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# Protein Profiling Analyses of the Outer Membrane of *Burkholderia cenocepacia* Reveal a Niche-Specific Proteome

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**Abstract** Outer membrane proteins (OMPs) are integral β-barrel proteins of the Gram-negative bacterial cell wall and are crucial to bacterial survival within the macrophages and for eukaryotic cell invasion. Here, we used liquid chromatography tandem mass spectrometry (LC-MS/MS) to comprehensively assess the outer membrane proteome of *Burkholderia cenocepacia*, an opportunistic pathogen causing cystic fibrosis (CF), in conditions mimicking four major ecological niches: water, CF sputum, soil, and plant leaf. Bacterial cells were harvested at late log phase, and OMPs were extracted following the separation of soluble proteins by one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (1D-SDS-PAGE). Protein bands were excised and identified by LC-MS/MS analysis. The proteins identified under various growth conditions were further subjected to in silico analysis of gene ontology (subcellular localization, structural, and functional analyses). Overall, 72 proteins were identified as common to the four culture conditions, while 33, 37, 20, and 10 proteins were exclusively identified in the water, CF sputum, soil, and plant leaf environments, respectively. The functional profiles of the majority of these proteins revealed significant diversity in protein expression between the four environments studied and may indicate that the protein expression profiles are unique for every condition. Comparison of OMPs from one strain in four distinct ecological niches allowed the elucidation of proteins that are essential for survival in each niche, while the commonly expressed OMPs, such as RND efflux system protein, TonB-dependent siderophore receptor, and ABC transporter-like protein, represent promising targets for drug or vaccine development.

## Introduction

The *Burkholderia cepacia* complex (Bcc, formerly *Pseudomonas cepacia*) was first described by Walter H. Burkholder as the causative agent of "sour skin" onion rot [1]. The bacterium responsible for rot in onion bulbs was referred to as *Pseudomonas multivorans* by Stanier et al. [2] and as *Pseudomonas kingii* by Jonsson [3]. Later, *P. kingii* was shown to be synonymous with *P. cepacia*, the name originally proposed by Burkholder. The genus *Burkholderia* was proposed in 1992 to accommodate the former rRNA group II pseudomonads [4]. The Bcc currently includes 17 Gram-negative species with similar morphological attributes but divergent genomes and capabilities [5, 6]. *Burkholderia cenocepacia*, which belongs to the Bcc, is a pathogen of both plants and humans and is ubiquitously found in water and soil [6].

Plethora of research conducted on membrane proteins and the availability of the chemical agents used for extraction have provided insight into a membrane topology and its evolution [7–11]. Several previous proteomic studies in Gram-negative bacteria, including

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*B. cenocepacia*, *Burkholderia vietnamiensis*, and other opportunistic pathogens of the genus *Burkholderia*, have revealed potential virulence factors [7, 11]. Many of these identified virulence factors were found on the cell surface or in the cell wall or extracellular fractions of the bacteria [7, 11]. Proteomic analysis of these bacterial fractions in human, animal, and plant environments under various growth conditions has revealed an extensive list of outer membrane proteins (OMPs). However, very few studies have focused on one strain under various growth conditions.

Because of the integral role of OMPs in the growth, survival, and pathogenicity of *Burkholderia* species, we analyzed purified fragments of OMPs from *B. cenocepacia* using liquid chromatography tandem mass spectrometry (LC-MS/MS), and the resultant peptides were identified using the MASCOT database. Functional annotation of purified peptides and characterization of *B. cenocepacia* OMPs were performed by in silico analysis.

## Material and Methods

### Culture Conditions and Growth Curve Analysis Under Simulated Host Environments

*B. cenocepacia* strain Y10 (EF426457) from cystic fibrosis (CF) patients was identified in our previous study [12]. The strain Y10 was stored in 20–30 % glycerol (Shanglin Industries, Hangzhou, China) at −80 °C. Bacterial cultures were maintained on Luria-Bertani agar medium and incubated at 30 °C for 24 h. For growth curve analyses, 2 ml of overnight culture was inoculated into 50 ml of host mimic media. Water-mimicking medium was prepared as described by Schell et al. [7]. Briefly, a minimal medium containing minimal salts, 3 % glycerol, 1× Basal Medium Eagle (BME), and Minimum Essential Medium (MEM) (20 amino acids; Sigma-Aldrich Germany) was prepared to mimic a nutrient-rich water environment. Artificial sputum medium was prepared as described by Dinesh [13] to mimic the sputum of CF patients. Apricot leaf extract medium was prepared as described by Tahara et al. [14] to mimic a plant host. The extract was added to the basal medium to obtain a final concentration of 1 mg/ml. Soil extract medium was prepared as described by Yoder-Himes et al. [11] to mimic soil conditions. Briefly, 400 g of sieved soil per liter was autoclaved and filtered through Whatman filter paper (0.45- and 0.22-μm pore size) consecutively. Glucose (1×) was then added. Bacteria were inoculated at an initial cell density of $OD_{600}$=0.1 and grown for 16–24 h, or until late log phase ($OD_{600}$=1.5–1.8).

### OMP Extraction and One-Dimensional Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

OMPs from *B. cenocepacia* cells were prepared using the method described by Jagannadham [8]. Briefly, a bacterial cell pellet was washed with membrane buffer (10 mM Tris, 2 mM ethylene-diaminetetraacetic acid, 0.75 M sucrose, 1 mM phenylmethylsulfonyl fluoride (PMSF) (pH 8.0) containing lysozyme (60 mg/ml)). The cell pellet was resuspended by sonication and then centrifuged at 8,000×*g* for 10 min at 4 °C. The supernatant, containing the inner and OMPs, was removed and centrifuged at 4 °C for 2 h at 30,000×*g*. The resultant cell pellet was resuspended in membrane buffer containing 2 % Triton X-100 and incubated for 30 min at room temperature, followed by centrifugation at 4 °C for 2 h at 30,000×*g*. The supernatant was removed, and pellets containing enriched OMPs were resuspended in lysis buffer (1.98 M thiourea, 8.5 M urea, 2 %*w*/*v* 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS). OMPs from *B. cenocepacia* were separated by one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (1D-SDS-PAGE) using 10 % acrylamide gels, which was conducted in a minigel apparatus vertical electrophoresis bath (VE-180; Tanon, Shanghai, China). Midrange protein molecular weight marker (14.4–97.4 kDa, Sangon Biotech) was used to calculate sample molecular weights. Silver staining was used to visualize the separated proteins.

### In-Gel Digestion and LC-MS/MS Analysis

In-gel digestion was performed as described by Wickramasekara et al. [15]. Briefly, gel bands were excised, transferred into 0.5-ml centrifuge tubes, destained, and then digested with a 50 % acetonitrile (*v*/*v*) and 2 % formic acid (*v*/*v*) solution using a Multiprobe II Plus Ex robotic liquid handling system (Perkin Elmer Waltham, MA, USA). The resultant tryptic peptides (10 μl) were separated using an UltiMate 3000 Nano LC system (Thermo Scientific Dionex, MA, USA). LC-MS/MS profiles of the peptides were used to identify the proteins by automated database searching (MASCOT Daemon, Matrix Science) against *B. cenocepacia*. The cross-correlation scores (X corr) were calculated by using SEQUEST [16] and fixed for protein identification. The X corr of singly, doubly, and triply charged peptides were greater than 1.8, 2.5, and 3.5, respectively, and peptide sequences with the highest X corr values than fixed were identified.

To increase the overall sequence coverage, peptides with values below the defined thresholds (with a cutoff value of 50) were also considered. The ultimate list of proteins was made by merging all the putative OMPs obtained from different LC-MS/MS runs following manual verification.

### In Silico Analysis and Characterization of Proteins

The subcellular localization of identified proteins was investigated using proteome analysis software PSORTb version 3.0.2 [17]. Parameters included Gram-negative species, normal format, and a significance score >7.5. The grand average of hydropathicity (GRAVY) of peptides was scored using ProtParam Expasy [18] to study the hydrophobic nature of the proteins. SignalP 3.0 [18] was used to predict any N-terminal secretary signal peptides among the identified proteins. Selected proteins were further structurally characterized using the I-TASSER server [19], an online platform for protein structure prediction. Three-dimensional protein structures were visualized and superimposed using UCSF Chimera [20]. The predicted structures evaluated and validated and errors in the models were analyzed using the Molprobity server [21]. Poor conformations were refined by UCSF Chimera for energy minimization, while geometry optimization was attained by Wincoot [22]. The best conformations were again validated by Molprobity. Structural alignment between all possible combinations of porins was calculated by superimposing of multiple porin protein structures based on their shape and three-dimensional conformation. It was carried out for both Cα-Cα backbone residues and side chain residues using UCSF Chimera. This analysis was performed to gain structural and functional insights into all of the identified porins.

## Results and Discussion

In the current study, we identified OMPs from *B. cenocepacia* that were expressed under simulated host and environmental growth conditions by applying a strategy combining 1D-SDS-PAGE with LC-MS/MS analysis and in silico characterization. Results of our study revealed the major shared proteins, as well as several niche-specific OMPs, indicating that a bacterial strain isolated from one environment may show diversity in its OMP expression during adaptation to other environments.

Growth curves were carried out to establish differences in growth of *B. cenocepacia* between the various independent niches. The growth curves showed notable differences, with the highest number of bacterial cells observed under CF-mimicking conditions ($p>0.05$), followed by simulated plant, water, and soil environments (Fig. 1). The results of SDS-PAGE also showed differences in the protein expression patterns under the four growth conditions, as shown in Fig. 2. All protein bands were then processed for LC-MS/MS analysis.

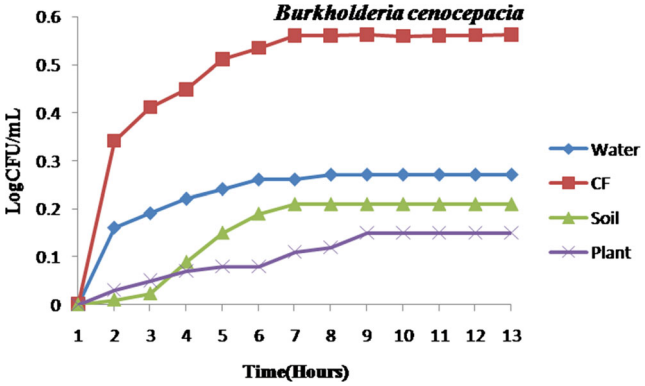



**Fig. 1** Growth of *B. cenocepacia*, in host-specific and host-independent growth conditions

### Global OMP Profiling

Proteins were pooled, duplicates were removed, and a final nonredundant list of putative OMPs was assembled for *B. cenocepacia* under simulated CF, plant, water, and soil conditions. Approximately 96 % of these proteins were detected in at least two replicates, and these nonredundant lists of OMPs with their in silico characterizations are shown in supplementary Tables S1, S2, S3, and S4, as well as in Fig. 3. We identified 72 proteins that were expressed (Table S5, Fig. 3) under all four conditions. Human bacterial pathogens have a various set of genetic components causative to pathogenicity, ranged from well-known secretion systems to adhesions and toxins, all of which are involved in circumventing or manipulating the human immune system [23, 24]. Similarly, several plant-associated pathogens have shown a wide specialization to plant systems, with numerous well-documented plant-specific virulence determinants, such as type 3 secretion system (T3SS), plant hormone analogs, and enzymes that invade plant-specific cell wall components [23, 24]. The large number of shared OMPs in *B. cenocepacia* among the different growth conditions, with nine proteins commonly identified under the CF and plant conditions alone, is consistent with the ability of cross-kingdom pathogens that can infect unrelated hosts [24, 25].

The identified proteins were categorized into various classes based on physical properties and clusters of orthologous groups (COGs). The majority of the proteins identified in this study by LC-MS/MS were OMPs; however, other cytoplasmic and cytoplasmic-associated proteins were also identified, including ribosomal proteins, glyoxalase resistance protein/dioxygenase, DNA topoisomerase, elongation factor, tetraacyl disaccharide, ATP synthase subunit beta, dead/death box helicase domain-containing protein, chaperonin GroEL, phosphoglucomutase, ribonuclease E, ribonuclease rng/rng family, 3-isopropylmalate dehydratase, gluconolactonase, and pyruvate kinase (data not shown).

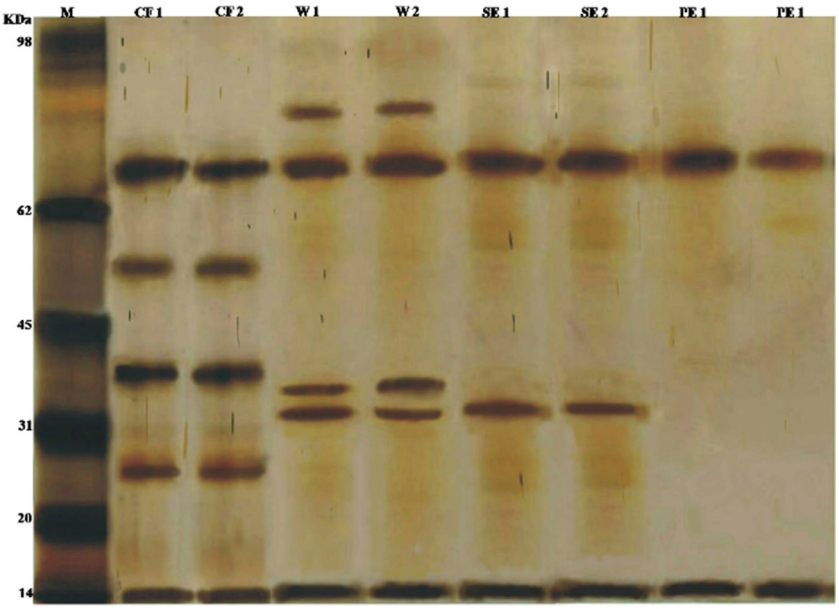



**Fig. 2** SDS-PAGE analysis of purified outer membrane proteins of *B. cenocepacia* Y10. *Lane 1* represents the protein marker. *Lanes 2* and *3* represent CF mimic condition, *lanes 4* and *5* water mimic (WE) condition, *lanes* 6 and *7* soil extract (SE) condition, and *lanes 8* and *9* represent the plant extract (PE) growth condition

## Analysis and Characterization of OMPs Expressed in Water-Mimicking Medium

Overall, 111 proteins (Table S1) were detected during growth in the water-mimicking medium, with 33 proteins determined to be exclusively expressed in those conditions (Table 1). There were 14 porin proteins (COG3203) among these differentially expressed proteins (Table 1), which was higher than those in the other growth conditions (Tables 3, 4, and 5). However, these findings are consistent with previous studies that showed that porins play an important role in bacterial survival in water [26, 27]. As not all identified porins have experimentally determined structures, a template identification search was performed to predict the three-dimensional models of these proteins. Because there was no suitable template, the three-dimensional structures of the porins were generated using the I-TASSER de novo prediction method. Table 2 shows Molprobity scores for the validated models included in the comparisons. All structural variations were highlighted (Fig. 4) in an attempt to predict functional variation of the porins. All these variations were evaluated by the root-mean-square deviation (RMSD) score, which was obtained following superimposition (Fig. 4). This analysis showed that all porins have structural variation that may possibly have functional variety. Porins allow the transport of medium-sized or charged molecules across the membrane,

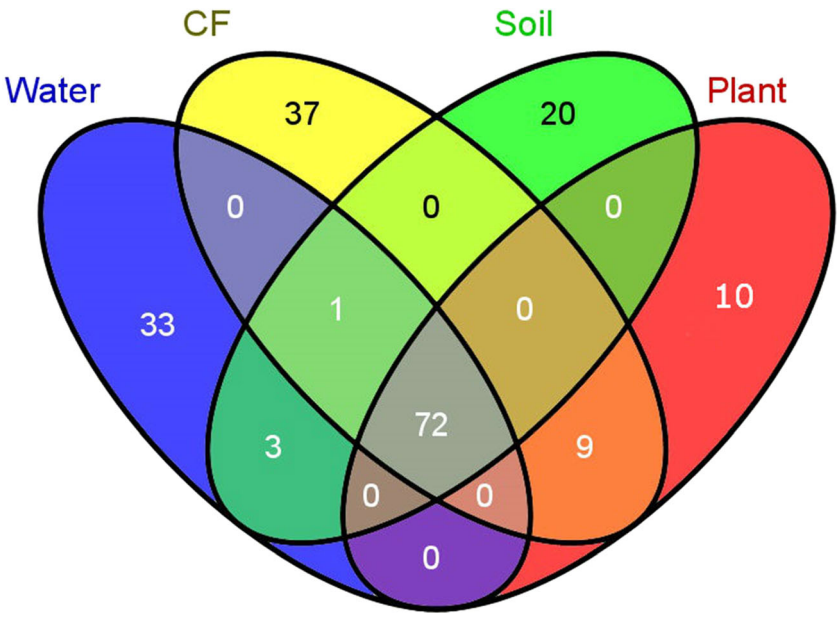



**Fig. 3** Culture-dependent and -independent distribution of outer membrane proteins in various growth conditions

**Table 1** List of LC-MS/MS differentially identified outer membrane proteins of *Burkholderia cenocepacia* under water niche

| Sr. no. | Locus tag | Protein name or predicted function/bacterial species |
|---|---|---|
| 1 | BCAL1368 | Porin |
| 2 | BCAL1692 | Iron complex receptor protein |
| 3 | BCAL1694 | Putative Fe transport |
| 4 | BCAL2696 | Copper resistance protein |
| 5 | BCAL2834 | Putative acylhydrolase |
| 6 | BCAL3412 | Membrane-bound lytic murein transglycosylase A |
| 7 | BCAL3514 | RND efflux system outer membrane lipoprotein |
| 8 | BCAL3526 | Type II secretory pathway component |
| 9 | BCAM0199 | RND efflux protein |
| 10 | BCAM0219 | YadA domain-containing protein |
| 11 | BCAM0267 | Porin |
| 12 | BCAM0407 | Porin |
| 13 | BCAM0473 | Porin |
| 14 | BCAM0652 | Porin |
| 15 | BCAM0779 | Methyl-accepting chemotaxis sensory transducer |
| 16 | BCAM1015 | Porin |
| 17 | BCAM1287 | Porin |
| 18 | BCAM1376 | Porin |
| 19 | BCAM1474 | Phosphoesterase |
| 20 | BCAM1497 | Hypothetical protein |
| 21 | BCAM1787 | Porin |
| 22 | BCAM1974 | Porin |
| 23 | BCAM2063 | Porin |
| 24 | BCAM2164 | Hypothetical protein |
| 25 | BCAM2223 | Iron uptake protein |
| 26 | BCAM2297 | Hypothetical Protein |
| 27 | BCAM2311 | Porin |
| 28 | BCAM2373 | Porin |
| 29 | BCAM2584 | Porin |
| 30 | BCAM2626 | ABC transporter iron complex receptor protein |
| 32 | BCAS0181 | Methyl-accepting chemotaxis sensory transducer |
| 32 | BCAS0237 | Putative outer membrane lipoprotein |
| 33 | BCAS0335 | YadA domain-containing protein |

and generally systemize the passive movement of small metabolites, like ions, amino acids, and sugars. Porins also function as receptors and pathogenicity effectors [27].

Following porins, the next most abundant proteins were acylhydrolase (COG3511), transglycosylase A (COG0744), a phosphoesterase-like protein (COG3144) involved in energy production, and various other biological functions [28]. We also identified an acylhydrolase (COG3511), a lipolytic patatin-like protein involved in host colonization [28] (Table 1). It is likely that *B. cenocepacia* constantly monitors the environment through an adaptive response by modulating the expression of various proteins involved in the survival.

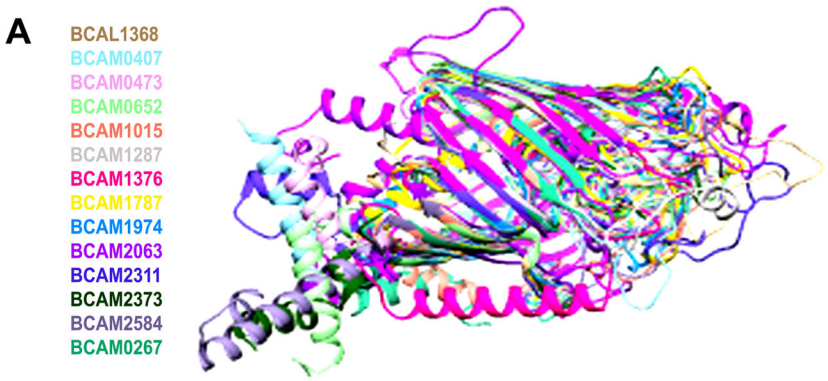
**Table 2** The structural characterization of porin proteins identified under water condition

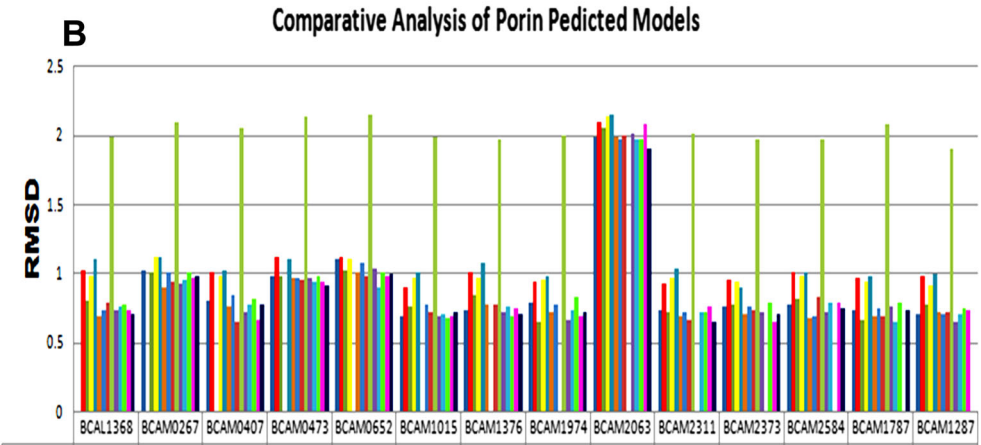
| Sr. no. | ORF name | Molprobity score |
|---|---|---|
| 1 | BCAL1368 | 2.29 |
| 2 | BCAM0267 | 2.40 |
| 3 | BCAM0407 | 2.51 |
| 4 | BCAM0473 | 2.77 |
| 5 | BCAM0652 | 2.42 |
| 6 | BCAM1015 | 2.47 |
| 7 | BCAM1287 | 2.64 |
| 8 | BCAM1376 | 2.59 |
| 9 | BCAM1787 | 2.76 |
| 10 | BCAM1974 | 2.31 |
| 11 | BCAM2063 | 2.43 |
| 12 | BCAM2311 | 2.34 |
| 13 | BCAM2373 | 2.12 |
| 14 | BCAM2584 | 2.33 |

## Analysis and Characterization of OMPs Expressed in the CF Niche

A total of 121 OMPs were identified in CF-mimicking medium (Table S2), with only 37 being uniquely identified under these conditions (Table 3). Several of these proteins were associated with energy conversion, translation, and energy production. Such a putative exported heme utilization protein (BCAL1522), a well recognized prokaryotic heme utilization systems and uptake systems [29], was identified in the simulated CF environment. Also identified in the CF-mimicking medium were a putative bound lytic murein transglycosylase (BCAL0403), a family M23 peptidase (BCAM0180), a short-chain dehydrogenase (BCAL0670), a M48B metal peptidase (BCAL0849), and DNA topoisomerase IV subunit A (BCAL2454) (Table 3). These proteins may play key roles in enzymatic activity, energy conversion, and hydrolytic activity in *B. cenocepacia* like in *Salmonella* [30], and it might be important to invade the host cells and survival in the hosts. We also identified several proteins determined to be pathogenicity factors in other bacterial pathogens. These included type VI secretion system proteins, BCAL0341 (TssB family), and BCAL0339 (lipoprotein/VasD). Physiological roles of TssB and lipoprotein/VasD proteins have been reported in other organisms specifically in virulence [31, 32]. We therefore speculate similar function of TssB and lipoprotein/VasD in *B. cenocepacia.*

Furthermore, an Flp-type pilus assembly protein, C flagellar hook protein FlgE, which is reported to be involved in twitching motility and biofilm formation in




| | BCAL1368 | BCAM0267 | BCAM0407 | BCAM0473 | BCAM0652 | BCAM1015 | BCAM1376 | BCAM1974 | BCAM2063 | BCAM2311 | BCAM2373 | BCAM2584 | BCAM1787 | BCAM1287 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BCAL1368 | 0 | 1.033 | 0.805 | 0.983 | 1.108 | 0.696 | 0.733 | 0.789 | 1.983 | 0.742 | 0.771 | 0.772 | 0.742 | 0.707 |
| BCAM0267 | 1.033 | 0 | 1.007 | 1.129 | 1.118 | 0.905 | 1.006 | 0.939 | 2.096 | 0.936 | 0.956 | 1.009 | 0.969 | 0.991 |
| BCAM0407 | 0.805 | 1.007 | 0 | 0.986 | 1.028 | 0.764 | 0.843 | 0.653 | 2.054 | 0.725 | 0.774 | 0.819 | 0.675 | 0.777 |
| BCAM0473 | 0.983 | 1.129 | 0.986 | 0 | 1.113 | 0.97 | 0.975 | 0.952 | 2.135 | 0.969 | 0.943 | 0.99 | 0.938 | 0.914 |
| BCAM0652 | 1.108 | 1.118 | 1.028 | 1.113 | 0 | 1.014 | 1.077 | 0.979 | 2.158 | 1.044 | 0.906 | 1.016 | 0.988 | 0.993 |
| BCAM1015 | 0.696 | 0.905 | 0.764 | 0.97 | 1.014 | 0 | 0.775 | 0.726 | 1.996 | 0.692 | 0.711 | 0.684 | 0.701 | 0.723 |
| BCAM1376 | 0.733 | 1.006 | 0.843 | 0.975 | 1.077 | 0.775 | 0 | 0.779 | 1.98 | 0.72 | 0.766 | 0.697 | 0.758 | 0.712 |
| BCAM1974 | 0.789 | 0.939 | 0.653 | 0.952 | 0.979 | 0.726 | 0.779 | 0 | 2.004 | 0.67 | 0.744 | 0.831 | 0.695 | 0.724 |
| BCAM2063 | 1.983 | 2.096 | 2.054 | 2.135 | 2.158 | 1.996 | 1.98 | 2.004 | 0 | 2.019 | 1.979 | 1.976 | 2.079 | 1.91 |
| BCAM2311 | 0.742 | 0.936 | 0.725 | 0.969 | 1.044 | 0.692 | 0.72 | 0.67 | 2.019 | 0 | 0.727 | 0.727 | 0.767 | 0.656 |
| BCAM2373 | 0.771 | 0.956 | 0.774 | 0.943 | 0.906 | 0.711 | 0.766 | 0.744 | 1.979 | 0.727 | 0 | 0.786 | 0.651 | 0.707 |
| BCAM2584 | 0.772 | 1.009 | 0.819 | 0.99 | 1.016 | 0.684 | 0.697 | 0.831 | 1.976 | 0.727 | 0.786 | 0 | 0.796 | 0.746 |
| BCAM1787 | 0.742 | 0.969 | 0.675 | 0.938 | 0.988 | 0.701 | 0.758 | 0.695 | 2.079 | 0.767 | 0.651 | 0.796 | 0 | 0.742 |
| BCAM1287 | 0.707 | 0.991 | 0.777 | 0.914 | 0.993 | 0.723 | 0.712 | 0.724 | 1.91 | 0.656 | 0.707 | 0.746 | 0.742 | 0 |

**Fig. 4** Structural comparison among all possible combinations of identified porins. **a** Structural alignment of 14 predicted models. **b** RMSD (root-mean-square deviation) values between all porins highlight structural diversity

Gram-negative bacteria [33], was also identified under CF growth conditions that seem to be important for *B. cenocepacia* in host. Zinc metalloprotease ZmpA (BCAS0409) was also identified in the CF-mimicking medium. ZmpA is a zinc metalloprotease originally described in *B. cenocepacia* Pc715j [34] and is an important virulence factor. It has the ability to deteriorate numerous biologically important substrates, such as $\alpha_2$-macroglobulin, neutrophil α-1 proteinase inhibitor, type IV collagen, and gamma interferon leading to cause tissue damage as well as modulate the host immune system [34, 35].

Moreover, capsular polysaccharide transport proteins, a well-known protein family with protein domain IPR003856, were also identified under CF-mimicking conditions. These proteins are involved in the synthesis of lipopolysaccharide, O-antigen polysaccharide, capsule polysaccharide, and exopolysaccharides and are associated with virulence in Gram-negative bacteria [36], which indicates that they may also play an important role in the virulence of *B. cenocepacia*. Two further uncharacterized proteins with locus tags BCAL1293 and BCAL0350 were also identified in the CF niche conditions and should be investigated further to elucidate their role in CF niche adaptation.

**Table 3** List of LC-MS/MS differentially identified outer membrane proteins of *Burkholderia cenocepacia* under CF niche

| Sr. no. | Locus tag | Protein name or predicted function/bacterial species |
|---|---|---|
| 1 | BCAM2282 | ABC transporter ATP-binding protein |
| 2 | BCAL0097 | Putative phage tail protein |
| 3 | BCAL0339 | Type VI secretion lipoprotein/VasD |
| 4 | BCAL0341 | Putative type VI secretion system protein TssB |
| 5 | BCAL0350 | Uncharacterized protein(T6SS) |
| 6 | BCAL0403 | Putative bound lytic murein transglycosylase |
| 7 | BCAL0570 | Flagellar basal body L-ring protein |
| 8 | BCAL0670 | Short chain dehydrogenase |
| 9 | BCAL0782 | Chitobiase |
| 10 | BCAL0849 | M48B metalopeptidase |
| 11 | BCAL0861 | Major Facilitator Superfamily protein |
| 12 | BCAL1293 | Uncharacterized protein |
| 13 | BCAL1522 | Putative exported heme utilisation related protein |
| 14 | BCAL1528 | Flp type pilus assembly protein |
| 15 | BCAL1828 | Putative fimbrial usher protein |
| 16 | BCAL1866 | Putative lipoprotein |
| 17 | BCAL1893 | Family M23 peptidase |
| 18 | BCAL2413 | Hypothetical protein |
| 19 | BCAL2452 | LysR family regulatory protein |
| 20 | BCAL2454 | DNA topoisomerase IV subunit A |
| 21 | BCAL2645 | OmpA |
| 22 | BCAL2648 | Lipoprotein |
| 23 | BCAL3243 | Putative capsular polysaccharide biosynthesis |
| 24 | BCAL3377 | Lipoprotein |
| 25 | BCAL3498 | Binding-protein-dependent transport systems |
| 26 | BCAM0081 | OmpW family |
| 27 | BCAM0180 | M23B metallopeptidase |
| 28 | BCAM0209 | Capsular polysaccharide transport protein |
| 29 | BCAM0858 | Putative polysaccharide export protein |
| 30 | BCAM0956 | Major facilitator superfamily protein |
| 31 | BCAM0987 | Flagellar hook protein FlgE |
| 32 | BCAM1330 | Putative polysaccharide export protein |
| 33 | BCAM1514 | Lipoprotien |
| 34 | BCAM2781 | Hypothetical protein |
| 35 | BCAS0409 | Zinc metalloprotease ZmpA |
| 36 | BCAS0450 | Putative binding-protein-dependent transport system |
| 37 | BCAS0625 | Flp type pilus assembly protein |

## Analysis and Characterization of OMPs Expressed in the Soil Extract Medium

Overall, 101 OMPs were identified by LC-MS/MS in the simulated soil environment (Table S3). Along with several uncharacterized proteins, we identified proteins involved in important cellular functions including signal transduction and transcription (Table 4). The response of cells to environmental changes by transduction of extracellular signals through well-structured circuits is important to the persistence and survival of microbes in various ecological niches [37]. The identification of signal-dependent transcriptional regulators, such as LysR (COG0583), along with extracellular solute-binding proteins under soil extract medium may be a key mechanism through which *B. cenocepacia* can incorporate environmental cues and mediate the suitable response for adaptation.

**Table 4** List of LC-MS/MS differentially identified outer membrane proteins of *Burkholderia cenocepacia* under soil growth conditions

| Sr. no | Locus tag | Protein name or predicted funtion/bacterial species |
|---|---|---|
| 1 | BCAL1679 | Fimbrial biogenesis usher protein |
| 2 | BCAL2206 | Phasin-like protein |
| 3 | BCAL0831 | Putative storage protein(Phasin) |
| 4 | BCAS0573 | hypothetical protein |
| 5 | BURPS1710b_0051 | Extracellular ligand-binding receptor |
| 6 | BCAS0722 | Putative patatin-like phospholipase |
| 7 | BCAL1954 | Putative exported protein |
| 8 | BCAL2083 | Surface antigen |
| 9 | BCAL3242 | Polysaccharide export protein |
| 10 | BCAM0371 | Hypothetical protein |
| 11 | BCAM2216 | Hypothetical protein |
| 12 | BCAM2813 | Hypothetical protein |
| 13 | BCAL0524 | Flagellar motor switch protein G |
| 14 | BCAL3505 | Flagellar protein export ATPase FliN |
| 15 | BCAL3506 | Flagellar hook-length control protein |
| 16 | BCAL3531 | FAD dependent oxidoreductase |
| 17 | BCAM0953 | Extracellular solute-binding protein |
| 18 | BCAM2075 | LysR family transcriptional regulator |
| 19 | BCAM2190 | LysR family regulatory protein |
| 20 | BCAM2365 | Fis family transcriptional regulator |

**Table 5** List of LC-MS/MS differentially identified outer membrane proteins of *Burkholderia cenocepacia* under plant niche

| Sr. no. | Locus tag | Protein name or predicted funtion/bacterial species |
|---|---|---|
| 1 | BCAL0278 | Type IV pilus secretin PilQ |
| 2 | BCAM0119 | Hypothetical protein |
| 3 | BCAM2048 | Type III secretion protein,YscJ/HrcJ family protein |
| 4 | BCAM2055 | Type II secretory pathway |
| 5 | BCAM2704 | Fis family transcriptional regulator |
| 6 | BCAL0126 | Fagellar motor switch protein |
| 7 | BCAL0343 | Type VI secretion system protein TssD |
| 8 | BCAM0408 | Phospholipase C |
| 9 | BCAM2140 | Secretion protein HlyD |
| 10 | BCAM2253 | Rhs element Vgr protein |

Bacterial flagellar proteins form complexes that provide swimming and swarming motilities but also play a central role in adhesion, biofilm formation, and host invasion [33]. Flagellar motor switch protein G (COG1536), flagellar export ATPase FliN (COG1886), and flagellar hook length control protein (COG1868) were identified under the simulated soil conditions (Table S4). These flagellar switch proteins may give *B. cenocepacia* strain Y10 a competitive advantage in soil environments by acclimatizing to a condition that is adaptable in microbial community in response to signal from others. Moreover, several hypothetical proteins were also identified in the soil extract medium conditions, including BCAM0371, BCAM2216, BCAM2813, and BCAS0573.

### Analysis and Characterization of OMPs Expressed Under Simulated Plant Niche Conditions

As shown in Fig. 2, an obvious distinction was noted between the SDS-PAGE profiles of *B. cenocepacia* grown in plant-mimicking medium, CF, and soil growth media. Overall, 104 OMPs were detected in the plant-mimicking medium, with 10 differentially expressed proteins identified (Table 5). These unique proteins included type IV pilus secretin PilQ, a type III secretion protein, and type VI secretion system proteins, including VgrG. Bacteria build a wide range of biotic associations, from pathogenic to mutualistic associations or biofilms formation with larger host organisms. It seems that in *B. cenocepacia*, protein secretary machinery plays a key role in modulating all of these interactions that corroborate the study of Tseng et al. [38]. Phospholipase C and flagellar motor switch protein were also identified in the plant host-mimicking conditions. The identification and further investigation of all of these genetic determinants in *B. cenocepacia*, a bacterium with both human and plant pathogenic potential, can provide a better understanding of the evolution of phytopathogenicity, as well as the role of plants as potential reservoirs for clinically relevant bacteria.

## Conclusions

The ability to mimic four distinct ecological niches for *B. cenocepacia* is substantial to demonstrate the important proteins for one strain and its survival in each niche. We have uncovered a large number of novel proteins among strain-specific responses to each environment, which may aid in the identification and understanding the role of OM proteins. Our study also showed that this is not only important to understand the necessitate of a set of pathogenicity factors to allow attachment and disease development but also to understand the universal disease strategies in which the same suite of pathogenicity factors is used for all hosts. Tracking the proteomic evolution of *B. cenocepacia* pathogen particularly OM during pathogenicity provides in vivo direct method for monitoring the evolutionary mechanism and identification of genes responsible for pathogenesis. OM proteomic analysis under host-specific growth conditions is a step toward a comprehensive understanding of genetic adaptation during pathogenesis or host adaptation.

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