Regulation of phenylacetic acid degradation genes of *Burkholderia cenocepacia* K56-2

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Abstract

**Background:** Metabolically versatile soil bacteria *Burkholderia cepacia* complex (Bcc) have emerged as opportunistic pathogens, especially of cystic fibrosis (CF). Previously, we initiated the characterization of the phenylacetic acid (PA) degradation pathway in *B. cenocepacia*, a member of the Bcc, and demonstrated the necessity of a functional PA catabolic pathway for full virulence in *Caenorhabditis elegans*. In this study, we aimed to characterize regulatory elements and nutritional requirements that control the PA catabolic genes in *B. cenocepacia* K56-2.

**Results:** Translational fusions of the PA degradation gene promoters with eGFP were constructed and introduced in *B. cenocepacia* K56-2. eGFP expression was observed when the reporter strains were grown in minimal media containing glycerol and PA or other compounds expected to proceed through the PA pathway, and in synthetic CF medium (SCFM). Addition of succinate or glucose to the PA containing medium repressed eGFP expression. To show that BCAL0210, a putative TetR-type regulator gene encodes a regulator for the PA genes in *B. cenocepacia*, we developed a BCAL0210 insertional mutant reporter strain. Results show that these strains exhibit fluorescence regardless of the presence of PA in the culture.

**Conclusion:** The PA catabolic genes of *B. cenocepacia* K56-2 are induced by PA and other related compounds, are negatively regulated by PaaR (named herein), a TetR-type regulator, and are subjected to catabolic repression by glucose and succinate. As the PA catabolic pathway of *B. cenocepacia* appears to be induced during growth in synthetic cystic fibrosis medium (SCFM), further research is necessary to determine the relevance of this pathway in CF-like conditions and in other host-pathogen interactions.

Background

The *Burkholderia cepacia* complex (Bcc) is a group of Gram negative bacteria that comprises at least fifteen taxonomically related species [1,2]. Bcc strains occupy multiple niches from soil to humans as they have emerged as opportunistic pathogens in patients with cystic fibrosis (CF), chronic granulomatous disease, and other medical conditions associated with a compromised immune system [1,3]. *Burkholderia* species have evolved large genomes that allow them to deal with a variety of nutrient sources, predation and competition. The three chromosomes of *B. cenocepacia*, one of the most common species
found in CF patients [4], encode a broad array of catabolic functions. Yet, the contribution of these metabolic capacities to colonization and survival in the host has not been established.

The phenylacetic acid (PA) catabolic pathway is the central route where catabolism of many aromatic compounds converge and are directed to the Krebs cycle [5]. It comprises of four steps, namely the PA-CoA ligation-activation performed by PaaK [6], the hydroxylation step for which the PaaABCDE enzymatic complex is responsible [7], the enoyl-CoA isomerization/hydration, ring opening performed by PaaG and PaaZ, [8], and the β-oxidation step carried out by PaaF and PaaH, [8].

Previously, we initiated the functional characterization of the PA catabolic pathway of *B. cenocepacia* K56-2 [9] and demonstrated that interruption of putative PA-CoA ring hydroxylation activity, but not the lower steps of PA degradation, resulted in an attenuated pathogenic phenotype in the *Caenorhabditis elegans* model of infection. Here, we report that the PA catabolic genes of *B. cenocepacia* K56-2 are induced by PA, are negatively regulated by PaaR, a TetR-type regulator and are subjected to catabolic repression by glucose and succinate.

**Results**

**Translational reporter plasmids containing PA catabolic gene promoters are responsive to PA and related compounds**

The PA degradation genes are arranged in three separate clusters in *B. cenocepacia*, namely paaABCDE, paaFZGJK1 and paaHK2, where the paaF gene is divergently orientated from the paaZGJK1 cluster [9]. To evaluate whether the upstream regions of the PA catabolic genes contain PA-inducible promoters, translational eGFP reporter plasmids containing DNA fragments upstream of the first gene of each of the three PA clusters: paaA, paaZ and paaH were constructed and introduced into *B. cenocepacia* K56-2. Previous results showed that eGFP is expressed and remains stable in *B. cenocepacia* [10]. Cells containing reporter plasmids with the paaA, paaH, and paaZ promoters (P\_paaA, P\_paaH, and P\_paaZ respectively) fused to the eGFP gene, exhibited increased fluorescence when grown in minimal media containing glycerol with PA in comparison with those grown in minimal media containing glycerol without PA (Figure 1). eGFP expression from P\_paaA was 5.7 fold higher when grown with PA compared to glycerol, while the ones from P\_paaH and P\_paaZ were each 2.9 fold higher.

According to the KEGG database [11-13] we expected phenylalanine, phenylacetamide and phenylethylamine to be degraded through the PA catabolic pathway in *B. cenocepacia* AU1054. To determine if these aromatic carbon sources induce the PA degradation pathway in *B. cenocepacia* K56-2, cells containing the P\_paaA reporter were grown in media containing these carbon sources. eGFP expression similar to the one shown with PA was observed with phenylalanine, phenylpyruvate or phenylacetamide (Figure 2). On the contrary, 2-hydroxy-phenylacetic acid did not induce eGFP expression, in accordance with this compound not being a true intermediate of the pathway [6].

In addition, we sought to determine whether the PA genes were activated in response to Synthetic Cystic Fibrosis Medium (SCFM), a chemically defined medium formulated according to the contents of CF sputum [14]. Our results show that P\_paaH reporter activity increases approximately 5-fold when cells are grown in SCFM (Figure 2).

**The PA catabolic genes are subject to catabolic repression by TCA intermediates and sugars**

Aromatic compound degradation is subject to catabolic repression in the presence of more readily usable carbon sources in other bacteria [15-17]. Therefore, the possible catabolic repression exerted by succinate and glucose was investigated. Strains containing the reporters P\_paaA, P\_paaZ and P\_paaH were grown in minimal medium containing PA with or without the additional carbon source and analyzed at one-hour intervals (Figure 3). *B. cenocepacia* K56-2 harbouring pJH1 was used as a
indicated the N-terminal region of BCAL0210 protein family regulatory protein. Results of our BLAST search superfamily) while BCAL0210 was annotated as a TetR conserved domain of unknown function (DUF1835 dicted to encode a 273 amino acid protein containing a media with different carbon sources

mately an O.D.600 of 0.3 where a shift in the slope towards was observed with reporters P

eGFP synthesis, was observed (Figure 3B). The same effect steady levels of fluorescence, suggesting lack of optical densities above 0.6, fluorescence varied slightly not affected with the different carbon sources, although at the different carbon sources used. Catabolic repression by glucose on the PA-inducible eGFP expression was observed in cells harbouring P\(_{paaA}\) at approximately an O.D.600 of 0.3 where a shift in the slope towards steady levels of fluorescence, suggesting lack of de novo eGFP synthesis, was observed (Figure 3B). The same effect was observed with reporters P\(_{paaZ}\) and P\(_{paaD}\) (Figure 3C and 3D respectively). This is contrasted with cells grown in succinate, which exhibited strong silencing of eGFP expression at all cell densities (Figure 3B-D). We concluded that glucose and succinate exert catabolic repression of the PA degradation pathway.

**Insertional mutagenesis of BCAL0210 results in increased expression of PA-inducible genes**

Located 128 bp downstream of the paaABCDE gene cluster and oriented in the same direction are genes BCAL0211 and BCAL0210 (Figure 4A). BCAL0211 is predicted to encode a 273 amino acid protein containing a conserved domain of unknown function (DUF1835 superfamily) while BCAL0210 was annotated as a TetR family regulatory protein. Results of our BLAST search indicated the N-terminal region of BCAL0210 protein shows 60% similarity to AcrR (Expect value = 5e-7), which is a TetR-like regulator of a multi-drug efflux pump of E. coli [19-21]. Given that a regulator protein homologous to PaaX, the GntR-type transcriptional regulator of PA degradation in E. coli [22] is not encoded in B. cenocepacia J2315 genome, we hypothesized that the BCAL0210 gene encoded the regulator of PA catabolism in B. cenocepacia. The effect of the loss of BCAL0210 function on the regulation on the PA genes was determined by insertional mutagenesis of the BCAL0210 gene to create the strain JNRH1. Reporter plasmids containing the P\(_{paaA}\) P\(_{paaH}\) and P\(_{paaZ}\) promoters were then conjugated into strain JNRH1 and eGFP expression was assessed in the presence and the absence of PA. Results show that these strains exhibit increased fluorescence regardless of the presence of PA in the culture (Figure 1). This PA independent activity suggests that BCAL0210 encodes for a negative regulator, whose regulatory ability is abolished in the JNRH1 mutant. Interestingly, eGFP expression driven by the P\(_{paaA}\) and P\(_{paaH}\) promoters in JNRH1 was higher in the presence of PA than in reporter strains grown with glycerol only (Figure 1) suggesting a BCAL0210 independent induction of gene expression in the presence of PA.

In order to determine if paaABCDE and BCAL0211-BCAL0210 were part of the same transcriptional unit, a transcriptional analysis was performed. Total RNA was isolated from B. cenocepacia cells grown with LB containing 1 mM PA and subjected to RT-PCR using specific primers. Results show that the paaA, paaB, paaC, paaD and paaE genes are contained on a single transcript and are thus co-regulated at the transcriptional level (Figure 4B). Primers were unable to generate an amplicon between paaE and BCAL0211 although an amplicon was generated between BCAL0211 and BCAL0210, indicating that they are located on the same transcript. Taken together these results demonstrate that paaABCDE and BCAL0211-BCAL0210 are two separate transcriptional units.

**A conserved Inverted Repeat is necessary for negative control of P\(_{paaA}\)**

Examination of upstream DNA sequences of the PA gene clusters identified near perfect 15 bp inverted repeat (IR) sequences located between the putative -10 and -35 core promoter signals (Figure 5) that resembled operator sites of a TetR regulatory protein [21]. In order to validate the IR sequences found in PA gene promoters as the operator sites of BCAL0210, translational fusion plasmids containing mutations in the paaA IR were created. We hypothesized that the sequence is a motif recognized by a TetR-like transcriptional regulator due to it being a dual overlapping inverted repeat, similar to the QacR operator [21].

Using plasmid mutagenesis with primers containing mismatched mutations on the 5’ ends (Additional file 1) that annealed to plasmid pJH7 containing the P\(_{paaA}\) reporter

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**Figure 2**

Activity of P\(_{paaA}\) as a result of growth in M9 minimal media with different carbon sources. B. cenocepacia K56-2 (WT) containing eGFP translational reporters P\(_{paaA}\) were grown for 18 hours in synthetic cystic fibrosis medium (SCFM) or M9 minimal media supplemented with various carbon sources. Gly, glycerol; PA, phenylacetic acid; 2-OHPA, 2-hydroxy-phenylacetic acid; Phe, L- phenylalanine; PhPy, phenylpyruvate; PhAc, phenylacetamide. Relative fluorescence was determined as described in methods. Data represent the mean from three independent experiments, with error bars signifying standard deviations.
we generated plasmids pJH10, pJH11 and pJH12 (Table 1). The plasmid pJH10 contains 14 mismatch mutations replacing nearly the entire IR within the paaA promoter. Plasmids pJH11 and pJH12 contain the mutations in the upstream or downstream half of the IR respectively. These plasmids were then transferred to B. cenocepacia K56-2 by triparental mating. Reporter strains were grown in minimal media supplemented with glycerol or PA. Cells harbouring plasmids pJH10, pJH11 and pJH12 exhibited higher levels of relative fluorescence in comparison with K56-2/pJH7 when grown with glycerol, demonstrating that the sequence is indeed required for negative control of paaA promoter activity (Table 2).

Because PaaX is involved in the regulation of upstream pathways of PA catabolism in other microorganisms through binding a conserved PaaX box [23,24] we searched for the consensus IR sequence in the genome of B. cenocepacia. A position weight matrix (PWM) [25] of the conserved IR present in the promoter regions of the

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**Figure 3**

Phenylacetic acid genes are subject to Carbon Catabolite Repression. B. cenocepacia K56-2 containing eGFP translational fusions with the *dhfr* promoter (A), *P_paaA* (B), *P_paaZ* (C), and *P_paaH* (D) were grown for 13 hours in M9 minimal media supplemented with the indicated carbon sources. Error bars represent the standard deviation of three independent cultures.
paaA, paaH and paaZ plus the divergent promoter of paaF and BCAL0211 was constructed (Additional file 2) and used to search the entire genome sequence of B. cenocepacia J2315. The coordinate positions of sequences detected up to a cut off score of 17.0 are listed (Additional file 3). The top scores for the search were the ones for the paaZ, paaF, paaA and paaH inverted repeats while BCAL0211 IR scored lower at 12.0. Other sequences with scores that ranked from 18.41 to 17.37 did not locate in putative promoters or between -10 and -35 regions, likely representing false positives. We concluded that the 15 bp IR sequences are specific to the PA catabolic gene clusters.

Discussion
In contrast to what has been observed in E. coli and Pseudomonas putida [5], the PA genes of B. cenocepacia K56-2 are organized into three gene clusters. We hypothesize that this arrangement may allow regulation of gene expression at different levels. The observation that eGFP expression driven by P\textsubscript{paaA} is roughly 3-fold stronger than either the P\textsubscript{paaF}, P\textsubscript{paaA} and P\textsubscript{paaH} inverted repeats while BCAL0211 IR scored lower at 12.0. Other sequences with scores that ranked from 18.41 to 17.37 did not locate in putative promoters or between -10 and -35 regions, likely representing false positives. We concluded that the 15 bp IR sequences are specific to the PA catabolic gene clusters.

Our results also show that BCAL0210 is necessary for repression of PA dependent activity of the paaA, paaH and paaZ gene promoters (Figure 1). Therefore, BCAL0210 (PaaR) encoding for a TetR-type transcriptional regulator is involved in negative regulation of the PA catabolic genes. Since a conserved inverted repeat DNA sequence is necessary for PA negative control of paaA gene expression (Table 2), we hypothesize that BCAL0210 binds the IRs located in the core promoter of the paaA, paaZ and paaH genes to negatively regulate transcription of the PA catabolic genes. It should be noted however, that the insertional mutagenesis system used to produce JNRH1 introduces polar mutations [27]. Although the possibility of polar effects on genes downstream BCAL0210 cannot be ruled out, the downstream gene BCAL0209, encoding a putative GNAT family acetyl transferase located several hundred base pairs downstream of BCAL0211 makes the
possibility of polar effects unlikely. On the other hand, BCAL0211 and BCAL0210 are located on the same transcript (Figure 4) and thus are co-regulated at the transcriptional level. TetR-type proteins are known to regulate their own transcription by self-repression [28]. Currently it is unknown if the conserved IR located in the DNA leader sequence of the BCAL0211 gene may be involved in regulation of this gene cluster. Whether BCAL0211, which encodes for a protein of unknown function (DUF1835) is involved in some fashion in the regulation of the PA genes remains to be determined.

Catabolic repression of aromatic compound degradation by TCA intermediates and glucose has been described in the β-proteobacterium Acidovorax sp. [29], and P. putida [15] respectively. In accordance with these data we found that the PA catabolic pathway of B. cenocepacia K56-2 is subject to catabolic repression by succinate and glucose (Figure 3). Interestingly, P_{paaA} is induced after 18 h of growth in SCFM probably as a result of the presence of phenylalanine (Figure 2). This observation is consistent with the recently reported B. cenocepacia global gene expression response to SCFM, which shows the induction of the PA catabolic pathway [30]. Whether this finding is relevant for pathogenesis of Bcc in the CF lung environment remains an unexplored point of interest.

**Conclusion**

We show that the PA gene promoters are responsive to PA, SCFM, and other compounds expected to proceed via the PA pathway. We also show the PA gene promoters are negatively regulated by PaaR, a TetR-type regulator, and are subjected to catabolic repression by succinate and glucose.

**Methods**

**Bacterial strains, nematode strains and growth conditions**

Bacterial strains and plasmids are listed in Table 1. B. cenocepacia K56-2 was grown at 37°C in Luria Bertani (LB) or M9 minimal medium with 5 mM PA or 25 mM of the indicated carbon sources, supplemented as required, with 100 μg/ml trimethoprim (Tp), 50 μg/ml gentamicin (Gm) and 200 μg/ml chloramphenicol (Cm). E. coli was grown...
at 37°C in LB medium supplemented with 50 μg/ml Tp, 40 μg/ml kanamycin (Km) or 20 μg/ml Cm.

**Bioinformatics analysis**

BLAST searches of the genome sequence of *B. cenocepacia* strain J2315 were performed with the *B. cenocepacia* Blast Server at Sanger Institute [http://www.sanger.ac.uk/cgi-bin/blast/submitblast/b_cenocepacia]. J2315 belongs to the same clonal lineage as strain K56-2 [32]. Gene clusters were visualized with Artemis software [33] and VectorNTI software (Invitrogen). PWM scores were calculated manually [25] (Additional file 2) as described by Hertz and Stormo [34] and Schnieder and Stephens [35]. Identification of binding sites using this PWM was achieved using the Target Explorer [36]. For TCOFFEE analysis [37] the default substitution matrix was used, with a gap opening penalty of -10 and a gap extension penalty of -1.

**Molecular Biology techniques**

Recombinant DNA techniques were carried out as previously described [38]. DNA ligase (New England Biolabs)
was used as recommended by the manufacturers. *E. coli* DH5α cells were transformed using the calcium chloride protocol [39] and electroporation was used for transformation of *E. coli* SY327 cells [40]. Reporter plasmids were constructed in *E. coli* and conjugation into *B. cenocepacia* K56-2 was accomplished by triparental mating [41] with *E. coli* DH5α carrying the helper plasmid pRK2013 [42]. DNA was amplified using a PTC-221 DNA engine (MJ Research) or an Eppendorf Mastercycler ep gradient S thermal cycler with Taq DNA polymerase, Phusion High-Fidelity PCR Kit or Proofstart DNA polymerase (Qiagen) (New England Biolabs). Amplification conditions were optimized for each primer pair and are available upon request. PCR products and plasmids were purified with QIAquick purification kit (Qiagen) and QIAprep Miniprep kit (Qiagen), respectively.

**RNA isolation methods and RT-PCR analysis**

For RNA isolation, bacteria were grown in LB supplemented with 1 mM PA. Cells were harvested during early log phase (O.D. 600 = 0.3) and lysed in TE buffer pH 8.0 containing 400 μl/ml lysozyme for 5 minutes at room temperature. RNA was recovered with the RNeasy Mini kit (Qiagen), and samples eluted into (Diethyl Pyrocarbonate) DEPC treated water. Total RNA was visualized in a 1% agarose gel in TAE buffer. Residual DNA was removed by on column treatment with DNase I (15 min, +72 (399 bp) of *B. cenocepacia* was sequenced (The Centre for Applied Genomics, Toronto). As *B. cenocepacia* is intrinsically resistant to Gm, in all conjugations Gm was added to the final transfer to eliminate donor *E. coli*. To create pJH2, pJH1 was then PCR amplified using divergently oriented primers (Additional file 1) containing multiple restriction sites on the 5' ends such that the self-ligated product of the reaction has a multiple cloning site in place of the original promoter. Growth rates for *B. cenocepacia* K56-2 with or without pJH2 were similar (data not shown). DNA fragments corresponding to *paaZ* from -420 to +90 (510 bp), *paaA* from -396 to +84 (480 bp), and *paaH* from -327 to +72 (399 bp) of *B. cenocepacia* K56-2 chromosomal DNA were amplified and cloned into pH2 to create pJH6, pJH7, and pJH8 respectively.

**Construction of *eGFP* translational fusion plasmids**

To create pJH1, digestion with Xbal/Ndel of pSCrhaB4 resulted in a 784 bp fragment containing *eGFP*, which was cloned into the same sites in pAP20 [9] such that *eGFP* is under control of the constitutive *dhfr* promoter. *E. coli* transformants were selected with 20 μg/ml chloramphenicol. The plasmid was conjugated into *B. cenocepacia* K56-2 by tri-parental mating with *E. coli* helper strain containing plasmid pRK2013. As *B. cenocepacia* is intrinsically resistant to Gm, in all conjugations Gm was added to the final transfer to eliminate donor *E. coli*. To create pJH2, pJH1 was then PCR amplified using divergently oriented primers (Additional file 1) containing multiple restriction sites on the 5' ends such that the self-ligated product of the reaction has a multiple cloning site in place of the original promoter. Growth rates for *B. cenocepacia* K56-2 with or without pJH2 were similar (data not shown). DNA fragments corresponding to *paaZ* from -420 to +90 (510 bp), *paaA* from -396 to +84 (480 bp), and *paaH* from -327 to +72 (399 bp) of *B. cenocepacia* K56-2 chromosomal DNA were amplified and cloned into pH2 to create pJH6, pJH7, and pJH8 respectively.

**Construction of site directed plasmid mutants**

The plasmids pJH10, pJH11 and pJH12 were constructed by plasmid PCR mutagenesis to contain mutations in the entire, left or right region of the conserved IR in the *paaA* core promoter. Appropriate phosphorylated primers (Additional file 1) were used to divergently amplify template pH7 (containing the *paaA* promoter), and each contained mismatch mutations on their 5' ends. Plasmids were self-ligated, transformed into *E. coli* DH5α and then conjugated into *B. cenocepacia* wild type. Mutations were verified by sequence analysis (The Centre for Applied Genomics, Toronto).

**Nucleotide accession number**

The nucleotide sequence of translational fusion vector pH2 is deposited in GenBank under accession no. JF607244.

**Authors’ contributions**

JNRH participated in the design of the study, carried out the majority of experiments and wrote the manuscript. RAMB was involved in plasmid construction, carried out some reporter analysis and critically read the manuscript. STC participated in the design and coordination of the study and final edition of the manuscript. All authors read and approved the final manuscript.
Additional material

Additional file 1
Primers used in this study.
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Additional file 2
Position Weight Matrix Calculations. A) The sequences used to generate the matrix of the conserved inverted repeat from the paaA, paaH, paa2, paaF and BCAL0211 genes. B) The sum the occurrence of nucleotides at each position. C) The formulas used to generate the PWM, modified from [25] p(b, i) = corrected probability of base b in position i; f(b, i) = counts of base b in position i; N = number of sites; p(b) = background probability of base b in B. cenocepacia J2315 genome as follows: p(A) or p(T) = 0.1665; p(C) or p(G) = 0.335; W(b, i) = PWM value of base b in position i. D) Resulting position weight matrix.

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Additional file 3
Position Weight Matrix scores in a genomic scan of B. cenocepacia. The position weight matrix calculated in Additional file 2 was used to scan the genome of Burkholderia cenocepacia K56-2. Genome co-ordinate is from the annotated sequence [4].

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1. Vanlaere E, Lipuma JJ, Baldwin A, Henry D, De Brandt E, Mahenthiralingam E, Speert D, Dowson C, Vandamme P. Burkholderia latens sp. nov., Burkholderia diffusa sp. nov., Burkholderia arboris sp. nov., Burkholderia senalis sp. nov. and Burkholderia metallica sp. nov., novel species within the Burkholderia cenocepacia complex. Int J Syst Evol Microbiol 2008, 58(Pt 7):1580-1590.

2. Valvano MA, Keith KE, Cardona ST: Survival and persistence of opportunistic Burkholderia species in host cells. Curr Opin Microbiol 2008, 5(1):99-105.

3. Hooken MT, Seth-Smith HM, Crossman LC, Seabia M, Bentley SD, Cerdeno-Tarraga AM, Thomson NR, Bason N, Quail MA, Sharp S, Cherevach I, Churcher C, Goodhead I, Hauser H, Holroyd N, Mungall K, Scott P, Walker D, White B, Rose H, Iversen P, Mil-Homens D, Rocha EP, Fialho AM, Baldwin A, Dowson C, Barrett BG, Govan JR, Vandamme P, Hart CA, Mahenthiralingam E, Parkhill J. The genome of Burkholderia cenocepacia J2315, an epidemic pathogen of cystic fibrosis patients. J Bacteriol 2009, 191(1):261-277.

4. Luengo JM, Garcia JL, Olivera ER: The phenylacetyl-CoA catabolon: a complex catabolic unit with broad biotechnological applications. Mol Microbiol 2001, 39(6):1434-1442.

5. Fernandez A, Minambres B, Garcia B, Olivera ER, Luengo JM, Garcia JL, Diaz E: Catabolism of phenylacetic acid in Escherichia coli. Characterization of a new aerobic hybrid pathway. J Biol Chem 1998, 273(40):25974-25986.

6. Fernandez C, Fernández A, Minambres B, Diaz E, Garcia JL: Genetic characterization of the phenylacetate-Coenzyme A oxygenase from the aerobic phenylacetic acid degradation pathway of Escherichia coli. Appl Environ Microbiol 2006, 72(11):7422-7426.

7. Ismail W, El-Said Mohamed M, Wanner BL, Datsenko KA, Eisenreich W, Rohdich F, Bacher A, Fuchs G. Functional genomics by NMR spectroscopy. Phenylacetate catabolism in Escherichia coli. Eur J Biochem 2003, 270(14):3047-3054.

8. Law RJ, Hamlin JN, Sivro A, McCrorrist SJ, Cardana GA, Cardona ST: A functional phenylacetic acid catabolic pathway is required for full pathogenicity of Burkholderia cenocepacia in the Caenorhabditis elegans host model. J Bacteriol 2008, 190(21):7209-7218.

9. Aranda-Olmedo I, Ramos JL, Marques S: Identification of signals through Crc and PtnS in catabolite repression of Pseudomonas aeruginosa multi-cellular behavior in cystic fibrosis sputum. J Bacteriol 2007, 189(22):8079-8087.

10. Martinez-Blanco H, Reglero A, Luengo JM: Carbon catabolite regulation of phenylacetate-CoA ligase from Pseudomonas putida. Biochem Biophys Res Commun 1990, 176(3):891-897.

11. Bruckner R, Tingemeyer F: Carbon catabolite repression in bacteria: choice of the carbon source and autoregulatory limitation of sugar utilization. FEMS Microbiol Lett 2002, 209(2):141-148.

12. Ferrandez A, Garcia JL, Diaz E: Transcriptional regulation of the divergent paa catabolic operons for phenylacetic acid degradation in Escherichia coli. J Biol Chem 2000, 275(16):12214-12222.

13. del Peso-Santos T, Bartolome-Martin D, Fernandez C, Alonso S, Garcia JL, Diaz E, Shinger V, Perera J: Coregulation by phenylacetyl-Coenzyme A-responsive PaaX integrates control of the upper and lower pathways for catabolism of styrene by Pseudomonas sp. strain Y2. J Bacteriol 2006, 188(13):4812-4821.

14. Kim HS, Kang TS, Hyun JS, Kang HS: Regulation of penicillin G acylase gene expression in Escherichia coli by repressor PaaX and the cAMP-cAMP receptor protein complex. J Bacteriol 2004, 186(27):3253-3262.

15. Wasserman WW, Sandelin A: Applied bioinformatics for the identification of regulatory elements. Nat Rev Genet 2004, 5(4):276-287.
26. Ross W, Gosink KK, Salomon J, Igarashi K, Zou C, Ishihama A, Sev-
ervinov K, Gourse RL: A third recognition element in bacterial
promoters: DNA binding by the alpha subunit of RNA
polymerase. Science 1993, 262(5138):1407-1413.
27. Flannagan RS, Aubert D, Kooi C, Sokol PA, Valvano MA: Burkholde-
ria cenocepacia requires a periplasmic HtrA protease for
growth under thermal and osmotic stress and for survival in
vivo. Infect Immun 2007, 75(4):1679-1689.
28. Saenger W, Orth P, Kisker C, Hillen W, Hinrichs W: The tetracy-
ccline repressor-A paradigm for a biological switch. Angew
Chem Int Ed Engl 2000, 39(12):2042-2052.
29. Ohtsubo Y, Goto H, Nagata Y, Kudo T, Tsuda M: Identification of
a response regulator gene for catabolite control from a PCB-
degrading beta-proteobacteria, Acidovorax sp. KKS102. Mol
Microbiol 2006, 60(6):1563-1575.
30. Yoder-Himes DR, Chain PS, Zhu Y, Wurtzel O, Rubin EM, Tiedje JM,
Sorek R: Mapping the Burkholderia cenocepacia niche
response via high-throughput sequencing. Proc Natl Acad Sci
USA 2009, 106(10):3976-3981.
31. Cormack BP, Valdivia RH, Falkow S: FACS-optimized mutants of
the green fluorescent protein (GFP). Gene 1996, 173(1 Spec
No):33-38.
32. Darling P, Chan M, Cox AD, Sokol PA: Siderophore production by
cystic fibrosis isolates of Burkholderia cenocepacia. Infect Immun
1998, 66(2):874-877.
33. Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream MA,
Barrell B: Artemis: sequence visualization and annotation. Bio-
informatics 2000, 16(10):944-5.
34. Hertz GZ, Stormo GD: Identifying DNA and protein patterns
with statistically significant alignments of multiple
sequences. Bioinformatics 1999, 15(7-8):563-577.
35. Schneider TD, Stephens RM: Sequence logos: a new way to display
consensus sequences. Nucleic Acids Res 1990, 18(20):6097-6100.
36. Cohen SN, Chang AC, Hsu L: Nonchromosomal antibiotic
resistance in bacteria: genetic transformation of Escherichia
coli by R-factor DNA. Proc Natl Acad Sci USA 1972,
69(8):2110-2114.
37. Miller VL, Mekalanos JJ: A novel suicide vector and its use in con-
struction of insertion mutations: osmoregulation of outer
membrane proteins and virulence determinants in Vibrio
cholerae requires toxR. J Bacterial 1988, 170(6):2575-2583.
38. Craig FF, Coote JG, Parton R, Freer JH, Gilmour NJ: A plasmid
which can be transferred between Escherichia coli and Pse-
udeilla haemolytica by electropropagation and conjugation. J
Gen Microbiol 1989, 135(1):2885-2890.
39. Fligurski DH, Helinski DR: Replication of an origin-containing
derivative of plasmid RK2 dependent on a plasmid function
provided in trans. Proc Natl Acad Sci USA 1979, 76(4):1648-1652.
40. Mahenthiralingam E, Coenye T, Chung JW, Speert DP, Govan JR, Tay-
lor P, Vandamme P: Diagnostically and experimentally useful
panel of strains from the Burkholderia cepacia complex. J Clin
Microbiol 2000, 38(2):910-913.