Exopolysaccharides produced by clinical strains belonging to the
Burkholderia cepacia complex

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Abstract

Background: In the frame of a research line dedicated to better clarify the role of exopolysaccharides (EPS) in bacterial virulence, EPS produced by species of the Burkholderia cepacia complex (Bcc), namely Burkholderia multivorans, Burkholderia cenocepacia, and a Bcc member of undetermined genomovar, all isolated at the Cystic Fibrosis Regional Centre of Florence (Italy), were investigated for they structural properties.

Methods: Three strains of B. multivorans, three of B. cenocepacia and one of a Bcc member of undetermined genomovar were isolated from CF patients. The reference strains C1576 and J2315, for genomovar II and III, respectively, were included in the study. The bacteria were grown on solid media, the exopolysaccharides produced were purified, and their structures were determined. In addition, sugar analysis of sputum samples was accomplished to search for EPS produced in vivo.

Results: Six strains out of seven produced the exopolysaccharide cepacian, while one strain of B. multivorans produced a completely different polymer, previously known in the literature as PS1. Two strains synthesised very small amounts of EPS. No definitive evidence for the presence of cepacian in sputum samples was found.

Conclusions: Most strains examined produced abundant amounts of polysaccharides. Cepacian was the most common EPS isolated and its production was not associated to a particular genomovar.

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Keywords: Cystic fibrosis; Burkholderia cepacia complex; Exopolysaccharide; Structure; Sputum samples

1. Introduction

Bacterial exopolysaccharides (EPS) are important factors contributing to infection maintenance. These biopolymers are excreted in the medium surrounding bacteria where they form a disperse coating around the cells. The macromolecular nature of the coating and its favourable interactions with the aqueous environment increase the medium viscosity immediately around bacterial cells. This property confers a protecting role against phagocytosis, opsonisation and dehydration [1,2]. In addition, a specific role against the action of antimicrobial peptides of the innate immune system has been proposed for bacterial EPS [3,4].

In cystic fibrosis (CF) patients, the composition of the fluid covering the lung epithelium is altered, and the mucociliary clearance is impaired, leading to colonisation by different bacteria. Pulmonary infections due to opportunistic pathogens are the major cause of death for CF patients and many reports evoke the important role of bacterial EPS in such infections. Studies carried out on Pseudomonas aeruginosa, one of the most important pathogen for CF
patients, clarified most of the biological role of its EPS, alginate [5]. More specifically, it was demonstrated that O-acetylation of alginate modulates the resistance of mucoid bacteria to antibody-independent opsonic killing [2]. In fact, acetate residues are bound via ester linkages to EPS hydroxyl groups that, when unsubstituted, may act as binding sites for the C3b and C4b opsonins. The above results clearly indicated a critical role for EPS in bacterial virulence and also suggested a specific role of acetylation in the modulation of bacterial resistance to the host immune defences. Therefore, the detailed knowledge of the EPS primary structure is a key factor in the investigation of bacterial infections.

The *Burkholderia cepacia* complex (Bcc) is a group of bacteria characterised by similar phenotype, but different genotype. The complex comprises nine different genomovars, each of which received a name species [6]. Although strains of each genomovar can colonise the lungs of CF patients, *Burkholderia multivorans* (genomovar II) and *Burkholderia cenocepacia* (genomovar III) are responsible for the majority of infections. The EPS produced by Bcc strains were investigated in several different laboratories, but usually these studies were not paralleled by the assignment of microbial species, except in a recent publication, where EPS from clinical and environmental strains of *B. cenocepacia* were compared [7]. Most of the Bcc strains investigated in different parts of the world produced only one type of EPS [8–12], to which the name cepacian was assigned [13]. The polysaccharide is constituted of a branched heptasaccharide repeating unit (Fig. 1), and is characterised by high molecular weight, a certain degree of chain rigidity and capacity of aggregates formation [13,14]. Only a small percentage of Bcc strains examined produced EPS completely different from cepacian [15,16] and their structures are reported in Fig. 1. The critical role of the Bcc EPS in CF infections was recently demonstrated in a mouse model, where a spontaneous variant of a Bcc strain persisted in the lungs longer than the parent strain and was characterised by abundant EPS production [12]. In addition, it was proved that the EPS of a *B. cenocepacia* isolate inhibited neutrophils chemotaxis and scavenged reactive oxygen species, which *in vivo* are produced by neutrophils to kill bacteria [17].

In the present paper, we further pursued the investigation of EPS from Bcc, taking into consideration clinical strains of *B. multivorans* and *B. cenocepacia* isolated from CF patients attending the Cystic Fibrosis Regional Centre of Florence. A strain of a Bcc member with unknown genomovar [18] isolated from a CF patient was included in the research work. This is the first investigation on the EPS produced by *B. multivorans* clinical isolates. Furthermore, with the aim of determining the presence of EPS *in vivo*, sputum samples were analysed for the presence of EPS.

### 2. Materials and methods

#### 2.1 Bacterial strains

Nine bacterial strains presumed to belong to the Bcc were isolated from 7 out of 167 (4%) patients attending the Cystic Fibrosis Regional Centre of Florence. All bacterial strains were isolated following international guidelines, and tentatively identified as *B. cepacia* complex using commercial tests. The isolates were stored at −80 °C. Bcc genomovar status was determined by the European Burkholderia

\[
\beta-D-Galp-(1\rightarrow2)-\alpha-D-Rhap
\]

Formula 1: cepacian [6-8]

\[
[5]-\beta-D-Kdo\beta-(2\rightarrow3)-\beta-D-Galp2Ac-(1\rightarrow4)-\alpha-D-Galp-(1\rightarrow3)-\beta-D-Galp-(1\rightarrow)n
\]

Formula 2 [14]

\[
[6]-\beta-D-Fru\beta-(2\rightarrow)n
\]

Formula 3 [14]

\[
[3]-\beta-D-Glc\beta-(1\rightarrow3)-[4,6-O-(1-carboxyethylidene)]-\alpha-D-Galp-(1\rightarrow)n
\]

Formula 4: PS1 [13]

![Fig. 1. Chemical structures of the EPS produced by Bcc.](image-url)
Cepacia Complex Referral Laboratory And Repository (Laboratorium voor Microbiologie, Universiteit, Gent) using a polyphasic approach, including whole cell protein electrophoresis, and recA restriction fragment length polymorphism (RFLP) analysis by means of the restriction enzymes HaeIII and MnlI [19,20]. Three isolates were B. multivorans, three were B. cenocepacia, while the genomovar status of one isolate remained undetermined. The latter isolate belongs to the Bcc, but its taxonomic status has not yet been clarified (Table 1) [18]. The reference strains C1576 (B. multivorans) and J2315 (B. cenocepacia) were also included in this study.

2.2. RAPD typing of Bcc isolates

DNA from each isolated colony was prepared by heating one colony at 95 °C for 15 min in 20 μl of lysis buffer containing 0.25% (wt./vol.) sodium dodecyl sulfate (SDS) and 0.05 M NaOH. Following lysis, 180 μl of distilled water was added and DNA solutions were stored at 4 °C. RAPD fingerprinting was performed with primer RAPD-270 (5′–TGCGCGCGGG–3′) as described previously [21] (PCR products were separated on 2% agarose gels in 0.5 TBE buffer (80 mA for 4 h at room temperature). Gels were stained with etidium bromide and visualized by UV illumination.

2.3. Patients

The 7 Bcc colonized patients were in a stable clinical condition except for one patient, colonised by B. multivorans, who died without showing symptoms of B. cepacia syndrome. Bcc colonization did not affect pulmonary function being the average annual decline of FEV1 of −1.9%, in agreement with the mean yearly decline reported for CF patients (−1.29% to −3%) [22].

2.4. Isolation and purification of the exopolysaccharides

Bacterial cells were grown for 4 or 5 days at 30 °C on a solid medium containing 2 g of yeast extract, 20 g of mannitol and 15 g of bacto agar/l [23] (medium YEM). The isolation and purification of the EPS was performed as already described [7].

2.5. General analytical methods

Analytical GLC was performed with an AutoSystem XL (Perkin Elmer) gas chromatograph equipped with a flame ionisation detector, using He as the carrier gas. GLC–MS analyses were carried out on a Hewlett-Packard 5890 gas chromatograph coupled to a Hewlett Packard 5971 mass selective detector.

Hydrolysis of the EPS was carried out in 2 M CF3CO2H at 125 °C for 1 h. The O-acetyl groups were removed by treatment with 0.01 M NaOH at room temperature for 5 h [24].

2.6. Composition analysis of exopolysaccharides

Alditol acetates were prepared as described previously [25] using inositol as internal standard. The derivatives were separated on 2% agarose gels in 0.5 TBE buffer (80 mA for 4 h at room temperature). Gels were stained with etidium bromide and visualized by UV illumination.

### Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Species</th>
<th>Genomovar</th>
<th>EPS/Plating dish (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF14</td>
<td>nd</td>
<td>nd</td>
<td>42</td>
</tr>
<tr>
<td>BF16</td>
<td>B. multivorans</td>
<td>II</td>
<td>5</td>
</tr>
<tr>
<td>BF18</td>
<td>B. cenocepacia</td>
<td>III</td>
<td>48</td>
</tr>
<tr>
<td>BF19</td>
<td>B. multivorans</td>
<td>II</td>
<td>46</td>
</tr>
<tr>
<td>BF111</td>
<td>B. multivorans</td>
<td>II</td>
<td>32</td>
</tr>
<tr>
<td>BF112</td>
<td>B. cenocepacia</td>
<td>III</td>
<td>27</td>
</tr>
<tr>
<td>BF113</td>
<td>B. cenocepacia</td>
<td>III</td>
<td>nm</td>
</tr>
<tr>
<td>C1576</td>
<td>B. multivorans</td>
<td>II</td>
<td>8</td>
</tr>
<tr>
<td>J2315</td>
<td>B. cenocepacia</td>
<td>III</td>
<td>nm</td>
</tr>
</tbody>
</table>

nd=not determined.
nm=non-mucoid.

2.7. Linkage analysis

The samples were sonicated to reduce the molecular weight and exchanged into the protonated form prior to chemical reactions. Methyllations were performed according to Dell [26] but using potassium methylsulfinyl–methanide [27]. After methylation, the permethylated samples were purified on Sep-Pak C18 cartridge [28], prior to hydrolysis, and derivatisation into alditol acetates. The products were analysed by GLC and GLC–MS using an SP2330 capillary column (Supelco, 30 m), and the temperature program: 1 min at 200 °C, 200–245 °C at 4 °C/min, 20 min at 245 °C.

2.8. 1H NMR spectroscopy

NMR spectra were recorded on a Varian UNITY INOVA NMR spectrometer operating at 500 MHz (1H) at 50 °C. Samples, previously sonicated to decrease the molecular weight, were exchanged three times with 99.9% deuterium oxide by lyophilisation, and finally dissolved in 99.96% deuterium oxide. Chemical shifts are expressed in ppm using as reference the HOD signal at 4.57 ppm at 50 °C.

2.9. Sugar analysis of CF sputum

Eight sputum samples from CF patients infected by Bec strains were available for sugar analysis in two years span. Three sputum samples from patients infected only by P.
Aeruginosa were used as control. Several different protocols were tested; here the one that gave the best results is reported and it was successfully applied to the available samples. The samples were suspended in the minimum amount of water in order to have a volume suitable for ultracentrifugation (105,000×g, at 4 °C for 4 h). The supernatants were then heated at 80 °C for 15 min, followed by centrifugation at 17,000×g, at room temperature for 10 min. To the supernatants an equal volume of a 1% solution of N-acetyl-L-cystein was added, the solutions were thoroughly mixed and let them stand for 10 min. After dialysis against water, they were treated with DNAse, RNAse and protease in 0.05 M Tris–HCl, pH 7.5, 0.01 M MgCl2. After a further dialysis, the samples were hydrolysed, derivatised and analysed as described in Sections 2.5 and 2.6.

3. Results

3.1. Exopolysaccharides production

The bacteria were grown on solid medium containing an excess of mannitol as carbon source [23] (medium YEM), which efficiently discriminated mucoid from non-mucoid strains. Strains BFI4, BFI6, BFI8, BFI9, BFI11, BFI12 and C1576 were mucoid and most produced abundant quantity of EPS (Table 1). In particular, two isolates of BFI8 had a different phenotype: the one isolated in 2001 was non-mucoid, while the one isolated in 2004 showed a highly mucoid character, and was used in this study. However, these two isolates showed a unique RAPD fingerprint revealing that the patient was persistently colonized by the same clone. Isolate BFI13 and the reference strain J2315 were non-mucoid, therefore they were not investigated further.

3.2. Composition analysis of exopolysaccharides

The composition of the exopolysaccharides was obtained after hydrolysis of the polymers and derivatisation into alditol acetates. As reported in Table 2, where data for cepacian [9] are also shown, most of the EPS contained rhamnose, mannose, galactose and glucose in amounts similar to cepacian. The reference strain C1576 was composed of the same sugars, but the molar ratios were quite different from those of cepacian. Strain BFI6 was composed of equimolar amounts of galactose and glucose, thus indicating a polymer completely different from cepacian.

3.3. Linkage analysis

The EPS were subjected to linkage analysis and the results are reported in Table 3, in comparison with the data for cepacian [9]. Sample BFI6 was composed of equimolar amounts of 3-linked glucose and 3,4,6-linked galactose, thus revealing a polysaccharide with two sugar residues per repeating unit. Moreover, the multiply substituted galactose residue not accompanied by nonreducing terminal sugars indicated the presence of a non-carbohydrate substituent. The polysaccharides BFI4, BFI8, BFI11 and C1576 contained 2-linked rhamnose, non-reducing terminal-galactose, 3-linked glucose and 3,6-linked mannose, the same residues found in cepacian. On the contrary, samples BFI9 and BFI12 contained, apart from the residues mentioned above, also 6-linked galactose, thus indicating the co-production of a polysaccharide different from cepacian. Sugar residues present in less than 10% were not considered.

3.4. 1H NMR and 13C NMR spectroscopy

All EPS, both in the native and de-acetylated form, were studied by means of NMR spectroscopy. The 1H NMR spectra recorded on the native samples showed signals compatible with the polysaccharide cepacian, except for BFI6, whose spectrum (Fig. 2a) was deeply different from

Table 2

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Cep</th>
<th>BFI4</th>
<th>BF16</th>
<th>BF18</th>
<th>BF19</th>
<th>BF111</th>
<th>BF112</th>
<th>C1576</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rha</td>
<td>0.81</td>
<td>0.80</td>
<td>0.04</td>
<td>0.85</td>
<td>0.81</td>
<td>0.73</td>
<td>0.79</td>
<td>2.22</td>
</tr>
<tr>
<td>Man</td>
<td>0.55</td>
<td>0.44</td>
<td>0.03</td>
<td>0.41</td>
<td>0.34</td>
<td>0.43</td>
<td>0.52</td>
<td>1.64</td>
</tr>
<tr>
<td>Gal</td>
<td>2.14</td>
<td>2.28</td>
<td>0.98</td>
<td>2.27</td>
<td>2.18</td>
<td>2.01</td>
<td>2.14</td>
<td>2.60</td>
</tr>
<tr>
<td>Glc</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

a Cep = data obtained on cepacian in a previous investigation [9].

Table 3

<table>
<thead>
<tr>
<th>Linked</th>
<th>Relative molar ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>RRT</td>
</tr>
<tr>
<td>2-Rha</td>
<td>0.81</td>
</tr>
<tr>
<td>t-Gal</td>
<td>0.88</td>
</tr>
<tr>
<td>3-Glc</td>
<td>1.00</td>
</tr>
<tr>
<td>6-Gal</td>
<td>1.14</td>
</tr>
<tr>
<td>3,6-Man</td>
<td>1.26</td>
</tr>
<tr>
<td>3,4,6-Gal</td>
<td>1.37</td>
</tr>
</tbody>
</table>

a The numbers indicate the position of the glycosidic linkage, t stands for non-reducing terminal.
b Retention time relative to 3-linked glucose.
c Cep = data obtained on cepacian in a previous investigation [9].
that one of cepacian (Fig. 2c) [9]. In the anomeric region of the BFI6 $^1$H NMR spectrum two signals at 5.42 and 4.72 ppm, with identical integration values, were present, indicating a polysaccharide constituted of a disaccharidic repeating unit. In the high field region of the spectrum the signal at 1.47 ppm was assigned to the $-\text{CH}_3$ protons of pyruvyl groups; its integration value, equal to 3.4 with respect to each of the anomeric signals, showed that there was one pyruvyl group per repeating unit. $^1$H and $^{13}$C NMR (Fig. 2b) spectra, together with compositional and linkage analysis, showed that the primary structure of the EPS BFI6 is identical to that one of the polysaccharide PS1 produced by a Bcc clinical strain and described by Cérantola et al. [15].

The $^1$H NMR spectra of all the other polysaccharides showed signals attributable to $-\text{CH}_3$ of rhamnose residues ($\sim\delta 1.3$) and $-\text{CH}_3$ of $\text{O}$-acetyl groups ($\delta 2.2$–$2.0$). Integration values of the methyl signals of the acetyl groups were calculated with respect to the methyl signal for the rhamnose residue. The data showed a content of acetyl substituents in the range 2.3–3.4, indicating the presence of

Fig. 2. $^1$H and $^{13}$C NMR spectra of the sample BFI6 recorded at 50 °C (a and b), and $^1$H NMR spectrum of the polysaccharide cepacian recorded at 80 °C (c). The principal regions of the spectrum are shown.

Fig. 3. Partial anomeric regions of the $^1$H NMR spectra of the de-acetylated exopolysaccharides BFI11, BFI4, and BFI8.
more than two and up to three acetyl groups per repeating unit. Only the sample C1576 had a content of acetyl groups equal to 1 substituent per repeating unit. The $^1$H NMR spectra of the de-acetylated polysaccharides BFI4, BFI8, BFI9, BFI11, BFI12, and C1576 were recorded and their anomeric regions are reported in Figs. 3 and 4. The EPS BFI11 (Fig. 3) showed only signals identical to those of cepacian, except for two small shoulders, at 5.51 and 5.20 ppm, and a very small signal at 5.46 ppm, indicating that the polysaccharide consists of almost pure cepacian. Samples BFI4 and BFI8 (Fig. 3), showed the signals typical of cepacian, but some of them differed for their intensity. Moreover, further anomeric signals at 5.53, 5.44 and 5.00 ppm established that the two samples were a mixture of polymers, one of which was identical to cepacian. The polymers BFI9 and BFI12 (Fig. 4) had the same characteristic of BFI4 and BFI8, but they were even more stressed, thus suggesting a relatively higher amount of polysaccharides different from cepacian. Finally, the EPS C1576 had the most complicated anomeric region among the samples examined, with signals belonging to cepacian and other proton signals attributable to different exopolysaccharides.

3.5. Sugar analysis of CF sputum

Eight samples of sputum from CF patients were subjected to purification in order to determine the presence of EPS. Two samples were from patients infected only with BFI4 (S4-1 and S4-2), two were co-infected with BFI8 and $P$. aeruginosa (S8-1 and S8-2), and one with BFI11 and $P$. aeruginosa (S11-1). It is worth stressing that these strains produced mainly the EPS cepacian in vitro. The other three samples gave poor results and therefore were not reported. Hydrolysis of the purified sputum and derivatisation of the neutral sugars to alditol acetates was followed by GLC analysis. The results are reported in Table 4. The sugars detected in the hydrolysates were rhamnose, fucose, arabinose, mannose, galactose, and glucose; in one sample xylose was also present. The same purification procedure and analysis were applied to three samples from patients colonised only with $P$. aeruginosa, used as control. The results indicated the presence of the same sugars, although in different amounts (Table 4).

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Sputum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S4-1</td>
</tr>
<tr>
<td>Rha</td>
<td>3.74</td>
</tr>
<tr>
<td>Fuc</td>
<td>14.18</td>
</tr>
<tr>
<td>Ara</td>
<td>17.46</td>
</tr>
<tr>
<td>Xyl</td>
<td>1.66</td>
</tr>
<tr>
<td>Man</td>
<td>15.38</td>
</tr>
<tr>
<td>Gal</td>
<td>42.96</td>
</tr>
<tr>
<td>Glc</td>
<td>4.62</td>
</tr>
</tbody>
</table>

4. Discussion

This study deals with the isolation and identification of EPS produced by clinical isolates of $B$. multivorans and $B$. cenocepacia; EPS from the former species has never been investigated before. The reference strains C1576 ($B$. multivorans) and J2315 ($B$. cenocepacia) and a Bcc strain of unknown genomovar were included in this research work. For the first time, sputum samples of CF patients infected with some of the strains reported above were analysed for the presence of EPS.

The EPS structure was determined by use of chemical analysis coupled to gas chromatography and mass spectrometry, and by $^1$H and $^{13}$C NMR spectroscopy analysis. The data showed that strain BF16 produced a polysaccharide identical to PS1 [15] and this is the first time that the
production of PS1 by a Bcc strain, different from the one investigated by Cérantola et al., is reported. All the other strains produced the EPS cepacian, almost pure in one case (BFI11) or together with small amounts of other polysaccharides whose primary structure was not identified. Only the strain C1576 produced a mixture of EPS where the unknown component was rather abundant. As previously reported for other strains belonging to the Bcc [7], no connection between type of EPS produced and Bcc species was observed, cepacian remaining as the most common EPS produced.

Sputum samples from CF patients infected by Bcc strains were analysed for the presence of EPS, via hydrolysis of the polysaccharides and GLC analysis of the derivatives. Besides the experimental protocol reported in Section 2.9, other attempts to purify the EPS from the biological samples were made. In particular, affinity and cation exchange chromatography were experimented to eliminate the mucin component, but both proved unsuccessful. Detection of uronic acids, not present in glycoproteins, as performed with CF sputum from patients infected by *P. aeruginosa* [30], was not suitable for two reasons. One is that a colorimetric method is rather unspecific in this case, because Bcc microorganisms produced different EPS, some of them containing no uronic acids. The second reason is the detection limit of the colorimetric method. In fact, not only the uronic acid concentration in cepacian is rather low (~16% w/w), but the concentration of EPS in sputum is also rather low, as determined for alginate (average concentration = 37 μg/ml) [30]. The sugars that constitute the EPS cepacian, were all present in the sputum samples, even if in different amounts. However, other sugars like fucose, arabinose and xylose, never found to be part of EPS produced by Bcc, were present. The data can be explained by the presence of mucins and contamination with blood. Bronchial mucins are highly glycosylated and their sugar composition consists mainly of fucos, galactose, N-acetyl glucosamine, N-acetyl galactosamine and sialic acid [31–35]. Therefore, the high content of fucose and galactose could derive from the carbohydrate moiety of mucins. At the same time it was reported that the seromucoid fraction of human serum contained fucose, arabinose, mannose, glucose, galactose, glucosamine and galactosamine [36]. Rhamnose was not reported to be a component of the macromolecules mentioned above and therefore it is reasonable to correlate its presence with that of cepacian. In particular, in the samples S4-1 and S4-2, which were contaminated exclusively by Bcc, the average value of rhamnose content was 4.90. The molar percentages of rhamnose are higher in these samples than in PA-1, PA-2, and PA-3 (average value 1.96), where most likely it derived from hydrolysis of the rhamnolipid component, since these samples were contaminated only by *P. aeruginosa*. Samples S8-1, S8-2 and S11-1, which were contaminated by Bcc and *P. aeruginosa*, also contained rhamnose, as expected, and interestingly the average value (3.33) was in between those of the previous samples (Table 4). In conclusion, the data obtained do not allow an unambiguous proof of the presence of cepacian in vivo due to the interference of other carbohydrates in sputum, like rhamnolipids and those contained in mucin. Nevertheless, the amount of rhamnose in sputum samples contaminated only by Bcc was higher than that found in samples contaminated only by *P. aeruginosa* suggesting the presence of Cepacian in vivo. However, the number of biological samples examined was not high enough to obtain a good statistics, a problem related to their availability from CF medical centres and to the patients’ clinical conditions.

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## References


[4] Cérantola S, Lemassu-Jacquier A, Montrozier H. Structural elucidation of the rhamnolipid component, since these samples were contaminated only by *P. aeruginosa*. Samples S8-1, S8-2 and S11-1, which were contaminated by Bcc and *P. aeruginosa*, also contained rhamnose, as expected, and interestingly the average value (3.33) was in between those of the previous samples (Table 4). In conclusion, the data obtained do not allow an unambiguous proof of the presence of cepacian in vivo due to the interference of other carbohydrates in sputum, like rhamnolipids and those contained in mucin. Nevertheless, the amount of rhamnose in sputum samples contaminated only by Bcc was higher than that found in samples contaminated only by *P. aeruginosa* suggesting the presence of Cepacian in vivo. However, the number of biological samples examined was not high enough to obtain a good statistics, a problem related to their availability from CF medical centres and to the patients’ clinical conditions.


