol* 79:487–503
- Moulin L, Munive A, Dreyfus B, Boivin-Masson C (2001) Nodulation of legumes by members of the  $\beta$ -subclass of Proteobacteria. *Nature* 411:948–950
- Mueller J, Devereux R, Santavy D, Lantz S, Willis S, Pritchard P (1997) Phylogenetic and physiological comparisons of PAH-degrading bacteria from geographically diverse soils. *Antonie van Leeuwenhoek* 71:329–343
- Naveed M, Qureshi M, Zahir ZA, Hassan MB, Sessitch A, Mitter B (2014) L-Tryptophan-dependent biosynthesis of indole-3-acetic acid (IAA) improves plant growth and colonization of maize by *Burkholderia phytofirmans* PsJN. *Ann Microbiol* 65:1381–1389
- Nierman WC, DeShazer D, Kim HS, Tettelin H, Nelson KE, Feldblyum T, Ulrich RL, Ronning CM, Brinkac LM, Daugherty SC (2004) Structural flexibility in the *Burkholderia mallei* genome. *Proc Natl Acad Sci USA* 101:14246–14251
- Pérez-Pantoja D, Donoso R, Agulló L, Córdova M, Seeger M, Pieper DH, González B (2012) Genomic analysis of the potential for aromatic compounds biodegradation in Burkholderiales. *Environ Microbiol* 14:1091–1117
- Perin L, Martínez-Aguilar L, Paredes-Valdez G, Baldani J, Estrada-de los Santos P, Reis V, Caballero-Mellado J (2006) *Burkholderia silvatlantica* sp. nov., a diazotrophic bacterium associated with sugar cane and maize. *Int J Syst Evol Microbiol* 56:1931–1937
- Postgate JR (1982) The fundamentals of nitrogen fixation. *Phil Trans R Soc Lond B* 296(1082) 375–385
- Reis V, Estrada-de los Santos P, Tenorio-Salgado S, Vogel J, Stoffels M, Guyon S, Manvingui P, Baldani VLD, Schmid M, Baldani JI, Balandreau J, Harmann A, Caballero-Mellado J (2004) *Burkholderia tropica* sp. nov., a novel nitrogen-fixing, plant-associated bacterium. *Int J Syst Evol Microbiol* 54:2155–2162
- Roselló-Mora R, Amann R (2001) The species concept for prokaryotes. *FEMS Microbiol Rev* 25:39–67
- Sawana A, Adeolu M, Gupta RS (2014) Molecular signatures and phylogenomic analysis of the genus *Burkholderia*: proposal for division of this genus into the emended genus *Burkholderia* containing pathogenic organisms and a new genus *Paraburkholderia* gen. nov. harboring environmental species. *Front Gen* 5:429. doi:10.3389/fgene.2014.00429
- Seeger M, Zielinski M, Timmis KN, Hofer B (1999) Regiospecificity of dioxygenation of di- to pentachlorobiphenyls and their degradation to chlorobenzoates by the bph-encoded catabolic pathway of *Burkholderia* sp. strain LB400. *Appl Environ Microbiol* 65:3614–3621
- Sessitch A, Coenye T, Sturz A, Vandamme P, Barka EA, Salles J, Van Elsas J, Faure D, Reiter B, Glick B (2005) *Burkholderia phytofirmans* sp. nov., a novel plant-associated bacterium with plant-beneficial properties. *Int J Syst Evol Microbiol* 55:1187–1192
- Sheu SY, Chou JH, Bontemps C, Elliott GN, Gross E, James EK, Sprent JI, Young JPW, Chen WM (2012) *Burkholderia symbiotica* sp. nov., isolated from root nodules of *Mimosa* spp. native to north-east Brazil. *Int J Syst Evol Microbiol* 62:2272–2278
- Sheu SY, Chen MH, Liu WYY, Andrews M, James EK, Ardley JK, De Meyer SE, James TK, Howieson JG, Coutinho BG, Chen WM (2015) *Burkholderia dipogonis* sp. nov., isolated from root nodules of *Dipogon lignosus* in New Zealand and Western Australia. *Int J Syst Evol Microbiol*. doi:10.1099/ijsem.0.000639
- Singh RK, Nalik N, Singh S (2013) Improved nutrient use efficiency increases plant growth with the use of IAA-overproducing strains of endophytic *Burkholderia cepacia* strain RR25. *Microb Ecol* 66:375–384
- Somtrakoon K, Suanjit S, Pokethitiyook P, Kruatrachue M, Lee H, Upatham S (2008a) Enhanced biodegradation of anthracene in acidic soil by inoculated *Burkholderia* sp. VUN10013. *Curr Microbiol* 57:102–106
- Somtrakoon K, Suanjit S, Pokethitiyook P, Kruatrachue M, Lee H, Upatham S (2008b) Phenanthrene stimulates the degradation of pyrene and fluoranthene by *Burkholderia* sp. VUN10013. *World J Microbiol Biotechnol* 24:523–531
- Sprague L, Neubauer H (2004) Melioidosis in animals: a review on epidemiology, diagnosis and clinical presentation. *J Vet Med* 51:305–320
- Srinivasan S, Kim J, Kang S-R, Jheong W-H, Lee S-S (2013) *Burkholderia humi* sp. nov., isolated from peat soil. *Curr Microbiol* 66:300–305
- Steenkamp ET, van Zyl E, Beukes CW, Avontuur JR, Chan WY, Palmer M, Mthombeni LS, Phalane FL, Sereme TK, Venter SN (2015) *Burkholderia kirstenboschensis* sp. nov. nodulated papilionoid legumes indigenous to South Africa. *Syst Appl Microbiol* 38(8):545–554

- Suarez-Moreno ZR, Caballero-Mellado J, Coutinho BG, Mendonca-Previato L, James EK, Venturi V (2012) Common features of environmental and potentially beneficial plant-associated *Burkholderia*. *Microb Ecol* 63:249–266
- Tamura K, Stecher G, Peterson D, Filipiński A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729
- Tindall BJ, Rossello-Mora R, Busse HJ, Ludwig W, Kämpfer P (2010) Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 60:249–266
- Urakami T, Ito-Yoshida C, Araki H, Kijima T, Suzuki K-I, Komagata K (1994) Transfer of *Pseudomonas plantarii* and *Pseudomonas glumae* to *Burkholderia* as *Burkholderia* spp. and description of *Burkholderia vandii* sp. nov. *Int J Syst Evol Microbiol* 44:235–245
- Van VT, Berge O, Ke SN, Balandreau J, Heulin T (2000) Repeated beneficial effects of rice inoculation with a strain of *Burkholderia vietnamiensis* on early and late yield components in low fertility sulphate acid soils of Vietnam. *Plant Soil* 218:273–284
- Vandamme P, Dawyndt P (2011) Classification and identification of the *Burkholderia cepacia* complex: past, present and future. *Syst Appl Microbiol* 34:87–95
- Vandamme P, Peeters C (2014) Time to revisit polyphasic taxonomy. *Antonie van Leeuwenhoek* 106:57–65
- Vandamme P, Goris J, Chen W-M, De Vos P, Willems A (2002) *Burkholderia tuberum* sp. nov. and *Burkholderia phymatum* sp. nov., nodulate the roots of tropical legumes. *Syst Appl Microbiol* 25:507–512
- Vandamme P, De Brandt E, Houf K, Salles JF, van Elsas JD, Spilker T, LiPuma JJ (2013) *Burkholderia humi* sp. nov., *Burkholderia choica* sp. nov., *Burkholderia telluris* sp. nov., *Burkholderia terrestris* sp. nov. and *Burkholderia udeis* sp. nov.: *Burkholderia glathei*-like bacteria from soil and rhizosphere soil. *Int J Syst Evol Microbiol* 63:4707–4718
- Vanlaere E, van der Meer JR, Falsen E, Salles JF, De Brandt E, Vandamme P (2008) *Burkholderia sartisoli* sp. nov., isolated from a polycyclic aromatic hydrocarbon-contaminated soil. *Int J Syst Evol Microbiol* 58:420–423
- Viallard V, Poirier I, Coumoyer B, Haurat J, Wiebkin S, Ophel-Keller K, Balandreau J (1998) *Burkholderia graminis* sp. nov., a rhizospheric *Burkholderia* species, and reassessment of [*Pseudomonas*] *phenazinium*, [*Pseudomonas*] *pyrrocinia* and [*Pseudomonas*] *glathei* as *Burkholderia*. *Int J Syst Bacteriol* 48:549–563
- Vinuesa P, Leon-Barrios M, Silva C, Willems A, Jarabo-Lorenzo A, Perez-Galdona R, Werner D, Martinz-Romero E (2005) *Bradyrhizobium canariense* sp. nov., an acid-tolerant endosymbiont that nodulates endemic genistoid legumes (Papilionoideae: Genisteae) from the Canary Islands, along with *Bradyrhizobium japonicum* bv. *genistearum*, *Bradyrhizobium* genospecies alpha and *Bradyrhizobium* genospecies beta. *Int J Syst Evol Microbiol* 55:569–575
- Vu H, Mu A, Moreau J (2013) Biodegradation of thiocyanate by a novel strain of *Burkholderia phytofirmans* from soil contaminated by gold mine tailings. *Lett Appl Microbiol* 57:368–372
- Wang L, Zhang L, Liu Z, Zhao D, Liu X et al (2013) A minimal nitrogen fixation gene cluster from *Paenibacillus* sp. WLY78 enables expression of active nitrogenase in *Escherichia coli*. *PLoS Genet* 9: e1003865. doi:10.1371/journal.pgen.1003865
- Xu L, Ge C, Cui Z, Li J, Fan H (1995) *Bradyrhizobium liaoningense* sp. nov., isolated from the root nodules of soybeans. *Int J Syst Bacteriol* 45:706–711
- Yabuuchi E, Kosako Y, Oyaizu H, Yano I, Hotta H, Hashimoto Y, Ezaki T, Arakawa M (1992) Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. *Microbiol Immunol* 36:1251–1275
- Yao ZY, Kan FL, Wang ET, Wei GH, Chen WX (2002) Characterization of rhizobia that nodulate legume species of the genus *Lespedeza* and description of *Bradyrhizobium yuanmingense* sp. nov. *Int J Syst Evol Microbiol* 52:2219–2230
- Yarza P, Yilmaz P, Glockner FO, Ludwig W, Schleifer KH, Whitman WB, Euzéby J, Amann R, Rosello-Mora R (2014) Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 12:635–645
- Zhang H, Hanada S, Shigematsu T, Shibuya K, Kamagata Y, Kanagawa T, Kurane R (2000) *Burkholderia kururiensis* sp. nov., a trichloroethylene (TCE)-degrading bacterium isolated from an aquifer polluted with TCE. *Int J Syst Evol Microbiol* 50:743–749
- Zuleta LF, de Cunha C, De Carvalho FM, Ciapina LP, Souza RC, Mercante FM, De Faria SM, Baldani JI, Straliootto R, Hungria M (2014) The complete genome of *Burkholderia phenoliruptrix* strain BR3459a, a symbiont of *Mimosa flocculosa*: highlighting the coexistence of symbiotic and pathogenic genes. *BMC Genomics* 15:535