Original article

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rpsU-BASED DISCRIMINATION WITHIN THE GENUS *BURKHOLDERIA*

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Sequencing of the gene *rpsU* reliably delineates saprophytic *Burkholderia* (*B.*) *thailandensis* from highly pathogenic *B. mallei* and *B. pseudomallei*. We analyzed the suitability of this technique for the delineation of the *B. pseudomallei* complex from other *Burkholderia* species.

Both newly recorded and previously deposited sequences of well-characterized or reference strains (n = 84) of *Azoarcus* spp., *B. ambifaria*, *B. anthina*, *B. caledonica*, *B. caribensis*, *B. caryophylli*, *B. cenocepacia*, *B. cepacia*, *B. cocovenenans*, *B. dolosa*, *B. fungorum*, *B. gladioli*, *B. glathei*, *B. glumae*, *B. graminis*, *B. hospita*, *B. kururensis*, *B. mallei*, *B. multivorans*, *B. phenazinium*, *B. phenoliruptrix*, *B. phymatum*, *B. phytofirmans*, *B. plantarii*, *B. pseudomallei*, *B. pyrrocinia*, *B. stabilis*, *B. thailandensis*, *B. ubonensis*, *B. vietnamiensis*, *B. xenovorans*, not further defined *Burkholderia* spp., and the outliers *Cupriavidus metallidurans*, *Laribacter hongkongensis*, *Pandorea norimbergensis*, and *Ralstonia pickettii* were included in a multiple sequence analysis.

Multiple sequence alignments led to the delineation of four major clusters, *rpsU-I* to *rpsU-IV*, with a sequence homology >92%. The *B. pseudomallei* complex formed the complex *rpsU-II*. Several *Burkholderia* species showed 100% sequence homology.

This procedure is useful for the molecular confirmation or exclusion of glanders or melioidosis from primary patient material. Further discrimination within the *Burkholderia* genus requires other molecular approaches.

Keywords: *Burkholderia*, *rpsU*, ribosomal subunit protein S21, discrimination, sequencing

Introduction

Burkholderia (*B.*) species are gram-negative, nonfermentative rod-shaped bacteria [1]. The genus *Burkholderia* includes highly pathogenic species, e.g., *B. mallei* and *B. pseudomallei*, the causative agents of glanders and melioidosis, respectively [2], various facultatively pathogenic species of the *B. cepacia* complex which endanger cystic fibrosis patients [3, 4], and saprophytic species such as *B. thailandensis* or others [5–8]. Accordingly, reliable identification is mandatory to discriminate harmful agents from harmless colonizers in severely ill patients. During outbreak investigations or epidemiological screening, there is also the need for a reliable discrimination of strains isolated from environmental samples such as food, drinking water, or soil.

Due to the close phylogenetic relatedness of various species of the *Burkholderia* genus, biochemical discrimination alone is usually insufficient for identification at species level [9–12], occasionally resulting in fatal outcome [12]. Accordingly, matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI–TOF–MS) [13–16] or sequence-based procedures such as multilocus sequence typing (MLST) [17] are applied. Besides the laborious and time-consuming MLST for the discrimination within the *B. cepacia* complex [17], less complex single-gene-based approaches have been described. They include *fur* sequencing [18], *hisA* sequencing [19], and *recA* sequencing [20, 21], the latter being applicable to the whole *Burkholderia* genus [20]. Within the *B. cepacia* complex, MLST can increase the identification rate at species level by 20% compared to *recA* typing [21]. Species-level identification based on 16S rRNA sequencing, in contrast, often fails due to high sequence homology [22].

Fluorescence *in situ* hybridization (FISH)-based discrimination has been described for individual *Burkholderia* species [23]. However, the evaluation of a commercial FISH kit for the detection of pathogens of the *B. cepacia* complex (seaFAST Cystic Fibrosis I kit) showed that common species such as *B. multivorans* and *B. cenocepacia*

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were correctly identified, but not all of the other species of the complex. Furthermore, the interpretability was limited by nonspecific background fluorescence. In addition, the sensitivity in relation to the pathogen density was – as expected – less than that of specific polymerase chain reaction (PCR) [24].

Recently, sequence analysis of a 120-base-pair fragment of *rpsU* coding for a ribosomal protein S21 homolog with a length of 70 amino acids upstream of the *B. pseudomallei fliC* [25] was described as a method for discriminating *B. mallei* and *B. pseudomallei* from apathogenic *B. thailandensis* within the *B. pseudomallei* complex [26]. The protein belongs to the eubacterial macromolecular synthesis (MMS) operon playing a role in the initiation of protein, DNA, and RNA synthesis. The species-specific variations within the *Burkholderia* genus seem to be low [26, 27]. So far, the power of *rpsU* sequencing to discriminate agents of the *B. pseudomallei* complex from other *Burkholderia* spp. and its discriminatory power within the *Burkholderia* genus in general are unknown.

In this study, *rpsU* sequencing was applied to a broad spectrum of *Burkholderia* spp. in an attempt to close this information gap.

Materials and Methods

rpsU *PCR and sequencing*

PCR was performed from DNA preparations of pure bacterial cultures. DNA preparation of the heat-inactivated strains was performed as previously described [26, 27]. The *rpsU* PCR using the primers *fup1* 5′-GTG-GAG-CTT-CTT-CGG-CAG-CAT-3′ and *fup2* 5′-ATG-ACG-ACG-ATT-CTT-TTG-AA-3′ specific for *Burkholderia* spp. and phylogenetically closely related bacteria [27] was performed according to the published protocol [26, 27]. Amplicons were purified using the NAT Clean-up/Nucleospin® Extrackt II kit (Macherey & Nagel, Düren, Germany) according to the manufacturer's instructions. Forward and reverse strands of each amplicon were sequenced using an ABI 377 Prism™ Dye Sequencing Apparatus and the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit™ (Perkin Elmer, Weiterstadt, Germany) as described [26]. A 169-bp sequence was analyzed.

New sequences of Burkholderia *spp. reference strains and sequences obtained from NCBI GenBank*

New *rpsU* sequences were generated from DNA of 36 reference strains of *Burkholderia* spp. and phylogenetically related outliers which were obtained from the strain collections ATCC (American Type Culture Collection, Manassas, Virginia, USA), DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany), JCM (Japan Collection of Microorganisms, Tsukuba, Ibaraki Prefecture, Japan), BCCM/LMG (Bacteria Collection, Ghent, Belgium), and NCTC (National Collection of Type Cultures, Porton Down, UK) *(Table 1)*. In addition, deposited *rpsU* sequences of 48 were downloaded and included in the analysis [1, 5, 6, 28–49] *(Table 1)*.

Table 1. Characteristics of the analyzed reference strains

Species	Strain	Database accession number	Ref.
Azoarcus sp.	BH72	emb/AM406670.1	–
B. ambifaria	AMMD	gb/CP000440.1	[28]
B. ambifaria	LMG 19182	–	[28]
B. ambifaria	LMG 19466	–	[28]
B. ambifaria	LMG 19467	–	[28]
B. ambifaria	MC40-6	gb/CP001025.1	[28]
B. anthina	LMG 20980	–	[38]
B. caledonica	LMG 19076	–	[39]
B. caribensis	LMG 18531	–	[6]
B. caryophylli	ATCC 25418	–	[1]
B. cenocepacia	AU 1054	gb/CP000378.1	[29]
B. cenocepacia	HI2424	gb/CP000458.1	[29]
B. cenocepacia	J2315	emb/AM747720.1	[29]
B. cenocepacia	LMG 12614	–	[29]
B. cenocepacia	LMG 12615	–	[29]
B. cenocepacia	MC0-3	gb/CP000958.1	[29]
B. cepacia	ATCC 17759	–	[1]
B. cepacia	ATCC 25416	gb/AF447444.1	[1]

Table 1. *(cont.)*

Species	Strain	Database accession number	Ref.
B. cepacia	DSM 9241	–	[1]
B. cepacia	GG4	gb/CP003774.1	[1]
B. cepacia	NCTC 10744	–	[1]
B. cocovenenans	ATCC 33664	–	[34]
B. dolosa	LMG 18941	–	[40]
B. fungorum	LMG 16225	–	[39]
B. fungorum	LMG 16307	–	[39]
B. gladioli	ATCC 10248	gb/AF447445.1	[1]
B. gladioli	BSR3	gb/CP002599.1	[1]
B. glathei	ATCC 29195	–	[31]
B. glumae	ATCC 33617	–	[30]
B. glumae	BGR1	gb/CP001503.2	[30]
B. graminis	LMG 18924	–	[41]
B. graminis	LMG 18947	–	[41]
B. graminis	LMG 18948	–	[41]
B. hospita	DSM 7336	–	[42]
B. kururensis	JCM 10599	–	[43]
B. mallei	ATCC 15310	gb/AF084814.1/AF084814	[1]
B. mallei	ATCC 23344	gb/CP000010.1	[1]
B. mallei	NCTC 10229	gb/CP000546.1	[1]
B. mallei	NCTC 10247	gb/CP000548.1	[1]
B. mallei	NCTC 10248	–	[1]
B. mallei	SAVP1	gb/CP000526.1	[1]
B. multivorans	ATCC 17616	gb/CP000868.1	[31]
B. multivorans	DSM 13243	–	[31]
B. multivorans	LMG 13010	–	[31]
B. phenazinium	ATCC 33666	–	[41]
B. phenoliruptrix	BR3459a	gb/CP003863.1	[32]
B. phymatum	STM815	gb/CP001043.1	[33]
B. phytofirmans	PsJN	gb/CP001052.1	[8]
*B. plantarii (*synonym: *B. vandii)*	ATCC 51545	gb/AF447449.1	[30]
B. plantarii	LMG 9035	gb/AF447446.1	[30]
B. pseudomallei	668	gb/CP000570.1	[1]
B. pseudomallei	1026b	gb/CP002833.1	[1]
B. pseudomallei	1106a	gb/CP000572.1	[1]
B. pseudomallei	1710b	gb/CP000124.1	[1]
B. pseudomallei	6068VIR	gb/AF447447.1	[1]
B. pseudomallei	ATCC 15682	gb/AF084812.1/AF084812	[1]
B. pseudomallei	ATCC 23343	gb/AF084813.1/AF084813	[1]
B. pseudomallei	BPC006	gb/CP003781.1	[1]
B. pseudomallei	K96243	emb/BX571965.1	[1]
B. pseudomallei	MSHR305	gb/CP006470.1	[1]
B. pseudomallei	MSHR346	gb/CP001408.1	[1]
B. pseudomallei	isolate	gb/U73848.1/BPU73848	[1]

Table 1. *(cont.)*

Species	Strain	Database accession number	Ref.
B. pyrrocinia	ATCC 15958	–	[44]
B. sacchari	LMG 19450	–	[7]
B. stabilis	LMG 6997	–	[45]
B. stabilis	LMG 14294	–	[45]
B. stabilis	LMG 15949	–	[45]
B. thailandensis	ATCC 700388	gb/AF447448.1	[5]
B. thailandensis	E264	gb/CP000086.1	[5]
B. thailandensis	MSMB121	gb/CP004095.1	[5]
B. ubonensis	NCTC 13147	–	[46]
B. vietnamiensis	DSM 11319	–	[34]
B. vietnamiensis	G4	gb/CP000614.1	[34]
B. vietnamiensis	LMG 10929	gb/AF447450.1	[34]
B. xenovorans	LB400	gb/CP000270.1	[35]
Burkholderia sp.	383	(gb/CP000151.1)	–
Burkholderia sp.	CCGE1001	gb/CP002519.1	–
Burkholderia sp.	CCGE1003	gb/CP002217.1	–
Burkholderia sp.	KJ006	gb/CP003514.1	–
Burkholderia sp.	YI23	gb/CP003087.1	–
Cupriavidus metallidurans	CH34	gb/CP000352.1	[36]
Laribacter hongkongensis	HLHK9	gb/CP001154.1	[37]
Pandorea norimbergensis	DSM 11628	–	[47, 48]
Ralstonia pickettii	ATCC 27511	–	[49]

ATCC = American Type Culture Collection, Manassas, Virginia, USA; DSMZ = German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany; JCM = Japan Collection of Microorganisms, Tsukuba, Ibaraki Prefecture, Japan; BCCM/LMG = Bacteria Collection, Ghent, Belgium; NCTC = National Collection of Type Cultures, Porton Down, UK; References (Ref.) provided, if at least identification on species level was guaranteed

Table 2. Characteristics of the analyzed clinical isolates. Gen. = genomovar

Species (confirmed by *recA* sequencing)	Strain	Source	Donated by
B. cepacia (gen. III)	P407	Infected mucoviscidosis patient	Pneumology Clinics Heckeshorn, Berlin, Germany
B. cepacia (gen. III-A)	CF976-1-02	Infected mucoviscidosis patient	Max von Pettenkofer Institute for Hygiene and Medical Microbiology, Munich, Germany
B. cepacia (gen. III-B)	CF669-1-02	Infected mucoviscidosis patient	Max von Pettenkofer Institute for Hygiene and Medical Microbiology, Munich, Germany
B. multivorans (gen. II)	CF670-1-02	Infected mucoviscidosis patient	Max von Pettenkofer Institute for Hygiene and Medical Microbiology, Munich, Germany
B. multivorans (gen. II)	CF670-2-02	Infected mucoviscidosis patient	Max von Pettenkofer Institute for Hygiene and Medical Microbiology, Munich, Germany
B. multivorans (gen. II)	CF879-1-02	Infected mucoviscidosis patient	Max von Pettenkofer Institute for Hygiene and Medical Microbiology, Munich, Germany
B. multivorans (gen. II)	CF932-3-02	Infected mucoviscidosis patient	Max von Pettenkofer Institute for Hygiene and Medical Microbiology, Munich, Germany
B. multivorans (gen. II)	P403	Infected mucoviscidosis patient	Pneumology Clinics Heckeshorn, Berlin, Germany
B. thailandensis	E216	Colonized patient	Mahidol University, Bangkok, Thailand

Assessment of the clinical impact of the procedure with clinical isolates

To assess the clinical impact of the evaluated procedure, clustering of the reference strains with clinical strains was analyzed. Altogether, eight clinical *B. cepacia* complex isolates from mucoviscidosis patients were obtained from the Max von Pettenkofer Institute, Munich, Germany, and the Pneumology Clinics Heckeshorn, Berlin, Germany *(Table 2)*. The species identity had been ensured by *recA* sequencing [20, 21] prior to shipping. In addition, a colonizing *B. thailandensis* isolate was provided by the Faculty of Tropical Medicine of the Mahidol University, Bangkok, Thailand *(Table 2)*.

Multiple alignment of the rpsU *sequences*

Sequences were aligned using BioNumerics 7.1 software (Applied Maths, Sint-Martens-Latem, Belgium). The alignment settings were as follows: open gap penalty, 100%; unit gap penalty, 0%; match score, 100%; and fast algorithm (= minimum match sequence: 2, maximum number of: 98). Because of the minimum requirement of a 200-bp fragment length for sequences (without primers) to be deposited, 169-base-pair-sequences cannot be deposited at NCBI GenBank. Accordingly, the new sequences are presented in the supplementary material (*Supporting information 1*). Clusters resulting from the *rpsU*-based multiple alignments were characterized. A cluster was defined as having a sequence identity >92% in concordance with the visually observed grouping.

Ethics

No ethical clearance was necessary because this study did not include patients, or patient data, or patient materials.

Results

Multiple sequence alignment resulted in four visually distinguishable major clusters of species with sequence homology >92% *(Fig. 1)*.

B. plantarii, *B. glumae*, *B. cocovenenans*, and *B. gladioli* formed the cluster *rpsU-I*. Within this cluster, *B. cocovenenans*, and *B. gladioli* showed 100% homology. This finding is in concordance with the already described high genetic similarity of these species [50].

The *Burkholderia pseudomallei* complex (*B. mallei*, *B. pseudomallei*, and *B. thailandensis*) formed the cluster *rpsU-II*, clearly delineable from all other analyzed species. As previously shown [26], *rpsU*-based discrimination of *B. thailandensis* from *B. mallei* and *B. pseudomallei* is possible, while several strains of the latter two species cluster identically. Different *B. pseudomallei* strains showed genetic variability within the *rpsU* sequence.

A relatively large third cluster, *rpsU-III*, comprises the species *B. caryophylli*, *B. multivorans*, *Pandorea norimbergensis*, *B. ubonensis*, *B. stabilis*, *B. cenocepacia*, *B. cepacia*, *B. pyrrocinia*, *B. ambifaria*, *B. anthina*, *B. vietnamiensis*, *B. dolosa*, and the strains "*Burkholderia* sp. 383" and "*Burkholderia* sp. KJ006." Within this cluster, *B. caryophylli*, *B. multivorans*, and *P. norimbergensis* had identical sequences. *B. cenocepacia* clustered with *B. cepacia*. Clustering was found for *B. ambifaria* and *B. anthina*, formerly known as *B. cepacia* genomovars VII and VIII [51], as well as for one *B. vietnamiensis* strain and "*Burkholderia* sp. KJ006." Variability within the analyzed *rpsU* sequence further resulted in clustering of *B. cepacia* and *B. vietnamiensis* strains.

Cluster *rpsU-IV* comprises the species *B. sacchari*, *B. graminis*, *B. fungorum*, *B. phytofirmans*, *B. xenovorans*, *B. phenoliruptrix*, *B. phenazinium*, *B. caribensis*, *B. hospita*, *B. phymatum*, and the strains "*Burkholderia* sp. CCGE1001" and "*Burkholderia* sp. CCGE1003." Identical sequences were found for *B. fungorum*, *B. phytofirmans*, and *B. xenovorans,* and for *B. caribensis* and *B. hospita*, respectively.

Apart from these clusters, *B. glathei*, *B. caledonica*, *B. kururensis*, and "*Burkholderia* sp. Y123" remained outliers.

The minimum sequence homology within the analyzed strains of the genera *Burkholderia* and *Pandorea* was >86%. *Laribacter hongkongensis* was the most closely related outlier regarding its *rpsU* sequence, with a sequence homology <80%. Homologies for "*Azoarcus* sp. BH72," *Cupriavidus metallidurans* and *Ralstonia pickettii* were even lower.

Intraspecies variability less than 1% was observed for strains of *B. plantarii* (ATCC 51545, synonym *B. vandii* [30]), *B. pseudomallei*, and *B. vietnamiensis*. The analyzed *B. cepacia* reference strains showed an intraspecies variability of >3%.

All tested clinical isolates of the *B. cepacia* complex clustered in the *rpsU*-III cluster as expected *(Fig. 2)*. Similarly, the clinical *B. thailandensis* strain from Bangkok clustered with *B. thailandensis* reference strains in the *rpsU*-II cluster *(Fig. 3)*. Close sequence similarity of different species did not allow for reliable assigning on species level within the *rpsU*-III cluster.

Discussion

This study assessed the suitability of *rpsU* sequencing for reliable diagnostic delineation of the *B. pseudomallei* complex from other *Burkholderia* spp. and for further discrimination within the *Burkholderia* genus. The suitability of the procedure as a diagnostic tool in addition to alternative molecular techniques for the discrimination within the *Burkholderia* genus like well-established MLST typing [17] or *recA* sequencing [20, 21] was evaluated.

rpsU is usually the first gene of the *rpsU–dnaG–rpoD* operon, which is highly conserved in many gram-negative

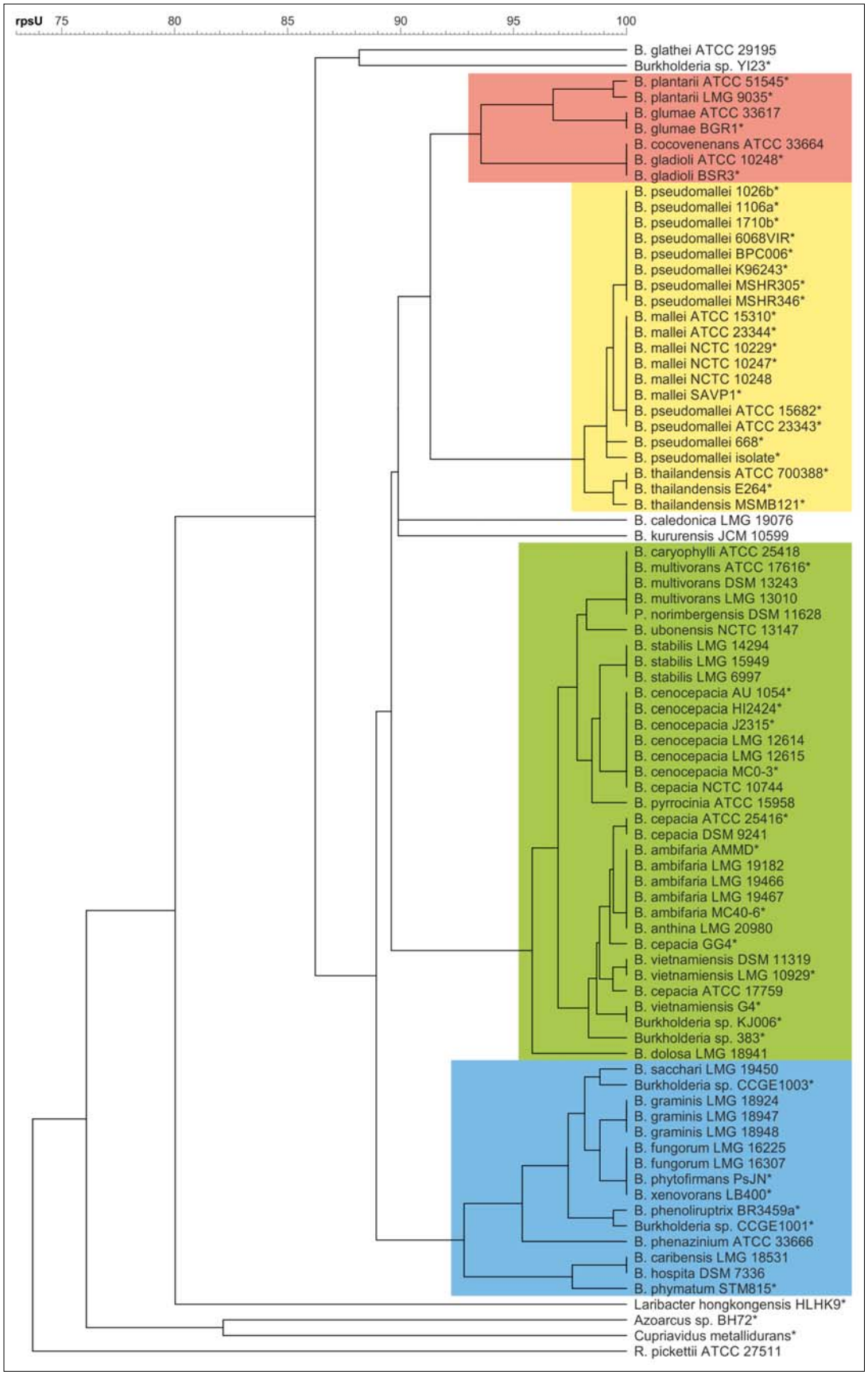

Fig. 1. *rpsU*-based clustering of reference strains or other well-characterized strains of the genus *Burkholderia* and of related genera. A clustering of >92% sequence homology was considered as a distinct *rpsU* cluster. Asterisks (*) mark sequences that were downloaded from the NCBI database (www.ncbi.nlm.nih.gov/genbank/). Red rectangle: *rpsU*-I cluster. Yellow rectangle: *rpsU*-II-cluster. Green rectangle: *rpsU*-III cluster. Blue rectangle: *rpsU*-IV cluster

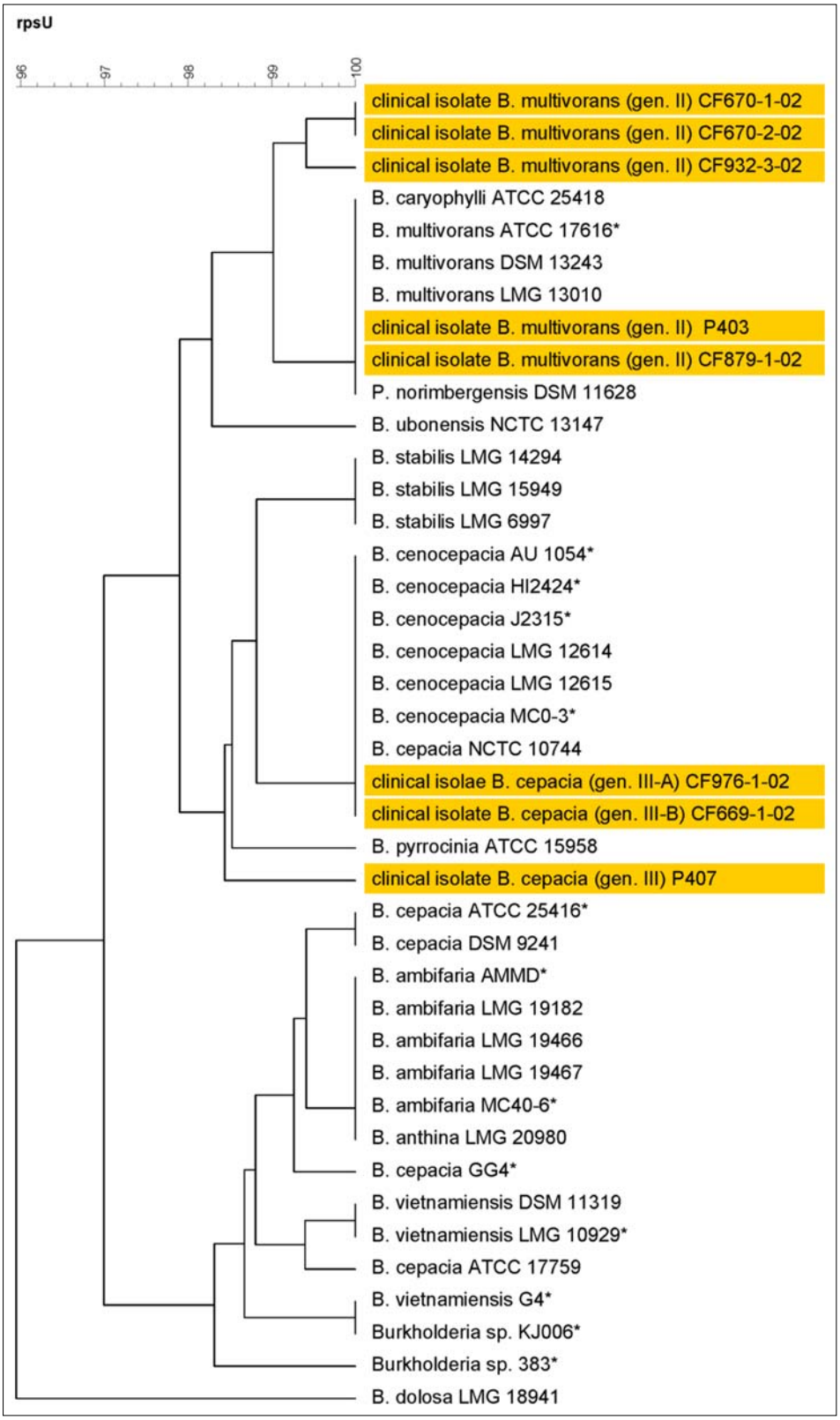

Fig. 2. Clustering of eight clinical isolates of the *B. cepacia* complex with the *rpsU*-III cluster. Yellow rectangles: clinical strains

bacteria [52]. However, the high specificity of the *rpsU* PCR for *Burkholderia* spp. and closely related genera [27] made *rpsU* a valuable target for the identification of *Burkholderia* spp. from clinical samples [12]. Consecutive sequencing of the amplicons facilitates sequence-based typing even when classical microbiology fails.

As demonstrated in this study, the *B. pseudomallei* complex is a very distinct cluster clearly separated from

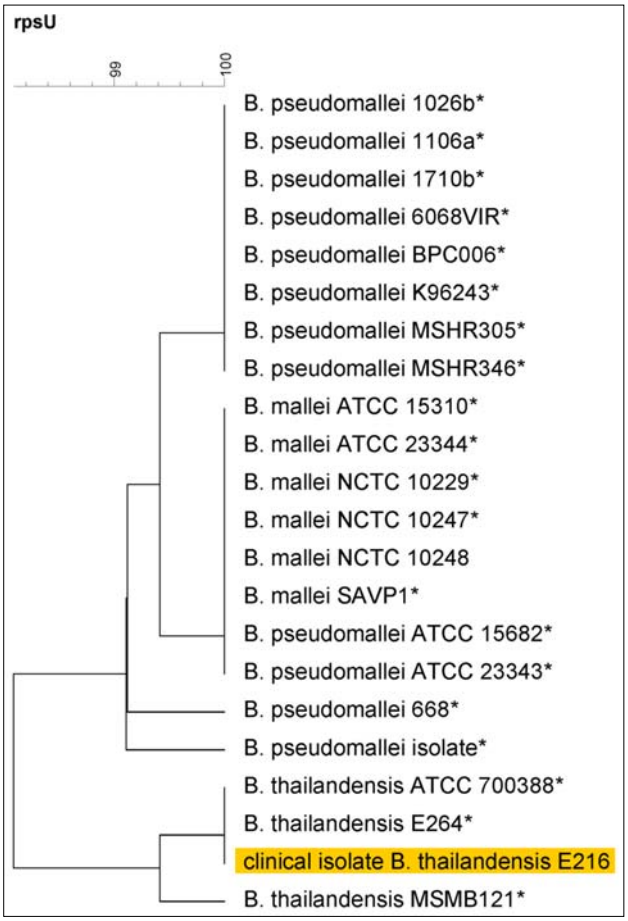

Fig. 3. Clustering of a clinical *B. thailandensis* isolate with the *rpsU*-II cluster. Yellow rectangle: clinical strain

the other clusters of the genus. Accordingly, the *rpsU* PCR with consecutive sequencing described here proved to be useful for the reliable identification of bacteria of the *B. pseudomallei* complex and their delineation from other *Burkholderia* species. Its potency to discriminate saprophytic *B. thailandensis* from highly pathogenic *B. mallei* and *B. pseudomallei* [53] has been shown previously [26]. Thus, the procedure allows for the discrimination of *B. pseudomallei/B. mallei* from colonizing or contaminating less pathogenic *Burkholderia* spp. in clinical samples of severely ill patients. As previously demonstrated, the *rpsU* sequence seems to be quite stabile [26], although *Burkholderia* spp. in general and *B. mallei* in particular are otherwise known to be littered with insertion sequence (IS) elements and prone to mutations, even from one generation to the next. Nevertheless, a low risk of unnecessary exposure to a biosafety level (BSL)-3 pathogen remains if potentially inaccurate sequencing results serve as the only identification procedure. Regarding this aspect, it is an undeniable limitation of this study that the *rpsU* sequence of *B. oklahomensis*, a further species of the *B. pseudomallei* complex, could not be obtained.

In contrast, *rpsU* sequencing does not allow the unambiguous assignment of an isolate to a species. Four distinct clusters, *rpsU-I* to *rpsU-IV*, with high sequential similarity within the clusters could be identified. Several *Burkholderia* species even showed identical *rpsU* sequences that do not allow any further discrimination. This finding was observed within all *rpsU* clusters. Intraspecies variability posed another problem: for example, it is not possible to discriminate *B. pseudomallei* from *B. mallei* [26]. For *B. cepacia* and *B. vietnamiensis*, this problem was so pronounced that even individual clustering with other species was observed. It has to be kept in mind that the formerly classified "*B. cepacia*" strains comprised a broad range of different genomovars [51] of which new species were subsequently defined (e.g., *B. cenocepacia* [29]).

Accordingly, *rpsU* sequencing does not allow for a reliable discrimination of *Burkholderia* spp. at the species level as single target, although its implementation in a MLST scheme might be considered. It is not unlikely that other *Burkholderia* spp. that were not available to the authors for sequencing might have sequences identical to species investigated in this study. Consequently, no further efforts were made to obtain rare *Burkholderia* spp. for further investigation of this limitation of the technique.

If discrimination beyond the power of *rpsU* sequencing is needed, alternative approaches including MLST [17], *fur* sequencing [18], *hisA* sequencing [19], or *recA* sequencing [20, 21] might be applied from pure cultures. In contrast, 16S rRNA sequencing shows too weak a discriminative power, not even allowing for a reliable discrimination within the *B. pseudomallei* complex due to close sequence homology [22]. Accordingly, 16S rRNA sequencing is not suitable for discriminating within the genus *Burkholderia* on species level.

In spite of the limitations mentioned, the big advantage of *rpsU* PCR and sequencing is that the technique is robust and works reliably even from primary material such as human tissue [12]. As well as agents of the *B. pseudomallei* complex, clinically important agents of the *B. cepacia* complex such as *B. cenocepacia* and *B. multivorans,* which spread epidemically between cystic fibrosis patients [3, 4, 24, 54], can be found. In case of a detected melioidosis, a well-timed start of therapy is possible [55–57].

Nevertheless, the discriminative power of *rpsU* sequencing is – in general – insufficient for a reliable discrimination within the *B. cepacia* complex. Recently, a multiplex PCR has been described for this purpose [58] and *recA* sequencing [20, 21] also shows a higher discriminative power. Several discrepancies between *rpsU* clustering and *recA* clustering remain puzzling and are not completely resolved, e.g., *rpsU* of *B. caryophilli* is identical to *B. multivorans*. However, based on *recA* sequencing [20, 21], *B. caryophilli* should be in a cluster different from the *B. cepacia* complex together with *B. glumae* and *B. glathei*. Similarly, it remains unclear why the *rpsU* of the outlier *P. norimbergensis* clusters identical to *B. multivorans*. In contrast, three different *Pandorea* species are in a *recA* cluster distinct from the *B. pseudomallei* complex and *B. cepacia* complex clusters [20]. As *B. caryophilli*, *B. norimbergensis*, and *B. multivorans* are phylogenetically very different, lateral gene transfer might be a possible explanation for the observed clustering results. Two cases

of lateral gene transfer within a set of less than 100 strains examined would, however, considerably reduce the value of *rpsU* sequencing for identification or taxonomic purposes. Future typing approaches based on whole genome assessments by next generation sequencing might help to explain the unexpected clustering.

Conclusions

In summary, *rpsU* sequencing allows a reliable delineation of the *B. pseudomallei* complex from other *Burkholderia* spp. A detailed discrimination at species level, e.g., within the *B. cepacia* complex, however, requires the application of alternative molecular procedures. The latter usually requires pure colony material from culture, which can be performed under BSL-2 laboratory conditions if *B. mallei* and *B. pseudomallei* are excluded on the basis of the *rpsU* sequence.

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Declaration of interest

The authors declare that there are no conflicts of interest.

Disclaimer

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