Appl Microbiol Biotechnol (2016) 100:5215–5229
DOI 10.1007/s00253-016-7520-x

MINI-REVIEW

Burkholderia: an update on taxonomy and biotechnological potential as antibiotic producers

Eliza Depoorter[1,2] · **Matt J. Bull**[3] · **Charlotte Peeters**[1] · **Tom Coenye**[2] · **Peter Vandamme**[1] · **Eshwar Mahenthiralingam**[3]

Received: 12 February 2016 / Revised: 29 March 2016 / Accepted: 31 March 2016 / Published online: 26 April 2016

Abstract *Burkholderia* is an incredibly diverse and versatile Gram-negative genus, within which over 80 species have been formally named and multiple other genotypic groups likely represent new species. Phylogenetic analysis based on the 16S rRNA gene sequence and core genome ribosomal multilocus sequence typing analysis indicates the presence of at least three major clades within the genus. Biotechnologically, *Burkholderia* are well-known for their bioremediation and biopesticidal properties. Within this review, we explore the ability of *Burkholderia* to synthesise a wide range of antimicrobial compounds ranging from historically characterised antifungals to recently described antibacterial antibiotics with activity against multiresistant clinical pathogens. The production of multiple *Burkholderia* antibiotics is controlled by quorum sensing and examples of quorum sensing pathways found across the genus are discussed. The capacity for antibiotic biosynthesis and secondary metabolism encoded within *Burkholderia* genomes is also evaluated. Overall, *Burkholderia* demonstrate significant biotechnological potential as a source of novel antibiotics and bioactive secondary metabolites.

✉ Peter Vandamme
Peter.Vandamme@UGent.be

✉ Eshwar Mahenthiralingam
MahenthiralingamE@cardiff.ac.uk

[1] Laboratory of Microbiology, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium

[2] Laboratory of Pharmaceutical Microbiology, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium

[3] Organisms and Environment Division, Cardiff School of Biosciences, Cardiff University, Main Building, Museum Avenue, Cardiff, UK

Keywords *Burkholderia* · Taxonomy · Phylogeny · Genomics · Antibiotic biosynthesis · Secondary metabolism

Introduction

The genus *Burkholderia* represents a rapidly expanding group of Gram-negative non-fermenting bacteria that occur worldwide in virtually all possible environments. Some species occur in plain soil or in planktonic form in fresh water, but most occur in association with an ever-increasing number of hosts including humans, animals (both vertebrates and invertebrates), plants and fungi. The type of interaction with these hosts is often not known, but a growing body of literature demonstrates that these interactions can be beneficial, harmful or both. Within the genus *Burkholderia*, a cluster of closely related species is known as the *Burkholderia cepacia* complex (Bcc) and presents particular challenges. Bcc bacteria are indeed well-known as rare but potentially life-threatening pathogens in patients with cystic fibrosis (CF) but simultaneously have been studied intensively for their biotechnological applications in plant growth promotion, biological control of plant pests and bioremediation.

The present mini-review provides an update on the taxonomy of these bacteria and addresses recent developments in terms of their capacity for secondary metabolism and antibiotic biosynthesis. The interested reader is referred to recent reviews on *Burkholderia* and Bcc that focused on extracellular products (Vial et al. 2007), relevance as contaminants in the pharmaceutical industry (Torbeck et al. 2011), conflicting life styles (Vial et al. 2011), potential for aromatic compound degradation (Pérez-Pantoja et al. 2012), common characteristics of plant-associated species (Suarez-Moreno et al. 2012), melioidosis (Currie 2015) and to the book entitled '*Burkholderia*: from genomes to function' (2014).

Taxonomy

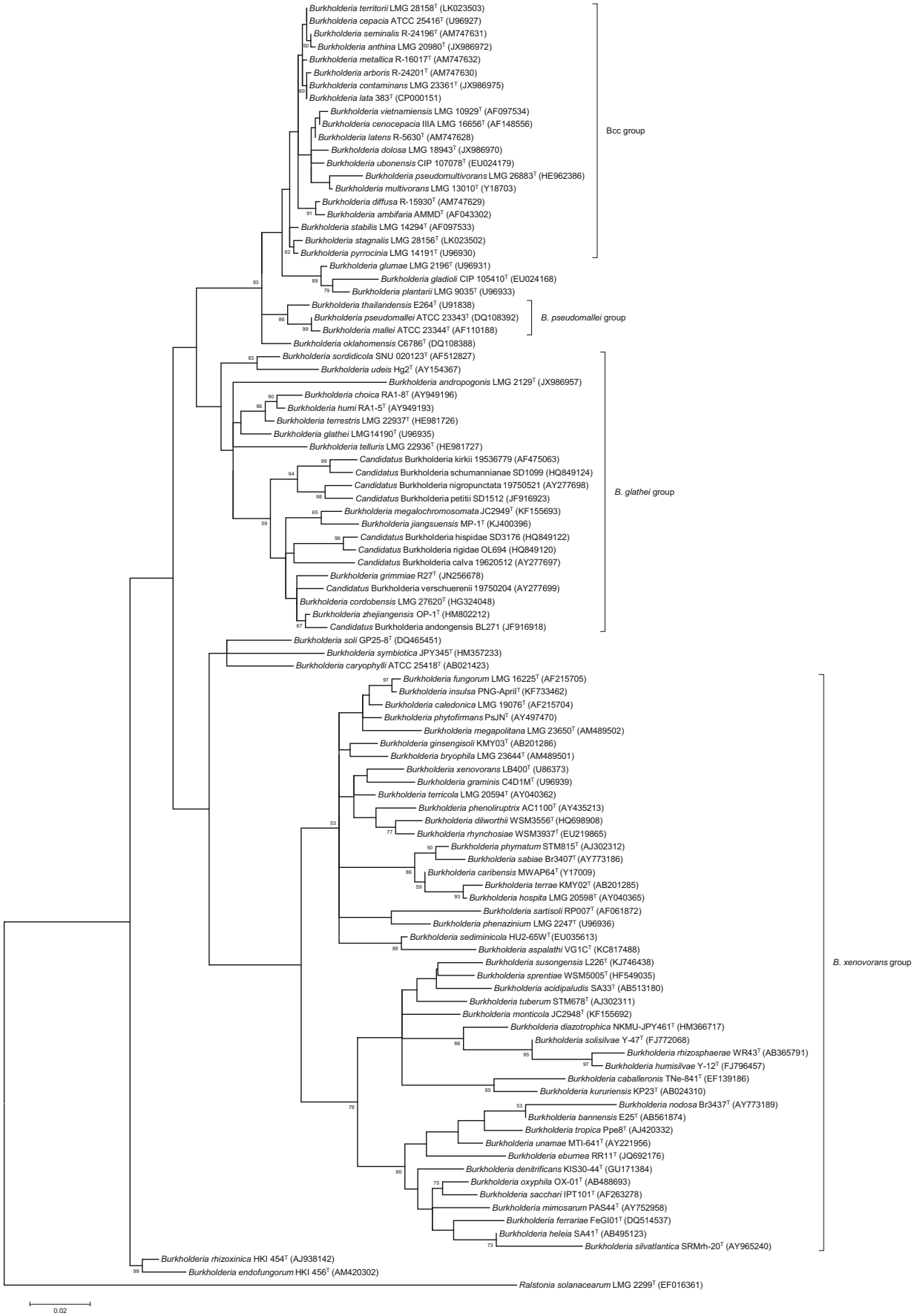
The genus *Burkholderia* belongs to the *Betaproteobacteria* class within the phylum of the *Proteobacteria*. When first described in 1992, it comprised seven species, two of which were subsequently reclassified into another novel genus, *Ralstonia* (Yabuuchi et al. 1992; Yabuuchi et al. 1995). During the past two decades, a large number of novel *Burkholderia* species have been reported and validly named. Yet, several species proved poorly characterised and needed further reclassification (Coenye et al. 1999; Coenye et al. 2000); at present (January 2016), the genus consists of 90 validly named species (Parte 2014) and a large number of uncultivated candidate species (van Oevelen et al. 2004; Verstraete et al. 2011; Lemaire et al. 2011; Lemaire et al. 2012). However, literature data and an analysis of publicly available 16S rRNA gene sequences suggest that many additional *Burkholderia* species await formal description. In addition, mining the Bcc PubMLST database (Jolley and Maiden 2010) using the 3 % threshold value of average concatenated allele sequence divergence for species delineation (Vanlaere et al. 2009; Peeters et al. 2013) demonstrated that also within the Bcc, a substantial number of additional *Burkholderia* species awaits formal naming (Vandamme and Peeters 2014).

The genus *Burkholderia* is phylogenetically diverse and consists of multiple deep-branching 16S rRNA lineages (Fig. 1). The first deep-branching *Burkholderia* clade comprises the type species, *Burkholderia cepacia*, and consists of all Bcc species, a group of plant-pathogenic species that includes *Burkholderia gladioli*, *Burkholderia plantarii* and *Burkholderia glumae* and a group of species closely related to the risk class 3 pathogens *Burkholderia mallei* and *Burkholderia pseudomallei*, the causative agents of glanders in *Equidae* and melioidosis in humans, respectively (Fig. 1). This first deep-branching *Burkholderia* clade comprises the majority of well-known pathogens in this genus but also includes many strains that have been used for plant growth promotion or biological control, such as *Burkholderia vietnamiensis* TVV74 and *Burkholderia ambifaria* AMMD^T, respectively (Parke and Gurian-Sherman 2001). The former is a rice isolate which, when inoculated in field studies on rice, increased grain yield by 13 to 22 % (Tran Van et al. 2000). The latter was isolated from the rhizosphere of peas and has activity against *Pythium aphanidermatum* (responsible for pre- and post-emergence damping-off in peas) and *Aphanomyces euteiches* (responsible for root rot in peas) (Parke 1990; Bowers and Parke 1993; Heungens and Parke 2000; Heungens and Parke 2001; Parke and Gurian-Sherman 2001). In addition, although *Burkholderia cenocepacia* is generally considered the most problematic Bcc species in patients with CF (Lipuma 2010), recently, a genome sequence of a plant-beneficial endophytic *B. cenocepacia* strain with both biocontrol and plant growth-promoting characteristics was reported (Ho and Huang 2015).

Fig 1 Phylogenetic tree based on partial 16S rRNA gene sequences of *Burkholderia* species. Sequences (1125–1610 bp) were aligned against the SILVA SSU reference database using SINA v1.2.11 (http://www.arb-silva.de/aligner/) (Pruesse et al. 2012). Phylogenetic analysis was conducted using MEGA6 (Tamura et al. 2013). All positions containing gaps and missing data were eliminated, resulting in a total of 1087 positions in the final dataset. The optimal tree (highest log likelihood) was constructed using the maximum likelihood method and Tamura-Nei model (Tamura and Nei 1993). A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories [+G, parameter = 0.3498]) and allowed for some sites to be evolutionarily invariable ([+I], 68.6154 % sites). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches if greater than 50 %. The sequence of *Ralstonia solanacearum* LMG 2299^T was used as outgroup. The *scale bar* indicates the number of substitutions per site

The second deep-branching *Burkholderia* clade comprises *Burkholderia glathei* (Zolg and Ottow 1975) and 11 recently named *Burkholderia* species (Fig. 1). Several of these species were isolated from polluted soils. For instance, *Burkholderia udeis* comprises naphthalene-degrading isolates from a polycyclic aromatic hydrocarbon-contaminated hillside soil (Wilson et al. 2003; Vandamme et al. 2013), while *Burkholderia jiangsuensis* and *Burkholderia zhejiangensis* are methyl parathion-degrading bacteria isolated from methyl parathion-contaminated soil and a wastewater treatment system, respectively (Lu et al. 2012; Liu et al. 2014). Other species in this clade have been isolated from less studied sources such as fungal mycelia and mosses (*Burkholderia sordidicola* and *Burkholderia grimmiae*, respectively) (Lim et al. 2003; Tian et al. 2013). This clade also includes isolates from insect guts (Kikuchi et al. 2011; Shibata et al. 2013) and several candidate species with an endophytic lifestyle in plants (Carlier and Eberl 2012; Verstraete et al. 2013). Although these bacteria show a remarkable diversity in terms of ecological niches, to our knowledge, none of the present species within this group has been involved in human or animal infections. However, at least two novel *B. glathei* group species have been isolated from human sources including pleural fluid and blood, and await formal classification (own unpublished data).

The third deep-branching *Burkholderia* clade comprises more than 40 primarily environmental and plant-associated species, many of which are diazotrophic and have been documented as beneficial to their host (Suarez-Moreno et al. 2012) (Fig. 1). Among these species, *Burkholderia fungorum* is a most striking exception, as it has been isolated from a wide range of human and veterinary samples including human blood, cerebrospinal fluid, vaginal secretions, sputum and lavage samples of CF patients, the brain of a pig with neurological deficit, the brain stem of an injured deer and the nose of


Burkholderia territorii LMG 28158T (LK023503)
Burkholderia cepacia ATCC 25416T (U96927)
Burkholderia seminalis R-24196T (AM747631)
Burkholderia anthina LMG 20980T (JX986972)
Burkholderia metallica R-16017T (AM747632)
Burkholderia arboris R-24201T (AM747630)
Burkholderia contaminans LMG 23361T (JX986975)
Burkholderia lata 383T (CP000151)
Burkholderia vietnamiensis LMG 10929T (AF097534)
Burkholderia cenocepacia IIIA LMG 16656T (AF148556)
Burkholderia latens R-5630T (AM747628)
Burkholderia dolosa LMG 18943T (JX986970)
Burkholderia ubonensis CIP 107078T (EU024179)
Burkholderia pseudomultivorans LMG 26883T (HE962386)
Burkholderia multivorans LMG 13010T (Y18703)
Burkholderia diffusa R-15930T (AM747629)
Burkholderia ambifaria AMMDT (AF043302)
Burkholderia stabilis LMG 14294T (AF097533)
Burkholderia stagnalis LMG 28156T (LK023502)
Burkholderia pyrrocinia LMG 14191T (U96930)
Bcc group
Burkholderia glumae LMG 2196T (U96931)
Burkholderia gladioli CIP 105410T (EU024168)
Burkholderia plantarii LMG 9035T (U96933)
Burkholderia thailandensis E264T (U91838)
Burkholderia pseudomallei ATCC 23343T (DQ108392)
Burkholderia mallei ATCC 23344T (AF110188)
B. pseudomallei group
Burkholderia oklahomensis C6786T (DQ108388)
Burkholderia sordidicola SNU 020123T (AF512827)
Burkholderia udeis Hg2T (AY154367)
Burkholderia andropogonis LMG 2129T (JX986957)
Burkholderia choica RA1-8T (AY949196)
Burkholderia humi RA1-5T (AY949193)
Burkholderia terrestris LMG 22937T (HE981726)
Burkholderia glathei LMG14190T (U96935)
Burkholderia telluris LMG 22936T (HE981727)
Candidatus Burkholderia kirkii 19536779 (AF475063)
Candidatus Burkholderia schumannianae SD1099 (HQ849124)
Candidatus Burkholderia nigropunctata 19750521 (AY277698)
Candidatus Burkholderia petitii SD1512 (JF916923)
Burkholderia megalochromosomata JC2949T (KF155693)
Burkholderia jiangsuensis MP-1T (KJ400396)
Candidatus Burkholderia hispidae SD3176 (HQ849122)
Candidatus Burkholderia rigidae OL694 (HQ849120)
Candidatus Burkholderia calva 19620512 (AY277697)
Burkholderia grimmiae R27T (JN256678)
Candidatus Burkholderia verschuerenii 19750204 (AY277699)
Burkholderia cordobensis LMG 27620T (HG324048)
Burkholderia zhejiangensis OP-1T (HM802212)
Candidatus Burkholderia andongensis BL271 (JF916918)
B. glathei group
Burkholderia soli GP25-8T (DQ465451)
Burkholderia symbiotica JPY345T (HM357233)
Burkholderia caryophylli ATCC 25418T (AB021423)
Burkholderia fungorum LMG 16225T (AF215705)
Burkholderia insulsa PNG-AprilT (KF733462)
Burkholderia caledonica LMG 19076T (AF215704)
Burkholderia phytofirmans PsJNT (AY497470)
Burkholderia megapolitana LMG 23650T (AM489502)
Burkholderia ginsengisoli KMY03T (AB201286)
Burkholderia bryophila LMG 23644T (AM489501)
Burkholderia xenovorans LB400T (U86373)
Burkholderia graminis C4D1MT (U96939)
Burkholderia terricola LMG 20594T (AY040362)
Burkholderia phenoliruptrix AC1100T (AY435213)
Burkholderia dilworthii WSM3556T (HQ698908)
Burkholderia rhynchosiae WSM3937T (EU219865)
Burkholderia phymatum STM815T (AJ302312)
Burkholderia sabiae Br3407T (AY773186)
Burkholderia caribensis MWAP64T (Y17009)
Burkholderia terrae KMY02T (AB201285)
Burkholderia hospita LMG 20598T (AY040365)
Burkholderia sartisoli RP007T (AF061872)
Burkholderia phenazinium LMG 2247T (U96936)
Burkholderia sediminicola HU2-65WT (EU035613)
Burkholderia aspalathi VG1CT (KC817488)
Burkholderia susongensis L226T (KJ746438)
Burkholderia sprentiae WSM5005T (HF549035)
Burkholderia acidipaludis SA33T (AB513180)
Burkholderia tuberum STM678T (AJ302311)
Burkholderia monticola JC2948T (KF155692)
Burkholderia diazotrophica NKMU-JPY461T (HM366717)
Burkholderia solisilvae Y-47T (FJ772068)
Burkholderia rhizosphaerae WR43T (AB365791)
Burkholderia humisilvae Y-12T (FJ796457)
Burkholderia caballeronis TNe-841T (EF139186)
Burkholderia kururiensis KP23T (AB024310)
Burkholderia nodosa Br3437T (AY773189)
Burkholderia bannensis E25T (AB561874)
Burkholderia tropica Ppe8T (AJ420332)
Burkholderia unamae MTI-641T (AY221956)
Burkholderia eburnea RR11T (JQ692176)
Burkholderia denitrificans KIS30-44T (GU171384)
Burkholderia oxyphila OX-01T (AB488693)
Burkholderia sacchari IPT101T (AF263278)
Burkholderia mimosarum PAS44T (AY752958)
Burkholderia ferrariae FeGI01T (DQ514537)
Burkholderia heleia SA41T (AB495123)
Burkholderia silvatlantica SRMrh-20T (AY965240)
B. xenovorans group
Burkholderia rhizoxinica HKI 454T (AJ938142)
Burkholderia endofungorum HKI 456T (AM420302)
Ralstonia solanacearum LMG 2299T (EF016361)
0.02

mice (Coenye et al. 2001b; Coenye et al. 2002; Gerrits et al. 2005) (own unpublished data). In addition, *Burkholderia tropica* has been isolated from a neonatal patient with necrotizing enterocolitis and bowel perforation, who developed septicaemia (Deris et al. 2010).

In addition to these three main clades, several *Burkholderia* species represent unique deep-branching 16S rRNA lineages (Fig. 1). These include *Burkholderia rhizoxinica* and *Burkholderia endofungorum* (two endosymbionts of the plant-pathogenic fungus *Rhizopus microsporus*) (Partida-Martinez et al. 2007) and a group consisting of *Burkholderia caryophylli* (a pathogen of carnations and onions) (Ballard et al. 1970), *Burkholderia symbiotica* (a root nodule endosymbiont of *Mimosa* species) (Sheu et al. 2012) and *Burkholderia soli* (a soil bacterium) (Yoo et al. 2007). These species do not cluster closely with any other *Burkholderia* species and their 16S rRNA sequence-based phylogenetic position appears variable and dependent on the other taxa included in a phylogenetic analysis. Finally, *Burkholderia andropogonis*, a pathogen causing stripe disease of sorghum and leaf spot of velvet bean (Coenye et al. 2001a), clustered within the *B. glathei* group clade in the present analysis (Fig. 1); yet it often occupies a distinct position in 16S rRNA-based phylogenetic trees as well (see e.g. Sawana et al. 2014; Estrada-de los Santos et al. 2015).

Today, the availability of whole genome sequences enables phylogenetic analyses based on the entire part of the genome that is shared between organisms, a discipline referred to as phylogenomics (Yutin et al. 2012; Wang and Wu 2013). Although the number of *Burkholderia* species for which whole genome sequences are available is still limited, phylogenomics only partially reveals the same major subdivisions as the 16S rRNA tree (Fig. 1). Analysing the diversity across the *Burkholderia* genus using complete genome comparison is difficult given their inherent genetic diversity. Nevertheless, comparison of the 53 ribosomal protein-encoding genes within the current ribosomal multilocus sequence typing (rMLST) scheme provides a robust and biologically meaningful representation of the core *Burkholderia* genome (Fig. 2) (Jolley et al. 2012). A first well-supported rMLST *Burkholderia* clade also comprises the Bcc, plant-pathogenic and *B. pseudomallei* species groups, though with greater support for their divergence (Fig. 2). The second 16S rRNA sequence-based branch including *B. glathei* group species is also distinct when analysed by rMLST (Fig. 2). However, it clusters among species belonging to the third 16S rRNA-based lineage. In the rMLST analysis too, *B. rhizoxinica* and *B. symbiotica* occupy very distinct positions each. In contrast to what is observed in the 16S rRNA-based phylogeny, *B. andropogonis* represents a unique, very deep-branching lineage in the rMLST tree, illustrating its isolated taxonomic position. It will be interesting to see how other *Burkholderia* species will group when additional whole genome sequences become available.

Fig 2 *Burkholderia* phylogeny reconstructed from concatenated ribosomal protein gene sequences. Aligned, concatenated gene sequences from defined ribosomal multilocus sequence typing (rMLST) loci were downloaded from the rMLST database at http://pubMLST.org (Jolley et al. 2012). Low confidence regions of the alignment were removed with Gblocks (Talavera and Castresana 2007), resulting in a total of 18,490 positions in the final dataset. A phylogeny was reconstructed with FastTree (Price et al. 2010) using the generalised time-reversible (GTR) model of nucleotide evolution, and the resulting tree was visualised with FigTree (http://tree.bio.ed.ac.uk/software/figtree). Type strains are indicated in *bold type*. Node confidence is shown if less than 80 %. The sequence of *Ralstonia solanacearum* PSI07 was used as outgroup. The *scale bar* indicates the number of substitutions per site

The phylogenetic heterogeneity described above inspired several researchers to suggest (Gyaneshwar et al. 2011; Suarez-Moreno et al. 2012; Estrada-de los Santos et al. 2013; Angus et al. 2014) and eventually propose (Sawana et al. 2014) a taxonomic subdivision of the genus *Burkholderia*. Although it is clear that one can distinguish beneficial and harmful interactions of *Burkholderia* strains, there is no such phylogenetic subdivision in this genus. In a study of whole genome sequences of 45 *Burkholderia* strains representing some 25 formally named species and several unclassified strains, species belonging to the first *Burkholderia* clade were characterised by a percentage guanine plus cytosine content in their genomes of 65 to 69 %, while all other *Burkholderia* strains examined had a percentage guanine plus cytosine content in their genomes of 61 to 65 %. In addition, species belonging to the first *Burkholderia* clade shared six conserved sequence indels. The remaining *Burkholderia* strains represented species belonging to 16S rRNA clades 2 and 3 described above and one of the ungrouped species (*B. rhizoxinica*), and shared two conserved sequence indels. However, the phylogenetic diversity among the clade 2 and 3 species and *B. rhizoxinica* as revealed by 16S rRNA-based divergence and by differences in the distribution of 22 additional conserved sequence indels was ignored, as the authors proposed to restrict the name *Burkholderia* to 16S rRNA clade 1 species while reclassifying all other species into a single novel genus, *Paraburkholderia* (Sawana et al. 2014). These novel names were subsequently validated (Garrity and Oren 2015). Clearly, rMLST analysis is also supportive of greater evolutionary divergence between *Burkholderia senso strictu* (i.e. 16S rRNA clade 1 species) and *Paraburkholderia* but excludes *B. rhizoxinica*, *B. symbiotica* and *B. andropogonis* from the latter (Figs. 1 and 2). It will be up to the scientific community to adopt these novel names or not. According to the International Code of Nomenclature of Bacteria, researchers who are convinced that these name changes are ill-founded can continue to work with the original species names as these all were validly published.


Burkholderia cenocepacia PT15
Burkholderia cenocepacia S2AES
Burkholderia cenocepacia PC184
Burkholderia cenocepacia MC0-3
Burkholderia cenocepacia AU1054
Burkholderia cenocepacia HI2424
Burkholderia cenocepacia D2AES
Burkholderia cenocepacia J2315T
Burkholderia cenocepacia BC7
Burkholderia cenocepacia K56-2Valvano
Burkholderia cenocepacia H111
Burkholderia cepacia ATCC 25416T
Burkholderia cepacia AU4i
Burkholderia lata 383T
Burkholderia sp. RB-39
Burkholderia ambifaria AMMDT
Burkholderia ambifaria MC40-6
Burkholderia ambifaria IOP40-10
Burkholderia ambifaria MEX-5
Burkholderia vietnamiensis G4
Burkholderia vietnamiensis KJ006
Burkholderia stagnalis CH-67
Burkholderia ubonensis Bu
Burkholderia multivorans CF2
Burkholderia multivorans ATCC 17616
Burkholderia multivorans DWS 42B-1
Burkholderia multivorans CGD1
Burkholderia multivorans CGD2
Burkholderia multivorans LMG 13010T
Burkholderia dolosa AU0158
Burkholderia dolosa PC543
Burkholderia pseudomultivorans TJI49
Bcc group
Burkholderia mallei NCTC 10229
Burkholderia mallei NCTC 10247
Burkholderia mallei ATCC 23344T
Burkholderia mallei SAVP1
Burkholderia pseudomallei K96243
Burkholderia pseudomallei 1710b
Burkholderia pseudomallei 1106a
Burkholderia pseudomallei 668
Burkholderia thailandensis Bt4
Burkholderia thailandensis E264T
Burkholderia thailandensis TXDOH
Burkholderia thailandensis E555
"Burkholderia humptydooensis" MSMB43
"Burkholderia humptydooensis" MSMB121
Burkholderia oklahomensis C6786T
Burkholderia oklahomensis EO147
B. pseudomallei group
Burkholderia gladioli BSR3
Burkholderia gladioli 3848s-5
Burkholderia gladioli ERR353354
Burkholderia gladioli CIP 105410T
Burkholderia gladioli UCD-UG_CHAPALOTE
Burkholderia glumae BGR1
Burkholderia glumae LMG 2196T
Burkholderia glumae 336gr-1
Burkholderia glumae 3252-8
Burkholderia glumae AU6208
Burkholderia phenoliruptrix AC1100T
Burkholderia phenoliruptrix JPY366
Burkholderia phenoliruptrix CCGE1001
Burkholderia phenoliruptrix BR3459a
Burkholderia sp. CCGE1003
Burkholderia sp. WSM2232
Burkholderia sp. WSM2230
Burkholderia graminis URHA0054
Burkholderia bryophila 376MFSha3.1
Burkholderia caledonica LMG 19076T
Burkholderia dilworthii WSM3556T
Burkholderia xenovorans LB400T
Burkholderia sp. Ch1-1
Burkholderia phytofirmans PsJNT
Burkholderia fungorum LMG 16225T
Burkholderia sp. CCGE1002
Burkholderia sp. JPY251
Burkholderia tuberum WSM4176
Burkholderia sprentiae WSM5005T
Burkholderia sp. H160
Burkholderia terrae BS001
Burkholderia terrae BT03
Burkholderia terrae NBRC 100964T
Burkholderia caribensis MBA4
Burkholderia phymatum STM815T
Burkholderia sp. UYPR1.413
B. xenovorans group
Burkholderia cordobensis YI23
Burkholderia zhejiangensis SJ98
Candidatus Burkholderia kirkii UZHbot1
Burkholderia jiangsuensis MP-1T
Burkholderia sp. RPE64
Burkholderia grimmiae R27T
Burkholderia glathei LMG 14190T
B. glathei group
Burkholderia mimosarum LMG 23256
Burkholderia mimosarum NBRC 106338T
Burkholderia mimosarum STM 3621
Burkholderia nodosa DSM 21604T
Burkholderia oxyphila NBRC 105797T
Burkholderia bannensis NBRC 103871T
Burkholderia ferrariae NBRC 106233T
Burkholderia kururiensis M130
Burkholderia kururiensis JCM 10599T
Burkholderia kururiensis NBRC 107107
Burkholderia symbiotica JPY347T
Burkholderia rhizoxinica HKI 454T
Burkholderia andropogonis Ba3549
Burkholderia andropogonis ICMP 2807T
Ralstonia solanacearum PSI07
15
76
69
55
78
10
75
48
5
70
11
0.04

Capacity for secondary metabolism: quorum sensing and antibiotic biosynthesis

Burkholderia bacteria are incredibly versatile organisms, with a phenomenal capacity for secondary metabolite production (Cimermancic et al. 2014), among which many with antifungal, antibacterial, herbicidal or insecticidal properties. Strains of *Burkholderia* are known to produce pyrrolnitrin (El-Banna and Winkelmann 1998), xylocandins (Meyers et al. 1987), cepafungins/glidobactins (Schellenberg et al. 2007), altericidins (Kirinuki et al. 1984), cepacins (Parker et al. 1984), cepaciamides (Jiao et al. 1996), phenazines (Cartwright et al. 1995) and quinoline derivatives (Moon et al. 1996). Although historical interest in *Burkholderia* secondary metabolites was largely focused on these primarily antifungal compounds, there is now growing evidence that *Burkholderia* also produce a range of potent antibacterial antibiotics, such as enacyloxin IIa (Mahenthiralingam et al. 2011). The following discussion will provide an overview of the state of the art on antimicrobial products from *Burkholderia*, as an update to the literature review by Vial et al. (2007) and genome-driven analysis of Liu and Cheng (2014). Biosynthesis of multiple *Burkholderia* antibiotics is controlled by quorum sensing (QS). For example, production of enacyloxin IIa and the resultant bioactivity of *B. ambifaria* AMMD^T against *B. multivorans* is lost when the QS system is genetically disrupted (Mahenthiralingam et al. 2011) and the production of multiple other *Burkholderia* antibiotics is regulated in a similar way (Schmidt et al. 2009; Seyedsayamdost et al. 2010). Since QS and the signalling molecules produced by this process represent a major class of *Burkholderia* secondary metabolites, this process will be discussed first.

Quorum sensing in the genus *Burkholderia*

The expression of extracellular products is tightly regulated in bacteria. The immediate environment of the organism has a major influence on the production and secretion of extracellular products, but bacteria themselves also have a global regulation system in place to coordinate their behaviour. This QS system, as it is known, allows bacteria to alter their gene expression according to population density, as a form of cell-to-cell communication. In Gram-negative bacteria, *N*-acyl homoserine lactones (AHLs) are the most commonly used signal molecules, usually produced by an autoinducer synthase of the LuxI protein family and perceived by a transcriptional regulator belonging to the LuxR family (Whitehead et al. 2001). Bcc bacteria contain a LuxI/R type QS system, known as CepI/R, which was first discovered in *B. cenocepacia* K56-2 (Lewenza et al. 1999). The CepI AHL synthase is responsible for the production of *N*-octanoyl homoserine lactone and, as a minor by-product, *N*-hexanoyl homoserine lactone, whereas CepR acts as a transcriptional regulator. This CepI/R QS system is highly conserved among members of the Bcc (Sokol et al. 2007) and has been shown to regulate the production of a variety of extracellular products, including siderophores, fungicides and proteases (Lewenza et al. 1999; Zhou et al. 2003; Malott et al. 2005), as well numerous antibiotics, as discussed below.

Certain Bcc members harbour additional QS systems, such as BviI/R in *B. vietnamiensis* (Conway and Greenberg 2002) and CciI/R in *B. cenocepacia* strains belonging to the epidemic ET12 lineage (Malott et al. 2005). In addition, Boon et al. (2008) described another QS system in *B. cenocepacia*, which uses cis-2-dodecenoic acid (also known as *Burkholderia* diffusible signal factor or BDSF) as a signal molecule. BDSF is structurally similar to the diffusible signal factor (DSF) produced by the plant pathogen *Xanthomonas campestris* pv. *campestris* in order to regulate virulence. A homologue of the *rpfF* gene, the key enzyme in DSF biosynthesis, has been identified in *B. cenocepacia* and appears to be conserved throughout the Bcc (Deng et al. 2010). *RpfF* deletion mutants show reduced motility and adherence to porcine mucin, decreased extracellular polysaccharide production and diminished biofilm formation (Ryan et al. 2009). BDSF thus acts as an intraspecies signal in *B. cenocepacia*, yet it is also involved in interspecies and interkingdom communication, as antagonistic effects on *Candida albicans* have been observed (Boon et al. 2008).

Although QS has been less explored outside the Bcc, it appears to be widespread in the genus *Burkholderia*. For example, the plant pathogens *B. glumae* and *B. plantarii* are known to have QS systems similar to CepI/R, known as TofI/R and PlaI/R, respectively (Kim et al. 2004; Solis et al. 2006). Another distinct AHL-based QS system, designated BraI/R, is present in *Burkholderia kururiensis* and other members of 16S rRNA clade 3 (Suarez-Moreno et al. 2008). This QS system is similar to the LasI/R system found in *Pseudomonas aeruginosa* and relies on *N*-3-oxo-dodecanoyl homoserine lactone as main signal molecule. Despite the high conservation of the BraI/R system in this *Burkholderia* clade, most phenotypes (including biofilm formation, plant colonisation and degradation of aromatic compounds) seem to be regulated in a species-specific manner, suggesting that its role has evolved to suit the niche-specific needs of each species (Coutinho et al. 2013). Finally, several highly conserved and complex QS systems are present in members of the *B. pseudomallei* group. Both *B. pseudomallei* and *Burkholderia thailandensis* contain three complete QS circuits (QS-1, QS-2 and QS-3) and at least two orphan *luxR* homologues, whereas *B. mallei* has lost a large genomic region containing the QS-2 system through reductive evolution (Majerczyk et al. 2014).

Antibiotic biosynthesis by members of the Bcc

Members of the Bcc are known to produce multiple antimicrobial products, as described below. Pyrrolnitrin is a potent antifungal and antibacterial metabolite produced by strains of *Burkholderia*, *Pseudomonas*, *Myxococcus*, *Serratia* and *Enterobacter* (El-Banna and Winkelmann 1998), and it plays a role in the biocontrol activity of Bcc strains against phytopathogenic fungi such as *Rhizoctonia solani* and *Fusarium* spp. (Burkhead et al. 1994; Hwang et al. 2002). Pyrrolnitrin also inhibits growth of *C. albicans* and several Gram-positive bacteria, whereas Gram-negative organisms, except *Proteus vulgaris*, are not affected (El-Banna and Winkelmann 1998). A recent study determined the distribution of *prnD*, the gene responsible for the last step of pyrrolnitrin biosynthesis, within the genus *Burkholderia* (Schmidt et al. 2009). The pyrrolnitrin operon was found in strains belonging to eight Bcc species (*Burkholderia pyrrocinia*, *B. cepacia*, *B. cenocepacia*, *B. ambifaria*, *Burkholderia ubonensis*, *Burkholderia lata* and two novel Bcc groups) and in three species from the *B. pseudomallei* group (*B. pseudomallei*, *B. thailandensis* and *Burkholderia oklahomensis*). In addition, pyrrolnitrin production was shown to be under the control of the CepI/R QS system in *B. lata* 383^{T}, as both *cepI* and *cepR* mutants lost inhibitory activity against *R. solani*, which could be restored in the *cepI* mutant through the addition of exogenous AHLs.

El-Banna and Winkelmann (1998) previously reported that glycerol strongly enhanced the production of pyrrolnitrin by *B. cepacia* NB-1. This finding was later confirmed in *Burkholderia* sp. O33, which produced increased amounts of pyrrolnitrin and polyhydroxyalkanoates in the presence of glycerol (Keum 2009). Based on these observations, Mahenthiralingam and colleagues used a basal salt medium, supplemented with glycerol as the only carbon source, to screen members of the Bcc for antimicrobial production (Mahenthiralingam et al. 2011). This led to the discovery that several *B. ambifaria* isolates show strong antimicrobial activity against pan-resistant Gram-negative pathogens, including *Acinetobacter baumannii* and two closely related Bcc species, *B. multivorans* and *Burkholderia dolosa*. The compounds responsible for this activity, enacyloxin IIa and the novel isomer cis-enacyloxin IIa, are produced by an unusual hybrid modular polyketide synthase (PKS) gene cluster. The fact that this cluster contains two orphan *luxR*-type homologues, disruption of which abolishes enacyloxin production, combined with the observation that *cepI* mutants no longer produce the enacyloxins, also indicates that QS plays a key role in regulation of this antibiotic biosynthesis cluster.

Another group of potent antifungals, named occidiofungins, was recently isolated from cultures of *Burkholderia contaminans* MS14 (Lu et al. 2009). These compounds display potent antifungal activity against a range of animal- and plant-pathogenic fungi, including *Alternaria alternata*, *Aspergillus fumigatus*, *R. solani* and several *Phytium* species. Occidiofungins are cyclic glycosylated oligopeptides, synthesised by a nonribosomal peptide synthetase (NRPS) and are structurally similar to the fungicidal xylocandins (Meyers et al. 1987) and the newly described burkholdines from *B. ambifaria* 2.2N (Tawfik et al. 2010). An NRPS gene cluster with close homology to the occidiofungin biosynthetic cluster was recently identified in *B. ambifaria* AMMD^{T} and *B. vietnamiensis* DBO1. This gene cluster was responsible for hemolytic and insecticidal activity in *B. vietnamiensis* DBO1, suggesting that occidiofungins/burkholdines are not strictly fungicidal, but rather cytotoxic (Thomson and Dennis 2012). The synthesis of this group of bioactive oligopeptides is likely also QS-regulated, as the occidiofungin/burkholdine gene cluster was identified among several QS-controlled loci in *B. ambifaria* (Chapalain et al. 2013).

Finally, *B. ambifaria* is known to produce a range of bioactive volatile compounds that inhibit growth of the phytopathogenic fungi *A. alternata* and *R. solani* (Groenhagen et al. 2013). In addition to exhibiting fungicidal activity, the volatiles also increased biomass in the model plant *Arabidopsis thaliana* and were able to induce increased levels of antibiotic resistance in *Escherichia coli*.

An unidentified *Burkholderia* species related to the Bcc produces cepafungins, also known as glidobactins, which exhibit broad-spectrum antifungal and antitumor activities (Schellenberg et al. 2007). Similar gene clusters were found in *B. pseudomallei*, and glidobactins were recently identified as strong inhibitors of the eukaryotic proteasome (Groll et al. 2008).

Antibiotic biosynthesis by non-Bcc *Burkholderia*

Although a wide variety of antimicrobial compounds have been isolated from Bcc bacteria, several non-Bcc members of the genus *Burkholderia* are also known to exhibit antimicrobial activity. Species belonging to the *B. pseudomallei* group in particular appear to be excellent sources of antimicrobial natural products such as betulinans (Biggins et al. 2011), malleilactone/burkholderic acid (Franke et al. 2012; Biggins et al. 2012), thailandamides (Nguyen et al. 2008), thailandepsins (Wang et al. 2011), capistruin (Knappe et al. 2008) and bactobolins (Seyedsayamdost et al. 2010). Three of these bioactive compounds, capistruin, bactobolin and thailandamide, were discovered through genome mining of *B. thailandensis* E264^{T}, which indicated the presence of a gene cluster involved in the synthesis of a lasso peptide, a type of ribosomally assembled bioactive peptide frequently isolated from *Actinobacteria*. The predicted molecule, capistruin, could be isolated from culture supernatant and demonstrated antibacterial activity against closely related *Burkholderia* and *Pseudomonas* strains (Knappe et al. 2008). Indication for the

production of another antibacterial molecule came from a study investigating the role of the second QS system (QS-2) in *B. thailandensis*, encoded by BtaI2/R2, as these genes were found in clusters predicted to be involved in antibiotic biosynthesis. Supernatants from stationary-phase cultures of *B. thailandensis* E264^T, but not a *btaR2* mutant or a strain defective in AHL production, showed inhibitory activity against Gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes* (Duerkop et al. 2009). This inhibitory activity was later attributed to a mixture of polar antibiotic compounds, known as bactobolins A–D, synthesised by a NRPS/PKS hybrid gene cluster (Seyedsayamdost et al. 2010).

The link between *Burkholderia* antibiotic production and QS was confirmed by Ishida et al. (2010). In this study, induction of thailandamide lactone A production in *B. thailandensis* was achieved by genetic manipulation of QS. Bioinformatic mining of the *B. thailandensis* E264^T genome identified biosynthetic gene clusters which were congruent with the biosynthesis of polyketide metabolites. One of the metabolites, thailandamide A, was inconsistently detected in minute quantities within the supernatants of early-stage *B. thailandensis* cultures. Examination of the putative thailandamide biosynthetic cluster demonstrated the presence of an orphan *luxR* gene, encoding a transcriptional regulator (designated *pthaA*), which was phylogenetically distinct from other *Burkholderia* LuxR homologues. Disruption of the *pthaA* transcription factor binding region by mutagenesis resulted in a strain that accumulated large quantities of yellow pigment. This pigment was subsequently identified as thailandamide A and displayed antiproliferative, cytotoxic and anticancer activities (Ishida et al. 2010). Contrastingly, production of the cytotoxin malleilactone is regulated by an orphan *luxR* homologue, known as *malR*, which is not responsive to AHLs (Truong et al. 2015).

Identification and characterisation of bioactive natural products outside of the Bcc and *B. pseudomallei* group has not been as extensive. The plant pathogen *B. plantarii* produces tropolone, a compound with antibacterial, antifungal and phytotoxic properties (Azegami et al. 1987). The observation that a sesquiterpene signal molecule from *Trichoderma virens* PS1–7, a biocontrol agent of *B. plantarii*, represses tropolone production via transcriptional suppression of the AHL synthase *plaI* suggests that tropolone production is at least partially QS-regulated (Wang et al. 2013). Another plant pathogen, *B. glumae*, is known for the production of the phytotoxin toxoflavin (Jeong et al. 2003), which is regulated by the TofI/R QS system (Kim et al. 2004). Recently, another TofI/R-independent regulatory mechanism for toxoflavin production was discovered. Deletion mutants of this regulatory factor, *tofM*, produced lower levels of toxoflavin and showed reduced virulence, suggesting that *tofM* is a positive regulator of toxoflavin production (Chen et al. 2012). Besides this well-known phytotoxin, *B. glumae* also produces a bioactive pyrazole with antibacterial activity against *Erwinia amylovora* and several other *Erwinia* and *Pseudomonas* species (Mitchell et al. 2008). *B. gladioli*, a third plant pathogen in the genus *Burkholderia*, is known to produce several toxic and antimicrobial metabolites. The respiratory toxin, bongkrekic acid, associated with the fermented coconut-based Indonesian food, tempe bongkrek, was identified as the product of a polyketide biosynthesis cluster in *B. gladioli* pv. *cocovenenans* (Moebius et al. 2012). Tempe bongkrek is produced via fermentation by the mould *Rhizopus oligosporus*, which was initially thought to be the source of bongkrekic acid. Later, it was shown that *B. gladioli* pv. *cocovenans*, which was found as a contaminant of the fungal cultures and fermentations, was responsible for the production of this toxin (Moebius et al. 2012). Further evidence that *B. gladioli* can secrete bioactive molecules has been observed by Bharti et al. (2012), who noted that strain OR1 produces a range of, as yet uncharacterised, antimicrobial compounds with activity against *Staphylococcus* and *Candida* species. Finally, *B. gladioli* was shown to produce a polyketide of the enacyloxin family with antibacterial and antifungal activities when grown in co-culture with *R. microsporus* (Ross et al. 2014). *B. rhizoxinica*, an endosymbiont of the plant-pathogenic fungus *R. microsporus*, was also shown to be the source of a polyketide toxin, rhizoxin (Partida-Martinez and Hertweck 2005; Scherlach et al. 2012). Interkingdom interactions, in particular with plants and fungi, are key aspects of the natural biology of *Burkholderia* and it will be interesting to see if further bioactive natural products will be discovered as these complex environmental lifestyles are explored.

A genomic perspective on the capacity of *Burkholderia* for antibiotic biosynthesis

Greater access to complete microbial genome sequences facilitates the discovery of novel antibiotics via genome mining (Zerikly and Challis 2009). Several genomic approaches have been used to identify multiple antibiotics within *Burkholderia*, and particularly within the *B. pseudomallei* group (Fig. 1), as recently reviewed by Liu and Cheng (2014). In the last decade, hundreds of genomes were obtained for this group of potential bioterrorism agents, facilitating the application of genome mining for nondefence-related research. With the recent availability of genome sequences of other *B. pseudomallei*-related *Burkholderia* strains (Figs. 1 and 2), spanning the phylogenetic diversity of this group, it appears that the genomic capacity for antibiotic biosynthesis is an intrinsic feature of this group of organisms. Several bioinformatics tools are available for identifying secondary metabolite pathways within microbial genomes, the antibiotics Secondary Metabolite Analysis Shell (antiSMASH) being

among the most advanced and well-curated (Blin et al. 2013; Weber et al. 2015). To provide a perspective on the genomic capacity for antibiotic biosynthesis within the genus *Burkholderia*, 15 complete genomes of strains representative of current diversity (Fig. 2) were analysed using antiSMASH 2.2.1 (Blin et al. 2013). The metrics for overall secondary metabolism and for the encoded PKS, NRPS, terpene and homoserine lactone biosynthetic capacities encoded in these 15 genomes are summarised in Table 1. The use of predictive biology software such as antiSMASH greatly accelerates the potential for novel secondary metabolite pathway discovery and was able to identify known PKS and NRPS pathways within the *Burkholderia* genomes analysed (Table 1). However, structure-pathway correlation and conventional chemical characterisation will still be required to characterise the wealth of potential bioactive molecules encoded by *Burkholderia*.

A large genome size is a well-recognised feature of *Burkholderia* bacteria, which stand out in terms of their general functional versatility (Suarez-Moreno et al. 2012): the mean genome size of the strains included in the analysis is nearly 7 Mb (Table 1). The number of secondary metabolite clusters encoded within *Burkholderia* genomes varies greatly: for the 15 genomes analysed, a mean of 13 clusters was observed (range 7 to 22). On average, this equates to more than 7 % of the *Burkholderia* genome (>450 kb) being devoted to secondary metabolism. Another key feature of this genomic capacity for secondary metabolism is the substantial size of the encoded pathways, at averages of 49.5, 58.7, 21.8 and 18.3 kb for PKS, NRPS, terpene and homoserine lactone biosynthetic loci, respectively (Table 1). Given the size of the identified clusters and the current knowledge about large multimodular pathways such as those found in PKS and NRPS operons, the majority of pathways are likely to be complete, functional and able to synthesise complex compounds, provided their expression is activated. With complex regulatory controls involving QS (Schmidt et al. 2009; Ishida et al. 2010), inducing carbon sources (El-Banna and Winkelmann 1998; Mahenthiralingam et al. 2011) and as yet uncharacterised environmental interactions as potential stimulants, understanding how to activate cryptic antibiotic biosynthetic pathways within *Burkholderia* will be key to unlocking their biotechnological potential as a source of fine pharmaceuticals.

Liu and Cheng (2014) suggested that *B. thailandensis* E264^T could be considered a champion *Burkholderia* in terms of its encoded capacity for natural product biosynthesis. From our preliminary analysis, the species within the *B. pseudomallei* group collectively encode the largest capacity for secondary metabolite biosynthesis (>11 % of their genomes). Evidence for significant antibiotic biosynthetic capacity is also present within the Bcc, with *B. ambifaria* currently leading the group, devoting over 9 % of its genome to secondary metabolism (Table 1). *B. gladioli* and *B. glumae* also dedicate 10 % or more of their genomes to antibiotic biosynthesis (Table 1). The potential of *Burkholderia* species belonging to the *B. glathei* and *B. xenovorans* clades (Fig. 1 and 2) appears less impressive, with fewer pathways and less than 5 % of their genomes in general being dedicated to antibiotic production (Table 1). However, within the latter group, fewer genomes have been characterised to date and the genomic distance between species is substantial as demonstrated by their deep-branching phylogenies, suggesting that greater diversity within these groups still remains to be characterised. *B. rhizoxinica* is an outlier, both in terms of its phylogenetic position (Figs. 1 and 2), as well as its capacity for antibiotic biosynthesis, which is substantial at 12.9 % of its relatively small endosymbiotic-adapted genome (Table 1).

In addition to the potential for the biosynthesis of antimicrobial compounds such as polyketide antibiotics and NRPS products, another highly conserved feature, observed across all *Burkholderia* genomes, is the significant potential for terpene production (Table 1). The well-known interactions of *Burkholderia* with plants (Suarez-Moreno et al. 2012) and the intrinsic ability of these bacteria to colonise the rhizosphere (Vidal-Quist et al. 2014) suggest that terpene biosynthesis and the potential interplay of these molecules during bacteria-plant interactions are areas worthy of future biotechnological exploration. Finally, another interesting feature of *Burkholderia* genomes is their organisation in a multireplicon structure. Species within the Bcc have a three replicon genome (Table 1) and can tolerate deletion of the smallest replicon (Agnoli et al. 2012). This results in strains which are highly attenuated in virulence as well as antibiotic production, especially in the case of *B. ambifaria* AMMD^T, where this deletion results in the loss of the enacyloxin pathway, encoded on the third replicon (Mahenthiralingam et al. 2011). The ability to colonise the rhizosphere is not affected by deletion of this third chromosome in Bcc strains (Vidal-Quist et al. 2014), suggesting that in the future, it may be possible to engineer biological control strains that contain the antimicrobial pathways required for biopesticidal activity but lack the virulence pathways associated with *Burkholderia* pathogenicity.

Conclusion

Burkholderia continue to fascinate as a diverse group of Gram-negative bacteria. They are arguably better known as primary pathogens such as *B. pseudomallei*, opportunistic pathogens such as members of the *B. cepacia* complex and plant pathogens such as *B. glumae*. However, the diverse environmental interactions of these bacteria are now pointing towards multiple beneficial properties extending beyond their known capacities for bioremediation and biological control, towards the significant biotechnological potential as antibiotic

Table 1 Genomic capacity for antibiotic biosynthesis in *Burkholderia*

Species	Strain (BioProject Accession)	Replicons	Genome size (Mbp)	Total secondary metabolism gene cluster size in basepair (number of clusters)	Genome % dedicated to secondary metabolism	Mean cluster size in bp (number of clusters)[a]							
						PKS		NRPS		Terpene synthase		Homoserine lactone synthase	
B. cepacia complex													
B. ambifaria	AMMD^T [PRJNA13490]	3	7.5	685,471 (19)	9.2	80,146.7 [enacyloxin IIa]	(3)	50,764.5 [occidiofungin]	(2)	22,011.0	(4)	20,607.5	(2)
B. cenocepacia	J2315^T [PRJNA339]	3	8.0	401,215 (13)	5.0	47,650.0	(1)	50,470.3	(3)	21,241.4	(5)	20,600.0	(2)
B. lata	383^T [PRJNA10695]	3	8.7	398,723 (13)	4.6	47,638.0	(1)	53,738.5	(2)	22,009.3	(4)	20,609.0	(1)
B. vietnamiensis	G4 [PRJNA10696]	3	7.3	235,114 (9)	3.2	0.0	(0)	54,724.0	(1)	22,000.3	(3)	20,629.0	(3)
B. multivorans	ATCC 17616 [PRJNA17407]	3	6.8	396,981 (13)	5.8	47,632.0	(1)	54,646.0	(1)	22,941.0	(5)	27,860.0	(1)
B. pseudomallei group													
B. mallei	NCTC 10229 [PRJNA13943]	2	5.7	708,969 (17)	12.3	72,748.0	(4)	54,930.3	(3)	20,809.0	(3)	20,610.5	(2)
B. pseudomallei	K96243 [PRJNA178]	2	7.2	1,012,801 (22)	14.0	76,664.2 [malleilactone]	(5)	60,664.4	(5)	20,957.0	(2)	20,610.5	(2)
'B. humptydooensis'	MSMB121 [PRJNA178701]	2	6.7	764,620 (17)	11.4	72,212.8	(6)	77,199.0	(1)	20,943.5	(2)	20,610.5	(2)
B. gladioli	BSR3 [PRJNA64503]	2	8.1	852,292 (20)	10.5	62,266.3	(3)	62,964.2	(6)	20,792.8	(4)	20,615.0	(1)
B. glumae	BGR1 [PRJNA33901]	2	6.7	642,600 (15)	9.5	67,284.0	(5)	64,370.5	(2)	20,932.0	(4)	20,612.0	(1)
B. xenovorans group													
B. phenoliruptrix	BR3459a [PRJNA174166]	2	6.9	237,360 (7)	3.5	0.0	(0)	65,879.0	(1)	22,022.0	(3)	20,594.0	(1)
B. phytofirmans	PsJN^T [PRJNA17463]	2	8.1	267,045 (9)	3.3	0.0	(0)	54,752.0	(1)	25,141.0	(3)	20,567.0	(2)
B. phymatum	STM815^T [PRJNA17409]	2	6.2	244,239 (8)	4.0	50,476.0	(1)	54,618.0	(1)	22,015.3	(3)	20,588.0	(1)
B. glathei group													
B. cordobensis	YI23 [PRJNA74517]	3	6.5	285,281 (7)	4.4	0.0	(0)	62,610.3	(3)	22,474.0	(2)	0.0	(0)
B. rhizoxinica	HKI 454^T [PRJNA74517]	1	2.8	355,337 (7)	12.9	118,192.0 [rhizoxin]	(1)	58,284.3	(3)	20,946.5	(2)	0.0	(0)
	Genus mean		6.9	(13)	7.57	49,527.3	(2.0)	58,707.7	(2.3)	21,815.7	(3.3)	18,340.9	(1.0)

Whole genome sequences were downloaded from NCBI (www.ncbi.nlm.nih.gov/) and analysed using antiSMASH 2.1.1 (Blin et al. 2013)

PKS polyketide synthase, *NRPS* nonribosomal peptide synthetase

[a] Names of characterised products of secondary metabolite gene clusters identified in genome sequences are given between square brackets

producers, as illustrated within this review. For most antimicrobial metabolites, the function in the natural environment is still not known. However, with insights into the ecological relevance of antibiotics, we could take advantage of the multifunctionality of these natural products, although positive exploitation of *Burkholderia* as biotechnological agents will have to balance against their potential pathogenicity. With the ability to rapidly define the entire functional content of *Burkholderia* strains using genomics, future exploitation of these organisms for biotechnological purposes will be greatly accelerated.

Acknowledgments MB acknowledges funding from the Cardiff University Synthetic Biology initiative. CP is indebted to the Special Research Council of Ghent University. TC acknowledges funding from the Fund for Scientific Research-Flanders and the Interuniversity Attraction Poles Programme initiated by the Belgian Science Policy Office. EM is a recipient of funding from the Biotechnology and Biological Sciences Research Council (Grant BB/L021692/1). PV and TC acknowledge funding from the Industrial Research Fund of Ghent University (Grant F2015/IOF-ConcepTT/142).

Compliance with ethical standards

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no conflict of interest.

References

Agnoli K, Schwager S, Uehlinger S, Vergunst A, Viteri DF, Nguyen DT, Sokol PA, Carlier A, Eberl L (2012) Exposing the third chromosome of *Burkholderia cepacia* complex strains as a virulence plasmid. Mol Microbiol 83:362–378. doi:10.1111/j.1365-2958.2011.07937.x

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. doi:10.1371/journal.pone.0083779

Azegami K, Nishiyama K, Watanabe Y, Kadota I, Ohuchi A, Fukazawa C (1987) *Pseudomonas plantarii* sp. nov., the causal agent of rice seedling blight. Int J Syst Bacteriol 37:144–152. doi:10.1099/00207713-37-2-144

Ballard RW, Palleroni NJ, Doudoroff M, Stanier RY, Mandel M (1970) Taxonomy of the aerobic pseudomonads: *Pseudomonas cepacia*, *P. marginata*, *P. alliicola* and *P. caryophylli*. J Gen Microbiol 60:199–214. doi:10.1099/00221287-60-2-199

Bharti P, Anand V, Chander J, Singh IP, Singh TV, Tewari R (2012) Heat stable antimicrobial activity of *Burkholderia gladioli* OR1 against clinical drug resistant isolates. Indian J Med Res 135:666–671

Biggins JB, Liu X, Feng Z, Brady SF (2011) Metabolites from the induced expression of cryptic single operons found in the genome of *Burkholderia pseudomallei*. J Am Chem Soc 133:1638–1641. doi:10.1021/ja1087369

Biggins JB, Ternei MA, Brady SF (2012) Malleilactone, a polyketide synthase-derived virulence factor encoded by the cryptic secondary metabolome of *Burkholderia pseudomallei* group pathogens. J Am Chem Soc 134:13192–13195. doi:10.1021/ja3052156

Blin K, Medema MH, Kazempour D, Fischbach MA, Breitling R, Takano E, Weber T (2013) antiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res 41:W204–W212. doi:10.1093/nar/gkt449

Boon C, Deng Y, Wang L-H, He Y, Xu J-L, Fan Y, Pan SQ, Zhang L-H (2008) A novel DSF-like signal from *Burkholderia cenocepacia* interferes with *Candida albicans* morphological transition. ISME J 2:27–36. doi:10.1038/ismej.2007.76

Bowers JH, Parke JL (1993) Epidemiology of *Pythium* damping-off and *Aphanomyces* root rot of peas after seed treatment with bacterial agents for biological control. Phytopathology 83:1466. doi:10.1094/Phyto-83-1466

Burkhead KD, Schisler DA, Slininger PJ (1994) Pyrrolnitrin production by biological control agent *Pseudomonas cepacia* B37w in culture and in colonized wounds of potatoes. Appl Environ Microbiol 60:2031–2039

Carlier AL, Eberl L (2012) The eroded genome of a *Psychotria* leaf symbiont: hypotheses about lifestyle and interactions with its plant host. Environ Microbiol 14:2757–2769. doi:10.1111/j.1462-2920.2012.02763.x

Cartwright DK, Chilton WS, Benson DM (1995) Pyrrolnitrin and phenazine production by *Pseudomonas cepacia*, strain 5.5B, a biocontrol agent of *Rhizoctonia solani*. Appl Microbiol Biotechnol 43:211–216. doi:10.1007/BF00172814

Chapalain A, Vial L, Laprade N, Dekimpe V, Perreault J, Déziel E (2013) Identification of quorum sensing-controlled genes in *Burkholderia ambifaria*. Microbiologyopen 2:226–242. doi:10.1002/mbo3.67

Chen R, Barphagha IK, Karki HS, Ham JH (2012) Dissection of quorum-sensing genes in *Burkholderia glumae* reveals non-canonical regulation and the new regulatory gene *tofM* for toxoflavin production. PLoS One 7:e52150. doi:10.1371/journal.pone.0052150

Cimermancic P, Medema MH, Claesen J, Kurita K, Wieland Brown LC, Mavrommatis K, Pati A, Godfrey PA, Koehrsen M, Clardy J, Birren BW, Takano E, Sali A, Linington RG, Fischbach MA (2014) Insights into secondary metabolism from a global analysis of prokaryotic biosynthetic gene clusters. Cell 158:412–421. doi:10.1016/j.cell.2014.06.034

Coenye T, Holmes B, Kersters K, Govan JRW, Vandamme P (1999) *Burkholderia cocovenenans* (van Damme et al. 1960) Gillis et al. 1995 and *Burkholderia vandii* Urakami et al. 1994 are junior synonyms of *Burkholderia gladioli* (Severini 1913) Yabuuchi et al. 1993 and *Burkholderia plantarii* (Azegami et al. 1987) Urakami et al. 1994, respectively. Int J Syst Bacteriol 49:37–42. doi:10.1099/00207713-49-1-37

Coenye T, Falsen E, Hoste B, Ohlen M, Goris J, Govan J, Gillis M, Vandamme P (2000) Description of *Pandoraea* gen. nov. with *Pandoraea apista* sp. nov., *Pandoraea pulmonicola* sp. nov., *Pandoraea pnomenusa* sp. nov., *Pandoraea sputorum* sp. nov. and *Pandoraea norimbergensis* comb. nov. Int J Syst Evol Microbiol 50:887–899. doi:10.1099/00207713-50-2-887

Coenye T, Laevens S, Gillis M, Vandamme P (2001a) 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. doi:10.1099/00207713-51-1-183

Coenye T, Laevens S, Willems A, Ohlen M, Hannant W, Govan J, Gillis M, Falsen E, Vandamme P (2001b) *Burkholderia fungorum* sp. nov. and *Burkholderia caledonica* sp. nov., two new species isolated from the environment, animals and human clinical samples. Int J Syst Evol Microbiol 51:1099–1107. doi:10.1099/00207713-51-3-1099

Coenye T, Goris J, Spilker T, Lipuma JJ, Vandamme P (2002) Characterization of unusual bacteria isolated from respiratory secretions of cystic fibrosis patients and description of *Inquilinus limosus* gen. nov., sp. nov. J Clin Microbiol 40:2062–2069. doi:10.1128/JCM.40.6.2062

Conway B-A, Greenberg EP (2002) Quorum-sensing signals and quorum-sensing genes in *Burkholderia vietnamiensis*. J Bacteriol 184:1187–1191. doi:10.1128/jb.184.4.1187-1191.2002

Coutinho BG, Mitter B, Talbi C, Sessitsch A, Bedmar EJ, Halliday N, James EK, Camara M, Venturi V (2013) Regulon studies and *in planta* role of the BraI/R quorum-sensing system in the plant-beneficial *Burkholderia* cluster. Appl Environ Microbiol 79:4421–4432. doi:10.1128/AEM.00635-13

Currie B (2015) Melioidosis: evolving concepts in epidemiology, pathogenesis, and treatment. Semin Respir Crit Care Med 36:111–125. doi:10.1055/s-0034-1398389

Deng Y, Wu J, Eberl L, Zhang L-H (2010) Structural and functional characterization of diffusible signal factor family quorum-sensing signals produced by members of the *Burkholderia cepacia* complex. Appl Environ Microbiol 76:4675–4683. doi:10.1128/AEM.00480-10

Deris ZZ, Van Rostenberghe H, Habsah H, Noraida R, Tan GC, Chan YY, Rosliza AR, Ravichandran M (2010) First isolation of *Burkholderia tropica* from a neonatal patient successfully treated with imipenem. Int J Infect Dis 14:e73–e74. doi:10.1016/j.ijid.2009.03.005

Duerkop BA, Varga J, Chandler JR, Peterson SB, Herman JP, Churchill ME, Parsek MR, Nierman WC, Greenberg EP (2009) Quorum-sensing control of antibiotic synthesis in *Burkholderia thailandensis*. J Bacteriol 191:3909–3918. doi:10.1128/JB.00200-09

El-Banna N, Winkelmann G (1998) Pyrrolnitrin from *Burkholderia cepacia*: antibiotic activity against fungi and novel activities against streptomycetes. J Appl Microbiol 85:69–78. doi:10.1046/j.1365-2672.1998.00473.x

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. doi:10.1007/s00284-013-0330-9

Estrada-de los Santos P, Rojas-Rojas FU, Tapia-García EY, Vásquez-Murrieta MS, Hirsch AM (2015) To split or not to split: an opinion on dividing the genus *Burkholderia*. Ann Microbiol. doi:10.1007/s13213-015-1183-1

Franke J, Ishida K, Hertweck C (2012) Genomics-driven discovery of burkholderic acid, a noncanonical, cryptic polyketide from human pathogenic *Burkholderia* species. Angew Chemie - Int Ed 51:11611–11615. doi:10.1002/anie.201205566

Garrity GM, Oren A (2015) List of new names and new combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol 65:2017–2025. doi:10.1099/ijs.0.000317

Gerrits GP, Klaassen C, Coenye T, Vandamme P, Meis JF (2005) *Burkholderia fungorum* septicemia. Emerg Infect Dis 11:1115–1117. doi:10.3201/eid1107.041290

Groenhagen U, Baumgartner R, Bailly A, Gardiner A, Eberl L, Schulz S, Weisskopf L (2013) Production of bioactive volatiles by different *Burkholderia ambifaria* strains. J Chem Ecol 39:892–906. doi:10.1007/s10886-013-0315-y

Groll M, Schellenberg B, Bachmann AS, Archer CR, Huber R, Powell TK, Lindow S, Kaiser M, Dudler R (2008) A plant pathogen virulence factor inhibits the eukaryotic proteasome by a novel mechanism. Nature 452:755–758. doi:10.1038/nature06782

Gyaneshwar P, Hirsch AM, Moulin L, Chen W-M, 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-Microbe Interact 24:1276–1288. doi:10.1094/MPMI-06-11-0172

Heungens K, Parke JL (2000) Zoospore homing and infection events: effects of the biocontrol bacterium *Burkholderia cepacia* AMMDR1 on two oomycete pathogens of pea (*Pisum sativum* L.). Appl Environ Microbiol 66:5192–5200. doi:10.1128/AEM.66.12.5192-5200.2000

Heungens K, Parke JL (2001) Postinfection biological control of oomycete pathogens of pea by *Burkholderia cepacia* AMMDR1. Phytopathology 91:383–391. doi:10.1094/PHYTO.2001.91.4.383

Ho Y-N, Huang C-C (2015) Draft genome sequence of *Burkholderia cenocepacia* strain 869T2, a plant-beneficial endophytic bacterium. Genome Announc 3:e01327–15. doi:10.1128/genomeA.01327-15

Hwang J, Chilton W, Benson D (2002) Pyrrolnitrin production by *Burkholderia cepacia* and biocontrol of *Rhizoctonia* stem rot of poinsettia. Biol Control 25:56–63. doi:10.1016/S1049-9644(02)00044-0

Ishida K, Lincke T, Behnken S, Hertweck C (2010) Induced biosynthesis of cryptic polyketide metabolites in a *Burkholderia thailandensis* quorum sensing mutant. J Am Chem Soc 132:13966–13968. doi:10.1021/ja105003g

Jeong Y, Kim J, Kim S, Kang Y, Nagamatsu T, Hwang I (2003) Toxoflavin produced by *Burkholderia glumae* causing rice grain rot is responsible for inducing bacterial wilt in many field crops. Plant Dis 87:890–895. doi:10.1094/PDIS.2003.87.8.890

Jiao Y, Yoshihara T, Ishikuri S, Uchino H, Ichihara A (1996) Structural identification of cepaciamide A, a novel fungitoxic compound from *Pseudomonas cepacia* D-202. Tetrahedron Lett 37:1039–1042. doi:10.1016/0040-4039(95)02342-9

Jolley KA, Maiden MC (2010) BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics 11:595. doi:10.1186/1471-2105-11-595

Jolley KA, Bliss CM, Bennett JS, Bratcher HB, Brehony C, Colles FM, Wimalarathna H, Harrison OB, Sheppard SK, Cody AJ, Maiden MCJ (2012) Ribosomal multilocus sequence typing: universal characterization of bacteria from domain to strain. Microbiology 158:1005–1015. doi:10.1099/mic.0.055459-0

Keum YS (2009) Effects of nutrients on quorum signals and secondary metabolite productions of *Burkholderia* sp. O33. J Microbiol Biotechnol. doi:10.4014/jmb.0901.465

Kikuchi Y, Hosokawa T, Fukatsu T (2011) An ancient but promiscuous host–symbiont association between *Burkholderia* gut symbionts and their heteropteran hosts. ISME J 5:446–460. doi:10.1038/ismej.2010.150

Kim J, Kim JG, Kang Y, Jang JY, Jog GJ, Lim JY, Kim S, Suga H, Nagamatsu T, Hwang I (2004) Quorum sensing and the LysR-type transcriptional activator ToxR regulate toxoflavin biosynthesis and transport in *Burkholderia glumae*. Mol Microbiol 54:921–934. doi:10.1111/j.1365-2958.2004.04338.x

Kirinuki T, Ichiba T, Katayama K (1984) General survey of action site of altericidins on metabolism of *Alternaria kikuchiana* and *Ustilago maydis*. J Pestic Sci 9:601–610. doi:10.1584/jpestics.9.601

Knappe TA, Linne U, Zirah S, Rebuffat S, Xie X, Marahiel MA (2008) Isolation and structural characterization of capistruin, a lasso peptide predicted from the genome sequence of *Burkholderia thailandensis* E264. J Am Chem Soc 130:11446–11454. doi:10.1021/ja802966g

Lemaire B, Robbrecht E, van Wyk B, Van Oevelen S, Verstraete B, Prinsen E, Smets E, Dessein S (2011) Identification, origin, and evolution of leaf nodulating symbionts of *Sericanthe* (*Rubiaceae*). J Microbiol 49:935–941. doi:10.1007/s12275-011-1163-5

Lemaire B, van Oevelen S, de Block P, Verstraete B, Smets E, Prinsen E, Dessein S (2012) Identification of the bacterial endosymbionts in leaf nodules of *Pavetta* (*Rubiaceae*). Int J Syst Evol Microbiol 62:202–209. doi:10.1099/ijs.0.028019-0

Lewenza S, Conway B, Greenberg EP, Sokol PA (1999) Quorum sensing in *Burkholderia cepacia*: identification of the LuxRI homologs CepRI. J Bacteriol 181:748–756

Lim YW, Baik KS, Han SK, Kim SB, Bae KS (2003) *Burkholderia sordidicola* sp. nov., isolated from the white-rot fungus *Phanerochaete sordida*. Int J Syst Evol Microbiol 53:1631–1636. doi:10.1099/ijs.0.02456-0

Lipuma JJ (2010) The changing microbial epidemiology in cystic fibrosis. Clin Microbiol Rev 23:299–323. doi:10.1128/CMR.00068-09

Liu X, Cheng Y-QQ (2014) Genome-guided discovery of diverse natural products from *Burkholderia* sp. J Ind Microbiol Biotechnol 41:275–284. doi:10.1007/s10295-013-1376-1

Liu X-Y, Li C-X, Luo X-J, Lai Q-L, Xu J-H (2014) *Burkholderia jiangsuensis* sp. nov., a methyl parathion degrading bacterium, isolated from methyl parathion contaminated soil. Int J Syst Evol Microbiol 64:3247–3253. doi:10.1099/ijs.0.064444-0

Lu S-E, Novak J, Austin FW, Gu G, Ellis D, Kirk M, Wilson-Stanford S, Tonelli M, Smith L (2009) Occidiofungin, a unique antifungal glycopeptide produced by a strain of *Burkholderia contaminans*. Biochemistry 48:8312–8321. doi:10.1021/bi900814c

Lu P, Zheng L-Q, Sun J-J, Liu H-M, Li S-P, Hong Q, Li W-J (2012) *Burkholderia zhejiangensis* sp. nov., a methyl-parathion-degrading bacterium isolated from a wastewater-treatment system. Int J Syst Evol Microbiol 62:1337–1341. doi:10.1099/ijs.0.035428-0

Mahenthiralingam E, Song L, Sass A, White J, Wilmot C, Marchbank A, Boaisha O, Paine J, Knight D, Challis GL (2011) Enacyloxins are products of an unusual hybrid modular polyketide synthase encoded by a cryptic *Burkholderia ambifaria* genomic island. Chem Biol 18:665–677. doi:10.1016/j.chembiol.2011.01.020

Majerczyk CD, Brittnacher MJ, Jacobs MA, Armour CD, Radey MC, Bunt R, Hayden HS, Bydalek R, Greenberg EP (2014) Cross-species comparison of the *Burkholderia pseudomallei*, *Burkholderia thailandensis*, and *Burkholderia mallei* quorum-sensing regulons. J Bacteriol 196:3862–3871. doi:10.1128/JB.01974-14

Malott RJ, Baldwin A, Mahenthiralingam E, Sokol PA (2005) Characterization of the *cciIR* quorum-sensing system in *Burkholderia cenocepacia*. Infect Immun 73:4982–4992. doi:10.1128/IAI.73.8.4982

Meyers E, Bisacchi GS, Dean L, Liu WC, Minassian B, Slusarchyk DS, Sykes RB, Tanaka SK, Trejo W (1987) Xylocandin: a new complex of antifungal peptides. I. Taxonomy, isolation and biological activity. J Antibiot (Tokyo) 40:1515–1519. doi:10.7164/antibiotics.40.1515

Mitchell RE, Greenwood DR, Sarojini V (2008) An antibacterial pyrazole derivative from *Burkholderia glumae*, a bacterial pathogen of rice. Phytochemistry 69:2704–2707. doi:10.1016/j.phytochem.2008.08.013

Moebius N, Ross C, Scherlach K, Rohm B, Roth M, Hertweck C (2012) Biosynthesis of the respiratory toxin bongkrekic acid in the pathogenic bacterium *Burkholderia gladioli*. Chem Biol 19:1164–1174. doi:10.1016/j.chembiol.2012.07.022

Moon SS, Kang PM, Park KS, Kim CH (1996) Plant growth promoting and fungicidal 4-quinolinones from *Pseudomonas cepacia*. Phytochemistry 42:365–368. doi:10.1016/0031-9422(95)00897-7

Nguyen T, Ishida K, Jenke-Kodama H, Dittmann E, Gurgui C, Hochmuth T, Taudien S, Platzer M, Hertweck C, Piel J (2008) Exploiting the mosaic structure of trans-acyltransferase polyketide synthases for natural product discovery and pathway dissection. Nat Biotechnol 26:225–233. doi:10.1038/nbt1379

Parke JL (1990) Population dynamics of *Pseudomonas cepacia* in the pea spermosphere in relation to biocontrol of *Pythium*. Phytopathology 80:1307. doi:10.1094/Phyto-80-1307

Parke JL, Gurian-sherman D (2001) Diversity of the *Burkholderia cepacia* complex and implications for risk assessment of biological control strains. Annu Rev Phytopathol 39:225–258. doi:10.1146/annurev.phyto.39.1.225

Parker WL, Rathnum ML, Seiner V, Trejo WH, Principe PA, Sykes RB (1984) Cepacin A and cepacin B, two new antibiotics produced by *Pseudomonas cepacia*. J Antibiot (Tokyo) 37:431–440

Parte AC (2014) LPSN—list of prokaryotic names with standing in nomenclature. Nucleic Acids Res 42:D613–D616. doi:10.1093/nar/gkt1111

Partida-Martinez LP, Hertweck C (2005) Pathogenic fungus harbours endosymbiotic bacteria for toxin production. Nature 437:884–888. doi:10.1038/nature03997

Partida-Martinez LP, Groth I, Schmitt I, Richter W, Roth M, Hertweck C (2007) *Burkholderia rhizoxinica* sp. nov. and *Burkholderia endofungorum* sp. nov., bacterial endosymbionts of the plant-pathogenic fungus *Rhizopus microsporus*. Int J Syst Evol Microbiol 57:2583–2590. doi:10.1099/ijs.0.64660-0

Peeters C, Zlosnik JEA, Spilker T, Hird TJ, LiPuma JJ, Vandamme P (2013) *Burkholderia pseudomultivorans* sp. nov., a novel *Burkholderia cepacia* complex species from human respiratory samples and the rhizosphere. Syst Appl Microbiol 36:483–489. doi:10.1016/j.syapm.2013.06.003

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. doi:10.1111/j.1462-2920.2011.02613.x

Price MN, Dehal PS, Arkin AP (2010) FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One 5:e9490. doi:10.1371/journal.pone.0009490

Pruesse E, Peplies J, Glockner FO (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823–1829. doi:10.1093/bioinformatics/bts252

Ross C, Opel V, Scherlach K, Hertweck C (2014) Biosynthesis of antifungal and antibacterial polyketides by *Burkholderia gladioli* in co-culture with *Rhizopus microsporus*. Mycoses 57:48–55. doi:10.1111/myc.12246

Ryan RP, McCarthy Y, Watt SA, Niehaus K, Dow JM (2009) Intraspecies signaling involving the diffusible signal factor BDSF (cis-2-dodecenoic acid) influences virulence in *Burkholderia cenocepacia*. J Bacteriol 191:5013–5019. doi:10.1128/JB.00473-09

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 Genet 5:1–22. doi:10.3389/fgene.2014.00429

Schellenberg B, Bigler L, Dudler R (2007) Identification of genes involved in the biosynthesis of the cytotoxic compound glidobactin from a soil bacterium. Environ Microbiol 9:1640–1650. doi:10.1111/j.1462-2920.2007.01278.x

Scherlach K, Busch B, Lackner G, Paszkowski U, Hertweck C (2012) Symbiotic cooperation in the biosynthesis of a phytotoxin. Angew Chemie Int Ed 51:9615–9618. doi:10.1002/anie.201204540

Schmidt S, Blom JF, Pernthaler J, Berg G, Baldwin A, Mahenthiralingam E, Eberl L (2009) Production of the antifungal compound pyrrolnitrin is quorum sensing-regulated in members of the *Burkholderia cepacia* complex. Environ Microbiol 11:1422–1437. doi:10.1111/j.1462-2920.2009.01870.x

Seyedsayamdost MR, Chandler JR, Blodgett JAV, Lima PS, Duerkop BA, Oinuma K-I, Greenberg EP, Clardy J (2010) Quorum-sensing-regulated bactobolin production by *Burkholderia thailandensis* E264. Org Lett 12:716–719. doi:10.1021/ol902751x

Sheu S-Y, Chou J-H, Bontemps C, Elliott GN, Gross E, James EK, Sprent JI, Young JPW, Chen W-M (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. doi:10.1099/ijs.0.037408-0

Shibata TF, Maeda T, Nikoh N, Yamaguchi K, Oshima K, Hattori M, Nishiyama T, Hasebe M, Fukatsu T, Kikuchi Y, Shigenobu S (2013)

Complete genome sequence of *Burkholderia* sp. strain RPE64, bacterial symbiont of the bean bug *Riptortus pedestris*. Genome Announc 1:e00441–13. doi:10.1128/genomeA.00441-13

Sokol PA, Malott RJ, Riedel K, Eberl L (2007) Communication systems in the genus *Burkholderia*: global regulators and targets for novel antipathogenic drugs. Future Microbiol 2:555–563. doi:10.2217/17460913.2.5.555

Solis R, Bertani I, Degrassi G, Devescovi G, Venturi V (2006) Involvement of quorum sensing and RpoS in rice seedling blight caused by *Burkholderia plantarii*. FEMS Microbiol Lett 259:106–112. doi:10.1111/j.1574-6968.2006.00254.x

Suarez-Moreno ZR, Caballero-Mellado J, Venturi V (2008) The new group of non-pathogenic plant-associated nitrogen-fixing *Burkholderia* spp. shares a conserved quorum-sensing system, which is tightly regulated by the RsaL repressor. Microbiology 154:2048–2059. doi:10.1099/mic.0.2008/017780-0

Suarez-Moreno ZR, Caballero-Mellado J, Coutinho BG, Mendonça-Previato L, James EK, Venturi V (2012) Common features of environmental and potentially beneficial plant-associated *Burkholderia*. Microb Ecol 63:249–266. doi:10.1007/s00248-011-9929-1

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol 56:564–577. doi:10.1080/10635150701472164

Tamura K, Nei M (1993) Estimation of the number of base nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. doi:10.1093/molbev/mst197

Tawfik KA, Jeffs P, Bray B, Dubay G, Falkinham JO, Mesbah M, Youssef D, Khalifa S, Schmidt EW (2010) Burkholdines 1097 and 1229, potent antifungal peptides from *Burkholderia ambifaria* 2.2N. Org Lett 12:664–666. doi:10.1021/ol9029269

Thomson ELS, Dennis JJ (2012) A *Burkholderia cepacia* complex non-ribosomal peptide-synthesized toxin is hemolytic and required for full virulence. Virulence 3:286–298. doi:10.4161/viru.19355

Tian Y, Kong BH, Liu SL, Li CL, Yu R, Liu L, Li YH (2013) *Burkholderia grimmiae* sp. nov., isolated from a xerophilous moss (*Grimmia montana*). Int J Syst Evol Microbiol 63:2108–3113. doi:10.1099/ijs.0.045492-0

Torbeck L, Raccasi D, Guilfoyle DE, Friedman RL, Hussong D (2011) *Burkholderia cepacia*: this decision is overdue. PDA J Pharm Sci Technol 65:535–543. doi:10.5731/pdajpst.2011.00793

Tran Van V, Berge O, Ngo Ke S, 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(2):273–284. doi:10.1023/A:1014986916913

Truong TT, Seyedsayamdost M, Greenberg EP, Chandler JR (2015) A *Burkholderia thailandensis* acyl-homoserine lactone-independent orphan LuxR homolog that activates production of the cytotoxin malleilactone. J Bacteriol 197:3456–3462. doi:10.1128/JB.00425-15

van Oevelen S, de Wachter R, Vandamme P, Robbrecht E, Prinsen E (2004) "Candidatus Burkholderia calva" and "Candidatus Burkholderia nigropunctata" as leaf gall endosymbionts of African *Psychotria*. Int J Syst Evol Microbiol 54:2237–2239. doi:10.1099/ijs.0.63188-0

Vandamme P, Peeters C (2014) Time to revisit polyphasic taxonomy. Antonie Van Leeuwenhoek 106:57–65. doi:10.1007/s10482-014-0148-x

Vandamme P, De Brandt E, Houf K, Salles JF, Dirk van Elsas J, 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. doi:10.1099/ijs.0.048900-0

Vanlaere E, Baldwin A, Gevers D, Henry D, De Brandt E, LiPuma JJ, Mahenthiralingam E, Speert DP, Dowson C, Vandamme P (2009) Taxon K, a complex within the *Burkholderia cepacia* complex, comprises at least two novel species, *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. nov. Int J Syst Evol Microbiol 59:102–111. doi:10.1099/ijs.0.001123-0

Verstraete B, Van Elst D, Steyn H, Van Wyk B, Lemaire B, Smets E, Dessein S (2011) Endophytic bacteria in toxic south African plants: identification, phylogeny and possible involvement in gousiekte. PLoS One 6:e19265. doi:10.1371/journal.pone.0019265

Verstraete B, Janssens S, Smets E, Dessein S (2013) Symbiotic ß-proteobacteria beyond legumes: *Burkholderia* in *Rubiaceae*. PLoS One 8:e55260. doi:10.1371/journal.pone.0055260

Vial L, Groleau M-C, Dekimpe V, Déziel E (2007) *Burkholderia* diversity and versatility: an inventory of the extracellular products. J Microbiol Biotechnol 17:1407–1429

Vial L, Chapalain A, Groleau MC, Déziel E (2011) The various lifestyles of the *Burkholderia cepacia* complex species: a tribute to adaptation. Environ Microbiol 13:1–12. doi:10.1111/j.1462-2920.2010.02343.x

Vidal-Quist JC, O'Sullivan LA, Desert A, Fivian-Hughes AS, Millet C, Jones TH, Weightman AJ, Rogers HJ, Berry C, Mahenthiralingam E (2014) *Arabidopsis thaliana* and *Pisum sativum* models demonstrate that root colonization is an intrinsic trait of *Burkholderia cepacia* complex bacteria. Microbiology 160:373–384. doi:10.1099/mic.0.074351-0

Wang Z, Wu M (2013) A phylum-level bacterial phylogenetic marker database. Mol Biol Evol 30:1258–1262. doi:10.1093/molbev/mst059

Wang C, Henkes LM, Doughty LB, He M, Wang D, Meyer-Almes F-J, Cheng Y-Q (2011) Thailandepsins: bacterial products with potent histone deacetylase inhibitory activities and broad-spectrum antiproliferative activities. J Nat Prod 74:2031–2038. doi:10.1021/np200324x

Wang M, Hashimoto M, Hashidoko Y (2013) Repression of tropolone production and induction of a *Burkholderia plantarii* pseudobiofilm by carot-4-en-9,10-diol, a cell-to-cell signaling disrupter produced by *Trichoderma virens*. PLoS One 8:e78024. doi:10.1371/journal.pone.0078024

Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Muller R, Wohlleben W, Breitling R, Takano E, Medema MH (2015) antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 1–7. doi: 10.1093/nar/gkv437

Whitehead NA, Barnard AML, Slater H, Simpson NJL, Salmond GPC (2001) Quorum-sensing in Gram-negative bacteria. FEMS Microbiol Rev 25:365–404. doi:10.1111/j.1574-6976.2001.tb00583.x

Wilson MS, Herrick JB, Jeon CO, Hinman DE, Madsen EL (2003) Horizontal transfer of *phnAc* dioxygenase genes within one of two phenotypically and genotypically distinctive naphthalene-degrading guilds from adjacent soil environments. Appl Environ Microbiol 69:2172–2181. doi:10.1128/AEM.69.4.2172-2181.2003

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. doi:10.1111/j.1348-0421.1992.tb02129.x

Yabuuchi E, Kosako Y, Yano I, Hotta H, Nishiuchi Y (1995) Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. Microbiol Immunol 39:897–904. doi:10.1111/j.1348-0421.1995.tb03275.x

Yoo S-H, Kim B-Y, Weon H-Y, Kwon S-W, Go S-J, Stackebrandt E (2007) *Burkholderia soli* sp. nov., isolated from soil cultivated with Korean ginseng. Int J Syst Evol Microbiol 57:122–125. doi:10.1099/ijs.0.64471-0

Yutin N, Puigbò P, Koonin EV, Wolf YI (2012) Phylogenomics of prokaryotic ribosomal proteins. PLoS One 7:e36972. doi:10.1371/journal.pone.0036972

Zerikly M, Challis GL (2009) Strategies for the discovery of new natural products by genome mining. ChemBioChem 10:625–633. doi:10.1002/cbic.200800389

Zhou H, Yao F, Roberts DP, Lessie TG (2003) AHL-deficient mutants of *Burkholderia ambifaria* BC-F have decreased antifungal activity. Curr Microbiol 47:174–179. doi:10.1007/s00284-002-3926-z

Zolg W, Ottow JCG (1975) *Pseudomonas glathei* sp. nov., a new nitrogen-scavenging rod isolated from acid lateritic relicts in Germany. Z Allg Mikrobiol 15:287–299. doi:10.1002/jobm.19750150410