ELSEVIER

FEMS Microbiology Letters 246 (2005) 39–45

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

Efficacy of species-specific *recA* PCR tests in the identification of *Burkholderia cepacia* complex environmental isolates

Claudia Dalmastri *, Luisa Pirone, Silvia Tabacchioni, Annamaria Bevivino, Luigi Chiarini

Ente Nazionale per le Nuove Tecnologie, l'Energia e l'Ambiente (ENEA), C.R. Casaccia Department of Biotechnologies, Protection of Health and Ecosystems, Plant Genetics and Genomics Section, Via Anguillarese 301, 00060 S. Maria di Galeria, Rome, Italy

Received 4 January 2005; received in revised form 25 February 2005; accepted 21 March 2005

First published online 1 April 2005

Edited by C.W. Penn

Abstract

In this study, we evaluated if *recA* species-specific PCR assays could be successfully applied to identify environmental isolates of the widespread *Burkholderia cepacia* complex (Bcc) species. A total of 729 Bcc rhizosphere isolates collected in different samplings were assigned to the species *B. cepacia* genomovar I (61), *B. cenocepacia recA* lineage IIIB (514), *B. ambifaria* (124) and *B. pyrrocinia* (30), by means of *recA* (RFLP) analysis, and PCR tests were performed to assess sensitivity and specificity of *recA* species-specific primers pairs. *B. cepacia* genomovar I specific primers produced the expected amplicon with all isolates of the corresponding species (sensitivity, 100%), and cross-reacted with all *B. pyrrocinia* isolates. On the contrary, *B. cenocepacia* IIIB primers did not give the expected amplicon in 164 *B. cenocepacia* IIIB isolates (sensitivity, 68.1%), and isolates of distinct populations showed different sensitivity. *B. ambifaria* primers failed to amplify a *recA*-specific fragment only in a few isolates of this species (sensitivity, 93.5%). The absence of specific amplification in a high number of *B. cenocepacia* rhizosphere isolates indicates that *recA* specific PCR assays can lead to an underestimation of environmental microorganisms belonging to this bacterial species.
© 2005 Federation of European Microbiological Societies. Published by Elsevier B.V. All rights reserved.

Keywords: *Burkholderia cepacia* complex identification; *recA* specific PCR; Cystic fibrosis; Rhizosphere environment

1. Introduction

Burkholderia cepacia complex (Bcc) is a heterogeneous group of bacteria, which includes nine closely related species: *B. cepacia* genomovar I, *B. multivorans*, *B. cenocepacia*, *B. stabilis*, *B. vietnamiensis*, *B. dolosa*, *B. ambifaria*, *B. anthina*, and *B. pyrrocinia* [1–9]. In addition, within the species *B. cenocepacia* four lineages based on *recA* gene polymorphism (IIIA, IIIB, IIIC, and IIID) have been found [8]. Bacteria belonging to Bcc, which comprises isolates originally described as plant pathogens [10], have emerged as opportunistic human pathogens in the 1980s, causing devastating lung infections in patients with cystic fibrosis (CF) and being responsible for various nosocomial infections in immunocompromised patients [11]. At the same time, Bcc organisms have been found widely distributed in natural habitats such as the rhizosphere of several crop plants, especially of maize [12,13], as well as river water [14,15] and some urban soils [16], suggesting that the natural environment may serve as a "reservoir" for pathogenic strains [17]. This hypothesis is reinforced by genotyping data, which demonstrated identity among certain isolates from CF patients and natural habitats [18,19]. Since new acquisitions of Bcc by CF patients after the introduction of infection control measures

* Corresponding author. Tel.: +39 06 30483196; fax: +39 06 30484808.
E-mail address: claudia.dalmastri@casaccia.enea.it (C. Dalmastri).

0378-1097/$22.00 © 2005 Federation of European Microbiological Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.femsle.2005.03.041

[20] could be due mainly to strains acquired from the environment [20,21], the ability to detect and identify bacteria belonging to all the Bcc species in natural habitats enables definition of the biologic origin of strains infecting CF patients and the risks posed to humans by naturally occurring strains.

Reliable detection of Bcc members requires efficient and rapid identification tools. Analysis based on polymorphisms of 16S rDNA and *recA* genes have been developed, but only *recA*-based tests have been proved to identify effectively most Bcc species [22,23]. Among these tests, specific PCR reactions for *B. cepacia* genomovar I, *B. multivorans*, *B. cenocepacia*, *B. stabilis*, *B. vietnamiensis*, *B. dolosa*, *B. ambifaria*, and *B. anthina*, can represent a valid alternative to restriction fragment length polymorphism (RFLP) analysis, because they are less time-consuming and simpler to use in routine analysis, compared to *recA* RFLP which is the basis for Bcc species identification. Indeed, specific PCR reactions have successfully been used to assess the presence of some of Bcc species in clinical samples [24]. As PCR specific primers have been mainly designed on the basis of *recA* sequence of clinical reference strains, it raises the issue of their efficacy in identification of environmental isolates. So far, very few studies have dealt with the assessment of Bcc species in the natural environment, and the efficacy of currently used tests has never been thoroughly investigated on natural Bcc populations. Where the efficacy of *recA* specific primers pairs was evaluated on both environmental and clinical isolates [25], it was not possible to assess the efficiency of the primers pairs on the basis of the origin of bacterial isolates as only the whole percentage of PCR positive strains was reported.

In the present work, we aimed at assessing the efficiency of *recA* species-specific PCR tests for the identification of Bcc bacteria isolated from the natural environment, in particular from the maize rhizosphere, where they show significant diversity [12,26,27]. Bcc rhizosphere isolates belonging to different populations were identified at the species level by means of *recA* RFLP analysis and then subjected to specific PCR assays in order to determine the sensitivity of *recA* specific primers, i.e., the ability to specifically react with organisms belonging to the corresponding species, and their specificity, i.e., the absence of cross-reaction with other species.

2. Materials and methods

2.1. Strains

A total of 729 Bcc isolates recovered from the maize rhizosphere were analysed. Among them, 465 were recovered by using the semi-selective PCAT medium [28] from the rhizosphere of maize plants cultivated in three fields located in different Italian geographic sites: S. Maria di Galeria, Rome (MC population), Pieve d'Olmi, Cremona (MVP population), and Dragoni, Caserta (MD population) [29–31]. The remaining 264 rhizosphere isolates were recovered by means of the selective TB-T medium [32] from the rhizosphere of maize plants cultivated in Dragoni (MDIII-T population) (Pirone et al., submitted). As two or three samplings were performed at each site by using PCAT medium, each bacterial population was divided into distinct sub-populations: MCI, MCII, and MCIII (MC population), MVPC1 and MVPC2 (MVPC population), and MDI, MDII, and MDIII-P (MD population).

2.2. RecA *RFLP analysis and identification*

DNA of each bacterial isolate was prepared by lysis of 2–3 colonies grown overnight on Nutrient Agar (NA, Difco), according to the method described by Vandamme et al. [7]. *RecA* DNA amplification with specific primer pairs for Bcc, BCR1 and BCR2, was performed to assign isolates to Bcc by using the procedure described by Mahenthiralingam et al. [22] with the following modifications. Two micro-litres of each bacterial lysate were used as template DNA, 0.5 U of *Taq* DNA polymerase (Polymed) and 1× Polymed PCR buffer were added, and the reaction mixture was cycled in a thermocycler (GeneAmp 9700 – Applied Biosystems). Amplified products were subjected to RFLP analysis with the restriction enzyme *Hae*III (Takara) [22]. The restriction patterns were manually analysed and compared to those shown by Bcc reference strains [33,34].

Some isolates had already been identified at the complex level and, in certain cases, also at the species level in previous work [26,27,29].

2.3. *Species-specific* recA *PCR assay*

Species-specific *recA* PCR were performed on cell lysates by using the procedure described by Mahenthiralingam et al. [22], with the above described modifications for *recA* DNA amplification of Bcc. Specific primer pairs for *B. cepacia* genomovar I (BCRG11 and BCRG12), *B. cenocepacia recA* lineages IIIA (BCRG3A1 and BCRG3A2) and IIIB (BCRG3B1 and BCRG3B2), and *B. ambifaria* (BCRGC1 and BCRGC2) were used [6,22], and amplification reactions were performed using the annealing temperature reported for each primer pair.

2.4. *DNA sequence analysis*

The reaction mixture containing the *rec*A gene amplified with primers BCR1 and BCR2 was purified using

the MinElute PCR purification kit (Qiagen) according to the supplier's instructions. Reactions were prepared using Applied Biosystems Big Dye® Terminator sequencing kit version 3.1, according to the manufacturer's instructions. Thermal cycling was performed with a gene Amp PCR System 9700 instrument (Applied Biosystems) and consisted of 30 cycles of 30 s at 94 °C, 30 s at 48 °C and 4 min at 60 °C. Raw sequences from both strands of the PCR products were visualized with Chromas software, then aligned, and a consensus sequence was derived using DNASTAR software (DNASTAR Inc., Madison, WI). Analyses of sequences were performed with the BLAST program run against the nr (non-redundant) database (http://www.ncbi.nlm.nih.gov/BLAST).

3. Results and discussion

According to previous work [15,22,35,36], *Hae*III *recA* RFLP patterns (Fig. 1) allowed the assignment of each Bcc isolate to its respective species. Isolates were identified as follows: 61 *B. cepacia* genomovar I, 514 *B. cenocepacia*, 124 *B. ambifaria*, and 30 *B. pyrrocinia*. All the 514 *B. cenocepacia* isolates belonged to the lineage IIIB. This result, according to another study in which the lineage IIIB was found highly diffuse in the rhizosphere (Pirone et al., submitted), suggests that it may be widespread in natural habitats. Species distribution among isolates recovered on both PCAT and TB-T media is summarized in Table 1; for PCAT isolates, species distribution among the different populations and sub-populations is reported in Table 2.

Table 1
Species distribution among *B. cepacia* complex (Bcc) rhizosphere isolates recovered on both PCAT and TB-T medium

	PCAT[a]	TB-T[b]
Total Bcc	465	264
B. cepacia genomovar I	14	47
B. cenocepacia IIIB	321	193
B. ambifaria	107	17
B. pyrrocinia	23	7

[a] Isolates recovered on PCAT medium belonged to different bacterial populations (see Table 2).
[b] Isolates recovered on TB-T medium belonged to one bacterial population, named MDII-T.

Given the presence of the species *B. cepacia* genomovar I, *B. cenocepacia*, *B. ambifaria* and *B. pyrrocinia* in the maize rhizosphere, we focused our investigation on *recA* specific PCR tests for their detection and attempted to correlate both their specificity and sensitivity to the distinct RFLP patterns observed.

3.1. B. cepacia *genomovar I and* B. pyrrocinia

PCR analysis with *B. cepacia* genomovar I specific primers BCRG11 and BCRG12 produced the expected amplicon in all of the 61 rhizosphere isolates of *B. cepacia* genomovar I (sensitivity, 100%). These primers also gave the same amplicon in all *B. pyrrocinia* isolates and did not amplify *recA* gene in isolates of other

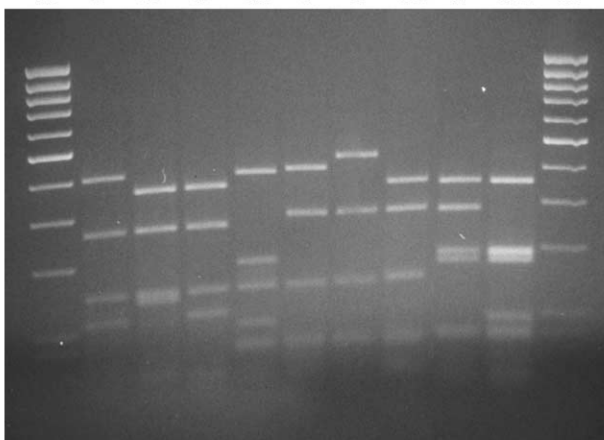

Fig. 1. RFLP analysis of the 1-kb *recA* gene amplified from *B. cepacia* complex strains: *Hae*III RFLP profiles. Lanes (left to right): "M", molecular size marker (100-bp ladder MBI-Fermentas); E, *B. cepacia* genomovar I LMG 2161; K, *B. cepacia* genomovar I MCII-61; D, *B. cepacia* genomovar I LMG 1222; M, *B. pyrrocinia* LMG 14191; J, *B. cenocepacia* IIIB LMG 16654; I, *B. cenocepacia* IIIB MCII-46; AD, *B. cenocepacia* IIIB MDIIR-102; N, *B. ambifaria* LMG 19466; AE, *B. ambifaria* MVPC1-3; "M", molecular size marker (100-bp ladder).

Table 2
Rhizosphere populations of the different Bcc species recovered on PCAT medium

Population MC	MC[a]	MCI[b]	MCII[b]	MCIII[b]
Total Bcc	79	10	23	46
B. cepacia genomovar I	2	0	2	0
B. cenocepacia IIIB	57	0	14	43
B. ambifaria	19	10	6	3
B. pyrrocinia	1	0	1	0
Population MVP	**MVP[a]**	**MVP-C1[b]**	**MVP-C2[b]**	
Total Bcc	138	61	77	
B. cepacia genomovar I	4	1	3	
B. cenocepacia IIIB	45	9	36	
B. ambifaria	81	44	37	
B. pyrrocinia	8	4	4	
Population MD	**MD[a]**	**MDI[b]**	**MDII[b]**	**MDIII-P[b]**
Total Bcc	248	12	23	213
B. cepacia genomovar I	8	1	0	7
B. cenocepacia IIIB	219	9	16	194
B. ambifaria	7	0	4	3
B. pyrrocinia	14	2	3	9

[a] Bcc populations MC, MVP, and MD were recovered from different fields.
[b] Bcc sub-populations represent distinct samplings performed for each population at each field.

Table 3
Percentage of isolates positive to specific PCR among rhizosphere Bcc isolates belonging to different species and showing different *recA* RFLP patterns

Species	RFLP pattern	No. of isolates	Specific PCR positive (%)
B. cepacia genomovar I	D	23	100
	E	21	100
	K	17	100
B. pyrrocinia[a]	Se13	30	100
B. cenocepacia	I	41	60.0
	J	40	97.6
	AD	433	66.6
B. ambifaria	N	77	98.7
	AE	47	85.1

[a] Specific PCR reactions were performed with primer pair specific for *B. cepacia* genomovar I.

species. Results achieved are in agreement with those reported by Vermis et al. [25], as far as both sensitivity and cross-reactivity with *B. pyrrocinia* are concerned. No cross-reactions were observed with primers specific for other species in either *B. cepacia* genomovar I or *B. pyrrocinia* isolates.

Three out of four *Hae*III RFLP patterns reported in the literature [22,25] were found among rhizosphere *B. cepacia* genomovar I isolates (patterns D, E, and K, Table 3) and a homogeneous distribution of all of them was observed among the distinct populations (data not shown).

Only one *Hae*III RFLP pattern –Se13– [36] was found to identify the 30 *B. pyrrocinia* isolates (23 from PCAT and seven from TB-T).

PCR reactions performed on *B. cepacia* genomovar I environmental populations revealed a low specificity of *recA* specific primers, that cross-reacted with all *B. pyrrocinia* isolates. Therefore, specific PCR for detection of *B. cepacia* genomovar I strains can only be used if associated with other analyses such as RFLP, and the development of more specific primers pairs is suggested.

3.2. B. cenocepacia recA *lineage IIIB*

Among the 514 rhizosphere Bcc isolates assigned to the species *B. cenocepacia recA* lineage IIIB, 350 gave the specific amplicon with the primers BCRG3B1 and BCRG3B2 (sensitivity, 68.1%). Sequence analysis of the *recA* gene was performed on representative isolates showing different RFLP patterns characteristic of *B. cenocepacia* IIIB, and those which were negative by PCR, in order to confirm their assignment to this species. Indeed, *recA* sequence data were perfectly in agreement with RFLP data, confirming the assignment of these isolates to the species *B. cenocepacia* IIIB.

Our sensitivity results are not in agreement with those obtained by Vermis et al. [25], who showed a combined sensitivity of 92% with specific primers for *B. cenocepacia recA* lineages IIIA and IIIB. In that study, PCR results achieved with specific IIIA and IIIB primers as well as with clinical and environmental isolates were not reported separately; on the contrary, here we focused exclusively on environmental *B. cenocepacia* IIIB isolates, showing the absence of specific amplification with IIIB specific primers in a relevant percentage (31.9%) of them. These primers did not amplify *recA* gene in isolates belonging to other species (specificity, 100%), and no cross-reactions were observed in *B. cenocepacia* isolates with primers specific for other species, neither with primers specific for *B. cenocepacia* lineage IIIA, as previously reported [25].

Among the four patterns (H, I, J and AD) actually reported to identify *B. cenocepacia* IIIB [22,25], three were observed in rhizosphere populations (patterns I, J, and AD, Table 3), with a high prevalence of the pattern AD which so far seems to be characteristic of environmental isolates. Differences in specific PCR sensitivity values were observed among isolates characterized by these distinct RFLP patterns: 60.0% for I, 97.6% for J, and 66.05% for AD (due to its highest prevalence, its sensitivity value is close to that found for the whole *B. cenocepacia* IIIB sample). It has to be noted that J, associated with the highest PCR sensitivity in *B. cenocepacia* IIIB rhizosphere isolates, was not abundant among them, whereas it was found to be spread among CF isolates (data not shown). The reduced PCR sensitivity associated with the most widespread pattern AD could be partly explained by the fact that no reference strains with the AD pattern were taken into account to set-up BCRG3B1 and BCRG3B2 primers as they had been designed before the discovery of this pattern in *B. cenocepacia* IIIB.

Some differences in sensitivity to IIIB specific primers were found among *B. cenocepacia* isolates depending on the sampling site. In fact, although the average sensitivity value calculated for all the 321 PCAT-isolates *B. cenocepacia* IIIB was 62.9%, the sensitivity varied among different bacterial populations examined. The MC population showed the highest sensitivity value (98.2%), as only one MCII isolate (having the pattern I) was negative by specific PCR. The MVP population showed the lowest sensitivity value among all the populations examined (20.0%); it has to be noted that the pattern I, according to its association with a low sensitivity value, was particularly widespread in this population, as it has been shown by 27.7% of PCR negative MVP isolates. Finally, the sensitivity value obtained in MD population was 62.5%. Differences in PCR test sensitivity have also been found within bacterial populations by comparing sub-populations to each other, such as MDI vs. MDII vs. MDIII-P (sensitivity values, 55.5%, 37.5% and 64.9%, respectively) and MVPC1 vs. MVPC2 (sensitivity values, 9% and 21.2%, respectively).

Concerning the sensitivity of specific primers for *B. cenocepacia* IIIB recovered by using different culture media, values of 64.9% and 76.7% were obtained by MDIII-P and MDIII-T isolates, respectively. The higher sensitivity value shown by TB-T isolates suggests that these two selective media, that have previously been found to exert some influence on biodiversity degree of bacterial populations at the complex level [37], could have some effect on the selection at the species level of populations with some differences in *recA* sequence, as revealed by both PCR sensitivity and RFLP pattern distribution (data not shown).

If compared to *B. cepacia* genomovar I specific primers, *B. cenocepacia* IIIB specific primers revealed some quite different features. In fact, the first showed low specificity and high sensitivity, whereas the latter showed high specificity and low sensitivity. Thus, the fact that one primer pair is not enough to detect all members belonging to *B. cenocepacia* IIIB suggests that other primer pairs should be developed to improve PCR tests for this species.

3.3. B. ambifaria

Among the 124 *B. ambifaria* isolates, the majority gave the amplicon of the expected size with the respective primers BCRGC1 and BCRGC2 (sensitivity, 93.5%). In fact, only eight PCAT isolates, among which seven belonged to the sub-population MVPC2 and one belonged to MCIII, gave a negative result in specific PCR. Therefore, although we found that *B. ambifaria* primers could result in a lack of amplification in a few environmental isolates, our results are very close to the 100% sensitivity value previously reported [25]. According to previous data, these primers did not give amplification with isolates of species different from *B. ambifaria* (specificity, 100%), and no cross-reactions were observed in *B. ambifaria* isolates with primers specific for other species.

Two *Hae*III patterns of the three known for *B. ambifaria* [7], were observed among rhizosphere isolates of this species (patterns N, and AE, Table 3). All MC isolates and all but one MD isolate had the pattern N, whereas a homogeneous distribution of both patterns N and AE was observed among MVP isolates. With regards to the absence of specific amplicons observed in the different RFLP patterns (Table 3), we can observe that seven out of eight PCR-negative isolates showed the pattern AE although it was also widely abundant among PCR-positive ones.

In conclusion, specific PCR assays performed on rhizosphere isolates resulted in both high specificity and sensitivity only in the species *B. ambifaria*. Therefore, the *B. ambifaria* primer pair seems to be appropriate to detect the environmental members belonging to this species.

4. Conclusions

The lack of *recA* specific amplification revealed by this study in a high number of environmental *B. cenocepacia* isolates indicates that species-specific PCR can lead to an underestimation of environmental microorganisms belonging to this species. In fact, as here pointed out by the analysis of a large number of rhizosphere isolates recovered in different samplings, bacterial populations characterized by different geographical origin and sampling time can exhibit differences in sensitivity to species-specific *recA* PCR assays, thus suggesting that the use of this method may cause a bias in analysis of natural populations. Although a reduced sensitivity to specific PCR was in some cases associated with distinct RFLP patterns, data obtained are not sufficient to establish any rules for PCR inefficiency, which probably simply depends on *recA* sequence variability. It is yet to be assessed whether isolates recovered from other natural habitats could show a reduced PCR sensitivity associated with different RFLP patterns.

Indeed, a proper detection of *B. cenocepacia* is fundamental, as it accounts for the majority of Bcc isolates in CF patients, with particular attention to the *recA* lineage IIIB since it has so far been recovered from both CF patients and natural habitats. Although *recA* RFLP analysis has so far been successfully employed to identify not only clinical isolates but also the environmental ones, the limits shown by *recA* specific PCR assays suggest that methods considered as useful tools for their rapidity and precision in the detection of clinical strains may fail when identifying Bcc isolates of natural origin. Therefore, the assessment and optimisation of new identification tests for Bcc species should be conducted not only on clinical strains, but also on environmental strains, to improve the detection of potential pathogenic strains in natural habitats.

Acknowledgements

The authors thank Dr. Annarita Tiberti for her valuable contribution in identification of bacterial isolates, Gabriella Seri for technical assistance, and Dr. Silvia Dalmastri for the careful revision of the text.

References

[1] Gillis, M., Tran Van, V., Bardin, R., Goor, M., Hebbar, P., Willems, A., Segers, P., Kersters, K., Heulin, T. and Fernandez, M.P. (1995) Polyphasic taxonomy in the genus *Burkholderia* leading to an emended description of the genus and proposition of *Burkholderia vietnamiensis* sp. nov. for N_2-fixing isolates from rice in Vietnam. Int. J. Syst. Bacteriol. 45, 274–289.

[2] Vandamme, P., Holmes, B., Vancanneyet, M., Coenye, T., Hoste, B., Coopman, R., Reverts, H., Lauwers, S., Gillis, M., Kersters,

K. and Govan, J.R.W. (1997) Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. Int. J. Syst. Bacteriol. 47, 1188–1200.

[3] Vandamme, P., Mahenthiralingam, E., Holmes, B., Coenye, T., Hoste, B., De Vos, P., Henry, D. and Speert, D.P. (2000) Identification and population structure of *Burkholderia stabilis* sp. nov. (formerly *Burkholderia cepacia* genomovar IV). J. Clin. Microbiol. 38, 1042–1047.

[4] Coenye, T., Vandamme, P., Govan, J.R.W. and LiPuma, J.J. (2001) Taxonomy and identification of the *Burkholderia cepacia* complex. J. Clin. Microbiol. 39, 3427–3436.

[5] Coenye, T., LiPuma, J.J., Henry, D., Hoste, B., Vandemeulebroucke, K., Gillis, M., Speert, D.P. and Vandamme, P. (2001) *Burkholderia cepacia* genomovar VI: a new member of the *Burkholderia cepacia* complex isolated from cystic fibrosis patients. Int. J. Syst. Evol. Microbiol. 51, 271–279.

[6] Coenye, T., Mahenthiralingam, E., Henry, D., LiPuma, J.J., Laevens, S., Gillis, M., Speert, D.P. and Vandamme, P. (2001) *Burkholderia ambifaria* sp. nov.: a novel member of the *Burkholderia cepacia* complex comprising biocontrol and cystic-fibrosis related isolates. Int. J. Syst. Evol. Microbiol. 51, 1481–1490.

[7] Vandamme, P., Henry, D., Coenye, T., Nuzla, S., Vancanneyt, M., LiPuma, J., Speert, D.P., Govan, J.R.W. and Mahenthiralingam, E. (2002) *Burkholderia anthina* sp. nov. and *Burkholderia pyrrocinia*: two additional *Burkholderia cepacia* complex bacteria, may confound results of new molecular diagnostic tools. FEMS Immunol. Med. Microbiol. 33, 143–149.

[8] Vandamme, P., Holmes, B., Coenye, T., Goris, J., Mahenthiralingam, E., LiPuma, J.J. and Govan, J.R.W. (2003) *Burkholderia cenocepacia* sp.nov. – a new twist to an old story. Res. Microbiol. 154, 91–96.

[9] Vermis, K., Coenye, T., LiPuma, J.J., Mahenthiralingam, E., Nelis, H.J. and Vandamme, P. (2004) Proposal to accommodate *Burkholderia cepacia* genomovar VI as *Burkholderia dolosa* sp. nov. Int. J. Syst. Evol. Microbiol. 54, 689–691.

[10] Burkholder, W.H. (1950) Sour skin, a bacterial rot of onion bulbs. Phytopathology 40, 115–117.

[11] Govan, J.R.W., Hughes, J.E. and Vandamme, P. (1996) *Burkholderia cepacia*: medical, taxonomic and ecological issues. J. Med. Microbiol. 45, 395–407.

[12] Hebbar, K.P., Martel, M.H. and Heulin, T. (1994) *Burkholderia cepacia*: a plant growth promoting rhizobacterial associate of maize In: Improving Plant Productivity with Rhizosphere Bacteria (Ryder, M.H., Stephens, P.M. and Bowen, G.D., Eds.), pp. 201–203. CSIRO Division of Soils, Adelaide, Australia.

[13] Balandreau, J., Viallard, V., Cournoyer, B., Coenye, T., Laevens, S. and Vandamme, P. (2001) *Burkholderia cepacia* genomovar III is a common plant-associated bacterium. Appl. Environ. Microbiol. 67, 982–985.

[14] Wise, M.G., Vaun McArthur, J., Wheat, C. and Shimkets, L.J. (1996) Temporal variation in genetic diversity and structure of a lotic population of *Burkholderia* (*Pseudomonas*) *cepacia*. Appl. Environm. Microbiol. 62, 1558–1562.

[15] Vermis, K., Brachkova, M., Vandamme, P. and Nelis, H.J. (2003) Isolation of *Burkholderia cepacia* complex genomovars from waters. Syst. Appl. Microbiol. 26, 595–600.

[16] Miller, S.C.M., LiPuma, J.J. and Parke, J.L. (2002) Culture-based and non-growth-dependent detection of the *Burkholderia cepacia* complex in soil environments. Appl. Environ. Microbiol. 68, 3750–3758.

[17] Coenye, T. and Vandamme, P. (2003) Diversity and significance of *Burkholderia* species occupying diverse ecological niches. Environ. Microbiol. 5, 719–729.

[18] Govan, J.R.W., Balendreau, J. and Vandamme, P. (2000) *Burkholderia cepacia* – friend and foe. Am. Soc. Microbiol. News 66, 124–125.

[19] LiPuma, J.J., Spilker, T., Coenye, T. and Gonzalez, C.F. (2002) An epidemic *Burkholderia cepacia* complex strain identified in soil. Lancet 359, 2002–2003.

[20] Speert, D.P., Henry, D., Vandamme, P., Corey, M. and Mahenthiralingam, E. (2002) Epidemiology of *Burkholderia cepacia* complex in patients with cystic fibrosis, Canada. Emerg. Infect. Dis. 8, 181–187.

[21] Manno, G., Dalmastri, C., Tabacchioni, S., Vandamme, P., Lorini, R., Minicucci, L., Romano, L., Giannattasio, A., Chiarini, L. and Bevivino, A. (2004) Epidemiology and clinical course of *Burkholderia cepacia* complex infections, particularly those caused by different *Burkholderia cenocepacia* strains, among patients attending an Italian Cystic fibrosis Center. J. Clin. Microbiol. 42, 1491–1497.

[22] Mahenthiralingam, E., Bischof, J., Byrne, S.K, Radomski, C., Davies, J.E., Av-Gay, Y. and Vandamme, P. (2000) DNA-based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, *Burkholderia cepacia* genomovars I and III. J. Clin. Microbiol. 38, 3165–3173.

[23] Vermis, K., Vandekerckhove, C., Nelis, H.J. and Vandamme, P. (2002) Evaluation of restriction fragment length polymorphism analysis of 16S rDNA as a tool for genomovar characterisation within the *Burkholderia cepacia* complex. FEMS Microb. Lett. 214, 1–5.

[24] Dřvínek, P., Hrbáčková, H., Cinek, O., Bartošova, J., Nyč, O., Nemec, A. and Pohunek, P. (2002) Direct PCR detection of *Burkholderia cepacia* complex and identification of its genomovars by using sputum as source of DNA. J. Clin. Microbiol. 40, 3485–3488.

[25] Vermis, K., Coenye, T., Mahenthiralingam, E., Nelis, H. and Vandamme, P. (2002) Evaluation of species-specific *recA*-based PCR tests for genomovar level identification within the *Burkholderia cepacia* complex. J. Med. Microbiol. 51, 937–940.

[26] Fiore, A., Laevens, S., Bevivino, A., Dalmastri, C., Tabacchioni, S., Vandamme, P. and Chiarini, L. (2001) *Burkholderia cepacia* complex: distribution of genomovars among isolates from the maize rhizosphere in Italy. Environ. Microbiol. 3, 137–143.

[27] Dalmastri, C., Fiore, A., Alisi, C., Bevivino, A., Tabacchioni, S., Giuliano, G., Sprocati, A., Segre, L., Mahenthiralingam, E., Chiarini, L. and Vandamme, P. (2003) A rhizospheric *Burkholderia cepacia* complex population: genotypic and phenotypic diversity of *Burkholderia cenocepacia* and *Burkholderia ambifaria*. FEMS Microbiol. Ecol. 46, 179–187.

[28] Burbage, D.A., Sasser, M. and Lumsden, R.D. (1982) A medium selective for *Pseudomonas cepacia*. Phytopathology 72, 706 (Abstracts).

[29] Di Cello, F., Bevivino, A., Chiarini, L., Fani, R., Paffetti, D., Tabacchioni, S. and Dalmastri, C. (1997) Biodiversity of a *Burkholderia cepacia* population isolated from maize rhizosphere at different plant growth stages. Appl. Environ. Microbiol. 63, 4485–4493.

[30] Bevivino, A., Sarrocco, S., Dalmastri, C., Tabacchioni, S., Cantale, C. and Chiarini, L. (1998) Characterization of a free-living maize-rhizosphere population of *Burkholderia cepacia*: effect of seed treatment on disease suppression and growth promotion of maize. FEMS Microbiol. Ecol. 27, 225–237.

[31] Dalmastri, C., Chiarini, L., Cantale, C., Bevivino, A. and Tabacchioni, S. (1999) Soil type and maize cultivar affect the genetic diversity of maize root-associated *Burkholderia cepacia* populations. Microb. Ecol. 38, 274–283.

[32] Hagerdon, C., Gould, W.D., Bardinelli, T.R. and Gustavson, D.R.A. (1987) Selective medium for enumeration and recovery of *Pseudomonas cepacia* biotypes from soil. Appl. Environ. Microbiol. 53, 2265–2268.

[33] Mahenthiralingam, E., Coenye, T., Chung, J.W., Speer, D.P., Govan, J.R.W., Taylor, P. and Vandamme, P. (2000)

Diagnostically and experimentally useful panel of strains from the *Burkholderia cepacia* complex. J. Clin. Microbiol. 38, 910–913.

[34] Coenye, T., Vandamme, P., LiPuma, J.J., Govan, J.R.W. and Mahenthiralingam, E. (2003) Updated version of the *Burkholderia cepacia* complex experimental strain panel. J. Clin. Microbiol. 41, 2797–2798.

[35] Seo, S.T. and Tsuchiya, K. (2004) PCR-based identification and characterization of *Burkholderia cepacia* complex bacteria from clinical and environmental sources. Lett. Appl. Microbiol. 39, 413–419.

[36] Storms, V., Van Den Vreken, N., Coenye, T., Mahenthiralingam, E., LiPuma, J.J., Gillis, M. and Vandamme, P. (2004) Polyphasic characterisation of *Burkholderia cepacia*-like isolates leading to the emended description of *Burkholderia pyrrocinia*. Syst. Appl. Microbiol. 27, 517–526.

[37] Tabacchioni, S., Chiarini, L., Bevivino, A., Cantale, C. and Dalmastri, C. (2000) The choice of an appropriate selective medium is critical for assessing the genetic diversity of a natural microbial population. Microb. Ecol. 40, 169–176.