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ORIGINAL PAPER

Characterization of *Burkholderia cepacia* complex from cystic fibrosis patients in China and their chitosan susceptibility

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Abstract A survey of *Burkholderia cepacia* complex (Bcc) species was conducted in sputum from cystic fibrosis (CF) patients in China. One hundred and four bacterial isolates were recovered on *B. cepacia* selective agar and 42 of them were assigned to Bcc by PCR assays. The species composition of the Bcc isolates from CF sputum was analyzed by a combination of *recA*-restriction fragment length polymorphism assays, species-specific PCR tests and *recA* gene sequencing. The results revealed that the 42 Bcc isolates belong to *B. cepacia*, *B. cenocepacia* and *B. contaminans* while predominant Bcc species was *B. cenocepacia*. This is the first report of *B. contaminans* from CF sputum in China. In addition, results from this study showed that chitosan solution at 10, 25, 50 and 100 μg/ml markedly inhibited the growth of the 16 representative isolates from the three different Bcc species, which indicated that chitosan was a potential bactericide against Bcc bacteria.

Keywords *Burkholderia cepacia* complex · Cystic fibrosis · Chitosan · Antibiotics · Antibacterial activity

Introduction

The *Burkholderia cepacia* complex (Bcc) is a collection of genetically distinct but phenotypically similar bacteria that have emerged as life-threatening pulmonary pathogens in immunocompromised patients, particularly individuals with cystic fibrosis (CF). Indeed, the Bcc currently comprises *B. cepacia*, *Burkholderia multivorans*, *Burkholderia cenocepacia*, *Burkholderia stabilis*, *Burkholderia vietnamiensis*, *Burkholderia dolosa*, *Burkholderia ambifaria*, *Burkholderia anthina* and *Burkholderia pyrrocinia* (Zhang and Xie 2007; Mahenthiralingam et al. 2008). In addition, Vermis et al. (2002) proposed *Burkholderia ubonensis* as the tenth species within the Bcc and while this has been confirmed via phylogenetic examination of the 16S rRNA gene sequence of this species (Coenye and Vandamme 2003), a formal polyphasic study of this species has yet to be published (Mahenthiralingam et al. 2008).

More recently, seven new species including *Burkholderia latens*, *Burkholderia diffusa*, *Burkholderia arboris*, *Burkholderia seminalis*, *Burkholderia metallica*, *Burkholderia contaminans* and *Burkholderia lata* have been proposed (Vanlaere et al. 2008, 2009). Interestingly, most current Bcc species have been isolated from clinical specimens (Isles et al. 1984; Coenye and Vandamme 2003; Mahenthiralingam et al. 2008). However, approximately 90% of the Bcc isolates cultured from CF patients belong to *B. multivorans* and *B. cenocepacia*. These two species account for most episodes of epidemic spread in CF and non-CF patients (LiPuma et al. 2001; Speert et al. 2002; Martins et al. 2008). In addition, *B. cenocepacia* is also the species most associated with the rapid pulmonary decline known as "cepacia syndrome", and with post-transplant mortality.

The numbers of infections caused by the Bcc have increased in China in the past decade (Lin et al. 2007). This increase is believed, at least in part, to be due to the increasing numbers of immunocompromised patients, increased population age of these patients, more accurate identification of Bcc bacteria, social and behavioural changes that have

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allowed for close contact between patients, and the increased use of antibiotics to which Bcc bacteria are resistant in patient therapy (Mahenthiralingam et al. 2005). However, little is known about species composition of Bcc bacteria from CF patients in China although it is fundamental to the treatment and management of CF patients.

Treatment of CF infections is very difficult due to the intrinsic resistance of Bcc bacteria to most clinically useful antibiotics (Aaron et al. 2000; Nzula et al. 2002). Most isolates of Bcc bacteria exhibit high-level resistance to all major classes of antibiotics. Some isolates of Bcc even can utilize penicillin G as a sole carbon source for growth (Beckman and Lessie 1979). Thus, it becomes important to identify newer and improved antibacterial therapies for CF patients. Interestingly, applications of chitosan to the fields of medicine and pharmaceuticals have received considerable attention in recent years (Simunek et al. 2006; Li et al. 2008). Thus, the use of chitosan as an antibacterial agent seems to be a promising approach for reducing the risk of CF patients.

Chitosan is a natural nontoxic biopolymer derived by deacetylation of chitin [poly-β-(1 $\rightarrow$ 4)-*N*-acetyl-D-glucosamine], a major component of the shells of crustacea such as crab, shrimp, and crawfish (Li et al. 2008). In addition, previous work had shown that chitosan have several advantages over other type of bactericide because it possesses a higher antibacterial activity, a broader spectrum of activity, a higher killing rate, and a lower toxicity toward mammalian cells (Simunek et al. 2006; Li et al. 2008). Recently, several studies have demonstrated that chitosan had strong antibacterial activity against human-associated bacteria (Simunek et al. 2006). However, the antimicrobial activity of chitosan against Bcc bacteria was not clear.

The aims of this study were to determine the species status of Bcc bacteria from CF patients in China and their chitosan susceptibility.

Materials and methods

Isolation of Bcc bacteria

The sputum samples were collected from 11 CF patients in First Affiliated Hospital of China Medical University during 2002–2005. Putative Bcc bacteria were isolated by streaking 0.01 ml of CF sputum onto *B. cepacia* selective agar (BCSA) (Henry et al. 1997). Individual bacterial colonies formed after 2–3 days of incubation at 28°C were isolated from culture plates, and stored in 30% aqueous glycerol at −80°C for further studies. The reference strain LMG 1222 of *B. cepacia* was provided by the Belgian Co-ordinated Collections of Microorganisms, BCCM, Gent, Belgium.

Identification of Bcc isolates

Total DNAs of bacterial isolates in this study were extracted according to the method of Vermis et al. (2003a). Amplification of the *recA* gene was carried out by PCR as described by Zhang and Xie (2007) using the specific primers for Bcc, BCR1 and BCR2. PCR reactions were performed with a Programmable Temperature Cycler (PTC-200, MJ Research, USA). The amplification conditions were as follows: initial denaturation 94°C for 3 min, followed by 30 cycles of 1 min at 94°C, 30 s at 62°C, and 1 min at 72°C and then a final extension step consisting of 10 min at 72°C.

Species status of Bcc isolates

The species composition of the Bcc isolates was analyzed by a combination of *recA*-restriction fragment length polymorphism (RFLP) assays and species-specific PCR tests (Bosch et al. 2008). RFLP analysis was performed according to the method of Mahenthiralingam et al. (2000). After amplification with primers BCR1 and BCR2, 8 µl of amplified product was digested by 1 U of either *Hae*III or *Mnl*I (Fermentas, USA) in a total volume of 15 µl and incubated at 37°C for 3 h. The RFLP products were separated by 2% agarose gel electrophoresis and visualized by staining with ethidium bromide. A 50-bp ladder (Fermentas, USA) was used as a molecular size marker. The RFLP patterns were manually analysed and compared to those shown by Bcc reference strains (Mahenthiralingam et al. 2000). Also, the putative species status of each Bcc isolate was determined by species-specific PCR tests, according to the procedures described previously (Mahenthiralingam et al. 2000; Vandamme et al. 2002; Pirone et al. 2005). Further resolution of the correct species was achieved with nucleotide sequence analysis of the Bcc *recA* gene.

DNA sequencing and phylogenetic analysis

PCR amplicons corresponding to fragments of the *recA* genes giving different RFLP patterns were excised from the gels, purified, ligated into PGEM-T Easy vector (Takara, Shanghai, China) according to the supplier's instructions and transferred into *Escherichia coli* cells (DH5α). Clones with the correct insert were sequenced by Huada Genomic Biotechnology Co., Ltd (Hangzhou, China). Raw sequences from both strands of the PCR products were then aligned, and a consensus sequence was derived using DNASTAR software (DNASTAR Inc.,

Madison, Wis.). Sequence identity was confirmed by analysis using the basic local alignment sequence tool (BLAST) at the National Center for Biotechnology Information (NCBI, Bethesda, Md.).

Phylogenetic analysis was performed on 6 novel *recA* sequences and 38 previously published Bcc *recA* gene (Mahenthiralingam et al. 2000; Vandamme et al. 2002; Coenye and Vandamme 2003; Baldwin et al. 2005; Payne et al. 2005; Zhang and Xie 2007). Nucleotides of the *recA* gene were aligned using CLUSTAL W. Phylogenetic and molecular evolutionary analyses were conducted using the genetic distance-based neighbor-joining algorithms within MEGA version 4.0 (http://www.megasoftware.net/). Bootstrap analysis for 1,000 replicates was performed to estimate the confidence of tree topology. *Pseudomonas aeruginosa* was used as the outgroup.

Chitosan susceptibility of Bcc isolates

Chitosan (degree of N-deacetylation no less than 85%, practical grade, from crab shells) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Cefoxitin and tetracycline were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China). Stock solution of chitosan (5 mg/ml) was prepared in 1% acetic acid (Sangon, Shanghai, China) with pH being adjusted to 6.0 with NaOH as described previously (Liu et al. 2006). After stirring (160 rpm) for 24 h at room temperature, the stock solution was autoclaved at 121°C for 20 min. Sterile deionized water of pH 6.0 was used as a control.

The 16 representative isolates of Bcc, including two *B. cepacia* isolates, nine *B. cenocepacia* IIIA isolates, two *B. cenocepacia* IIIB isolates and three *B. contaminans* isolates, were selected and cultured for 48 h on nutrient agar medium at 28°C. After incubation, each bacterial suspension was prepared in sterilized water, and the initial concentration of bacteria was adjusted to approximately 10^8 colony forming units (cfu)/ml. The bacterial cells were counted according to the method as described by Li et al. (2008).

The inhibitory effect of chitosan as well as two antibiotics cefoxitin and tetracycline against Bcc isolates was determined according to the method of Li et al. (2008). Chitosan solutions of 5 ml in volume were prepared by adding chitosan stock to deionized water to give a final chitosan concentration of 10, 25, 50 and 100 µg/ml. The final concentration of the antibiotics was 512 µg/ml. Bacterial suspension was added to 5 ml of chitosan or antibiotics solution to give a final bacterial concentration of 10^7 cfu/ml and then the mixture was incubated at 28°C on a rotary shaker (Hualida Company, Taicang, China) at 160 rpm. In the control treatment chitosan stock was replaced with sterile deionized water of pH 6.0 in order to obtain the same pH. Six hours later, samples were collected from each cell suspension and bacterial counting was followed as indicated above. The inhibition percentage (%) was evaluated by comparing cell numbers between the treatment with and without chitosan (Chung et al. 2003).

Statistic analysis

The software STATGRAPHICS Plus, version 4.0 (Copyright Manugistics Inc., Rockville, Md., USA) was used to perform the statistical analysis. Levels of significance ($P < 0.05$) of main treatments and their interactions were calculated by analysis of variance after testing for normality and variance homogeneity.

Nucleotide sequence accession numbers

The nucleotide sequences of the *recA* genes from the following 6 Bcc isolates have been determined and deposited in GenBank (GenBank accession number shown in brackets): Y4 (EU500764); Y5 (EU500765); Y6 (EF426456); Y10 (EF426457); Y17 (EU521725) and Y20 (EF426458).

Results and discussion

Isolation and identification of Bcc isolates

A total of 104 putative Bcc isolates were recovered on the semi-selective media BCSA. Among them, 42 isolates from CF sputum samples were identified as Bcc by PCR amplification of the *recA* gene with specific primers for Bcc, BCR1 and BCR2. An amplicon of about 1,041 bp was obtained from all Bcc isolates. In addition, the result from this study indicated that the Bcc recovery frequency on BCSA plates was higher than those on other growth agar (data not shown), which is different from the result of Zhang and Xie (2007), who found that environmental isolates of Bcc were unable to grow on BCSA medium.

Coenye et al. (2001) review the best methods for phenotypic identification, and recommend that these include the use of selective media such as BCSA for clinical isolates and respiratory specimens; and less selective media such as ''*P. cepacia*'' polycyclic hydrocarbon medium (PCAT) and Trypan Blue Tetracycline (TB-T) for environmental isolates (Ramette et al. 2005). However, misidentification or lack of identification of Bcc bacteria is still a problem that faces many diagnostic microbiology laboratories (Coenye et al. 2001). To confirm a presumptive phenotypic identification of Bcc bacteria, genetic methods are the most accurate and reliable (Mahenthiralingam et al. 2008).

Species status of Bcc isolates

The species composition of the 42 Bcc isolates from CF sputum was determined by a combination of RFLP assays of *recA* gene with the enzyme *Hae*III and *Mnl*I and species-specific PCR tests as well as *recA* gene sequencing. Digestion with *Hae*III of *recA* gene resulted in three different restriction patterns (K, G and H) while four distinct *Mnl*I RFLP patterns (d, f, g and h) were observed among the 42 Bcc isolates (Fig. 1). Each pattern was assigned an aphabetical code as described previously (Mahenthiralingam et al. 2000; Petrucca et al. 2003; Dalmastri et al. 2005; Pirone et al. 2005). This results revealed that the 42 Bcc isolates could be ascribed to *B. cepacia*, *B. cenocepacia* IIIA and *B. cenocepacia* IIIB based on the RFLP patterns of *recA* gene (Fig. 1; Table 1), which is consistent with the result of Mahenthiralingam et al. (2000), who found that RFLP with *Hae*III and *Mnl*I could be used to place the isolate within a specific species.

In agreement with the result of RFLP assay of *recA* gene, the species-specific PCR tests allowed us to assign most of the 42 Bcc isolates to respective species. However, three Bcc isolates Y1, Y2 and Y4 showing a unique RFLP pattern of *B. cenocepacia* IIIB gave positive amplification with primers specific for *B. cenocepacia* IIIA. This contrary result may reflect the fact that *recA* RFLP or *recA*-based species-specific PCR are not very accurate for all current Bcc species. However, positive amplification of a 1,040-bp *recA* product using primers BCR1 and BCR2 has remained 100% predictive that an isolate is a member of the Bcc.

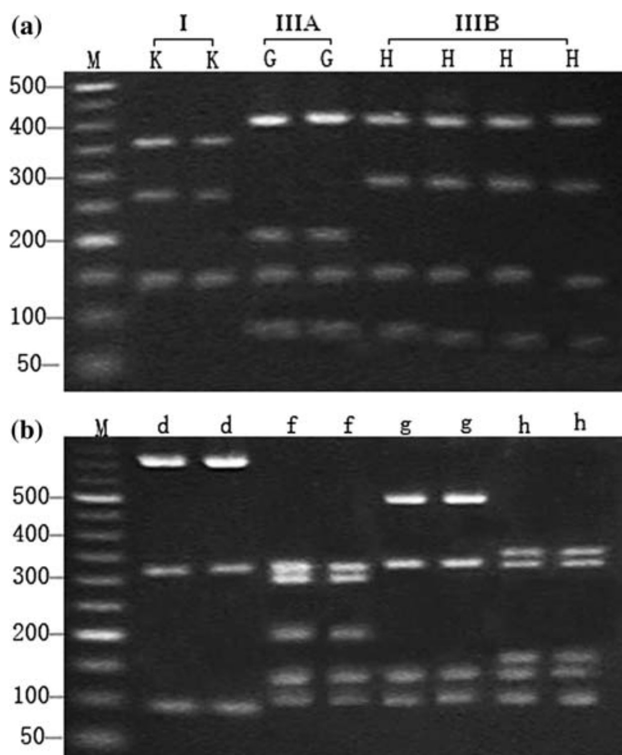

Fig. 1 RFLP assays of *B. cepacia* complex *recA* gene with the enzyme *Hae*III and *Mnl*I. **a** The alphabetical *Hae*III RFLP type is shown *above each lane*. The DNA molecular size standard is in *lane M*. The Bcc isolates in *each lane* are as follows (*second lane; left to right*): Y3; Y6; Y10; Y20; Y2; Y4; Y5 and Y17. The species status of each isolate is indicated *above the relevant lane*. **b** *Mnl*I RFLP analysis of *recA* gene. The designated *Mnl*I RFLP type is shown above *each lane*. The order of Bcc isolates in *each lane* is the same with that of *Hae*III RFLP

To solve these ambiguities and to definitively assess the species status of the Bcc isolates, the complete *recA* gene sequence of 6 Bcc isolates representative of different *recA* RFLP type were determined and aligned to other known Bcc sequences deposited in GenBank. In agreement with the result of *recA* RFLP and species-specific PCR, *recA* gene sequence analysis allowed us to assign the 6 Bcc isolates except Y4 to respective species. The phylogenetic analysis revealed that isolate Y4 and strains of *B. contaminans* clustered within a group and well separated from other species of Bcc (Fig. 2). It is therefore considered that the isolate should be identified as *B. contaminans*. This is consistent with previous studies that have shown that phylogenetic analysis of the *recA* gene sequence is discriminatory and can place an isolate within a named or novel Bcc group (Mahenthiralingam et al. 2000; Payne et al. 2005).

Overall, three Bcc species *B. cepacia*, *B. cenocepacia* IIIA and IIIB, and *B. contaminans* were recovered from the CF patients in China. To our knowledge, this is the first report of *B. contaminans* from CF patients in China. Predominant Bcc species was *B. cenocepacia* in CF sputum samples, which is consistent with previous studies that have shown that *B. cenocepacia* frequently caused highly virulent and transmissible infections in large numbers of individuals with CF. (Vandamme et al. 1997; Clode et al. 2000; LiPuma et al. 2001; Mahenthiralingam et al. 2008). However, this result is different from the study of Lin et al. (2007), who found that the outbreak of nosocomial bloodstream infection in China was caused by *B. stabilis*. This difference may be ascribed to the clinical state and predisposition of CF individuals at the time of infection (Mahenthiralingam et al. 2008).

Susceptibility of Bcc isolates to chitosan

Chitosan solution at four different concentrations showed effective antibacterial activity against the 16 representative isolates from three different species of Bcc compared to the corresponding control after 6 h of incubation (Table 2). Totally, chitosan solutions up to 100 μg/ml showed stronger antibacterial activity against the Bcc isolates compared with the remainder treatment, which is consistent with the

Table 1 Isolation, identification and species composition of *Burkholderia cepacia* complex from 11 cystic fibrosis patients in China during 2002–2005

Year	Patient[a]	Number of		Status of species	
		Bacterial isolates[b]	Bcc isolates[c]	PCR–RFLP identification	Species-specific PCR identification
2002	1	3	1	*B. cepacia* (1)	*B. cepacia* (1)
	2	10	4	*B. cepacia* (1)	*B. cepacia* (1)
				B. cenocepacia IIIA (3)	*B. cenocepacia* IIIA (3)
2003	3	9	2	*B. cepacia* (2)	*B. cepacia* (2)
	4	13	4	*B. cenocepacia* IIIA (4)	*B. cenocepacia* IIIA (4)
	5	8	3	*B. cenocepacia* IIIA (1)	*B. cenocepacia* IIIA (1)
				B. cenocepacia IIIB (2)	*B. cenocepacia* IIIB (2)
2004	6	15	3	*B. cenocepacia* IIIA (3)	*B. cenocepacia* IIIA (3)
	7	1	1	*B. cepacia* (1)	*B. cepacia* (1)
	8	20	11	*B. cepacia* (5)	*B. cepacia* (5)
				B. cenocepacia IIIA (6)	*B. cenocepacia* IIIA (6)
	9	5	3	*B. cenocepacia* IIIA (2)	*B. cenocepacia* IIIA (2)
				B. cenocepacia IIIB (1)	*B. cenocepacia* IIIB (1)
2005	10	14	6	*B. cenocepacia* IIIA (3)	*B. cenocepacia* IIIA (6)
				B. cenocepacia IIIB (3)[d]	
	11	6	4	*B. cenocepacia* IIIA (4)	*B. cenocepacia* IIIA (4)

[a] The patients were numbered in sequence using Arabic numerals

[b] A total of 104 bacterial isolates were recovered on BCSA medium

[c] A total of 42 Bcc isolates were determinated by PCR amplification with specific primers BCR1 and BCR2

[d] The Bcc isolates which showed a RFLP pattern of *B. cenocepacia* IIIB and gave positive amplification with primers specific for *B. cenocepacia* IIIA were finally identified as *B. contanimans* based on *recA* sequences analysis (Fig. 2)

result of Liu et al. (2006), who found that the antibacterial activity of chitosan was influenced by its concentration in the solution. At the concentration of 100 μg/ml, the growth of the 16 Bcc isolates except *B. cenocepacia* Y5 were inhibited more than 85% by chitosan. The highest inhibition percentages were 99.9% for *B. cepacia* Y3 and Y6.

The inhibition percentage of two antibiotics cefoxitin and tetracycline against Bcc bacteria depends on the tested isolate. The growths of the 5 Bcc isolates were inhibited more than 85% by cefoxitin while the inhibition percentage of cefoxitin against 9 Bcc isolates was lower than 50%. Similarly, the growths of the 3 Bcc isolates were inhibited more than 85% by tetracycline while the inhibition percentage of cefoxitin against 11 Bcc isolates was lower than 50%. In addition, tetracycline even stimulated the growth of the *B. cenocepacia* isolates Y7 and Y14. In contrast, the growth of the *B. cenocepacia* isolates Y7 and Y14 were inhibited more than 85% by chitosan.

The intrinsic resistance of the Bcc isolates from CF patients to cefoxitin and tetracycline has been well documented (Vermis et al. 2003b). In this study, the growths of the 16 Bcc isolates (100%) were inhibited by cefoxitin at 512 μg/ml while the growths of the 7 Bcc isolates (44%) were inhibited by tetracycline at 512 μg/ml compared to the corresponding control (data not shown), which is consistent with the result of Vermis et al. (2003b), who found that the 90% minimum inhibitory concentration (MIC90) of cefoxitin was 512 μg/ml while the MIC90 of tetracycline was more than 512 μg/ml. In contrast, chitosan solution at four different concentrations inhibited the growths of the 16 Bcc isolates (100%) compared to the corresponding control, which indicated that chitosan was a potential bactericide against Bcc bacteria. Therefore, it could be suggested that chitosan could be used as a natural disinfectant to reduce the risk of transmission of Bcc in CF patients.

From the study, it is evident that chitosan solution has strong antibacterial activity against Bcc bacteria irregardless of the species. As many Bcc isolates are resistant to multiple antibiotics (Aaron et al. 2000; Nzula et al. 2002), it represents a growing problem for public health. Considering the absence of any sort of bactericide for CF infections, the present investigation may prove helpful in a field. In addition, many attempts have also been taken up to improve the antimicrobial activity of chitosan, such as structural modification, adjustment of molecular factors, and forming complexes with other antimicrobial materials (Li et al. 2008; San et al. 2009). Interestingly, San et al. (2009) recently found that all the chitosans show synergistic activity with sulfamethoxazole, a sulfonamide antimicrobial agent, which

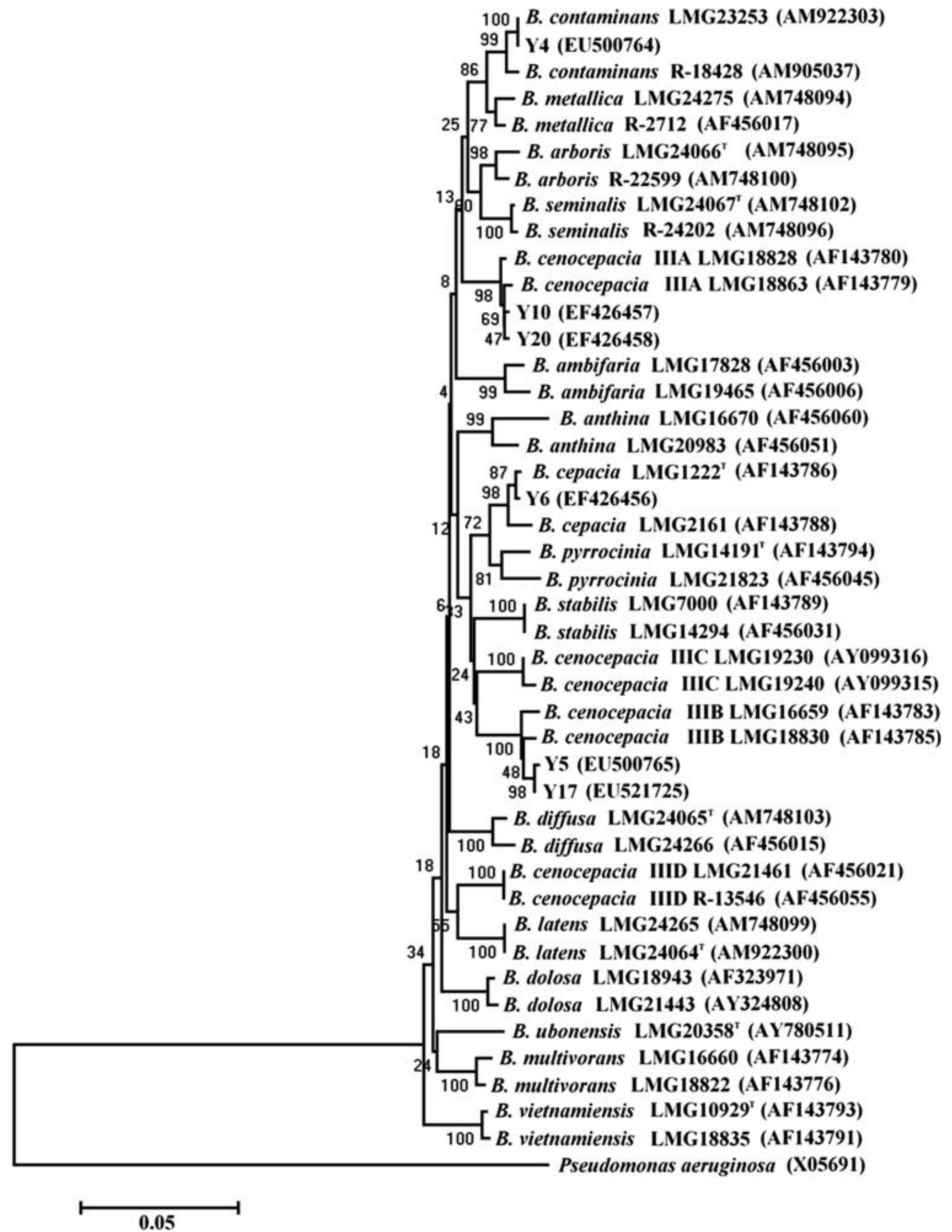

Fig. 2 Phylogenetic tree derived from the *recA* gene sequence analysis on reference strains of each *Burkholderia cepacia* complex species and representative isolates of different *B. cepacia* complex species from the CF patients in China. The tree was generated by the neighbor-joining method based on the two-parameter Kimura correction of evolutionary distances. Bootstrap analyses (1,000 replicates) for node values from 50% are indicated. *Pseudomonas aeruginosa* was used as the outgroup

suggest that the antibacterial activity of chitosan could be enhanced by combination with antibiotics in pharmaceutical preparations.

Most studies on the mode of action of chitosan have been conducted with fungal pathogens, and little is known about its action on bacteria (Li et al. 2008). Several studies have indicated that the interactions between positively charged chitosan molecules and negatively charged residues on the bacterial cell surface play an important role in the inhibitory effect of chitosan on gram-negative bacteria (Helander et al. 2001). In addition, it has been suggested that reduction of cell numbers is caused by cell surface alterations and loss of barrier functions (Helander et al. 2001). Chitosan with positive charges easily reacts with negatively charged bacteria and further inhibits bacterial growth. Surface interference may be the possible mechanism for the bactericidal properties (Helander et al. 2001). However, Raafat et al. (2008) have recently postulated that binding of chitosan to teichoic acids, coupled with a potential extraction of membrane lipids (predominantly lipoteichoic acid) results in a sequence of events, ultimately leading to bacterial death. Further, Chung and Chen (2008) found that the inactivation of *E. coli* by chitosan occurs via a two-step sequential mechanism: an initial separation of the cell wall from its cell membrane, followed by destruction of the cell membrane.

Table 2 Inhibition of chitosan and antibiotics against *Burkholderia cepacia* complex from cystic fibrosis patients in China

Strain	Species identity	Inhibition (%)[a]					
		Chitosan solution (μg/ml) at				Cefoxitin	Tetracycline
		10	25	50	100	(512 μg/ml)	(512 μg/ml)
Y3	*B. cepacia*	89.0 ± 4.5[b]	90.4 ± 3.8	95.4 ± 2.5	99.9 ± 0.0	37.9 ± 5.4	7.0 ± 8.6
Y6	*B. cepacia*	87.4 ± 4.6	92.7 ± 2.3	93.6 ± 2.5	99.9 ± 0.0	90.0 ± 2.9	95.4 ± 2.2
Y7	*B. cenocepacia* IIIA	81.3 ± 3.5	87.8 ± 4.1	89.6 ± 3.0	93.8 ± 2.8	46.7 ± 4.7	-0.3 ± 4.6
Y8	*B. cenocepacia* IIIA	77.9 ± 3.7	86.3 ± 3.8	89.5 ± 3.6	94.9 ± 2.2	99.4 ± 0.2	26.2 ± 3.3
Y9	*B. cenocepacia* IIIA	86.3 ± 4.1	88.9 ± 3.8	90.2 ± 2.9	90.2 ± 2.3	19.4 ± 3.7	42.7 ± 3.0
Y10	*B. cenocepacia* IIIA	98.6 ± 0.7	97.9 ± 1.8	95.2 ± 2.3	95.8 ± 1.8	94.9 ± 2.2	89.1 ± 1.8
Y11	*B. cenocepacia* IIIA	75.0 ± 3.9	76.1 ± 2.8	82.1 ± 3.1	94.1 ± 2.6	81.8 ± 3.5	12.3 ± 3.0
Y12	*B. cenocepacia* IIIA	87.8 ± 3.5	89.0 ± 3.8	91.6 ± 2.5	94.8 ± 2.3	28.1 ± 2.8	5.1 ± 2.1
Y14	*B. cenocepacia* IIIA	65.7 ± 3.5	72.6 ± 3.9	78.2 ± 3.0	85.3 ± 1.7	42.7 ± 2.1	−5.7 ± 4.0
Y16	*B. cenocepacia* IIIA	88.3 ± 3.6	91.7 ± 2.6	93.3 ± 2.5	99.4 ± 0.2	72.0 ± 3.7	88.6 ± 2.9
Y20	*B. cenocepacia* IIIA	72.7 ± 3.7	93.2 ± 2.1	95.5 ± 2.1	92.7 ± 2.2	37.7 ± 5.0	55.6 ± 5.6
Y5	*B. cenocepacia* IIIB	35.3 ± 2.4	44.2 ± 3.4	48.1 ± 3.4	49.0 ± 2.1	21.4 ± 3.5	0.1 ± 5.4
Y17	*B. cenocepacia* IIIB	76.3 ± 3.2	83.3 ± 1.3	86.4 ± 3.6	89.2 ± 2.2	9.6 ± 2.7	3.3 ± 3.9
Y1	*B. contaminans*	77.9 ± 3.5	84.3 ± 2.7	88.2 ± 2.9	90.9 ± 1.8	85.1 ± 2.9	0.3 ± 2.4
Y2	*B. contaminans*	67.1 ± 3.9	71.6 ± 2.8	79.1 ± 3.8	87.0 ± 4.0	91.8 ± 2.8	45.5 ± 4.8
Y4	*B. contaminans*	90.1 ± 3.1	89.8 ± 3.3	91.8 ± 3.0	94.2 ± 2.9	12.1 ± 2.8	50.3 ± 2.7

[a] Initial concentration of bacteria is 10^7 cfu/ml; the surviving cells in chitosan and antibiotics solution were counted after 6 h of incubation

[b] Data are presented as means ± standard error from a representative experiment repeated twice with similar results

In summary, our results indicate that species composition and abundance of Bcc among CF patients in China vary dramatically. Totally, *B. cenocepacia* IIIA was the most dominant in the sputum of CF patients, followed by *B. cepacia*. In addition, result from this study demonstrates that the chitosan could inhibit the growth of the Bcc isolates isolated from the sputum of CF patients. To the best of our knowledge, this is the first report about antibacterial activities of chitosan on Bcc bacteria, which showed chitosan may be a good candidate for novel antimicrobial agents against bacterial infections in CF patients.

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