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Synthetic cystic fibrosis sputum medium diminishes *Burkholderia cenocepacia* antifungal activity against *Aspergillus fumigatus* independently of phenylacetic acid production

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Abstract

Phenylacetic acid (PAA), an intermediate of phenylalanine degradation, is emerging as a signal molecule in microbial interactions with the host. In this work, we explore the presence of phenylalanine and PAA catabolism in three microbial pathogens of the cystic fibrosis (CF) lung microbiome: *Pseudomonas aeruginosa*, *Burkholderia cenocepacia* and *Aspergillus fumigatus*. While *in silico* analysis of *B. cenocepacia* J2315 and *A. fumigatus* Af293 genome sequences showed complete pathways from phenylalanine to PAA, the *P. aeruginosa* PAO1 genome lacked several coding genes for phenylalanine and PAA catabolic enzymes. HPLC analysis of supernatants from *B. cenocepacia* K56-2 detected PAA when grown in LB but not in synthetic cystic fibrosis sputum medium (SCFM). However, we were unable to identify PAA production by *A. fumigatus* or *P. aeruginosa* in any of the conditions tested. The inhibitory effect of *B. cenocepacia* on *A. fumigatus* growth was evaluated using agar plate interaction assays. Inhibition of fungal growth by *B. cenocepacia* was lessened in SCFM but this effect was not dependent on bacterial production of PAA. In summary, while we demonstrated PAA production by *B. cenocepacia*, we were not able to link this metabolite with the *B. cenocepacia*-*A. fumigatus* microbial interaction in CF nutritional conditions.

Key Words phenylacetic acid, *Burkholderia cenocepacia*, *Aspergillus fumigatus*, *Pseudomonas aeruginosa*, cystic fibrosis

Introduction

Cystic fibrosis (CF) is a human genetic disease characterized by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (Cheng et al. 1990; Riordan et al. 1989). CFTR malfunction results in an imbalance of electrolytes and water in the airways, leading to accumulation of thick dehydrated mucus in the patient's lungs (Matsui et al. 1998). The CF mucus is rich in nutrients, such as amino acids (Palmer et al. 2007), and supports the establishment of a polymicrobial community that leads to lung inflammation, chronic infection, and loss of lung function (Hauser et al. 2011).

Haemophilus influenzae and *Staphylococcus aureus* are generally the first to colonize the CF lung, followed by *Pseudomonas aeruginosa* and, as the disease progresses, members of the *Burkholderia cepacia* complex (Bcc) (Lipuma 2010; Mahenthiralingam et al. 2005). Although *P. aeruginosa* is the most common opportunistic pathogen in CF patients, the outcomes of Bcc infection are the most severe (Courtney et al. 2004; Courtney et al. 2007). Bacteria are not the only colonizers of the CF lung. Filamentous fungi, such as *Aspergillus fumigatus*, are very prevalent (Bakare et al. 2003; Mortensen et al. 2011; Paugam et al. 2010). While research and treatment of CF patients have traditionally focused on individual pathogens, there is accumulating evidence that the progression of CF lung disease is governed by the complex interactions of the CF polymicrobial community (Sibley et al. 2006; Sibley and Surette 2011). Of particular interest is the possibility of microbial beneficial interactions that may result in poorer outcomes for the patient.

Microbial production of phenylacetic acid (PAA) is emerging as a regulatory mechanism by which microorganisms may modulate their pathogenicity and interactions with the

host. PAA produced by a *paaABCDE* mutant of *B. cenocepacia* decreased *luxI/luxR*-type QS signaling. The *paaABCDE* mutant was defective in QS-related virulence as demonstrated in a *Caenorhabditis elegans* model of infection (Pribytkova et al. 2014). In line with our findings, exogenous addition of PAA inhibited QS-regulated virulence traits of *P. aeruginosa* PAO1, such as pyocyanin and exopolysaccharide production, protease and elastase activity, and swimming motility (Musthafa et al. 2012). Intriguingly, Wang et al. found that *P. aeruginosa* PAO1 produced PAA at high cell densities in LB, which in turn attenuated *P. aeruginosa*'s cytotoxicity (Wang et al. 2013). The ability of two prominent CF pathogens to use PAA to modify their QS-regulated virulence presents intriguing possibilities for a physiological role of PAA metabolism in microbial interactions as microbial production of PAA may result in intra- and interspecies regulation of virulence. This has been demonstrated in the rhizosphere where PAA, released as a phytotoxin by the root infecting fungus *Rhizoctonia solani*, can repress the biosynthesis of nematocidal compounds by *Pseudomonas fluorescens*, indirectly protecting the plant-parasitic nematode *Meloidogyne incognita* from biocontrol by *P. fluorescens* (Siddiqui and Shaukat 2004). PAA can also affect the host immune system directly. PAA excreted by *Bacillus fortis* can activate the induced systemic response of tomato plants, protecting the plants by priming them to respond to the plant pathogen responsible for *Fusarium* wilt disease (Akram et al. 2016). In zebrafish, PAA released by a mutant of *Acinetobacter baumannii* acted as a chemoattractant for neutrophils resulting in improved bacterial clearance (Bhuiyan et al. 2016). Understanding the links between bacterial metabolism, pathogenicity and host immune response may help in the development of treatments.

In *Escherichia coli* and *Pseudomonas putida*, PAA can be produced aerobically from phenylalanine (Supplemental Fig. S1 and S2), according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (Kanehisa and Goto 2000; Kanehisa et al. 2016) and accumulated experimental evidence (Teufel et al. 2010; Teufel et al. 2011). Phenylalanine can be converted to PAA through 2-phenylacetamide, which is then transformed to PAA by an amidase. Conversion of phenylalanine to PAA can also occur through synthesis of phenylethylamine by an aromatic-L-amino-acid decarboxylase followed by conversion to phenylacetaldehyde by a primary amine oxidase, such as the TynA (MaoA) enzyme of *E. coli* (Steinebach et al. 1996). Finally, phenylacetaldehyde is converted to PAA by a phenylacetaldehyde dehydrogenase, FeaB (PadA) (Ferrandez et al. 1997). In turn, PAA can be further degraded to succinyl-CoA and acetyl-CoA in aerobic conditions. In *E. coli* and *P. putida* (Supplemental Fig. S1 and S2), PAA is first converted to phenylacetyl-coenzyme A (PAA-CoA) by a CoA ligase, PaaK (Martinez-Blanco et al. 1990; Vitovski 1993). Next, the aromatic ring of PAA-CoA undergoes epoxidation by PaaABC(D)E, a four or five subunit-monooxygenase complex (Teufel et al. 2010; Teufel et al. 2012). Then, PaaG isomerizes the epoxide into an oxepin and the bifunctional enzyme PaaZ performs the hydrolysis of the oxepin and the subsequent oxidation of the terminal aldehyde group (Grishin and Cygler 2015; Teufel et al. 2010). The final steps of the PAA catabolic pathway, often referred to as the PAA catabolic lower pathway, are similar to the fatty acid β -degradation pathway. The product of PaaZ is cleaved by a CoA thiolase PaaJ, rearranged by the CoA isomerase PaaG, and hydrated by PaaF to form 3-hydroxyadipyl-CoA. This intermediate is then oxidized by PaaH,

before finally being converted to acetyl-CoA and succinyl-CoA by PaaJ (Grishin and Cygler 2015; Grishin and Cygler 2015; Nogales et al. 2007; Teufel et al. 2010).

The aim of this study was to examine three CF pathogens, *B. cenocepacia*, *P. aeruginosa* and *A. fumigatus*, for their ability to produce PAA in aerobic conditions by analyzing the presence of putative genes of phenylalanine and PAA catabolic pathways, the presence of PAA in culture supernatants, and the role of PAA in their interactions. We demonstrate that *B. cenocepacia* is able to release PAA when grown in LB medium. The antifungal activity of both *B. cenocepacia* and *P. aeruginosa* against *A. fumigatus* was decreased in conditions that mimic the nutrients of the CF sputum. However, these interactions were not mediated by bacterial PAA production.

Materials and methods

Strains, media preparation and growth conditions

Bacterial and fungal strains are listed in Table 1. Bacterial strains were grown overnight at 37°C in Luria-Bertani (LB) medium, unless otherwise specified. *A. fumigatus* UAMH 2978 was grown and maintained on potato dextrose agar (PDA) media at 37°C. The growth of bacterial cultures was measured by optical density at 600 nm (OD₆₀₀) using a Biotek Synergy 2 plate reader with values converted to 1-cm-path-length OD₆₀₀ by prior calibration with a GeneQuant™ III 4283, version 4283V1.6. Synthetic cystic fibrosis sputum medium (SCFM) (Palmer et al. 2007) agar plates were made using 2X SCFM pre-warmed to 55°C and mixed in equal volumes with 3% agar cooled to 55°C for a final concentration of 1X SCFM and 1.5% agar.

Computational analysis of the phenylalanine catabolic pathway in CF pathogens

The organism-specific pathway maps for phenylalanine catabolism to PAA for *E. coli* K-12 MG1655 (Supplemental Fig. S1 and Supplemental File S1) and *P. putida* KT2440 (Supplemental Fig. S2 and Supplemental File S1) were retrieved from the KEGG pathway database. Gene annotations were examined by performing bidirectional BLASTP searches using the BLAST server tool from NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). All BLASTP searches used the non-redundant protein sequences database (nr) and an expect threshold of 10^{-8} . All other algorithm parameters used were the default settings.

Next, the genes in the organism-specific pathway map of *B. cenocepacia* J2315, *P. aeruginosa* PAO1, and *A. fumigatus* Af293 were analyzed using KEGG. For the steps with no gene product assigned, the amino acid sequences of the corresponding genes from *P. putida* KT2440 and *E. coli* K-12 MG1655 were used as queries for a bidirectional BLASTP search against either *B. cenocepacia* J2315, *P. aeruginosa* PAO1, or *A. fumigatus* Af293. Genes that were identified as hits by bidirectional BLASTP searches against either *P. putida* KT2440 or *E. coli* K-12 MG1655 were considered as putative coding genes for the corresponding enzymes.

Examination of bacterial supernatants for the release of PAA in LB media

Using the method previously described (Pribytkova et al. 2014), cultures were grown overnight on LB agar and a full loopful was inoculated into 100 mL of LB in 250 mL flasks. Flasks were incubated at 37°C with agitation at 230 rpm for 24 hours and cells were pelleted at 7,000 rpm for 10 minutes. Supernatants were filtered through a 0.2 µm vacuum filter (Sarstedt, Montréal, Québec) and stored at 4°C overnight before extraction.

Supernatants were acidified to a pH of ≤ 2 with hydrochloric acid (HCl) and extracted twice with 50 ml of ethyl acetate (100 ml total). The organic phases were combined and sodium sulphate was added in excess. The organic phase was filtered using Whatman No. 1 filter paper and evaporated under reduced pressure. The crude extract was dissolved in methanol to a concentration of approximately 2 mg/ml. HPLC vials were filled in duplicate with 1 ml of suspended crude extract and evaporated under reduced pressure. Two mg of crude extract was dissolved in either 1 ml of acidified methanol (1 ml of HCl in 50 ml of methanol), for esterification, or 1 ml of neutral methanol (no acid added) and heated for 15 minutes at 50°C. Samples were cooled and analyzed by HPLC using a Waters HPLC Separation Module 2695, combined with PDA Detector Model 2996 and a μ BondapakTM Waters C₁₈ (3.9 X 300mm) column particle diameter of 15-20 μ m, with 125 Å pores. Several gradients and solvents were tested for optimal peak separation of extracted supernatants. For LB media extracts the solvents chosen were methanol and 0.075% aqueous trifluoroacetic acid. The gradient was held at 20% methanol in 0.075% aqueous trifluoroacetic acid for 10 minutes, increased to 100% methanol over 10 minutes following a linear gradient and held there for 20 minutes. Methanol was decreased to 20% using a linear gradient over 10 minutes and then held for 10 minutes. The total run time was 60 minutes with a flow rate of 1 mL/minute. The eluent was monitored continuously at 210-600 nm and HPLC traces were displayed at 210.3 nm. Experiments were performed a minimum of three times.

Examination of bacterial supernatants for PAA in SCFM media

Cultures were grown overnight on LB agar and a full loopful was inoculated into 100 mL of SCFM in 250 mL flasks. As described for LB media, supernatants were collected and extracted after 24 hours of growth. Crude extracts were dissolved in methanol to a concentration of approximately 2 mg/ml and analyzed on the same HPLC and column. The solvents selected for SCFM extracted supernatants were acetonitrile and 0.075% aqueous trifluoroacetic acid. The gradient was held at 5% acetonitrile in 0.075% aqueous trifluoroacetic acid for 10 minutes, increased to 20% acetonitrile over 10 minutes following a linear gradient and held there for 20 minutes. This was decreased to 5% acetonitrile for 10 minutes using a linear gradient and held for 10 minutes. The total run time was 60 minutes with a flow rate of 1 mL/minute. The eluent was monitored continuously at 210-600 nm and HPLC traces were displayed at 210.3 nm. The experiments were performed in triplicate.

Examination of fungal supernatants for PAA

A fungal plug of *A. fumigatus* UAMH 2978 was inoculated into 100 ml of potato dextrose broth (PDB or SCFM) and grown at 37°C with aeration at 150 rpm for 5 days. Cultures were filtered through cheesecloth and the supernatant was acidified to a pH of ≤ 2 . Extractions and analysis were performed as described for bacterial supernatants in SCFM and the experiments were performed in duplicate.

Fungal and bacterial agar plate interaction assay

A. fumigatus UAMH 2978 was grown for 5 days at 37°C in a conidial flask of Potato Dextrose Agar (PDA). Spores were collected in isotonic saline + 0.005% Tween 20. Fungal spores were counted using a haemocytometer and diluted to a concentration of 10^5 spores/ml. Bacterial cultures were standardized to 10^7 cfu/ml using a McFarland standard and dilutions were plated on LB agar for confirmation. Based on the experiments performed using *Candida albicans* (Morales et al. 2013), 10 µl of *A. fumigatus* spore suspension was streaked onto the agar plate (PDA or SCFM) and 10 µl of bacterial culture was spotted 0.75 cm from the end of the streak (3 replicates per plate). Plates were incubated right side up at 37°C overnight and then incubated upside down. Plates were observed every 24 hours and photos were taken using a Leica DFC425 digital camera attached to a Leica M80 stereomicroscope integrated using Leica Application Suites version 3.7. Photos of SCFM plates were taken against a black background for contrast. Experiments were performed in triplicate.

Results and Discussion

Computational analysis of the phenylalanine catabolic pathway in CF pathogens

We examined the phenylalanine and PAA aerobic catabolism of *P. aeruginosa* PAO1, *B. cenocepacia* J2315 and *A. fumigatus* Af293 using the KEGG database. Although *A. fumigatus* has not been described as a PAA producer, another fungal species belonging to the *Aspergillaceae*, *Penicillium chrysogenum*, was able to produce and release PAA (Veiga et al. 2012). According to KEGG, each of the selected CF pathogen genomes was missing genes encoding the enzymes responsible for various steps of the pathway. The

metabolism of phenylalanine through PAA and subsequent degradation of PAA to acetyl-CoA and succinyl-CoA of two model organisms, *P. putida* and *E. coli*, has been thoroughly studied (Ferrandez et al. 1998; Grishin et al. 2011; Ismail et al. 2003; Jimenez et al. 2002; Nogales et al. 2007; Teufel et al. 2010; Teufel et al. 2012). Therefore, for the steps with no gene product assigned, we used the genes from these model organisms to perform bidirectional BLASTP searches against the genomes of the selected CF pathogens.

Bidirectional BLASTP searches of *P. putida* KT2440 and *E. coli* K-12 MG1655 against each other were performed to confirm the completeness of the pathways in these microorganisms (Supplemental File S1). By performing this analysis, we were able to identify PP_3280 of *P. putida* KT2440 as a homolog to the *E. coli* K-12 MG1655 *paaJ* (b1397) gene (Supplemental File S1 and Supplemental Fig. S2, steps 15 and 18). PP_3280 was not indicated as a putative *paaJ* gene in KEGG, but is annotated as a phenylalanine degradation gene, *phaD*, from another study (Jimenez et al. 2002). We then used the amino acid sequences of the genes from these strains in bidirectional BLASTP searches against the genomes of the selected CF pathogens (Supplemental File S2).

B. cenocepacia J2315. The KEGG organism-specific pathway map of *B. cenocepacia* J2315 contains genes encoding enzymes that theoretically enable degradation of phenylalanine and the production of PAA. However, according to KEGG, the *B. cenocepacia* J2315 genome lacks some genes encoding enzymes for PAA degradation, such as PaaZ, which hydrolyzes the ring of 2-oxepin-2(3H)-ylideneacetyl-CoA and

subsequently oxidizes the aldehyde group to a carboxyl group (Fig. 1 and Supplemental Fig. S1 steps 13 and 14, respectively) and PaaJ, a 3-oxoadipyl-CoA/3-oxo-5,6-dehydrosuberyl-CoA thiolase (Fig. 1 and Supplemental Fig. S1, steps 15 and 18) (Teufel et al. 2010).

Although missing from KEGG annotations, BCAL0408 is annotated as *paaZ* in *B. cenocepacia* J2315 (Law et al. 2008). However, our bidirectional BLASTP search identified BCAS0364 as the best hit to b1387 (*paaZ*) from *E. coli* K-12 MG1655 (Fig. 1 and Supplemental Fig. S1, steps 13 and 14). This discrepancy may be explained by the fact that PaaZ in *E. coli* is a bifunctional enzyme with an N-terminal aldehyde dehydrogenase domain and a C-terminal hydratase domain. In many other bacteria the gene present in the cluster of *paa* genes comprises only an aldehyde dehydrogenase domain and the gene containing the hydratase domain is found elsewhere in the genome (Grishin and Cygler 2015; Teufel et al. 2011). Indeed, BCAL0408 and BCAS0364 code for an aldehyde dehydrogenase and a hydratase, respectively. Thus, it is likely that BCAS0364 and BCAL0408 are both responsible for fulfilling the functions of the *E. coli* PaaZ.

We also identified BCAM2568 as a homolog of b1397, *paaJ* of *E. coli* K-12 MG1655 (Fig. 1 and Supplemental Fig. S1, step 15 and 18), and BCAL0407 as a homolog of PP_3280, the putatively identified *paaJ* gene of *P. putida* KT2440 by our bi-directional BLASTP search. This corroborates our previous annotation where BCAM2568 and BCAL0407 were both identified as candidate *paaJ* genes (Law et al. 2008).

P. aeruginosa PAO1. According to the KEGG organism-specific pathway map, *P. aeruginosa* PAO1 lacks many coding genes for the conversion of phenylalanine to phenylacetic acid (Fig. 1). The KEGG organism-specific pathway map also shows that the PAA catabolic pathway lacks a *paaK* homolog for the conversion of PAA to PAA-CoA (Fig. 1 and Supplemental Fig. S1, step 10). Also the monooxygenase complex, *paaABCDE*, for the epoxidation of phenylacetyl-CoA to 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl-CoA (Fig. 1 and Supplemental Fig. S1, step 11) is not present, nor is *paaZ* (Fig. 1 and Supplemental Fig. S1, step 13 and 14) or *paaJ* (Fig. 1 and Supplemental Fig. S1, step 18).

The bidirectional BLASTP search for missing genes in *P. aeruginosa* PAO1 identified no alternative candidates for the conversion of phenylalanine to PAA, indicating that *P. aeruginosa* PAO1 should not be able to produce PAA from phenylalanine. For the catabolism of PAA, no *paaK* homolog (Fig. 1 and Supplemental Fig. S1, step 10) was identified although the gene encoding PaaI (Fig. 1 and Supplemental Fig. S1, step 9), which catalyzes the removal of CoA from PAA, is present according to KEGG. PA4889 was identified as a homolog of b1392 (PaaE) from *E. coli* (Fig. 1 and Supplemental Fig. S1, step 11), but no other genes from the monooxygenase complex were identified. PA3302 was identified in *P. aeruginosa* PAO1 as the best match for PaaZ from *E. coli* K-12 MG1655 (b1387) (Fig. 1, steps 13 and 14). PA3302 is a hypothetical protein with a hotdog-fold hydratase domain, but does not contain the aldehyde dehydrogenase domain, unlike the bifunctional *E. coli* PaaZ. Our bidirectional BLASTP search did not find any genes with the aldehyde dehydrogenase domains similar to that of the PaaZ from *P. putida* KT2440 and *E. coli* K-12 MG1655. Unlike the genes for the degradation of PAA,

which are mostly found clustered together in many bacterial genomes (Luengo et al. 2001), the putative genes in *P. aeruginosa* PAO1 do not seem to be in a cluster, therefore it is more difficult to identify a potential aldehyde dehydrogenase. Also identified by bidirectional BLASTP searches were PA0228 and PA3589, which are homologs of the *E. coli* K-12 MG1655 and *P. putida* KT2440 *paaJ* genes, b1397 and PP_3280, respectively. However, our analysis indicates that *P. aeruginosa* PAO1 does not have a complete pathway (Fig. 1) and would not be a good candidate for the production and degradation of PAA through this aerobic pathway.

A. fumigatus Af293. The KEGG organism-specific pathway map of *A. fumigatus* Af293 shows that phenylalanine can be converted by catalase-peroxidase to 2-phenylacetamide and then converted to PAA by an amidase (Fig. 1, steps 3 and 6). In *A. fumigatus* Af293, the aromatic-L-amino-acid decarboxylase is also present to catalyze the reaction of phenylalanine to phenylethylamine, which is then converted to phenylacetaldehyde by a monoamine oxidase (Fig. 1, steps 2 and 5). Lastly, the phenylacetaldehyde is converted to PAA by an aldehyde dehydrogenase (Fig. 1, step 8). However, for the degradation of PAA the only gene present, as identified by KEGG, is a homolog of the *paaH* gene responsible for the oxidation of 3-hydroxyadipyl-CoA to 3-oxyadipyl-CoA (Fig. 1 and Supplemental Fig. S1, step 17).

The bidirectional BLASTP search identified AFUA_8G02310 and AFUA_8G05520 as a homolog to the *P. putida* KT2440 gene and *E. coli* K-12 MG1655 genes, respectively, for the conversion of phenylacetaldehyde to PAA by a phenylacetaldehyde dehydrogenase (Fig. 1 and Supplemental Fig. S1, step 7), putatively providing a second method for *A.*

fumigatus Af293 to convert phenylacetaldehyde to PAA. No PAA-CoA ligase gene (Fig. 1 and Supplemental Fig. S1, step 10), *paaK*, was identified and the gene for the thioesterase (Fig. 1 and Supplemental Fig. S1, step 9), *paaI*, was absent as well. Without the PAA-CoA, further degradation of PAA by this pathway is unlikely, as it requires CoA-thioester intermediates. Similarly to *P. aeruginosa* PAO1, we identified a homolog of PaaE in *A. fumigatus* Af293, AFUA_5G10060, but no other genes of the monooxygenase complex (Fig. 1 and Supplemental Fig. S1, step 11) were identified. Homologs to PaaG (AFUA_6G04040) and PP_3726 (AFUA_1G01890) were identified by bidirectional BLASTP search (Fig. 1 and Supplemental Fig. S1, steps 12 and 15). PaaF (AFUA_2G10920 and AFUA_3G14520) (Fig. 1 and Supplemental Fig. S1, step 16) and PaaJ (AFUA_2G11350) (Fig. 1 and Supplemental Fig. S1, steps 15 and 18) homologs were also discovered by a bidirectional BLASTP search. However, no gene homologous to PaaZ was identified for *A. fumigatus* Af293. The absence of a *paaZ* gene means the ring of 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl-CoA cannot be cleaved leaving the pathway incomplete. As summarized in Fig. 1, the pathway for the degradation of PAA in *A. fumigatus* Af293 is incomplete. Nevertheless, the inability to degrade PAA could lead to the production of PAA by *A. fumigatus* Af293, which has the genes necessary to convert phenylalanine to PAA.

The release of PAA by the selected CF pathogens

Based on our bioinformatics analysis, we determined that *B. cenocepacia* K56-2 and *A. fumigatus* Af293 may be able to produce PAA. Our bidirectional BLASTP search did not identify the genes necessary to produce PAA through this pathway in *P. aeruginosa*

PAO1. However, we cannot rule out that other non-homologous enzymes could be involved in the production of PAA, because PAA was previously detected in *P. aeruginosa* PAO1 supernatants (Wang et al. 2013). To experimentally test the production of PAA by these CF pathogens, we tested spent media of *B. cenocepacia* K56-2, a clonal isolate of *B. cenocepacia* J2315, *A. fumigatus* UAMH 2978, a clinical isolate from a non-CF sputum sample, and *P. aeruginosa* PAO1 for any detectable PAA released by the organisms. Fig. 2A shows the HPLC chromatograms of bacterial extracted supernatants from LB media. The extracted LB media control had several peaks present in the chromatogram, especially from 16-22 minutes. As a positive control, 0.2 mM of PAA was added to LB media, extracted and esterified in the same manner as the rest of the samples. PAA had a large peak at a retention time of 18.3 minutes. In the extracted supernatant of *P. aeruginosa* PAO1, the peaks were examined for a similar retention time and UV trace as PAA, however no PAA was identified. A peak with a retention time of 18.3 minutes and the same UV trace as PAA was released in *B. cenocepacia* K56-2 when grown in LB.

The HPLC chromatograms of extracted *A. fumigatus* UAMH 2978 supernatants grown in PDB are shown in Fig. 2B. The extracted PDB media control had several small peaks between 2-6 minutes and a little peak at 10 minutes and then at 18 minutes. In the positive control, 0.2 mM of PAA was added to PDB media and then extracted. The peak of PAA was visible at 12.5 minutes. The *A. fumigatus* sample had a peak at 22 minutes and a peak at 24 minutes, but no peak was present with the same retention time and UV trace as PAA.

To investigate the CF pathogens for the production of PAA in media designed to reproduce nutritional conditions of the CF sputum, the supernatants of cultures grown in synthetic cystic fibrosis sputum media (SCFM) (Palmer et al. 2007) were extracted and analyzed (Fig. 3). The extracted SCFM media control had very few peaks and they were found between 2-4 minutes. There were no peaks near the expected retention time of PAA, which was at 12.2 minutes. The extracted supernatants of *A. fumigatus* and *B. cenocepacia* have some very small peaks around the expected retention time of PAA, but they were all examined and did not have a similar UV trace to PAA. *P. aeruginosa* had some slight peaks when zoomed in (data not shown), but none of the peaks matched the retention time or UV trace of PAA. Under the conditions tested we did not find evidence of PAA released by the wild type strains in SCFM. We also tested *B. cenocepacia* K56-2 $\Delta paaABCDE$, a mutant that is incapable of degrading PAA, which results in the accumulation and release of PAA in common lab media (Pribytkova et al. 2014). *B. cenocepacia* $\Delta paaABCDE$ produced a large peak with a retention time of 12.0 minutes and a UV trace that matched PAA.

B. cenocepacia released PAA in LB, but no PAA was produced in the CF nutritional conditions of SCFM. The lack of PAA production by *A. fumigatus* despite the presence of a pathway that putatively should convert phenylalanine to PAA, may indicate that phenylalanine degradation is performed by the homogentisate pathway (Fernandez-Canon and Penalva 1995). Conversely, PAA may be produced from phenylalanine, but used as an intermediate of secondary metabolism as in other fungal strains (Veiga et al. 2012). Regarding the absence of PAA in *P. aeruginosa* spent medium, our experimental results were consistent with our *in silico* analysis, but are in disagreement with those of

Wang *et al.* where they found production of PAA from *P. aeruginosa* PAO1 at high cell densities (Wang *et al.* 2013). Several efforts for reproducing the results of Wang *et al.* were attempted by collecting supernatants from *P. aeruginosa* PAO1 at different cell densities with no success in identifying PAA (data not shown). This discrepancy may be explained by different laboratory stocks of *P. aeruginosa* PAO1. *P. aeruginosa* PAO1 is commonly used as a reference strain, but genotypical and phenotypical differences have emerged amongst the sublines maintained worldwide (Klockgether *et al.* 2010). Also possible, is that limitations of our detection method or slight variations of the experimental conditions may have caused the discrepancy with the previous study. We consider these alternative explanations less likely as we made an effort to reproduce the same growing conditions as in Wang *et al.* (2013) and our method was able to detect 0.025 mM of PAA added to LB before extraction (Supplemental Fig. S3). Wang *et al.* (2013) purified ~20 μ M of PAA and predicted levels in bacterial supernatants are approximately 74-184 μ M, therefore our method is sufficient to detect the levels of PAA present. Future work may elucidate the reasons of this disagreement.

Examining fungal-bacterial interactions of CF pathogens and the role of the PAA pathway in these interactions

Analysis of soil samples revealed the co-occurrence of multiple fungi taxa with *Burkholderia*, and further investigation revealed that *Burkholderia* is able to attach to fungi and translocate using their hyphae (Stopnisek *et al.* 2016). It is known that Bcc, *P. aeruginosa* and *A. fumigatus* co-exist in the CF lung, but how they may interact with each other is unknown. The microbial interactions between *B. cenocepacia* and *P. aeruginosa*

are complex. While *B. cenocepacia* and *P. aeruginosa* have been isolated together from the CF sputum (Jacques et al. 1998), *P. aeruginosa* usually kills *B. cenocepacia* in laboratory conditions (Tomlin et al. 2001) by synthesizing cyanide (Bernier et al. 2016). In direct contact with *A. fumigatus*, *P. aeruginosa* releases small diffusible extracellular molecules (Mowat et al. 2010), such as phenazines (Briard et al. 2015), that inhibit *A. fumigatus*. Although members of the Bcc can also inhibit *A. fumigatus* (Kerr 1994), it is not known which species are inhibitory, what molecules are responsible for this inhibition or the influence of the environmental conditions on this relationship. In this study, we decided to focus on these bacterial-fungal interactions and the involvement of phenylalanine and PAA metabolism of *B. cenocepacia* in CF nutritional conditions. Whereas PAA production inhibited protease activity, increased siderophore production, downregulated production of nematicidal protein AidA, and decreased swarming in *B. cenocepacia* (Pribytkova et al. 2014), the effect on QS-regulated antifungal activity is not known.

Bacteria were spotted on an agar plate with fungal spores streaked next to them. The interface between the fungi and bacteria was then examined for any potential morphological changes and growth inhibition. After 48 hours of growth in common lab media (PDA) there were no observable morphological or growth changes between the bacteria grown as monocultures (Fig. 4, top panels) or in the presence of the fungus (Fig. 4, lower panels). This observation is suggestive of lack of antibacterial activity by *A. fumigatus*. When *B. cenocepacia* and *A. fumigatus* were cultured on the same plate, a slight zone of inhibition was observed. This zone of inhibition was more evident when *P. aeruginosa* PAO1 was spotted with *A. fumigatus*.

These results are in accordance with previous demonstrations of antifungal activity of *P. aeruginosa* and members of the Bcc against *A. fumigatus* (Kerr 1994). We chose *E. coli* OP50 as a negative control due to findings that another strain of *E. coli*, NCTC 10418, does not inhibit *A. fumigatus* (Kerr 1994). As expected, *E. coli* was unable to inhibit the growth of *A. fumigatus*.

After examining the interactions of these strains in common lab media, we used a CF sputum-mimicking medium, SCFM, to repeat the agar plate interaction method. As observed in PDA, there were no morphological or growth changes between the bacterial cultures grown alone (Fig. 5, top panels) compared to those grown in the presence of the fungus (Fig. 5, lower panels). The zone of inhibition previously seen between *B. cenocepacia* and *A. fumigatus* was absent in SCFM and *A. fumigatus* grew onto the edge of the *B. cenocepacia* spot. The interaction between *B. cenocepacia* Δ paaABCDE, which produced a large amount of PAA in SCFM (Fig. 3), and *A. fumigatus* did not appear any different than that between wild type *B. cenocepacia*, which did not produce a detectable amount of PAA in SCFM, and *A. fumigatus*. The antifungal activity of *P. aeruginosa* was also less noticeable than on PDA, although there was a slight zone of inhibition preventing *A. fumigatus* from contacting the *P. aeruginosa* spot.

It should be noted that *A. fumigatus* seemed to have thinner growth on SCFM than on PDA. Despite less growth of *A. fumigatus* on SCFM, the antifungal activity, evidenced as a halo of inhibition at the interface between the microbes, was lessened compared to PDA. This suggests that the negative interaction between these bacteria and the fungus is lessened in CF sputum nutritional conditions, likely due a decrease of the antifungal activities by the bacteria.

The production of pyrrolnitrin, an antifungal compound, is regulated by the CepIR QS system in members of the Bcc (Schmidt et al. 2009). Due to the inhibition of the CepIR QS system by PAA in *B. cenocepacia* (Pribytkova et al. 2014), we expected to see a difference in the interactions between wild type and mutant *B. cenocepacia* K56-2 and *A. fumigatus*. However, our results suggest that the diminished antifungal activity of *B. cenocepacia* in SCFM is independent of the PAA metabolic pathway in our experimental conditions, indicating that another non-QS-regulated antifungal compound may be responsible for the interaction.

Conclusion

We identified the presence of genes in *B. cenocepacia* J2315 and *A. fumigatus* Af293 necessary for the production of PAA through phenylalanine, but the pathway was not complete in *P. aeruginosa* PAO1. *B. cenocepacia* J2315 also has the genes for the degradation of PAA. Confirming our *in silico* findings, we demonstrated the release of PAA by *B. cenocepacia* K56-2 in LB and confirmed the absence of production of PAA by *P. aeruginosa* PAO1 under these conditions. Contrary to what the *in silico* analysis suggested, *A. fumigatus* UAMH 2978 did not release PAA in LB or PDA. We were unable to detect PAA release from any of the wild type strains tested in the CF nutritional conditions of SCFM. The antagonistic interactions between *P. aeruginosa* PAO1/*A. fumigatus* UAMH 2978 and *B. cenocepacia* K56-2/*A. fumigatus* UAMH 2978 were lessened in CF conditions when compared to common laboratory media. Using a *B. cenocepacia* K56-2 Δ *paaABCDE* mutant, deficient in the PAA catabolic pathway, we were able to demonstrate that PAA catabolic pathway dependent metabolites do not play

a role in the antifungal activity of *B. cenocepacia* against *A. fumigatus* under the conditions tested.

Acknowledgements

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Table 1. Bacterial and fungal strains used in this study.

Strains	Features	Reference or Source
<i>Burkholderia cenocepacia</i> K56-2 (LMG18863)	WT strain, ET12 clone related to a cystic fibrosis clinical isolate, J2315	(Mahenthiralingam et al. 2000)
$\Delta paaABCDE$	Deletion of <i>paaABCDE</i> operon in K56-2	(Pribytkova et al. 2014)
<i>Escherichia coli</i> OP50	Uracil auxotroph	<i>Caenorhabditis</i> Genetic Center (CGC, University of Minnesota, Minneapolis, USA)
<i>Pseudomonas aeruginosa</i> PAO1	Clinical isolate	Courtesy of Dr. Teresa deKievit, University of Manitoba, Canada
<i>Aspergillus fumigatus</i> UAMH 2978	Sputum ex adult, thermotolerant	University of Alberta Microfungus Collection and Herbarium (UAMH, Alberta, Canada)

Figure legends

Fig. 1. Putative phenylalanine (Phe) catabolic pathways of selected CF pathogens.

Using the KEGG database and bidirectional NCBI BLASTP searches, the phenylalanine catabolic pathway was determined for three CF pathogens. Numbered arrows correspond to those shown in Supplemental Fig. S1 and represent the steps in the pathway, which are catalyzed by enzymes encoded by one or more genes. Green arrows indicate enzymatic steps where the presence of the gene(s) has been identified by KEGG with the locus tags in green boxes. Red arrows are for steps with gene(s) absent as indicated by KEGG and bidirectional BLASTP search (this study). Blue arrows represent steps where the gene(s) were putatively identified by bidirectional BLASTP search in this study (locus tags in blue boxes). An asterisk marks steps where although a gene is present, the step cannot be completed due to missing genes.

Fig. 2. HPLC analysis of culture extracts grown in common lab media. A) Esterified ethyl acetate extracts from LB media were compared. The top trace (PAA) is the extract of 0.2 mM PAA in LB with the UV trace of PAA (inset) and the bottom trace (LB) is the extract of the media control. *B. cenocepacia* K56-2 (Bce) had a peak at 18.3 minutes with a UV trace (inset) that resembles that of PAA. *P. aeruginosa* PAO1 (Pae) was also tested and no PAA was detected. B) Ethyl acetate extracts from potato dextrose broth (PDB) were examined. The top trace (PAA) was the extract of 0.2 mM PAA in PDB and the bottom trace was the extract of the media (PDB). The extract of *A. fumigatus* UAMH2978 (Afu) had no peaks with a retention time similar to PAA. Chromatograms are from one replicate, but are representative of at least two biological replicates.

Fig. 3. HPLC analysis of culture extracts grown in SCFM. The HPLC traces at 210.3 nm were examined for the presence of PAA. The top trace (PAA) is the ethyl acetate extract of 0.2 mM PAA in SCFM and the bottom trace is the ethyl acetate extract from the media alone. The peak of PAA had a retention time of 12.2 minutes and the inset is the UV spectra recorded for this signal. *B. cenocepacia* K56-2 $\Delta paaABCDE$ (Bce Δpaa) is the only trace where a similar peak was found, with a retention time of 12.0 minutes and an identical UV spectra. All experiments were performed at least two times.

Fig. 4. Fungal interaction assay on potato dextrose agar. Bce: *B. cenocepacia* K56-2; Eco: *E. coli* OP50; Pae: *P. aeruginosa* PAO1; Afu: *A. fumigatus* UAMH 2978. The top row is the colony morphology of monocultures after 48 h. The bottom row is the colony morphology of each bacterial strain when grown next to *A. fumigatus* UAMH 2978 for 48 h. *B. cenocepacia* K56-2 slightly inhibited the growth of *A. fumigatus* UAMH 2978. *E. coli* OP50 was unable to inhibit *A. fumigatus* and *P. aeruginosa* PAO1 had a clear zone of inhibition. Images were obtained using a stereomicroscope (2x) and the experiment was repeated three times.

Fig. 5. Fungal interaction assay on SCFM. Bce: *B. cenocepacia* K56-2; Bce Δ paa: *B. cenocepacia* K56-2 Δ paaABCDE; Eco: *E. coli* OP50; Pae: *P. aeruginosa* PAO1; Afu: *A. fumigatus* UAMH 2978. The top row is the colony morphology of monocultures after 48 h. The bottom row is the colony morphology of each bacterial strain when grown next to *A. fumigatus* UAMH 2978 for 48 h. Both *B. cenocepacia* K56-2 wild type and mutant Δ paaABCDE had similar interactions with *A. fumigatus* UAMH 2978 as they were unable to inhibit the growth of *A. fumigatus* UAMH 2978 on SCFM. *E. coli* OP50 was unable to inhibit *A. fumigatus* UAMH 2978. *P. aeruginosa* PAO1 inhibited *A. fumigatus* UAMH 2978 slightly. Experiments were performed in triplicate and images were obtained using a stereomicroscope (2x).

Fig. 1. Putative phenylalanine (Phe) catabolic pathways of selected CF pathogens. Using the KEGG database and bidirectional NCBI BLASTP searches, the phenylalanine catabolic pathway was determined for three CF pathogens. Numbered arrows correspond to those shown in Supplemental Fig. S1 and represent the steps in the pathway, which are catalyzed by enzymes encoded by one or more genes. Green arrows indicate enzymatic steps where the presence of the gene(s) has been identified by KEGG with the locus tags in green boxes. Red arrows are for steps with gene(s) absent as indicated by KEGG and bidirectional BLASTP search (this study). Blue arrows represent steps where the gene(s) were putatively identified by bidirectional BLASTP search in this study (locus tags in blue boxes). An asterisk marks steps where although a gene is present, the step cannot be completed due to missing genes.

Fig. 1

199x217mm (300 x 300 DPI)

Fig. 2. HPLC analysis of culture extracts grown in common lab media. A) Esterified ethyl acetate extracts from LB media were compared. The top trace (PAA) is the extract of 0.2 mM PAA in LB with the UV trace of PAA (inset) and the bottom trace (LB) is the extract of the media control. *B. cenocepacia* K56-2 (Bce) had a peak at 18.3 minutes with a UV trace (inset) that resembles that of PAA. *P. aeruginosa* PAO1 (Pae) was also tested and no PAA was detected. B) Ethyl acetate extracts from potato dextrose broth (PDB) were examined. The top trace (PAA) was the extract of 0.2 mM PAA in PDB and the bottom trace was the extract of the media (PDB). The extract of *A. fumigatus* UAMH2978 (Afu) had no peaks with a retention time similar to PAA. Chromatograms are from one replicate, but are representative of at least two biological replicates.

Fig. 2

95x51mm (300 x 300 DPI)

Fig. 3. HPLC analysis of culture extracts grown in SCFM. The HPLC traces at 210.3 nm were examined for the presence of PAA. The top trace (PAA) is the ethyl acetate extract of 0.2 mM PAA in SCFM and the bottom trace is the ethyl acetate extract from the media alone. The peak of PAA had a retention time of 12.2 minutes and the inset is the UV spectra recorded for this signal. *B. cenocepacia* K56-2 $\Delta paaABCDE$ (BceΔpaa) is the only trace where a similar peak was found, with a retention time of 12.0 minutes and an identical UV spectra. All experiments were performed at least two times.

Fig. 3

172x200mm (300 x 300 DPI)

Fig. 4. Fungal interaction assay on potato dextrose agar. Bce: *B. cenocepacia* K56-2; Eco: *E. coli* OP50; Pae: *P. aeruginosa* PAO1; Afu: *A. fumigatus* UAMH 2978. The top row is the colony morphology of monocultures after 48 h. The bottom row is the colony morphology of each bacterial strain when grown next to *A. fumigatus* UAMH 2978 for 48 h. *B. cenocepacia* K56-2 slightly inhibited the growth of *A. fumigatus* UAMH 2978. *E. coli* OP50 was unable to inhibit *A. fumigatus* and *P. aeruginosa* PAO1 had a clear zone of inhibition. Images were obtained using a stereomicroscope (2x) and the experiment was repeated three

times.
 Fig. 4
 86x43mm (300 x 300 DPI)

Fig. 5. Fungal interaction assay on SCFM. Bce: *B. cenocepacia* K56-2; Bce Δ paa: *B. cenocepacia* K56-2 Δ paaABCDE; Eco: *E. coli* OP50; Pae: *P. aeruginosa* PAO1; Afu: *A. fumigatus* UAMH 2978. The top row is the colony morphology of monocultures after 48 h. The bottom row is the colony morphology of each bacterial strain when grown next to *A. fumigatus* UAMH 2978 for 48 h. Both *B. cenocepacia* K56-2 wild type and mutant Δ paaABCDE had similar interactions with *A. fumigatus* UAMH 2978 as they were unable to inhibit the growth of *A. fumigatus* UAMH 2978 on SCFM. *E. coli* OP50 was unable to inhibit the growth of *A. fumigatus* UAMH 2978. *P. aeruginosa* PAO1 inhibited *A. fumigatus* UAMH 2978 slightly. Experiments were performed in triplicate and images were obtained using a stereomicroscope (2x).

Fig. 5

78x35mm (300 x 300 DPI)

Supplemental File 1: Bi-directional BLASTP from *P. putida* KT2440 to *E. coli* K-12 MG1

Overview:

Each tab contains the results from a BLASTP search

Tab #	Tab Title	Contents
Tab 1	About File	Overview of Supplemental File 1
Tab 2	KT2440 to K12	Contains the results from a BLASTP search using <i>P. putida</i> KT2440 genes from the BLASTP search against <i>E. coli</i> K-12 MG1655 genes
Tab 3	K12 back to KT2440	Summary of the locus tag of the initial query gene (<i>P. putida</i> KT2440)
Tab 4	Bidirectional KT2440 to K12	Contains the results from a BLASTP search using <i>E. coli</i> K-12 MG1655 genes from the BLASTP search against <i>P. putida</i> KT2440 genes
Tab 5	K12 to KT2440	Summary of the locus tag of the initial query gene (<i>E. coli</i> K-12 MG1655)
Tab 6	KT2440 back to K12	Contains the results from a BLASTP search using <i>P. putida</i> KT2440 genes from the BLASTP search against <i>E. coli</i> K-12 MG1655 genes
Tab 7	Bidirectional K12 to KT2440	Summary of the locus tag of the initial query gene (<i>E. coli</i> K-12 MG1655)

1655 and vice-versa

e *P. putida* to *E. coli*)

ida KT2440 genes as queries against the *E. coli* K-12 MG1655 genome
 ainst the *E. coli* K-12 MG1655 genome were used in a BLASTP search against the *P. putida* KT2440 genome (the resulting hit from the *E. coli* K-12 MG1655 genome and the resulting hit of the *P. putida* KT2440 genome), the resulting hit from the *E. coli* K-12 MG1655 genome and the resulting hit of the *P. putida* KT2440 genome
 K12-MG1655 genes as queries against the *P. putida* KT2440 genome
 against the *P. putida* KT2440 genome were used in a BLASTP search against the *E. coli* K-12 MG1655 genome (the resulting hit from the *P. putida* KT2440 genome and the resulting hit of the *E. coli* K-12 MG1655 genome), the resulting hit from the *P. putida* KT2440 genome and the resulting hit of the *E. coli* K-12 MG1655 genome

Draft

40 genome

the *E. coli* K-12 MG1655 gene against the *P. putida* KT2440 genome

1655 genome

the *P. putida* KT2440 gene against the *E. coli* K-12 MG1655 genome

Pseudomonas putida KT2440 protein sequences from pseudomonas.com BLAST against *E. coli* M

Query		Result
<i>P. putida</i> gene name	Locus Tag	Description
	PP_3668	MULTISPECIES: catalase peroxidase [Proteobacteria]
	PP_2932	No Significant Similarity Found
		MULTISPECIES: ABC transporter ATPase
	PP_2552	[Proteobacteria]
		MULTISPECIES: N-methyl-L-tryptophan oxidase
	PP_4983	[Proteobacteria]
		MULTISPECIES: NAD-dependent phenylacetaldehyde
	PP_3463	dehydrogenase [Escherichia]
hisC	PP_0967	No Significant Similarity Found
		MULTISPECIES: aromatic amino acid aminotransferase
tyrB-1	PP_1972	[Proteobacteria]
		MULTISPECIES: aromatic amino acid aminotransferase
tyrB-2	PP_3590	[Proteobacteria]
		MULTISPECIES: phenylacetate--CoA ligase
phaE	PP_3279	[Enterobacteriaceae]
		MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase
phaI	PP_3274	subunit E [Enterobacteriaceae]
		MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase
phaH	PP_3275	[Enterobacteriaceae]
		MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase
	PP_3276	subunit C [Enterobacteriaceae]
		MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase
paaB	PP_3277	subunit B [Enterobacteriaceae]
		MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase
paaA	PP_3278	subunit A [Enterobacteriaceae]*
		MULTISPECIES: 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl-
phaB	PP_3283	CoA isomerase [Enterobacteriaceae]
		MULTISPECIES: 2,3-dehydroadipyl-CoA hydratase
	PP_3726	[Enterobacteriaceae]
		MULTISPECIES: phenylacetic acid degradation
phaL	PP_3270	bifunctional protein PaaZ [Escherichia]
	PP_1845	No Significant Similarity Found
		MULTISPECIES: 2,3-dehydroadipyl-CoA hydratase
fadB1x	PP_2217	[Enterobacteriaceae]
		MULTISPECIES: 2,3-dehydroadipyl-CoA hydratase
	PP_3284	[Enterobacteriaceae]

	PP_4030	MULTISPECIES: carnitinyI-CoA dehydratase [Proteobacteria]
paaC	PP_3282	MULTISPECIES: 3-hydroxyadipyl-CoA dehydrogenase [Enterobacteriaceae]
paaH	PP_3755	MULTISPECIES: 3-hydroxyadipyl-CoA dehydrogenase [Enterobacteriaceae]
	PP_0244	MULTISPECIES: acyl-CoA thioesterase [Enterobacteriaceae]
	PP_3281	MULTISPECIES: acyl-CoA thioesterase [Enterobacteriaceae]

*first hit was protein with ligand, used 2nd hit

Draft

G1655 (taxid:511145)

Max Score	Total Score	Query cover	E value	Ident	Accession	<i>E. coli</i> Locus
885	885	99%		0	63% WP_001295695.1	b3942
26.2	26.2	9%	5.4	37%	WP_001300558.1	b1756
30.4	30.4	4%	0.45	52%	WP_000872833.1	b1059
419	419	98%	8.00E-144	43%	WP_000138615.1	b1385
418	418	99%	3.00E-146	51%	WP_000486985.1	b4054
590	590	99%	0.00E+00	71%	WP_000486985.1	b4054
614	614	96%	0	68%	WP_000632280.1	b1398
350	350	99%	1.00E-120	49%	WP_000206388.1	b1392
176	176	88%	7.00E-58	54%	WP_001189201.1	b1391
258	258	98%	2.00E-91	54%	WP_001072837.1	b1390
125	125	96%	5.00E-40	61%	WP_000073393.1	b1389
456	456	94%	1.00E-163	68%	WP_000191077.1	b1388
308	308	99%	4.00E-107	62%	WP_000969784.1	b1394
142	142	98%	2.00E-42	36%	WP_001292353.1	b1393
732	732	98%	0	59%	WP_001186469.1	b1387
200	200	95%	7.00E-65	43%	WP_001292353.1	b1393
267	267	96%	3.00E-91	56%	WP_001292353.1	b1393

128	128	91%	2.00E-36	34%	WP_001295419.1	b0036
410	410	96%	2.00E-140	48%	WP_000973360.1	b1395
219	303	100%	3.00E-69	41%	WP_000973360.1	b1395
61.2	61.2	91%	8.00E-14	36%	WP_000018413.1	b1396
124	124	89%	5.00E-38	47%	WP_000018413.1	b1396

Draft

GenBank Accession *E. coli* Gene Name

AAC76924.1 katG

AAC74826.1 ynjD

AAC74143.1 solA

AAC74467.2 feaB

AAC77024.1 tyrB

AAC77024.1 tyrB

AAC74480.1 paaK

AAC74474.1 paaE

AAC74473.4 paaD

AAC74472.1 paaC

AAC74471.1 paaB

AAC74470.1 paaA

AAC74476.1 paaG

AAC74475.1 paaF

AAC74469.1 paaZ

AAC74475.1 paaF

AAC74475.1 paaF

AAC73147.2	caiD
AAC74477.1	paaH
AAC74477.1	paaH
AAC74478.1	paal
AAC74478.1	paal

Draft

Reverse Blast of *E. coli* K-12 gene results from
Query

<i>E. coli</i> gene name	<i>E. coli</i> Locus	<i>P. putida</i> Locus Initial Query
katG	b3942	PP_3668 PP_2932
ynjD	b1756	PP_2552
solA	b1059	PP_4983
feaB	b1385	PP_3463 PP_0967
tyrB	b4054	PP_1972
tyrB	b4054	PP_3590
paaK	b1398	PP_3279
paaE	b1392	PP_3274
paaD	b1391	PP_3275
paaC	b1390	PP_3276
paaB	b1389	PP_3277
paaA	b1388	PP_3278
paaG	b1394	PP_3283
paaF	b1393	PP_3726
paaZ	b1387	PP_3270 PP_1845
paaF	b1393	PP_2217
paaF	b1393	PP_3284
caiD	b0036	PP_4030
paaH	b1395	PP_3282

paaH	b1395	PP_3755
paal	b1396	PP_0244
paal	b1396	PP_3281

Draft

n *P. putida* KT2440 to *E. coli* K12 against *P. putida* KT2440 (taxid:160488)

Result

Description	Max Score	Total Score	Query cover
catalase peroxidase [<i>Pseudomonas putida</i>]	888	888	97%
MULTISPECIES: ABC transporter ATP-binding protein [<i>Pseudomonas</i>]	113	113	98%
MULTISPECIES: sarcosine oxidase [<i>Pseudomonas</i>]	112	112	92%
MULTISPECIES: aldehyde dehydrogenase [<i>Pseudomonas</i>]	415	415	98%
MULTISPECIES: aromatic amino acid aminotransferase [<i>Pseudomonas</i>]	590	590	99%
MULTISPECIES: aromatic amino acid aminotransferase [<i>Pseudomonas</i>]	590	590	99%
phenylacetate-CoA ligase [<i>Pseudomonas putida</i>]	614	614	97%
MULTISPECIES: phenylacetic acid degradation protein [<i>Pseudomonas</i>]	345	345	98%
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaJ [<i>Pseudomonas</i>]	176	176	93%
MULTISPECIES: phenylacetic acid degradation protein [<i>Pseudomonas</i>]	258	258	98%
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaB [<i>Gammaproteobacteria</i>]	125	125	94%
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaA [<i>Pseudomonas</i>]	456	456	100%
MULTISPECIES: 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl- CoA isomerase [<i>Pseudomonas</i>]	335	335	99%
enoyl-CoA hydratase [<i>Pseudomonas putida</i>]	255	255	96%
MULTISPECIES: bifunctional aldehyde dehydrogenase/enoyl-CoA hydratase [<i>Pseudomonas</i>]	724	724	98%
enoyl-CoA hydratase [<i>Pseudomonas putida</i>]	255	255	96%
enoyl-CoA hydratase [<i>Pseudomonas putida</i>]	255	255	96%
MULTISPECIES: enoyl-CoA hydratase [<i>Pseudomonas</i>]	158	158	99%
MULTISPECIES: 3-hydroxyacyl-CoA dehydrogenase PaaC [<i>Pseudomonas</i>]	387	387	97%

MULTISPECIES: 3-hydroxyacyl-CoA dehydrogenase			
PaaC [Pseudomonas]	387	387	97%
MULTISPECIES: phenylacetic acid degradation protein			
PaaD [Pseudomonas]	124	124	93%
MULTISPECIES: phenylacetic acid degradation protein			
PaaD [Pseudomonas]	124	124	93%

Draft

E value	Ident	Accession	<i>P. putida</i> Locus	GenBank Accession/NCBI reference sequence
0	63%	WP_010954507.1	PP_3668	NP_745804.1
1.00E-30	33%	WP_010952248.1	PP_1078	NP_743239.1
7.00E-29	29%	WP_010954605.1	PP_3775	NP_745905.1
6.00E-142	43%	WP_010954328.1	PP_3463	NP_745601.1
0	71%	WP_003247729.1	PP_3590	NP_745726.1
0	71%	WP_003247729.1	PP_3590	NP_745726.1
0	68%	WP_010954158.1	PP_3279	NP_745422.1
7.00E-119	49%	WP_010954154.1	PP_3274	NP_745417.1
7.00E-58	54%	WP_010954155.1	PP_3275	NP_745418.1
6.00E-88	54%	WP_010954156.1	PP_3276	NP_745419.1
6.00E-40	61%	WP_004374528.1	PP_3277	NP_745420.1
1.00E-163	68%	WP_010954157.1	PP_3278	NP_745421.1
6.00E-118	62%	WP_010954162.1	PP_3283	NP_745426.1
2.00E-86	56%	WP_010954163.1	PP_3284	NP_745427.1
0	59%	WP_010954151.1	PP_3270	NP_745413.1
2.00E-86	56%	WP_010954163.1	PP_3284	NP_745427.1
2.00E-86	56%	WP_010954163.1	PP_3284	NP_745427.1
2.00E-48	38%	WP_003250095.1	PP_2217	NP_744366.1
2.00E-131	48%	WP_010954161.1	PP_3282	NP_745425.1

2.00E-131	48% WP_010954161.1	PP_3282	NP_745425.1
5.00E-38	47% WP_010954160.1	PP_3281	NP_745424.1
5.00E-38	47% WP_010954160.1	PP_3281	NP_745424.1

Draft

***P. putida* Gene Name**

katG

peaE

amaC

amaC

paaK

paaE

paaD

paaC

paaB

paaA

paaG

paaF

paaZ

paaF

paaF

paaH

paaH

paal

paal

Draft

Results from *P. putida* KT2440 to *E. coli* K12 and *E. coli* K12 back to *P. putida* KT2440

<i>Pseudomonas</i>	Locus Tag	<i>E. coli</i>	Locus	<i>P. putida</i>	Locus
PP_3668		b3942		PP_3668	
PP_2932					
PP_2552		b1756		PP_1078	
PP_4983		b1059		PP_3775	
PP_3463		b1385		PP_3463	
PP_0967					
PP_1972		b4054		PP_3590	
PP_3590		b4054		PP_3590	
PP_3279		b1398		PP_3279	
PP_3274		b1392		PP_3274	
PP_3275		b1391		PP_3275	
PP_3276		b1390		PP_3276	
PP_3277		b1389		PP_3277	
PP_3278		b1388		PP_3278	
PP_3283		b1394		PP_3283	
PP_3726		b1393		PP_3284	
PP_3270		b1387		PP_3270	
PP_1845					
PP_2217		b1393		PP_3284	
PP_3284		b1393		PP_3284	
PP_4030		b0036		PP_2217	
PP_3282		b1395		PP_3282	
PP_3755		b1395		PP_3282	
PP_0244		b1396		PP_3281	
PP_3281		b1396		PP_3281	

E. coli K-12 MG1655 prot

Query

E. coli gene name Locus Tag

katG	b3942
tynA	b1386
feaB	b1385
aspC	b0928
hisC	b2021
tyrB	b4054
paaK	b1398
paaI	b1396
paaA	b1388
paaB	b1389
paaC	b1390
paaD	b1391
paaE	b1392
paaG	b1394
paaZ	b1387
paaJ	b1397
paaF	b1393
paaH	b1395

Draft

tein sequences from ecocyc.com BLAST against *P. putida* KT2440 (taxid:160488)

Result

Description	Max Score	Total Score
catalase peroxidase [<i>Pseudomonas putida</i>]	888	888
No Significant Similarity Found		
MULTISPECIES: aldehyde dehydrogenase [<i>Pseudomonas</i>]	415	415
MULTISPECIES: aspartate aminotransferase [<i>Pseudomonas</i>]	373	373
MULTISPECIES: succinyldiaminopimelate transaminase [<i>Pseudomonas</i>]	61.2	61.2
MULTISPECIES: aromatic amino acid aminotransferase [<i>Pseudomonas</i>]	590	590
phenylacetate-CoA ligase [<i>Pseudomonas putida</i>]	614	614
MULTISPECIES: phenylacetic acid degradation protein PaaD [<i>Pseudomonas</i>]	124	124
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaA [<i>Pseudomonas</i>]	456	456
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaB [Gammaproteobacteria]	125	125
MULTISPECIES: phenylacetic acid degradation protein [<i>Pseudomonas</i>]	258	258
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaJ [<i>Pseudomonas</i>]	176	176
MULTISPECIES: phenylacetic acid degradation protein [<i>Pseudomonas</i>]	345	345
MULTISPECIES: 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl-CoA isomerase [<i>Pseudomonas</i>]	335	335
MULTISPECIES: bifunctional aldehyde dehydrogenase/enoyl-CoA hydratase [<i>Pseudomonas</i>]	724	724
MULTISPECIES: acetyl-CoA acetyltransferase [<i>Pseudomonas</i>]	549	549
enoyl-CoA hydratase [<i>Pseudomonas putida</i>]	255	255
MULTISPECIES: 3-hydroxyacyl-CoA dehydrogenase PaaC [<i>Pseudomonas</i>]	387	387

Query cover	E value	Ident	Accession	<i>P. putida</i> Locus	NCBI Reference Sequence
97%	0	63%	WP_010954507.1	PP_3668	NP_745804.1
98%	6.00E-142	43%	WP_010954328.1	PP_3463	NP_745601.1
100%	1.00E-128	47%	WP_010952990.1	PP_1972	NP_744123.1
66%	4.00E-11	24%	WP_010952672.1	PP_1588	NP_743745.1
99%	0	71%	WP_003247729.1	PP_3590	NP_745726.1
97%	0	68%	WP_010954158.1	PP_3279	NP_745422.1
93%	5.00E-38	47%	WP_010954160.1	PP_3281	NP_745424.1
100%	1.00E-163	68%	WP_010954157.1	PP_3278	NP_745421.1
94%	6.00E-40	61%	WP_004374528.1	PP_3277	NP_745420.1
98%	6.00E-88	54%	WP_010954156.1	PP_3276	NP_745419.1
93%	7.00E-58	54%	WP_010954155.1	PP_3275	NP_745418.1
98%	7.00E-119	49%	WP_010954154.1	PP_3274	NP_745417.1
99%	6.00E-118	62%	WP_010954162.1	PP_3283	NP_745426.1
98%	0	59%	WP_010954151.1	PP_3270	NP_745413.1
99%	0	71%	WP_010954159.1	PP_3280	NP_745423.1
96%	2.00E-86	56%	WP_010954163.1	PP_3284	NP_745427.1
97%	2.00E-131	48%	WP_010954161.1	PP_3282	NP_745425.1

***P. putida* Gene Name**

katG

peaE

tyrB

dapC

amaC

paaK

paaI

paaA

paaB

paaC

paaD

paaE

paaG

paaZ

paaJ

paaF

paaH

Results from *E. coli* K12 to *P. putida* KT244

Query

<i>P. putida</i> gene name	<i>P. putida</i> Locus	<i>E. coli</i> Locus Initial query
katG	PP_3668	b3942 b1386
peaE	PP_3463	b1385
tyrB	PP_1972	b0928
dapC	PP_1588	b2021
amaC	PP_3590	b4054
paaK	PP_3279	b1398
paal	PP_3281	b1396
paaA	PP_3278	b1388
paaB	PP_3277	b1389
paaC	PP_3276	b1390
paaD	PP_3275	b1391
paaE	PP_3274	b1392
paaG	PP_3283	b1394
paaZ	PP_3270	b1387
paaJ	PP_3280	b1397
paaF	PP_3284	b1393
paaH	PP_3282	b1395

0 stored as FASTA files BLAST against *Escherichia coli* MG1655 (taxid:511145)

Result

Description	Max Score	Total Score	Query cover
MULTISPECIES: catalase-peroxidase [Proteobacteria]	885	885	99%
MULTISPECIES: NAD-dependent phenylacetaldehyde dehydrogenase [Escherichia]	419	419	98%
MULTISPECIES: aromatic amino acid aminotransferase [Proteobacteria]	418	418	99%
MULTISPECIES: glutamate--pyruvate aminotransferase AlaC [Proteobacteria]	141	141	98%
MULTISPECIES: aromatic amino acid aminotransferase [Proteobacteria]	590	590	99%
MULTISPECIES: phenylacetate--CoA ligase [Enterobacteriaceae]	614	614	96%
MULTISPECIES: acyl-CoA thioesterase [Enterobacteriaceae]	124	124	89%
MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase subunit A [Enterobacteriaceae]*	456	456	94%
MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase subunit B [Enterobacteriaceae]	125	125	96%
MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase subunit C [Enterobacteriaceae]	258	258	98%
MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase [Enterobacteriaceae]	176	176	88%
MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase subunit E [Enterobacteriaceae]	350	350	99%
MULTISPECIES: 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl-CoA isomerase [Enterobacteriaceae]	308	308	99%
MULTISPECIES: bifunctional aldehyde dehydrogenase/enoyl-CoA hydratase [Escherichia]	732	732	98%
MULTISPECIES: 3-oxoadipyl-CoA/3-oxo-5,6-dehydrosuberil-CoA thiolase [Enterobacteriaceae]	561	561	98%
MULTISPECIES: 2,3-dehydroadipyl-CoA hydratase [Enterobacteriaceae]	267	267	96%
MULTISPECIES: 3-hydroxyadipyl-CoA dehydrogenase [Enterobacteriaceae]	410	410	96%

*used 2nd hit because 1st hit was protein subcomplex

E value	Ident	Accession	<i>E. coli</i> Locus	GenBank Accession	<i>E. coli</i> Gene Name
0	63%	WP_001295695.1	b3942	AAC76924.1	katG
8.00E-144	43%	WP_000138615.1	b1385	AAC74467.2	feaB
3.00E-146	51%	WP_000486985.1	b4054	AAC77024.1	tyrB
7.00E-39	28%	WP_000785931.1	b2379	AAC75438.1	alaC
0	71%	WP_000486985.1	b4054	AAC77024.1	tyrB
0	68%	WP_000632280.1	b1398	AAC74480.1	paaK
5.00E-38	47%	WP_000018413.1	b1396	AAC74478.1	paal
1.00E-163	68%	WP_000191077.1	b1388	AAC74470.1	paaA
5.00E-40	61%	WP_000073393.1	b1389	AAC74471.1	paaB
6.00E-88	54%	WP_001072837.1	b1390	AAC74472.1	paaC
7.00E-58	54%	WP_001189201.1	b1391	AAC74473.4	paaD
1.00E-120	49%	WP_000206388.1	b1392	AAC74474.1	paaE
4.00E-107	62%	WP_000969784.1	b1394	AAC74476.1	paaG
0	59%	WP_001186469.1	b1387	AAC74469.1	paaZ
0	71%	WP_001206197.1	b1397	AAC74479.1	paal
3.00E-91	56%	WP_001292353.1	b1393	AAC74475.1	paaF
2.00E-140	48%	WP_000973360.1	b1395	AAC74477.1	paaH

Results from *E. coli* K12 to *P. putida* KT2440 and *P. putida* KT2440 back to *E. coli* K12

<i>E. coli</i> Locus	<i>P. putida</i> Locus	<i>E. coli</i> Locus
b3942	PP_3668	b3942
b1386		
b1385	PP_3463	b1385
b0928	PP_1972	b4054
b2021	PP_1588	b2379
b4054	PP_3590	b4054
b1398	PP_3279	b1398
b1396	PP_3281	b1396
b1388	PP_3278	b1388
b1389	PP_3277	b1389
b1390	PP_3276	b1390
b1391	PP_3275	b1391
b1392	PP_3274	b1392
b1394	PP_3283	b1394
b1387	PP_3270	b1387
b1397	PP_3280	b1397
b1393	PP_3284	b1393
b1395	PP_3282	b1395

Supplemental File 1: Bi-directional BLASTP from *P. putida* KT2440 to *E. coli* K-12 MG1

Overview:

Each tab contains the results from a BLASTP search

Tab #	Tab Title	Contents
Tab 1	About File	Overview of Supplemental File 1
Tab 2	KT2440 to K12	Contains the results from a BLASTP search using <i>P. putida</i> KT2440 genes from the BLASTP search against <i>E. coli</i> K-12 MG1655 genes
Tab 3	K12 back to KT2440	Summary of the locus tag of the initial query gene (<i>P. putida</i> KT2440)
Tab 4	Bidirectional KT2440 to K12	Summary of the locus tag of the initial query gene (<i>P. putida</i> KT2440)
Tab 5	K12 to KT2440	Contains the results from a BLASTP search using <i>E. coli</i> K-12 MG1655 genes from the BLASTP search against <i>P. putida</i> K-12 MG1655 genes
Tab 6	KT2440 back to K12	Summary of the locus tag of the initial query gene (<i>E. coli</i> K-12 MG1655)
Tab 7	Bidirectional K12 to KT2440	Summary of the locus tag of the initial query gene (<i>E. coli</i> K-12 MG1655)

1655 and vice-versa

e *P. putida* to *E. coli*)

ida KT2440 genes as queries against the *E. coli* K-12 MG1655 genome
 ainst the *E. coli* K-12 MG1655 genome were used in a BLASTP search against the *P. putida* KT2440 genome (the resulting hit from the *E. coli* K-12 MG1655 genome and the resulting hit of the *P. putida* KT2440 genome), the resulting hit from the *E. coli* K-12 MG1655 genome and the resulting hit of the *P. putida* KT2440 genome
 K12-MG1655 genes as queries against the *P. putida* KT2440 genome
 against the *P. putida* KT2440 genome were used in a BLASTP search against the *E. coli* K-12 MG1655 genome (the resulting hit from the *P. putida* KT2440 genome and the resulting hit of the *E. coli* K-12 MG1655 genome), the resulting hit from the *P. putida* KT2440 genome and the resulting hit of the *E. coli* K-12 MG1655 genome

Draft

40 genome

the *E. coli* K-12 MG1655 gene against the *P. putida* KT2440 genome

1655 genome

the *P. putida* KT2440 gene against the *E. coli* K-12 MG1655 genome

Pseudomonas putida KT2440 protein sequences from pseudomonas.com BLAST against *E. coli* M

Query		Result
<i>P. putida</i> gene name	Locus Tag	Description
	PP_3668	MULTISPECIES: catalase peroxidase [Proteobacteria]
	PP_2932	No Significant Similarity Found
		MULTISPECIES: ABC transporter ATPase
	PP_2552	[Proteobacteria]
		MULTISPECIES: N-methyl-L-tryptophan oxidase
	PP_4983	[Proteobacteria]
		MULTISPECIES: NAD-dependent phenylacetaldehyde
	PP_3463	dehydrogenase [Escherichia]
hisC	PP_0967	No Significant Similarity Found
		MULTISPECIES: aromatic amino acid aminotransferase
tyrB-1	PP_1972	[Proteobacteria]
		MULTISPECIES: aromatic amino acid aminotransferase
tyrB-2	PP_3590	[Proteobacteria]
		MULTISPECIES: phenylacetate--CoA ligase
phaE	PP_3279	[Enterobacteriaceae]
		MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase
phaI	PP_3274	subunit E [Enterobacteriaceae]
		MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase
phaH	PP_3275	[Enterobacteriaceae]
		MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase
	PP_3276	subunit C [Enterobacteriaceae]
		MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase
paaB	PP_3277	subunit B [Enterobacteriaceae]
		MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase
paaA	PP_3278	subunit A [Enterobacteriaceae]*
		MULTISPECIES: 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl-
phaB	PP_3283	CoA isomerase [Enterobacteriaceae]
		MULTISPECIES: 2,3-dehydroadipyl-CoA hydratase
	PP_3726	[Enterobacteriaceae]
		MULTISPECIES: phenylacetic acid degradation
phaL	PP_3270	bifunctional protein PaaZ [Escherichia]
	PP_1845	No Significant Similarity Found
		MULTISPECIES: 2,3-dehydroadipyl-CoA hydratase
fadB1x	PP_2217	[Enterobacteriaceae]
		MULTISPECIES: 2,3-dehydroadipyl-CoA hydratase
	PP_3284	[Enterobacteriaceae]

paaC	PP_4030	MULTISPECIES: carnitinyI-CoA dehydratase [Proteobacteria]
	PP_3282	MULTISPECIES: 3-hydroxyadipyl-CoA dehydrogenase [Enterobacteriaceae]
paaH		MULTISPECIES: 3-hydroxyadipyl-CoA dehydrogenase
	PP_3755	[Enterobacteriaceae]
	PP_0244	MULTISPECIES: acyl-CoA thioesterase [Enterobacteriaceae]
	PP_3281	MULTISPECIES: acyl-CoA thioesterase [Enterobacteriaceae]

*first hit was protein with ligand, used 2nd hit

Draft

G1655 (taxid:511145)

Max Score	Total Score	Query cover	E value	Ident	Accession	<i>E. coli</i> Locus
885	885	99%		0	63% WP_001295695.1	b3942
26.2	26.2	9%	5.4	37%	WP_001300558.1	b1756
30.4	30.4	4%	0.45	52%	WP_000872833.1	b1059
419	419	98%	8.00E-144	43%	WP_000138615.1	b1385
418	418	99%	3.00E-146	51%	WP_000486985.1	b4054
590	590	99%	0.00E+00	71%	WP_000486985.1	b4054
614	614	96%	0	68%	WP_000632280.1	b1398
350	350	99%	1.00E-120	49%	WP_000206388.1	b1392
176	176	88%	7.00E-58	54%	WP_001189201.1	b1391
258	258	98%	2.00E-91	54%	WP_001072837.1	b1390
125	125	96%	5.00E-40	61%	WP_000073393.1	b1389
456	456	94%	1.00E-163	68%	WP_000191077.1	b1388
308	308	99%	4.00E-107	62%	WP_000969784.1	b1394
142	142	98%	2.00E-42	36%	WP_001292353.1	b1393
732	732	98%	0	59%	WP_001186469.1	b1387
200	200	95%	7.00E-65	43%	WP_001292353.1	b1393
267	267	96%	3.00E-91	56%	WP_001292353.1	b1393

128	128	91%	2.00E-36	34%	WP_001295419.1	b0036
410	410	96%	2.00E-140	48%	WP_000973360.1	b1395
219	303	100%	3.00E-69	41%	WP_000973360.1	b1395
61.2	61.2	91%	8.00E-14	36%	WP_000018413.1	b1396
124	124	89%	5.00E-38	47%	WP_000018413.1	b1396

Draft

GenBank Accession *E. coli* Gene Name

AAC76924.1 katG

AAC74826.1 ynjD

AAC74143.1 solA

AAC74467.2 feaB

AAC77024.1 tyrB

AAC77024.1 tyrB

AAC74480.1 paaK

AAC74474.1 paaE

AAC74473.4 paaD

AAC74472.1 paaC

AAC74471.1 paaB

AAC74470.1 paaA

AAC74476.1 paaG

AAC74475.1 paaF

AAC74469.1 paaZ

AAC74475.1 paaF

AAC74475.1 paaF

AAC73147.2	caiD
AAC74477.1	paaH
AAC74477.1	paaH
AAC74478.1	paal
AAC74478.1	paal

Draft

Reverse Blast of *E. coli* K-12 gene results from
Query

<i>E. coli</i> gene name	<i>E. coli</i> Locus	<i>P. putida</i> Locus Initial Query
katG	b3942	PP_3668 PP_2932
ynjD	b1756	PP_2552
solA	b1059	PP_4983
feaB	b1385	PP_3463 PP_0967
tyrB	b4054	PP_1972
tyrB	b4054	PP_3590
paaK	b1398	PP_3279
paaE	b1392	PP_3274
paaD	b1391	PP_3275
paaC	b1390	PP_3276
paaB	b1389	PP_3277
paaA	b1388	PP_3278
paaG	b1394	PP_3283
paaF	b1393	PP_3726
paaZ	b1387	PP_3270 PP_1845
paaF	b1393	PP_2217
paaF	b1393	PP_3284
caiD	b0036	PP_4030
paaH	b1395	PP_3282

paaH	b1395	PP_3755
paal	b1396	PP_0244
paal	b1396	PP_3281

Draft

n *P. putida* KT2440 to *E. coli* K12 against *P. putida* KT2440 (taxid:160488)

Result

Description	Max Score	Total Score	Query cover
catalase peroxidase [<i>Pseudomonas putida</i>]	888	888	97%
MULTISPECIES: ABC transporter ATP-binding protein [<i>Pseudomonas</i>]	113	113	98%
MULTISPECIES: sarcosine oxidase [<i>Pseudomonas</i>]	112	112	92%
MULTISPECIES: aldehyde dehydrogenase [<i>Pseudomonas</i>]	415	415	98%
MULTISPECIES: aromatic amino acid aminotransferase [<i>Pseudomonas</i>]	590	590	99%
MULTISPECIES: aromatic amino acid aminotransferase [<i>Pseudomonas</i>]	590	590	99%
phenylacetate-CoA ligase [<i>Pseudomonas putida</i>]	614	614	97%
MULTISPECIES: phenylacetic acid degradation protein [<i>Pseudomonas</i>]	345	345	98%
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaJ [<i>Pseudomonas</i>]	176	176	93%
MULTISPECIES: phenylacetic acid degradation protein [<i>Pseudomonas</i>]	258	258	98%
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaB [<i>Gammaproteobacteria</i>]	125	125	94%
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaA [<i>Pseudomonas</i>]	456	456	100%
MULTISPECIES: 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl- CoA isomerase [<i>Pseudomonas</i>]	335	335	99%
enoyl-CoA hydratase [<i>Pseudomonas putida</i>]	255	255	96%
MULTISPECIES: bifunctional aldehyde dehydrogenase/enoyl-CoA hydratase [<i>Pseudomonas</i>]	724	724	98%
enoyl-CoA hydratase [<i>Pseudomonas putida</i>]	255	255	96%
enoyl-CoA hydratase [<i>Pseudomonas putida</i>]	255	255	96%
MULTISPECIES: enoyl-CoA hydratase [<i>Pseudomonas</i>]	158	158	99%
MULTISPECIES: 3-hydroxyacyl-CoA dehydrogenase PaaC [<i>Pseudomonas</i>]	387	387	97%

MULTISPECIES: 3-hydroxyacyl-CoA dehydrogenase			
PaaC [Pseudomonas]	387	387	97%
MULTISPECIES: phenylacetic acid degradation protein			
PaaD [Pseudomonas]	124	124	93%
MULTISPECIES: phenylacetic acid degradation protein			
PaaD [Pseudomonas]	124	124	93%

Draft

E value	Ident	Accession	<i>P. putida</i> Locus	GenBank Accession/NCBI reference sequence
0	63%	WP_010954507.1	PP_3668	NP_745804.1
1.00E-30	33%	WP_010952248.1	PP_1078	NP_743239.1
7.00E-29	29%	WP_010954605.1	PP_3775	NP_745905.1
6.00E-142	43%	WP_010954328.1	PP_3463	NP_745601.1
0	71%	WP_003247729.1	PP_3590	NP_745726.1
0	71%	WP_003247729.1	PP_3590	NP_745726.1
0	68%	WP_010954158.1	PP_3279	NP_745422.1
7.00E-119	49%	WP_010954154.1	PP_3274	NP_745417.1
7.00E-58	54%	WP_010954155.1	PP_3275	NP_745418.1
6.00E-88	54%	WP_010954156.1	PP_3276	NP_745419.1
6.00E-40	61%	WP_004374528.1	PP_3277	NP_745420.1
1.00E-163	68%	WP_010954157.1	PP_3278	NP_745421.1
6.00E-118	62%	WP_010954162.1	PP_3283	NP_745426.1
2.00E-86	56%	WP_010954163.1	PP_3284	NP_745427.1
0	59%	WP_010954151.1	PP_3270	NP_745413.1
2.00E-86	56%	WP_010954163.1	PP_3284	NP_745427.1
2.00E-86	56%	WP_010954163.1	PP_3284	NP_745427.1
2.00E-48	38%	WP_003250095.1	PP_2217	NP_744366.1
2.00E-131	48%	WP_010954161.1	PP_3282	NP_745425.1

2.00E-131	48% WP_010954161.1	PP_3282	NP_745425.1
5.00E-38	47% WP_010954160.1	PP_3281	NP_745424.1
5.00E-38	47% WP_010954160.1	PP_3281	NP_745424.1

Draft

***P. putida* Gene Name**

katG

peaE

amaC

amaC

paaK

paaE

paaD

paaC

paaB

paaA

paaG

paaF

paaZ

paaF

paaF

paaH

paaH

paal

paal

Draft

Results from *P. putida* KT2440 to *E. coli* K12 and *E. coli* K12 back to *P. putida* KT2440

<i>Pseudomonas</i>	Locus Tag	<i>E. coli</i>	Locus	<i>P. putida</i>	Locus
PP_3668		b3942		PP_3668	
PP_2932					
PP_2552		b1756		PP_1078	
PP_4983		b1059		PP_3775	
PP_3463		b1385		PP_3463	
PP_0967					
PP_1972		b4054		PP_3590	
PP_3590		b4054		PP_3590	
PP_3279		b1398		PP_3279	
PP_3274		b1392		PP_3274	
PP_3275		b1391		PP_3275	
PP_3276		b1390		PP_3276	
PP_3277		b1389		PP_3277	
PP_3278		b1388		PP_3278	
PP_3283		b1394		PP_3283	
PP_3726		b1393		PP_3284	
PP_3270		b1387		PP_3270	
PP_1845					
PP_2217		b1393		PP_3284	
PP_3284		b1393		PP_3284	
PP_4030		b0036		PP_2217	
PP_3282		b1395		PP_3282	
PP_3755		b1395		PP_3282	
PP_0244		b1396		PP_3281	
PP_3281		b1396		PP_3281	

E. coli K-12 MG1655 prot

Query

E. coli gene name Locus Tag

katG	b3942
tynA	b1386
feaB	b1385
aspC	b0928
hisC	b2021
tyrB	b4054
paaK	b1398
paaI	b1396
paaA	b1388
paaB	b1389
paaC	b1390
paaD	b1391
paaE	b1392
paaG	b1394
paaZ	b1387
paaJ	b1397
paaF	b1393
paaH	b1395

Draft

tein sequences from ecocyc.com BLAST against *P. putida* KT2440 (taxid:160488)

Result

Description	Max Score	Total Score
catalase peroxidase [<i>Pseudomonas putida</i>]	888	888
No Significant Similarity Found		
MULTISPECIES: aldehyde dehydrogenase [<i>Pseudomonas</i>]	415	415
MULTISPECIES: aspartate aminotransferase [<i>Pseudomonas</i>]	373	373
MULTISPECIES: succinyldiaminopimelate transaminase [<i>Pseudomonas</i>]	61.2	61.2
MULTISPECIES: aromatic amino acid aminotransferase [<i>Pseudomonas</i>]	590	590
phenylacetate-CoA ligase [<i>Pseudomonas putida</i>]	614	614
MULTISPECIES: phenylacetic acid degradation protein PaaD [<i>Pseudomonas</i>]	124	124
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaA [<i>Pseudomonas</i>]	456	456
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaB [Gammaproteobacteria]	125	125
MULTISPECIES: phenylacetic acid degradation protein [<i>Pseudomonas</i>]	258	258
MULTISPECIES: phenylacetate-CoA oxygenase subunit PaaJ [<i>Pseudomonas</i>]	176	176
MULTISPECIES: phenylacetic acid degradation protein [<i>Pseudomonas</i>]	345	345
MULTISPECIES: 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl-CoA isomerase [<i>Pseudomonas</i>]	335	335
MULTISPECIES: bifunctional aldehyde dehydrogenase/enoyl-CoA hydratase [<i>Pseudomonas</i>]	724	724
MULTISPECIES: acetyl-CoA acetyltransferase [<i>Pseudomonas</i>]	549	549
enoyl-CoA hydratase [<i>Pseudomonas putida</i>]	255	255
MULTISPECIES: 3-hydroxyacyl-CoA dehydrogenase PaaC [<i>Pseudomonas</i>]	387	387

Query cover	E value	Ident	Accession	<i>P. putida</i> Locus	NCBI Reference Sequence
97%	0	63%	WP_010954507.1	PP_3668	NP_745804.1
98%	6.00E-142	43%	WP_010954328.1	PP_3463	NP_745601.1
100%	1.00E-128	47%	WP_010952990.1	PP_1972	NP_744123.1
66%	4.00E-11	24%	WP_010952672.1	PP_1588	NP_743745.1
99%	0	71%	WP_003247729.1	PP_3590	NP_745726.1
97%	0	68%	WP_010954158.1	PP_3279	NP_745422.1
93%	5.00E-38	47%	WP_010954160.1	PP_3281	NP_745424.1
100%	1.00E-163	68%	WP_010954157.1	PP_3278	NP_745421.1
94%	6.00E-40	61%	WP_004374528.1	PP_3277	NP_745420.1
98%	6.00E-88	54%	WP_010954156.1	PP_3276	NP_745419.1
93%	7.00E-58	54%	WP_010954155.1	PP_3275	NP_745418.1
98%	7.00E-119	49%	WP_010954154.1	PP_3274	NP_745417.1
99%	6.00E-118	62%	WP_010954162.1	PP_3283	NP_745426.1
98%	0	59%	WP_010954151.1	PP_3270	NP_745413.1
99%	0	71%	WP_010954159.1	PP_3280	NP_745423.1
96%	2.00E-86	56%	WP_010954163.1	PP_3284	NP_745427.1
97%	2.00E-131	48%	WP_010954161.1	PP_3282	NP_745425.1

***P. putida* Gene Name**

katG

peaE

tyrB

dapC

amaC

paaK

paaI

paaA

paaB

paaC

paaD

paaE

paaG

paaZ

paaJ

paaF

paaH

Results from *E. coli* K12 to *P. putida* KT244

Query

<i>P. putida</i> gene name	<i>P. putida</i> Locus	<i>E. coli</i> Locus Initial query
katG	PP_3668	b3942 b1386
peaE	PP_3463	b1385
tyrB	PP_1972	b0928
dapC	PP_1588	b2021
amaC	PP_3590	b4054
paaK	PP_3279	b1398
paal	PP_3281	b1396
paaA	PP_3278	b1388
paaB	PP_3277	b1389
paaC	PP_3276	b1390
paaD	PP_3275	b1391
paaE	PP_3274	b1392
paaG	PP_3283	b1394
paaZ	PP_3270	b1387
paaJ	PP_3280	b1397
paaF	PP_3284	b1393
paaH	PP_3282	b1395

0 stored as FASTA files BLAST against *Escherichia coli* MG1655 (taxid:511145)

Result

Description	Max Score	Total Score	Query cover
MULTISPECIES: catalase-peroxidase [Proteobacteria]	885	885	99%
MULTISPECIES: NAD-dependent phenylacetaldehyde dehydrogenase [Escherichia]	419	419	98%
MULTISPECIES: aromatic amino acid aminotransferase [Proteobacteria]	418	418	99%
MULTISPECIES: glutamate--pyruvate aminotransferase AlaC [Proteobacteria]	141	141	98%
MULTISPECIES: aromatic amino acid aminotransferase [Proteobacteria]	590	590	99%
MULTISPECIES: phenylacetate--CoA ligase [Enterobacteriaceae]	614	614	96%
MULTISPECIES: acyl-CoA thioesterase [Enterobacteriaceae]	124	124	89%
MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase subunit A [Enterobacteriaceae]*	456	456	94%
MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase subunit B [Enterobacteriaceae]	125	125	96%
MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase subunit C [Enterobacteriaceae]	258	258	98%
MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase [Enterobacteriaceae]	176	176	88%
MULTISPECIES: 1,2-phenylacetyl-CoA epoxidase subunit E [Enterobacteriaceae]	350	350	99%
MULTISPECIES: 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl-CoA isomerase [Enterobacteriaceae]	308	308	99%
MULTISPECIES: bifunctional aldehyde dehydrogenase/enoyl-CoA hydratase [Escherichia]	732	732	98%
MULTISPECIES: 3-oxoadipyl-CoA/3-oxo-5,6-dehydrosuberil-CoA thiolase [Enterobacteriaceae]	561	561	98%
MULTISPECIES: 2,3-dehydroadipyl-CoA hydratase [Enterobacteriaceae]	267	267	96%
MULTISPECIES: 3-hydroxyadipyl-CoA dehydrogenase [Enterobacteriaceae]	410	410	96%

*used 2nd hit because 1st hit was protein subcomplex

E value	Ident	Accession	<i>E. coli</i> Locus	GenBank Accession	<i>E. coli</i> Gene Name
0	63%	WP_001295695.1	b3942	AAC76924.1	katG
8.00E-144	43%	WP_000138615.1	b1385	AAC74467.2	feaB
3.00E-146	51%	WP_000486985.1	b4054	AAC77024.1	tyrB
7.00E-39	28%	WP_000785931.1	b2379	AAC75438.1	alaC
0	71%	WP_000486985.1	b4054	AAC77024.1	tyrB
0	68%	WP_000632280.1	b1398	AAC74480.1	paaK
5.00E-38	47%	WP_000018413.1	b1396	AAC74478.1	paal
1.00E-163	68%	WP_000191077.1	b1388	AAC74470.1	paaA
5.00E-40	61%	WP_000073393.1	b1389	AAC74471.1	paaB
6.00E-88	54%	WP_001072837.1	b1390	AAC74472.1	paaC
7.00E-58	54%	WP_001189201.1	b1391	AAC74473.4	paaD
1.00E-120	49%	WP_000206388.1	b1392	AAC74474.1	paaE
4.00E-107	62%	WP_000969784.1	b1394	AAC74476.1	paaG
0	59%	WP_001186469.1	b1387	AAC74469.1	paaZ
0	71%	WP_001206197.1	b1397	AAC74479.1	paal
3.00E-91	56%	WP_001292353.1	b1393	AAC74475.1	paaF
2.00E-140	48%	WP_000973360.1	b1395	AAC74477.1	paaH

Results from *E. coli* K12 to *P. putida* KT2440 and *P. putida* KT2440 back to *E. coli* K12

<i>E. coli</i> Locus	<i>P. putida</i> Locus	<i>E. coli</i> Locus
b3942	PP_3668	b3942
b1386		
b1385	PP_3463	b1385
b0928	PP_1972	b4054
b2021	PP_1588	b2379
b4054	PP_3590	b4054
b1398	PP_3279	b1398
b1396	PP_3281	b1396
b1388	PP_3278	b1388
b1389	PP_3277	b1389
b1390	PP_3276	b1390
b1391	PP_3275	b1391
b1392	PP_3274	b1392
b1394	PP_3283	b1394
b1387	PP_3270	b1387
b1397	PP_3280	b1397
b1393	PP_3284	b1393
b1395	PP_3282	b1395

Supplemental File 2: Identification of putative genes of the phenylalanine catabolic pathway in 3 CF pathogens by bi-directional BLASTP searches using *P. putida* KT2440 and *E. coli* K-12 MG1655 genes against the genome of each CF pathogen

Overview:

Using the KEGG database the phenylalanine catabolic pathway for each CF strain was elucidated. (See CF Strain Pathways Tab)
Steps where no gene has been identified by KEGG were selected for further investigation (Highlighted Red in CF Strain Pathways Tab).
Create a FASTA File (one for each strain) containing the amino acid sequences for the genes (from *P. putida* KT2440 and *E. coli* K-12 MG1655 responsible for the steps with genes absent in each)
These FASTA files were used to BLAST against each genome
The accession number of the resulting hits from each genome was used as a query in a BLASTP search back against the initial genome (*P. putida* or *E. coli*)
If the locus tag of the initial query matched the locus tag returned from the bidirectional BLASTP search, it was considered a putative gene for the step.

Tab #	Tab Title	Contents
Tab 1	About File	Overview of the contents of Supplemental File 2
Tab 2	CF Strain Pathways	The locus tags of the KEGG identified genes in each strain and the steps with no identified genes have been highlighted for further study.
Tab 3	BLAST against B. cenocepacia	Using the genes from <i>P. putida</i> and <i>E. coli</i> for the steps with no identified genes in <i>B. cenocepacia</i> a BLASTP search was performed.
Tab 4	B. cenocepacia Back to genome	The accession numbers of the genes identified by BLAST against <i>B. cenocepacia</i> were used as queries back against the initial <i>P. putida</i> or <i>E. coli</i> genome
Tab 5	BLAST against P. aeruginosa	Using the genes from <i>P. putida</i> and <i>E. coli</i> for the steps with no identified genes in <i>P. aeruginosa</i> a BLASTP search was performed.
Tab 6	P. aeruginosa back to genome	The accession numbers of the genes identified by BLAST against <i>P. aeruginosa</i> were used as queries back against the initial <i>P. putida</i> or <i>E. coli</i> genome
Tab 7	BLAST against A. fumigatus	Using the genes from <i>P. putida</i> and <i>E. coli</i> for the steps with no identified genes in <i>A. fumigatus</i> a BLASTP search was performed.
Tab 8	A. fumigatus back to genome	The accession numbers of the genes identified by BLAST against <i>A. fumigatus</i> were used as queries back against the initial <i>P. putida</i> or <i>E. coli</i> genome

Draft

h CF strain

Step in Pathway	Locus Tag(s) in <i>P. putida</i>	Locus Tag(s) in <i>E. coli</i>	<i>B. cenocepacia</i>	<i>P. aeruginosa</i>	<i>A. fumigatus</i>
1	PP_0967 PP_1972 PP_3590	b0928 b2021 b4054	BCAL0313 BCAL0797 BCAL2303 BCAM1478	PA2531 PA3165 PA4447 PA0870 PA3139	AFUA_1G04160 AFUA_2G09650 AFUA_4G10410 AFUA_6G02490 AFUA_1G14090 AFUA_2G13630
2	PP_2552				AFUA_3G02240
3	PP_3668	b3942	BCAL3299 BCAM2107		AFUA_8G01670
4					
5	PP_4983	b1386	BCAM0112 BCAM0123	PA0421	AFUA_3G00100 AFUA_1G13440 AFUA_3G00680 AFUA_3G14590 AFUA_5G01470 AFUA_5G07360 AFUA_7G04180 AFUA_7G08470
6	PP_2932		BCAL0148 BCAM0538 BCAM0641 BCAM1315 BCAM1544 BCAM1828	PA0202 PA0704 PA3366 PA4163 PA4342	AFUA_1G14880 AFUA_5G00470 AFUA_5G09140 AFUA_6G08000 AFUA_6G14410 AFUA_8G05220
7	PP_3463	b1385	BCAM1979 BCAM2298	PA4073	
8					
9	PP_0244 PP_3281	b1396	BCAL0405	PA5202	
10	PP_3279	b1398	BCAL0404 BCAM1711		
11	PP_3274 PP_3275 PP_3276 PP_3277 PP_3278	b1388 b1389 b1390 b1391 b1392	BCAL0212 BCAL0213 BCAL0214 BCAL0215 BCAL0216		
12	PP_3283 PP_3726	b1394	BCAL0406	PA4980	
13	PP_3270	b1387			
14	PP_3270	b1387			
15	PP_3283 PP_3726	b1394 b1397	BCAL0406	PA4980	
16	PP_1845 PP_2217 PP_3284 PP_4030	b1393	BCAL0409 BCAL0687 BCAM1632 BCAM1634	PA1748 PA1821 PA3426	
17	PP_3282 PP_3755	b1395	BCAM0656 BCAM1712 BCAS0607	PA1628 PA3590	AFUA_4G04410
18	PP_3280	b1397			

B. cenocepacia using e value threshold 10 ⁻⁸								
Step in Pathway	Locus Tag(s) in <i>P. putida</i>	Locus Tag(s) in <i>E. coli</i>	Description	Max Score	Total Score	Query conE value	Ident	Accession
2	PP_2552		No Significant Similarity Found					
	PP_3270	Absent	MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	95.9	95.9	39%	2.00E-21	31% WP_006488133.1
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	89.4	89.4	42%	2.00E-19	28% WP_006484845.1
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	87.8	87.8	52%	7.00E-19	31% WP_006489660.1
			Chain A, Crystal Structure Of A Putative Aldehyde Dehydrogenase From Burkholderia Cenocepacia	87.8	87.8	52%	7.00E-19	31% 4YWE_A
			Chain A, Crystal Structure Of A Putative Aldehyde Dehydrogenase From Burkholderia Cenocepacia	87.8	87.8	72%	9.00E-19	27% 4O6R_A
			MULTISPECIES: betaine-aldehyde dehydrogenase [Burkholderia cepacia complex]	87.4	87.4	72%	1.00E-18	27% WP_006484608.1
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	87	87	52%	1.00E-18	26% WP_006487205.1
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	82.8	82.8	51%	3.00E-17	28% WP_034178280.1
			betaine aldehyde dehydrogenase [Burkholderia cenocepacia J2315]	82.8	82.8	51%	3.00E-17	28% CAR54273.1
			MULTISPECIES: 5-carboxymethyl-2-hydroxymuconate semialdehyde dehydrogenase [Burkholderia cepacia complex]	81.6	81.6	50%	8.00E-17	24% WP_006490715.1
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	79	79	52%	5.00E-16	25% WP_006481882.1
			MULTISPECIES: aldehyde dehydrogenase (NAD) family protein [Burkholderia cepacia complex]	78.2	78.2	52%	1.00E-15	28% WP_006484740.1
			putative phenylacetaldehyde dehydrogenase [Burkholderia cenocepacia J2315]	78.2	78.2	52%	1.00E-15	28% CAR56157.1
			Chain A, X-ray Crystal Structure Of A Putative Phenylacetaldehyde Dehydrogenase From Burkholderia Cenocepacia	77.8	77.8	52%	1.00E-15	28% 4O5H_A
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	73.2	73.2	51%	4.00E-14	26% WP_012493562.1
			MULTISPECIES: phosphonoacetaldehyde dehydrogenase [Burkholderia cepacia complex]	70.5	70.5	42%	2.00E-13	23% WP_006491137.1
			MULTISPECIES: phosphonoacetaldehyde dehydrogenase [Burkholderia cepacia complex]	70.5	70.5	42%	3.00E-13	23% WP_006485777.1
			Chain A, The Crystal Structure Of Gamma-glutamyl-gamma-aminobutyraldehyde Dehydrogenase From Burkholderia Cenocepacia J2315	70.5	70.5	41%	3.00E-13	26% 4LIH_A
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	70.1	70.1	41%	3.00E-13	25% WP_006490987.1
13 and 14			MULTISPECIES: gamma-glutamyl-gamma-aminobutyraldehyde dehydrogenase [Burkholderia cepacia complex]	70.5	70.5	41%	3.00E-13	26% WP_006488053.1
			MULTISPECIES: NAD-dependent succinate-semialdehyde dehydrogenase [Burkholderia cepacia complex]	69.7	69.7	42%	5.00E-13	26% WP_012493582.1
			MULTISPECIES: acyl dehydratase [Burkholderia cepacia complex]	64.7	64.7	21%	6.00E-13	30% WP_006483300.1
			MULTISPECIES: methylmalonate-semialdehyde dehydrogenase (acylating) [Burkholderia cepacia complex]	67.4	67.4	44%	2.00E-12	25% WP_006488924.1
			MULTISPECIES: aldehyde dehydrogenase (NAD) family protein [Burkholderia cepacia complex]	66.6	66.6	49%	4.00E-12	26% WP_006481750.1
			MULTISPECIES: NAD-dependent succinate-semialdehyde dehydrogenase [Burkholderia cepacia complex]	60.1	60.1	52%	5.00E-10	26% WP_006489632.1
	b1387		MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	100	100	52%	5.00E-23	26% WP_006488133.1
			MULTISPECIES: NAD-dependent succinate-semialdehyde dehydrogenase [Burkholderia cepacia complex]	97.1	97.1	69%	1.00E-21	24% WP_012493582.1
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	95.5	95.5	51%	3.00E-21	28% WP_006487205.1
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	87.8	87.8	51%	9.00E-19	26% WP_006481882.1
			MULTISPECIES: phosphonoacetaldehyde dehydrogenase [Burkholderia cepacia complex]	87	87	56%	1.00E-18	24% WP_006491137.1
			MULTISPECIES: phosphonoacetaldehyde dehydrogenase [Burkholderia cepacia complex]	86.7	86.7	56%	2.00E-18	24% WP_006485777.1
			MULTISPECIES: phosphonoacetaldehyde dehydrogenase [Burkholderia cepacia complex]	85.9	85.9	55%	3.00E-18	24% WP_006493412.1
			putative NADP-dependent glyceraldehyde-3-phosphate dehydrogenase [Burkholderia cenocepacia J2315]	85.9	85.9	55%	3.00E-18	24% CAR56358.1
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	85.5	85.5	51%	5.00E-18	28% WP_012493562.1
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	81.3	81.3	37%	9.00E-17	26% WP_006490987.1
			MULTISPECIES: 5-carboxymethyl-2-hydroxymuconate semialdehyde dehydrogenase [Burkholderia cepacia complex]	77.8	77.8	42%	1.00E-15	25% WP_006490715.1
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	72.8	72.8	41%	5.00E-14	25% WP_006484845.1
			Chain A, The Crystal Structure Of Gamma-glutamyl-gamma-aminobutyraldehyde Dehydrogenase From Burkholderia Cenocepacia J2315	70.9	70.9	40%	2.00E-13	25% 4LIH_A
			MULTISPECIES: gamma-glutamyl-gamma-aminobutyraldehyde dehydrogenase [Burkholderia cepacia complex]	70.9	70.9	40%	2.00E-13	25% WP_006488053.1
			MULTISPECIES: methylmalonate-semialdehyde dehydrogenase (acylating) [Burkholderia cepacia complex]	68.9	68.9	42%	8.00E-13	26% WP_006488924.1
	PP_3280		MULTISPECIES: acyl dehydratase [Burkholderia cepacia complex]	62.8	62.8	19%	3.00E-12	32% WP_006483300.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	570	570	98%	0	73% WP_006491099.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	552	552	98%	0	70% WP_006482586.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	307	307	98%	1.00E-102	46% WP_006485256.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	307	307	98%	2.00E-102	46% WP_012492402.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	296	296	98%	3.00E-98	46% WP_006485733.1
			MULTISPECIES: 3-ketoacyl-CoA thiolase [Burkholderia]	287	287	98%	9.00E-95	44% WP_006481441.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	270	270	97%	2.00E-88	43% WP_006493096.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	254	254	98%	9.00E-82	42% WP_006483911.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	249	249	97%	6.00E-80	42% WP_006493100.1
18		b1397	MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	242	242	97%	5.00E-77	39% WP_006491472.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	541	541	100%	0	69% WP_006482586.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	535	535	100%	0	69% WP_006491099.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	323	323	100%	6.00E-109	46% WP_012492402.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	321	321	99%	4.00E-108	46% WP_006485256.1
			MULTISPECIES: 3-ketoacyl-CoA thiolase [Burkholderia]	310	310	99%	8.00E-104	45% WP_006481441.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	299	299	100%	2.00E-99	44% WP_006485733.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	265	265	100%	2.00E-86	40% WP_006493096.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	262	262	99%	4.00E-85	42% WP_006483911.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	252	252	99%	6.00E-81	41% WP_006493100.1
			MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	229	229	98%	4.00E-72	37% WP_006491472.1

B. cenocepacia using e value threshold 10 ⁻⁸																				
Step in Pathway	Locus Tag(s)	Locus Tag(s) in E. coli	Description	Max Score	Total Score	Query cover	E value	Ident	Accession	Results from BLAST against P. putida or E. coli genome	Description	Max Score	Total Score	Query cover	E value	Ident	Accession	P. putida/E. coli Locus Tag	B. cenocepac	
2	PP_2552	Absent	No Significant Similarity Found																	
13 and 14	PP_3270		MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	95.9	95.9	39%	2.00E-21	31%	WP_006488133.1		aldehyde dehydrogenase [Pseudomonas putida]	322	322	97%	5.00E-106	40%	WP_010953423.1	PP_2487		
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	89.4	89.4	42%	2.00E-19	28%	WP_006484845.1		MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	305	305	96%	2.00E-99	38%	WP_010954328.1	PP_1461		
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	87.8	87.8	52%	7.00E-19	31%	WP_006489660.1		aldehyde dehydrogenase [Pseudomonas putida]	363	363	98%	5.00E-122	44%	WP_010952968.1	PP_1548		
			Chain A, Crystal Structure Of A Putative Aldehyde Dehydrogenase From Burkholder	87.8	87.8	52%	7.00E-19	31%	4YWE_A		aldehyde dehydrogenase [Pseudomonas putida]	363	363	97%	5.00E-122	44%	WP_010952968.1	PP_1548		
			Chain A, Crystal Structure Of A Putative Aldehyde Dehydrogenase From Burkholder	87.8	87.8	72%	9.00E-19	27%	4O6R_A											
			MULTISPECIES: betaine-aldehyde dehydrogenase [Burkholderia cepacia complex]	87.4	87.4	72%	1.00E-18	27%	WP_006484608.1		aldehyde dehydrogenase [Pseudomonas putida]	437	437	97%	8.00E-151	45%	WP_010953423.1	PP_2487		
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	87	87	52%	1.00E-18	26%	WP_006487205.1		MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	403	403	96%	2.00E-137	44%	WP_010954488.1	PP_1646		
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	82.8	82.8	51%	3.00E-17	28%	WP_034178280.1		betaine-aldehyde dehydrogenase [Pseudomonas putida]	383	383	89%	6.00E-130	46%	WP_010951956.1	PP_0708		
			betaine aldehyde dehydrogenase [Burkholderia cenocepacia J2315]	82.8	82.8	51%	3.00E-17	28%	CAR54273.1		betaine-aldehyde dehydrogenase [Pseudomonas putida]	384	384	89%	5.00E-130	46%	WP_010951956.1	PP_0708		
			MULTISPECIES: 5-carboxymethyl-2-hydroxyruconate semialdehyde dehydrogenase	81.6	81.6	50%	8.00E-17	24%	WP_006490715.1		aldehyde dehydrogenase [Pseudomonas putida]	375	375	95%	9.00E-127	40%	WP_010953423.1	PP_2487		
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	79	79	52%	5.00E-16	25%	WP_006481882.1		aldehyde dehydrogenase [Pseudomonas putida]	323	323	94%	2.00E-106	39%	WP_010953423.1	PP_2487		
			MULTISPECIES: aldehyde dehydrogenase (NAD) family protein [Burkholderia cepaci	78.2	78.2	52%	1.00E-15	28%	WP_006484740.1		MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	622	622	98%	0.00E+00	61%	WP_010954328.1	PP_2463		
			putative phenylacetaldehyde dehydrogenase [Burkholderia cenocepacia J2315]	78.2	78.2	52%	1.00E-15	28%	CAR56157.1		MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	622	622	97%	0.00E+00	61%	WP_010954328.1	PP_2463		
			Chain A, X-ray Crystal Structure Of A Putative Phenylacetaldehyde Dehydrogenase I	77.8	77.8	52%	1.00E-15	28%	4DSH_A											
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	73.2	73.2	51%	4.00E-14	26%	WP_012493562.1		MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	675	675	94%	0.00E+00	68%	WP_003253547.1	PP_5258		
			MULTISPECIES: phosphonoacetaldehyde dehydrogenase [Burkholderia cepacia corr	70.5	70.5	42%	2.00E-13	23%	WP_006491137.1		MULTISPECIES: glyceraldehyde-3-phosphate dehydrogenase [	295	295	93%	9.00E-96	38%	WP_010951923.1	PP_0665		
			MULTISPECIES: phosphonoacetaldehyde dehydrogenase [Burkholderia cepacia corr	70.5	70.5	42%	3.00E-13	23%	WP_006485777.1		MULTISPECIES: glyceraldehyde-3-phosphate dehydrogenase [	296	296	93%	3.00E-96	37%	WP_010951923.1	PP_0665		
			Chain A, The Crystal Structure Of Gamma-glutamyl-gamma-aminobutyraldehyde De	70.5	70.5	41%	3.00E-13	26%	4LH_A											
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	70.1	70.1	41%	3.00E-13	25%	WP_006490987.1		MULTISPECIES: glyceraldehyde-3-phosphate dehydrogenase [	299	29900%	94%	1.00E-97	36%	WP_010951923.1	PP_0665		
			MULTISPECIES: gamma-glutamyl-gamma-aminobutyraldehyde dehydrogenase [Bur	70.5	70.5	41%	3.00E-13	26%	WP_006488053.1		MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	635	635	99%	0.00E+00	64%	WP_010955785.1	PP_5278		
			MULTISPECIES: NAD-dependent succinate-semialdehyde dehydrogenase [Burkhold	69.7	69.7	42%	5.00E-13	26%	WP_012493582.1		MULTISPECIES: NAD-dependent succinate-semialdehyde dehy	696	696	98%	0.00E+00	70%	WP_010951596.1	PP_0211		
			MULTISPECIES: acyl dehydratase [Burkholderia cepacia complex]	64.7	64.7	21%	6.00E-13	30%	WP_006483300.1		MULTISPECIES: acyl dehydratase [Pseudomonas]	268	268	99%	8.00E-95	83%	WP_010953900.1	PP_3000		
			MULTISPECIES: methylmalonate-semialdehyde dehydrogenase [acylating] [Burkhol	67.4	67.4	44%	2.00E-12	25%	WP_006488924.1		MULTISPECIES: methylmalonate-semialdehyde dehydrogenas	600	600	97%	0.00E+00	58%	WP_003255276.1	PP_0597		
			MULTISPECIES: aldehyde dehydrogenase (NAD) family protein [Burkholderia cepaci	66.6	66.6	49%	4.00E-12	26%	WP_006481750.1		aldehyde dehydrogenase [Pseudomonas putida]	410	410	99%	2.00E-141	46%	WP_010954040.1	PP_3151		
			MULTISPECIES: NAD-dependent succinate-semialdehyde dehydrogenase [Burkhold	60.1	60.1	52%	5.00E-10	26%	WP_006489632.1		MULTISPECIES: NAD-dependent succinate-semialdehyde dehy	444	444	96%	9.00E-154	47%	WP_010951596.1	PP_0213		
	b1387		MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	100	100	52%	5.00E-23	26%	WP_006488133.1		MULTISPECIES: aldehyde dehydrogenase PvuC [Proteobacteri	322	322	98%	4.00E-106	38%	WP_001009090.1	B1300		
			MULTISPECIES: NAD-dependent succinate-semialdehyde dehydrogenase [Burkhold	97.1	97.1	69%	1.00E-21	24%	WP_012493582.1		MULTISPECIES: NAD-dependent succinate-semialdehyde dehy	702	702	98%	0.00E+00	70%	WP_000772831.1	B2661		
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	95.5	95.5	51%	3.00E-21	28%	WP_006487205.1		betaine-aldehyde dehydrogenase [Escherichia coli str. K-12 su	341	341	96%	3.00E-113	41%	AA818038.1		betB	
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	87.8	87.8	51%	9.00E-19	26%	WP_006481862.1		MULTISPECIES: aldehyde dehydrogenase PvuC [Proteobacteri	306	306	95%	1.00E-99	40%	WP_001009090.1	B1300		
			MULTISPECIES: phosphonoacetaldehyde dehydrogenase [Burkholderia cepacia corr	87	87	56%	1.00E-18	24%	WP_006491137.1		MULTISPECIES: NAD-dependent succinate-semialdehyde dehy	251	251	96%	1.00E-78	32%	WP_000772831.1	B2661		
			MULTISPECIES: phosphonoacetaldehyde dehydrogenase [Burkholderia cepacia corr	86.7	86.7	56%	2.00E-18	24%	WP_006485777.1		MULTISPECIES: NAD-dependent succinate-semialdehyde dehy	248	248	96%	8.00E-78	32%	WP_000772831.1	B2661		
			MULTISPECIES: phosphonoacetaldehyde dehydrogenase [Burkholderia cepacia corr	85.9	85.9	55%	3.00E-18	24%	WP_006493412.1		MULTISPECIES: NAD-dependent succinate-semialdehyde dehy	248	248	98%	2.00E-77	33%	WP_000772831.1	B2661		
			putative NADP-dependent glyceraldehyde-3-phosphate dehydrogenase [Burkholde	85.9	85.9	55%	3.00E-18	24%	CAR56358.1		MULTISPECIES: NAD-dependent succinate-semialdehyde dehy	248	248	98%	2.00E-77	33%	WP_000772831.1	B2661		
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	85.5	85.5	51%	5.00E-18	28%	WP_012493562.1		MULTISPECIES: lactaldehyde dehydrogenase [Enterobacteriac	197	197	93%	5.00E-58	30%	WP_000115943.1	B1415		
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	81.3	81.3	37%	9.00E-17	26%	WP_006490987.1		MULTISPECIES: NAD-dependent succinate-semialdehyde dehy	238	238	95%	4.00E-74	35%	WP_000772831.1	B2661		
			MULTISPECIES: 5-carboxymethyl-2-hydroxyruconate semialdehyde dehydrogenase	77.8	77.8	42%	1.00E-15	25%	WP_006490715.1		MULTISPECIES: aldehyde dehydrogenase PvuC [Proteobacteri	348	348	94%	3.00E-116	40%	WP_001009090.1	B1300		
			MULTISPECIES: aldehyde dehydrogenase [Burkholderia cepacia complex]	72.8	72.8	41%	5.00E-14	25%	WP_006484845.1		MULTISPECIES: aldehyde dehydrogenase PvuC [Proteobacteri	297	297	96%	2.00E-96	37%	WP_001009090.1	B1300		
			Chain A, The Crystal Structure Of Gamma-glutamyl-gamma-aminobutyraldehyde De	70.9	70.9	40%	2.00E-13	25%	4LH_A											
			MULTISPECIES: gamma-glutamyl-gamma-aminobutyraldehyde dehydrogenase [Bur	70.9	70.9	40%	2.00E-13	25%	WP_006488053.1		MULTISPECIES: aldehyde dehydrogenase PvuC [Proteobacteri	525	525	99%	0.00E+00	54%	WP_001009090.1	B1300		
			MULTISPECIES: methylmalonate-semialdehyde dehydrogenase [acylating] [Burkhol	68.9	68.9	42%	8.00E-13	26%	WP_006488924.1		MULTISPECIES: NAD-dependent phenylacetaldehyde dehydro	242	242	95%	6.00E-75	33%	WP_000138615.1	B1385		
			MULTISPECIES: acyl dehydratase [Burkholderia cepacia complex]	62.8	62.8	19%	3.00E-12	32%	WP_006483300.1		MULTISPECIES: bifunctional aldehyde dehydrogenase/enoyl-C	62.8	62.8	82%	5.00E-13			B1387	BCAS0364	
	PP_3280		MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	570	570	98%	0	73%	WP_006491099.1		MULTISPECIES: acetyl-CoA acetyltransferase [Pseudomonas]	569	569	99%	0	73%	WP_010954159.1	PP_3280	BCAL0407	
	18	b1397		MULTISPECIES: acetyl-CoA acetyltransferase [Burkholderia cepacia complex]	541	541	100%	0	69%	WP_006482586.1		MULTISPECIES: 3-oxoadipyl-CoA(3-oxo-5,6-dehydrosheryl-Co	557	557	100%	0	69%	WP_001206197.1	B1397	BCAM2568

for Locus tag

Draft

			<i>P. aeruginosa</i> using e value threshold 10 ⁻⁸							
Step in Pathway	Locus Tag(s) in <i>P. putida</i>	Locus Tag(s) in <i>E. coli</i>	Description	Max Score	Total Score	Query cover	E value	Ident	Accession	
3	PP_3668	b3942	No Significant Similarity Found							
			No Significant Similarity Found							
2	PP_2552	Absent	No Significant Similarity Found							
10	PP_3279		No Significant Similarity Found							
	PP_3274	b1398	No Significant Similarity Found							
			MULTISPECIES: hybrid-cluster NAD(P)-dependent oxidoreductase [Pseudomonas]	143	143	98%	7.00E-40	29%	WP_003096769.1	
			MULTISPECIES: flavohemoprotein [Pseudomonas]	93.2	93.2	59%	1.00E-21	30%	WP_003113387.1	
			vanillate O-demethylase [Pseudomonas aeruginosa]	88.6	88.6	84%	3.00E-20	29%	WP_003112295.1	
			oxidoreductase [Pseudomonas aeruginosa]	82.8	82.8	79%	4.00E-18	27%	WP_003112305.1	
			ferredoxin [Pseudomonas aeruginosa]	81.6	81.6	79%	1.00E-17	27%	WP_038404013.1	
			Vanillate O-demethylase oxidoreductase [Pseudomonas aeruginosa]	79	79	84%	6.00E-17	29%	WP_038404026.1	
			MULTISPECIES: toluate 1,2-dioxygenase electron transfer subunit [Pseudomonas]	67.8	67.8	59%	4.00E-13	26%	WP_003109537.1	
			anthranilate dioxygenase reductase [Pseudomonas aeruginosa]	61.6	61.6	68%	2.00E-11	25%	WP_047696755.1	
			anthranilate dioxygenase reductase [Pseudomonas aeruginosa]	62	62	68%	4.00E-11	25%	WP_003113312.	
	PP_3275	Absent	No Significant Similarity Found							
	PP_3276		No Significant Similarity Found							
	PP_3276		No Significant Similarity Found							
	PP_3277		No Significant Similarity Found							
11	PP_3278		No Significant Similarity Found							
		b1388	No Significant Similarity Found							
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		b1389	No Significant Similarity Found							
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		b1390	No Significant Similarity Found							
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		b1391	No Significant Similarity Found							
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		b1392	No Significant Similarity Found							
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			No Significant Similarity Found							
		b1392	MULTISPECIES: hybrid-cluster NAD(P)-dependent oxidoreductase [Pseudomonas]	129	129	97%	1.00E-34	26%	WP_003096769.1	
			MULTISPECIES: flavohemoprotein [Pseudomonas]	87.4	87.4	66%	2.00E-19	30%	WP_003113387.1	
			oxidoreductase [Pseudomonas aeruginosa]	85.9	85.9	78%	4.00E-19	28%	WP_003112305.1	
			ferredoxin [Pseudomonas aeruginosa]	85.9	85.9	78%	4.00E-19	28%	WP_038404013.1	
			vanillate O-demethylase [Pseudomonas aeruginosa]	70.5	70.5	88%	5.00E-14	26%	WP_003112295.1	
			Vanillate O-demethylase oxidoreductase [Pseudomonas aeruginosa]	67.4	67.4	88%	5.00E-13	26%	WP_038404026.1	
			MULTISPECIES: oxidoreductase [Pseudomonas]	66.2	66.2	91%	1.00E-12	26%	WP_003112912.1	
			anthranilate dioxygenase reductase [Pseudomonas aeruginosa]	65.1	65.1	59%	1.00E-12	26%	WP_047696755.1	
			anthranilate dioxygenase reductase [Pseudomonas aeruginosa]	65.9	65.9	59%	2.00E-12	26%	WP_003113312.1	
			MULTISPECIES: toluate 1,2-dioxygenase electron transfer subunit [Pseudomonas]	60.5	60.5	19%	1.00E-10	42%	WP_003109537.1	
		b1392	aldehyde dehydrogenase [Pseudomonas aeruginosa]	95.1	95.1	39%	4.00E-21	33%	WP_047697426.1	
			aldehyde dehydrogenase [Pseudomonas aeruginosa]	94.7	94.7	39%	5.00E-21	33%	WP_003114713.1	
			MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	94.7	94.7	39%	9.00E-21	33%	WP_003101377.1	
			MULTISPECIES: betaine-aldehyde dehydrogenase [Pseudomonas]	91.7	91.7	72%	8.00E-20	27%	WP_003114436.1	
			betaine-aldehyde dehydrogenase [Pseudomonas aeruginosa]	90.1	90.1	72%	2.00E-19	27%	WP_038404164.1	
			MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	82	82	45%	8.00E-17	29%	WP_003102149.1	
			aldehyde dehydrogenase [Pseudomonas aeruginosa]	82	82	45%	9.00E-17	29%	WP_038403199.1	
			MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	77	77	44%	4.00E-15	26%	WP_003112906.1	
			MULTISPECIES: NAD-dependent succinate-semialdehyde dehydrogenase [Pseudomonas]	76.6	76.6	60%	4.00E-15	23%	WP_003106269.1	
			MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	70.9	70.9	63%	3.00E-13	26%	WP_003107137.1	
		b1392	aldehyde dehydrogenase [Pseudomonas aeruginosa]	70.9	70.9	63%	3.00E-13	25%	WP_038404021.1	
			putative aldehyde dehydrogenase [Pseudomonas aeruginosa PAO1H2O]	68.6	68.6	28%	4.00E-13	33%	AID75383.1	
			aldehyde dehydrogenase [Pseudomonas aeruginosa]	70.1	70.1	54%	5.00E-13	26%	WP_047697654.1	
			aldehyde dehydrogenase [Pseudomonas aeruginosa]	70.1	70.1	44%	6.00E-13	28%	WP_038403762.1	
			MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	69.7	69.7	43%	7.00E-13	28%	WP_003118247.1	
			aldehyde dehydrogenase [Pseudomonas aeruginosa]	69.7	69.7	44%	8.00E-13	28%	WP_038403149.1	
			aldehyde dehydrogenase [Pseudomonas aeruginosa]	69.3	69.3	43%	1.00E-12	28%	WP_003115507.1	
			MULTISPECIES: 5-carboxy-2-hydroxymuconate semialdehyde dehydrogenase [Pseudomonas]	67.8	67.8	50%	3.00E-12	23%	WP_003110130.1	
			5-carboxymethyl-2-hydroxymuconate semialdehyde dehydrogenase [Pseudomonas aeruginosa]	67.8	67.8	50%	3.00E-12	23%	WP_003115626.1	
			MULTISPECIES: hypothetical protein [Pseudomonas]	62.4	62.4	14%	6.00E-12	36%	WP_003093220.1	
		b1392	aldehyde dehydrogenase [Pseudomonas aeruginosa]	66.2	66.2	51%	9.00E-12	26%	WP_003112530.1	
			aldehyde dehydrogenase [Pseudomonas aeruginosa]	66.2	66.2	51%	9.00E-12	26%	WP_003112530.1	

PP_3280

18

b1387

b1397

aldehyde dehydrogenase [Pseudomonas aeruginosa]	65.9	65.9	42%	1.00E-11	27% WP_010895535.1
MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	65.5	65.5	51%	1.00E-11	26% WP_003086325.1
MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	62	62	52%	2.00E-10	26% WP_003113043.1
aldehyde dehydrogenase [Pseudomonas aeruginosa]	61.6	61.6	52%	2.00E-10	26% WP_038403780.1
NADP-dependent glyceraldehyde-3-phosphate dehydrogenase [Pseudomonas aeruginosa]	59.7	59.7	45%	9.00E-10	23% WP_047705006.1
MULTISPECIES: glyceraldehyde-3-phosphate dehydrogenase [Pseudomonas]	60.1	60.1	45%	9.00E-10	23% WP_003115067.1
NADP-dependent glyceraldehyde-3-phosphate dehydrogenase [Pseudomonas aeruginosa]	59.7	59.7	45%	1.00E-09	23% WP_038403238.1
MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	97.4	97.4	65%	1.00E-21	27% WP_003107137.1
MULTISPECIES: NAD-dependent succinate-semialdehyde dehydrogenase [Pseudomonas]	95.1	95.1	65%	5.00E-21	24% WP_003106269.1
aldehyde dehydrogenase [Pseudomonas aeruginosa]	94.7	94.7	65%	9.00E-21	26% WP_038404021.1
MULTISPECIES: betaine-aldehyde dehydrogenase [Pseudomonas]	91.3	91.3	67%	1.00E-19	27% WP_003114436.1
betaine-aldehyde dehydrogenase [Pseudomonas aeruginosa]	90.1	90.1	67%	2.00E-19	27% WP_038404164.1
MULTISPECIES: aldehyde dehydrogenase [Pseudomonas]	88.2	88.2	42%	1.00E-18	28% WP_003112906.1
aldehyde dehydrogenase [Pseudomonas aeruginosa]	87.8	87.8	45%	1.00E-18	27% WP_047697654.1
MULTISPECIES: 5-carboxy-2-hydroxymuconate semialdehyde dehydrogenase [Pseudomonas]	78.6	78.6	42%	1.00E-15	27% WP_003110130.1
5-carboxymethyl-2-hydroxymuconate semialdehyde dehydrogenase [Pseudomonas aeruginosa]	78.6	78.6	42%	1.00E-15	27% WP_003115626.1
MULTISPECIES: hypothetical protein [Pseudomonas]	57.8	57.8	16%	3.00E-10	32% WP_003091647.1
MULTISPECIES: hypothetical protein [Pseudomonas]	57.4	57.4	12%	4.00E-10	35% WP_003093220.1
MULTISPECIES: beta-ketoadipyl CoA thiolase [Pseudomonas]	541	541	98%	0	68% WP_003101891.1
MULTISPECIES: acetyl-CoA acetyltransferase [Pseudomonas]	387	387	98%	2.00E-133	56% WP_003098466.1
beta-ketoadipyl CoA thiolase [Pseudomonas aeruginosa]	384	384	98%	3.00E-132	56% WP_038403640.1
MULTISPECIES: acetyl-CoA acetyltransferase [Pseudomonas]	324	324	98%	6.00E-109	48% WP_003121716.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	323	323	98%	1.00E-108	48% WP_003113500.1
3-ketoacyl-CoA thiolase [Pseudomonas aeruginosa]	287	287	98%	2.00E-94	41% WP_003115356.1
MULTISPECIES: 3-ketoacyl-CoA thiolase [Pseudomonas]	286	286	98%	4.00E-94	41% WP_003113978.1
MULTISPECIES: acetyl-CoA acetyltransferase [Pseudomonas]	278	278	98%	5.00E-91	43% WP_003087747.1
MULTISPECIES: acetyl-CoA acetyltransferase [Pseudomonas]	276	276	93%	3.00E-90	45% WP_003101465.1
MULTISPECIES: acyl-CoA thiolase [Pseudomonas]	275	275	93%	4.00E-90	45% WP_003114002.1
MULTISPECIES: acyl-CoA thiolase [Pseudomonas]	244	244	97%	1.00E-77	40% WP_003114496.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	234	234	98%	9.00E-74	39% WP_038403740.1
MULTISPECIES: acyl-CoA thiolase [Pseudomonas]	234	234	98%	1.00E-73	39% WP_003103022.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	233	233	98%	1.00E-73	39% WP_003114307.1
MULTISPECIES: acyl-CoA thiolase [Pseudomonas]	220	220	97%	2.00E-68	38% WP_003108823.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	210	210	78%	1.00E-65	40% WP_047697289.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	203	203	85%	2.00E-62	38% WP_003115775.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	200	200	80%	1.00E-61	39% WP_047705128.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	196	196	78%	3.00E-60	38% WP_047696772.1
MULTISPECIES: acetyl-CoA acetyltransferase [Pseudomonas]	125	125	97%	1.00E-32	29% WP_003102247.1
putative acetyl-CoA acetyltransferase [Pseudomonas aeruginosa MPAO1/P1]	94.7	94.7	24%	2.00E-23	53% EHS33070.1
MULTISPECIES: beta-ketoadipyl CoA thiolase [Pseudomonas]	531	531	99%	0	65% WP_003101891.1
MULTISPECIES: acetyl-CoA acetyltransferase [Pseudomonas]	389	389	99%	1.00E-134	54% WP_003098466.1
beta-ketoadipyl CoA thiolase [Pseudomonas aeruginosa]	387	387	99%	1.00E-133	53% WP_038403640.1
MULTISPECIES: acetyl-CoA acetyltransferase [Pseudomonas]	310	310	99%	2.00E-103	46% WP_003121716.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	309	309	99%	5.00E-103	46% WP_003113500.1
3-ketoacyl-CoA thiolase [Pseudomonas aeruginosa]	300	300	99%	1.00E-99	42% WP_003115356.1
MULTISPECIES: 3-ketoacyl-CoA thiolase [Pseudomonas]	300	300	99%	2.00E-99	42% WP_003113978.1
MULTISPECIES: acetyl-CoA acetyltransferase [Pseudomonas]	284	284	100%	4.00E-93	42% WP_003087747.1
MULTISPECIES: acetyl-CoA acetyltransferase [Pseudomonas]	282	282	95%	7.00E-93	45% WP_003101465.1
MULTISPECIES: acyl-CoA thiolase [Pseudomonas]	282	282	95%	8.00E-93	45% WP_003114002.1
MULTISPECIES: acyl-CoA thiolase [Pseudomonas]	254	254	100%	9.00E-82	40% WP_003114496.1
MULTISPECIES: acyl-CoA thiolase [Pseudomonas]	231	231	99%	6.00E-73	39% WP_003103022.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	231	231	99%	7.00E-73	39% WP_003114307.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	231	231	99%	7.00E-73	39% WP_038403740.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	201	201	78%	4.00E-62	39% WP_047697289.1
MULTISPECIES: acyl-CoA thiolase [Pseudomonas]	201	201	98%	3.00E-61	36% WP_003108823.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	186	186	86%	3.00E-56	36% WP_003115775.1

acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	180	180	81%	4.00E-54	35% WP_047705128.1
acetyl-CoA acetyltransferase [Pseudomonas aeruginosa]	177	177	79%	5.00E-53	36% WP_047696772.1
MULTISPECIES: acetyl-CoA acetyltransferase [Pseudomonas]	136	136	99%	1.00E-36	29% WP_003102247.1
putative acetyl-CoA acetyltransferase [Pseudomonas aeruginosa MPAO1/P1]	97.8	97.8	24%	1.00E-24	51% EHS33070.1

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<https://mc06.manuscriptcentral.com/cjm-pubs>

			A. fumigatus Af293 using e value threshold 10 ⁻⁸						
Step in Pathway	Locus Tag(s) in <i>P. putida</i>	Locus Tag(s) in <i>E. coli</i>	Description	Max Score	Total Score	Query cover	E value	Ident	Accession
7	PP_3463		aldehyde dehydrogenase AldA [Aspergillus fumigatus Af293]	420	420	96%	8.00E-143	45% XP_751026.1	
			aldehyde dehydrogenase ALDH [Aspergillus fumigatus Af293]	404	404	95%	3.00E-136	44% XP_746975.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	400	400	98%	5.00E-135	42% XP_751967.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	396	396	96%	2.00E-134	43% XP_746831.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	363	363	94%	2.00E-121	43% XP_749209.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	282	282	91%	4.00E-90	36% XP_746469.1	
			betaine aldehyde dehydrogenase (BadH) [Aspergillus fumigatus Af293]	283	283	94%	4.00E-90	38% XP_747215.1	
10	PP_3279	b1385	succinate-semialdehyde dehydrogenase [Aspergillus fumigatus Af293]	281	281	94%	1.00E-89	37% XP_748332.1	
			betaine aldehyde dehydrogenase [Aspergillus fumigatus Af293]	278	278	94%	2.00E-88	37% XP_749799.1	
			succinate-semialdehyde dehydrogenase Uga2 [Aspergillus fumigatus Af293]	270	270	98%	4.00E-85	35% XP_752009.1	
			succinate-semialdehyde dehydrogenase [Aspergillus fumigatus Af293]	266	266	94%	8.00E-84	34% XP_754891.1	
			aldehyde dehydrogenase [AldH12] [Aspergillus fumigatus Af293]	265	265	93%	2.00E-83	35% XP_756081.1	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	253	253	91%	1.00E-78	34% XP_748644.1	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	244	244	90%	2.00E-75	34% XP_746990.1	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	216	216	92%	7.00E-65	33% XP_747354.1	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	213	213	93%	7.00E-64	31% XP_746556.2	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	212	212	93%	2.00E-63	30% XP_755901.1	
			methylmalonate-semialdehyde dehydrogenase [Aspergillus fumigatus Af293]	201	201	97%	3.00E-58	29% XP_751545.1	
			vanillin dehydrogenase [Aspergillus fumigatus Af293]	184	184	91%	6.00E-53	32% XP_750112.1	
			oxidoreductase (Msc7) [Aspergillus fumigatus Af293]	180	180	96%	4.00E-50	27% XP_747700.1	
			delta-1-pyrroline-5-carboxylate dehydrogenase PrnC [Aspergillus fumigatus Af293]	133	133	93%	8.00E-34	27% XP_750764.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	130	130	84%	2.00E-33	28% XP_746302.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	127	127	88%	6.00E-32	26% XP_751481.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	367	367	95%	6.00E-122	40% XP_751967.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	358	358	95%	1.00E-119	39% XP_746831.1	
			aldehyde dehydrogenase AldA [Aspergillus fumigatus Af293]	348	348	98%	1.00E-114	37% XP_751026.1	
			aldehyde dehydrogenase ALDH [Aspergillus fumigatus Af293]	335	335	95%	2.00E-109	40% XP_746975.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	331	331	95%	6.00E-109	39% XP_749209.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	280	280	93%	3.00E-89	36% XP_746469.1	
			betaine aldehyde dehydrogenase (BadH) [Aspergillus fumigatus Af293]	280	280	95%	7.00E-89	36% XP_747215.1	
			betaine aldehyde dehydrogenase [Aspergillus fumigatus Af293]	277	277	95%	6.00E-88	36% XP_749799.1	
			aldehyde dehydrogenase (AldH12) [Aspergillus fumigatus Af293]	276	276	94%	1.00E-87	36% XP_756081.1	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	276	276	95%	2.00E-87	35% XP_748644.1	
			succinate-semialdehyde dehydrogenase [Aspergillus fumigatus Af293]	266	266	90%	1.00E-83	34% XP_754891.1	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	254	254	90%	4.00E-79	33% XP_746990.1	
			succinate-semialdehyde dehydrogenase Uga2 [Aspergillus fumigatus Af293]	254	254	94%	6.00E-79	35% XP_752009.1	
			succinate-semialdehyde dehydrogenase [Aspergillus fumigatus Af293]	252	252	94%	2.00E-78	33% XP_748332.1	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	242	242	91%	8.00E-75	35% XP_747354.1	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	221	221	92%	6.00E-67	29% XP_746556.2	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	217	217	92%	2.00E-65	29% XP_755901.1	
9	PP_0244 PP_3281	b1398	vanillin dehydrogenase [Aspergillus fumigatus Af293]	214	214	92%	4.00E-64	35% XP_750112.1	
			methylmalonate-semialdehyde dehydrogenase [Aspergillus fumigatus Af293]	201	201	94%	4.00E-58	30% XP_751545.1	
			oxidoreductase (Msc7) [Aspergillus fumigatus Af293]	183	183	93%	2.00E-51	30% XP_747700.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	160	160	84%	5.00E-44	30% XP_746302.1	
			delta-1-pyrroline-5-carboxylate dehydrogenase PrnC [Aspergillus fumigatus Af293]	143	143	88%	2.00E-37	28% XP_750764.1	
			aldehyde dehydrogenase [Aspergillus fumigatus Af293]	141	141	67%	5.00E-37	30% XP_751481.1	
			No Significant Similarity Found						
			No Significant Similarity Found						
			No Significant Similarity Found						
			No Significant Similarity Found						
11	PP_3274 PP_3275 PP_3276 PP_3277 PP_3278	b1396	cytochrome b5 reductase [Aspergillus fumigatus Af293]	75.1	75.1	65%	3.00E-15	26% XP_748717.1	
			flavohemoprotein [Aspergillus fumigatus Af293]	70.5	70.5	59%	1.00E-13	27% XP_747410.1	
			No Significant Similarity Found						
			No Significant Similarity Found						
			No Significant Similarity Found						
			No Significant Similarity Found						
			No Significant Similarity Found						
11		b1388	No Significant Similarity Found						
			No Significant Similarity Found						

	b1389	No Significant Similarity Found							
	b1390	No Significant Similarity Found							
	b1391	No Significant Similarity Found							
	b1392	cytochrome b5 reductase [Aspergillus fumigatus Af293]	70.1	70.1	55%	1.00E-13	24%	XP_753636.1	
		NADH-cytochrome b5 reductase [Aspergillus fumigatus Af293]	63.9	63.9	65%	9.00E-12	24%	XP_755738.2	
12	PP_3283	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	121	121	98%	2.00E-33	29%	XP_755425.1	
		enoyl-CoA hydratase [Aspergillus fumigatus Af293]	103	103	97%	2.00E-26	30%	XP_755398.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	92.4	92.4	93%	1.00E-22	27%	XP_747085.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	92	92	98%	2.00E-22	28%	XP_748661.1	
		mitochondrial methylglutaconyl-CoA hydratase (Auh) [Aspergillus fumigatus Af293]	78.6	78.6	95%	2.00E-17	26%	XP_754471.1	
		mitochondrial 3-hydroxyisobutyryl-CoA hydrolase [Aspergillus fumigatus Af293]	76.6	76.6	73%	3.00E-16	28%	XP_753374.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	67	67	77%	2.00E-13	29%	XP_754855.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	106	106	96%	1.00E-27	31%	XP_755425.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	105	105	92%	1.00E-27	31%	XP_748661.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	102	102	91%	2.00E-26	31%	XP_747085.1	
13 and 14	b1394	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	100	100	93%	3.00E-25	32%	XP_749938.1	
		mitochondrial methylglutaconyl-CoA hydratase (Auh) [Aspergillus fumigatus Af293]	95.9	95.9	94%	1.00E-23	31%	XP_754471.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	89.7	89.7	93%	2.00E-21	28%	XP_754174.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	88.6	88.6	69%	4.00E-21	34%	XP_754855.1	
		enoyl-CoA hydratase [Aspergillus fumigatus Af293]	86.7	86.7	98%	3.00E-20	28%	XP_755398.1	
		mitochondrial 3-hydroxyisobutyryl-CoA hydrolase [Aspergillus fumigatus Af293]	81.3	81.3	74%	9.00E-18	31%	XP_753374.1	
		peroxisomal D3,D2-enoyl-CoA isomerase [Aspergillus fumigatus Af293]	67	67	84%	2.00E-13	28%	XP_747673.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	125	125	99%	1.00E-34	31%	XP_755425.1	
		enoyl-CoA hydratase [Aspergillus fumigatus Af293]	117	117	97%	5.00E-32	31%	XP_755398.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	112	112	96%	3.00E-30	32%	XP_747085.1	
15	PP_3270	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	105	105	94%	5.00E-27	31%	XP_749938.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	99.4	99.4	97%	3.00E-25	29%	XP_748661.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	89.7	89.7	96%	1.00E-21	26%	XP_754174.1	
		peroxisomal D3,D2-enoyl-CoA isomerase [Aspergillus fumigatus Af293]	89.4	89.4	89%	2.00E-21	29%	XP_747673.1	
		mitochondrial 3-hydroxyisobutyryl-CoA hydrolase [Aspergillus fumigatus Af293]	80.9	80.9	71%	1.00E-17	31%	XP_753374.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	67.8	67.8	78%	1.00E-13	28%	XP_754855.1	
		aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	76.6	76.6	41%	4.00E-15	25%	XP_746556.2	
		aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	68.2	68.2	42%	2.00E-12	27%	XP_755901.1	
		aldehyde dehydrogenase [Aspergillus fumigatus Af293]	62.8	62.8	66%	1.00E-10	23%	XP_751481.1	
		succinate-semialdehyde dehydrogenase [Aspergillus fumigatus Af293]	83.2	83.2	65%	5.00E-17	25%	XP_754891.1	
15	b1387	aldehyde dehydrogenase family protein [Aspergillus fumigatus Af293]	72.4	72.4	44%	1.00E-13	25%	XP_755901.1	
	PP_3283	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	121	121	98%	2.00E-33	29%	XP_755425.1	
		enoyl-CoA hydratase [Aspergillus fumigatus Af293]	103	103	97%	2.00E-26	30%	XP_755398.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	92.4	92.4	93%	1.00E-22	27%	XP_747085.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	92	92	98%	2.00E-22	28%	XP_748661.1	
		mitochondrial methylglutaconyl-CoA hydratase (Auh) [Aspergillus fumigatus Af293]	78.6	78.6	95%	2.00E-17	26%	XP_754471.1	
		mitochondrial 3-hydroxyisobutyryl-CoA hydrolase [Aspergillus fumigatus Af293]	76.6	76.6	73%	3.00E-16	28%	XP_753374.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	67	67	77%	2.00E-13	29%	XP_754855.1	
	PP_3276	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	106	106	96%	1.00E-27	31%	XP_755425.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	105	105	92%	1.00E-27	31%	XP_748661.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	102	102	91%	2.00E-26	31%	XP_747085.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	100	100	93%	3.00E-25	32%	XP_749938.1	
		mitochondrial methylglutaconyl-CoA hydratase (Auh) [Aspergillus fumigatus Af293]	95.9	95.9	94%	1.00E-23	31%	XP_754471.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	89.7	89.7	93%	2.00E-21	28%	XP_754174.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	88.6	88.6	69%	4.00E-21	34%	XP_754855.1	
		enoyl-CoA hydratase [Aspergillus fumigatus Af293]	86.7	86.7	98%	3.00E-20	28%	XP_755398.1	
		mitochondrial 3-hydroxyisobutyryl-CoA hydrolase [Aspergillus fumigatus Af293]	81.3	81.3	74%	9.00E-18	31%	XP_753374.1	
		peroxisomal D3,D2-enoyl-CoA isomerase [Aspergillus fumigatus Af293]	67	67	84%	2.00E-13	28%	XP_747673.1	
15	b1394	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	125	125	99%	1.00E-34	31%	XP_755425.1	
		enoyl-CoA hydratase [Aspergillus fumigatus Af293]	117	117	97%	5.00E-32	31%	XP_755398.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	112	112	96%	3.00E-30	32%	XP_747085.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	105	105	94%	5.00E-27	31%	XP_749938.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	99.4	99.4	97%	3.00E-25	29%	XP_748661.1	
		enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	99.4	99.4	97%	3.00E-25	29%	XP_748661.1	

	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	89.7	89.7	96%	1.00E-21	26% XP_754174.1
	peroxisomal D3,D2-enoyl-CoA isomerase [Aspergillus fumigatus Af293]	89.4	89.4	89%	2.00E-21	29% XP_747673.1
	mitochondrial 3-hydroxyisobutyryl-CoA hydrolase [Aspergillus fumigatus Af293]	80.9	80.9	71%	1.00E-17	31% XP_753374.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	67.8	67.8	78%	1.00E-13	28% XP_754855.1
b1397	3-ketoacyl-CoA thiolase (POT1) [Aspergillus fumigatus Af293]	224	224	98%	2.00E-69	39% XP_749100.1
	acetyl-CoA-acetyltransferase [Aspergillus fumigatus Af293]	221	221	100%	3.00E-68	37% XP_751294.1
	peroxisomal 3-ketoacyl-coA thiolase (Kat1) [Aspergillus fumigatus Af293]	212	212	97%	4.00E-65	37% XP_755468.1
	3-ketoacyl-coA thiolase peroxisomal A precursor [Aspergillus fumigatus Af293]	205	205	97%	1.00E-62	36% XP_751733.1
	3-ketoacyl-CoA ketothiolase (Kat1) [Aspergillus fumigatus Af293]	201	201	98%	7.00E-61	35% XP_752635.1
	acetyl-CoA acetyltransferase [Aspergillus fumigatus Af293]	200	200	98%	1.00E-60	33% XP_747207.1
PP_1845	No Significant Similarity Found					
PP_2217	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	270	270	93%	3.00E-91	56% XP_755425.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	183	183	93%	1.00E-57	43% XP_747085.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	137	137	93%	7.00E-40	33% XP_748661.1
	enoyl-CoA hydratase [Aspergillus fumigatus Af293]	136	136	93%	3.00E-39	33% XP_755398.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	115	115	95%	5.00E-31	30% XP_754174.1
	mitochondrial methylglutaconyl-CoA hydratase (Auh) [Aspergillus fumigatus Af293]	106	106	95%	2.00E-27	30% XP_754471.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	92.4	92.4	94%	2.00E-22	29% XP_749938.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	82.8	82.8	73%	5.00E-19	33% XP_754855.1
	peroxisomal D3,D2-enoyl-CoA isomerase [Aspergillus fumigatus Af293]	79	79	68%	1.00E-17	27% XP_747673.1
	mitochondrial 3-hydroxyisobutyryl-CoA hydrolase [Aspergillus fumigatus Af293]	75.9	75.9	63%	5.00E-16	33% XP_753374.1
PP_3284	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	213	213	99%	4.00E-69	46% XP_755425.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	175	175	96%	2.00E-54	41% XP_747085.1
	enoyl-CoA hydratase [Aspergillus fumigatus Af293]	136	136	98%	4.00E-39	33% XP_755398.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	134	134	96%	1.00E-38	33% XP_748661.1
	mitochondrial methylglutaconyl-CoA hydratase (Auh) [Aspergillus fumigatus Af293]	112	112	97%	5.00E-30	34% XP_754471.1
	mitochondrial 3-hydroxyisobutyryl-CoA hydrolase [Aspergillus fumigatus Af293]	73.6	73.6	64%	3.00E-15	28% XP_753374.1
16	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	67	67	72%	2.00E-13	31% XP_754855.1
	peroxisomal D3,D2-enoyl-CoA isomerase [Aspergillus fumigatus Af293]	63.9	63.9	64%	3.00E-12	27% XP_747673.1
PP_4030	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	241	241	90%	7.00E-80	46% XP_754174.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	126	126	91%	3.00E-35	32% XP_748661.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	119	119	91%	4.00E-32	28% XP_755425.1
	enoyl-CoA hydratase [Aspergillus fumigatus Af293]	118	118	90%	4.00E-32	30% XP_755398.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	90.9	90.9	89%	7.00E-22	26% XP_747085.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	87.8	87.8	89%	2.00E-20	29% XP_749938.1
	mitochondrial 3-hydroxyisobutyryl-CoA hydrolase [Aspergillus fumigatus Af293]	65.1	65.1	72%	3.00E-12	24% XP_753374.1
	peroxisomal D3,D2-enoyl-CoA isomerase [Aspergillus fumigatus Af293]	58.5	58.5	64%	2.00E-10	24% XP_747673.1
b1393	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	197	197	98%	1.00E-62	40% XP_755425.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	170	170	96%	2.00E-52	41% XP_747085.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	161	161	95%	4.00E-49	40% XP_748661.1
	enoyl-CoA hydratase [Aspergillus fumigatus Af293]	122	122	94%	6.00E-34	32% XP_755398.1
	mitochondrial methylglutaconyl-CoA hydratase (Auh) [Aspergillus fumigatus Af293]	121	121	96%	4.00E-33	34% XP_754471.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	97.1	97.1	94%	5.00E-24	31% XP_749938.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	94.4	94.4	96%	2.00E-23	27% XP_754174.1
	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus Af293]	85.5	85.5	90%	4.00E-20	30% XP_754855.1
	mitochondrial 3-hydroxyisobutyryl-CoA hydrolase [Aspergillus fumigatus Af293]	67	67	74%	5.00E-13	26% XP_753374.1
PP_3280	3-ketoacyl-CoA thiolase (POT1) [Aspergillus fumigatus Af293]	239	239	98%	3.00E-75	40% XP_749100.1
	peroxisomal 3-ketoacyl-coA thiolase (Kat1) [Aspergillus fumigatus Af293]	220	220	97%	3.00E-68	39% XP_755468.1
	acetyl-CoA-acetyltransferase [Aspergillus fumigatus Af293]	214	214	98%	2.00E-65	38% XP_751294.1
	acetyl-CoA acetyltransferase [Aspergillus fumigatus Af293]	201	201	97%	3.00E-61	35% XP_747207.1
	3-ketoacyl-coA thiolase peroxisomal A precursor [Aspergillus fumigatus Af293]	202	202	97%	4.00E-61	36% XP_751733.1
18	3-ketoacyl-CoA ketothiolase (Kat1) [Aspergillus fumigatus Af293]	201	201	96%	8.00E-61	36% XP_752635.1
b1397	3-ketoacyl-CoA thiolase (POT1) [Aspergillus fumigatus Af293]	224	224	98%	2.00E-69	39% XP_749100.1
	acetyl-CoA-acetyltransferase [Aspergillus fumigatus Af293]	221	221	100%	3.00E-68	37% XP_751294.1
	peroxisomal 3-ketoacyl-coA thiolase (Kat1) [Aspergillus fumigatus Af293]	212	212	97%	4.00E-65	37% XP_755468.1
	3-ketoacyl-coA thiolase peroxisomal A precursor [Aspergillus fumigatus Af293]	205	205	97%	1.00E-62	36% XP_751733.1
	3-ketoacyl-CoA ketothiolase (Kat1) [Aspergillus fumigatus Af293]	201	201	98%	7.00E-61	35% XP_752635.1
	acetyl-CoA acetyltransferase [Aspergillus fumigatus Af293]	200	200	98%	1.00E-60	33% XP_747207.1

A. fumigatus AF293 using e value threshold 10 ⁻⁸										Results from BLAST against P. putida or E. coli genome									
Step in Pathway	Locus Tag(s) in P. putida	Locus Tag(s) in E. coli	Description	Max Score	Total Score	Query cover	E value	Ident	Accession	Description	Max Score	Total Score	Query cover	E value	Ident	Accession	P. putida/E. coli Locus Tag	P. putida/E. coli Gene Name	A. fumigatus locus tag
7	PP_3463	b1385	aldehyde dehydrogenase Alox [Aspergillus fumigatus AF293]	420	420	96%	8.00E-143	45%	XP_753026.1	aldehyde dehydrogenase [Pseudomonas]	432	432	89%	8.00E-148	45%	WP_010954231.1	PP_2487	hda	
			aldehyde dehydrogenase Alox2 [Aspergillus fumigatus AF293]	404	404	95%	3.00E-136	44%	XP_746075.1	MULTISPECIES: aldehyde dehydrogenase	401	401	83%	1.00E-135	44%	WP_010954231.1	PP_3463	hda	AFUA_0020310
			aldehyde dehydrogenase [Aspergillus fumigatus AF293]	367	367	95%	6.00E-122	40%	XP_753027.1	MULTISPECIES: aldehyde dehydrogenase	410	410	86%	2.00E-139	44%	WP_001009060.1	PP_2217	hda	
			aldehyde dehydrogenase [Aspergillus fumigatus AF293]	358	358	95%	1.00E-119	39%	XP_746831.1	MULTISPECIES: aldehyde dehydrogenase	413	413	94%	2.00E-141	44%	WP_001009060.1	PP_2217	hda	
			aldehyde dehydrogenase Alox [Aspergillus fumigatus AF293]	348	348	98%	1.00E-114	37%	XP_751026.1	MULTISPECIES: aldehyde dehydrogenase	427	427	83%	8.00E-146	45%	WP_001009060.1	PP_2217	hda	
			aldehyde dehydrogenase Alox2 [Aspergillus fumigatus AF293]	335	335	95%	2.00E-109	34%	XP_746075.1	MULTISPECIES: aldehyde dehydrogenase	374	374	84%	4.00E-125	40%	WP_001009060.1	PP_2217	hda	
			aldehyde dehydrogenase [Aspergillus fumigatus AF293]	331	331	95%	6.00E-109	39%	XP_749209.1	betaine-aldehyde dehydrogenase [Esche	364	364	95%	2.00E-122	43%	AA081838.1		hda	
			aldehyde dehydrogenase [Aspergillus fumigatus AF293]	280	280	93%	3.00E-89	36%	XP_746469.1	MULTISPECIES: NAD-dependent succinat	281	281	93%	4.00E-80	35%	WP_00772831.1	PP_3061	hda	
			betaine-aldehyde dehydrogenase [Aspergillus fumigatus AF293]	280	280	95%	7.00E-89	36%	XP_747215.1	betaine-aldehyde dehydrogenase [Esche	348	348	96%	0	59%	AA081838.1		hda	
			betaine-aldehyde dehydrogenase [Aspergillus fumigatus AF293]	277	277	95%	6.00E-88	36%	XP_749791.1	MULTISPECIES: betaine-aldehyde dehydro	356	356	95%	0	59%	WP_000809110.1	PP_3012	hda	
			aldehyde dehydrogenase [Aspergillus fumigatus AF293]	276	276	94%	1.00E-87	36%	XP_746831.1	MULTISPECIES: NAD-dependent succinat	284	284	94%	2.00E-91	35%	WP_00772831.1	PP_3061	hda	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus AF293]	276	276	95%	2.00E-87	36%	XP_746831.1	MULTISPECIES: betaine-aldehyde dehydro	300	300	92%	5.00E-97	37%	WP_000809110.1	PP_3012	hda	
			succinate-semialdehyde dehydrogenase [Aspergillus fumigatus AF293]	266	266	90%	1.00E-83	34%	XP_754801.1	MULTISPECIES: NAD-dependent succinat	434	434	96%	1.00E-149	44%	WP_00772831.1	PP_3061	hda	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus AF293]	254	254	90%	4.00E-79	33%	XP_746800.1	MULTISPECIES: NAD-dependent succinat	254	254	94%	1.00E-79	34%	WP_00772831.1	PP_3061	hda	
			succinate-semialdehyde dehydrogenase Ugg2 [Aspergillus fumigatus AF293]	254	254	94%	6.00E-79	35%	XP_752009.1	MULTISPECIES: NAD-dependent succinat	556	556	90%	0	55%	WP_00772831.1	PP_3061	hda	
succinate-semialdehyde dehydrogenase [Aspergillus fumigatus AF293]	252	252	94%	2.00E-78	33%	XP_748332.1	MULTISPECIES: NAD-dependent succinat	527	527	96%	0	52%	WP_00772831.1	PP_3061	hda				
aldehyde dehydrogenase family protein [Aspergillus fumigatus AF293]	242	242	91%	8.00E-75	35%	XP_753543.1	MULTISPECIES: NAD-dependent phenyla	242	242	92%	3.00E-75	35%	WP_000138615.1	PP_3038	hda	AFUA_0005520			
10	PP_3279	b1398	No Significant Similarity Found																
			No Significant Similarity Found																
9	PP_0344 PP_3281	b1396	No Significant Similarity Found																
			No Significant Similarity Found																
11	PP_3274 PP_3275 PP_3277 PP_3278		cytochrome b5 reductase [Aspergillus fumigatus AF293]	75.1	75.1	65%	3.00E-15	26%	XP_748717.1	hybrid-cluster NAD(P) dependent oxidor	84.3	84.3	45%	2.00E-18	30%	WP_010954678.1	PP_3274	hcb	
			flavohemoprotein [Aspergillus fumigatus AF293]	70.5	70.5	59%	1.00E-13	27%	XP_747410.1	MULTISPECIES: flavohemoprotein [Pant	300	300	92%	2.00E-99	42%	WP_010952044.1	PP_3274	hcb	
			No Significant Similarity Found																
			No Significant Similarity Found																
			No Significant Similarity Found																
13 and 14	PP_3283	b1388 b1389 b1390 b1391 b1392	No Significant Similarity Found																
			No Significant Similarity Found																
			No Significant Similarity Found																
			No Significant Similarity Found																
			No Significant Similarity Found																
			cytochrome b5 reductase [Aspergillus fumigatus AF293]	70.1	70.1	55%	1.00E-13	24%	XP_753636.1	MULTISPECIES: 1,2-phenylacetyl-CoA ep	70.1	70.1	40%	7.00E-14	24%	WP_000206388.1	PP_3392	hda	AFUA_0610060
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	121	121	98%	2.00E-13	29%	XP_755425.1	MULTISPECIES: enoyl-CoA hydratase [Pu	270	270	82%	1.00E-91	56%	WP_003250095.1	PP_2217	hcb	
			enoyl-CoA hydratase [Aspergillus fumigatus AF293]	103	103	97%	3.00E-16	30%	XP_753538.1	MULTISPECIES: enoyl-CoA hydratase [Pu	139	139	91%	1.00E-10	32%	WP_001292533.1	PP_2217	hcb	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	92.4	92.4	93%	1.00E-12	27%	XP_747085.1	MULTISPECIES: enoyl-CoA hydratase [Pu	183	183	89%	4.00E-58	43%	WP_003250095.1	PP_2217	hcb	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	92	92	98%	2.00E-12	28%	XP_748661.1	MULTISPECIES: enoyl-CoA hydratase [Pu	137	137	90%	3.00E-40	33%	WP_003250095.1	PP_2217	hcb	
			mitochondrial methylglutacetyl-CoA hydratase [Auh] [Aspergillus fumigatus AF293]	78.6	78.6	95%	2.00E-17	26%	XP_754771.1	MULTISPECIES: enoyl-CoA hydratase [Pu	106	106	84%	7.00E-28	30%	WP_003250095.1	PP_2217	hcb	
			mitochondrial 3-hydroxyisobutyryl-CoA hydratase [Aspergillus fumigatus AF293]	76.6	76.6	73%	3.00E-16	28%	XP_753741.1	MULTISPECIES: crotonase [Pseudomonas	220	220	58%	3.00E-67	37%	WP_010954541.1	PP_3481	hcb	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	67	67	77%	2.00E-13	29%	XP_748455.1	MULTISPECIES: enoyl-CoA hydratase [Pu	86.3	86.3	70%	1.00E-20	35%	WP_010954561.1	PP_3481	hcb	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	106	106	96%	1.00E-17	31%	XP_755425.1	MULTISPECIES: enoyl-CoA hydratase [Pu	270	270	82%	1.00E-91	56%	WP_003250095.1	PP_2217	hcb	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	105	105	95%	1.00E-17	31%	XP_748661.1	MULTISPECIES: enoyl-CoA hydratase [Pu	137	137	90%	3.00E-40	33%	WP_003250095.1	PP_2217	hcb	
enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	102	102	91%	2.00E-16	31%	XP_747085.1	MULTISPECIES: enoyl-CoA hydratase [Pu	183	183	89%	4.00E-58	43%	WP_003250095.1	PP_2217	hcb				
enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	100	100	93%	3.00E-15	32%	XP_749398.1	MULTISPECIES: enoyl-CoA hydratase [Pu	88.2	88.2	78%	1.00E-24	31%	WP_010954561.1	PP_3726	hcb	AFUA_1001890			
15	PP_3276	b1394	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	125	125	99%	1.00E-14	31%	XP_755425.1	MULTISPECIES: 2,3-dehydroacyl-CoA h	197	197	87%	5.00E-63	40%	WP_001292533.1	PP_3893	hda	
			enoyl-CoA hydratase [Aspergillus fumigatus AF293]	117	117	97%	5.00E-12	31%	XP_753538.1	MULTISPECIES: 2,3-dehydroacyl-CoA h	125	125	91%	1.00E-10	32%	WP_001292533.1	PP_3893	hda	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	112	112	96%	3.00E-10	32%	XP_747085.1	MULTISPECIES: 2,3-dehydroacyl-CoA h	170	170	91%	7.00E-53	41%	WP_001292533.1	PP_3893	hda	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	105	105	94%	5.00E-17	31%	XP_749398.1	MULTISPECIES: 2,3-dehydroacyl-CoA h	97.1	97.1	78%	2.00E-24	31%	WP_001292533.1	PP_3893	hda	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	99.4	99.4	97%	3.00E-15	29%	XP_748661.1	MULTISPECIES: 2,3-dehydroacyl-CoA h	161	161	91%	1.00E-49	40%	WP_001292533.1	PP_3893	hda	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	89.7	89.7	96%	1.00E-21	26%	XP_754774.1	MULTISPECIES: carnitine-CoA dehydrata	127	127	91%	6.00E-36	32%	WP_001295419.1	PP_3893	hda	
			peroxisomal DLDL enoyl-CoA isomerase [Aspergillus fumigatus AF293]	89.4	89.4	89%	2.00E-21	29%	XP_747673.1	MULTISPECIES: 2-(L)-apoyl-L-2-dihydro	72.8	72.8	88%	8.00E-16	29%	WP_00069784.1	PP_3894	hda	AFUA_0604040
			aldehyde dehydrogenase family protein [Aspergillus fumigatus AF293]	76.6	76.6	61%	4.00E-15	25%	XP_746556.1	MULTISPECIES: NAD-dependent succinat	233	233	98%	4.00E-72	33%	WP_010953586.1	PP_3913	hda	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus AF293]	68.2	68.2	42%	2.00E-12	27%	XP_755001.1	aldehyde dehydrogenase [Pseudomonas]	219	219	98%	2.00E-66	31%	WP_010953586.1	PP_3913	hda	
			aldehyde dehydrogenase [Aspergillus fumigatus AF293]	62.8	62.8	66%	1.00E-10	23%	XP_754181.1	MULTISPECIES: coniferyl aldehyde dehydro	237	237	90%	4.00E-73	33%	WP_010953586.1	PP_3913	hda	
			succinate-semialdehyde dehydrogenase [Aspergillus fumigatus AF293]	83.2	83.2	65%	5.00E-17	25%	XP_754801.1	MULTISPECIES: NAD-dependent succinat	434	434	96%	1.00E-149	44%	WP_00772831.1	PP_3961	hda	
			aldehyde dehydrogenase family protein [Aspergillus fumigatus AF293]	72.4	72.4	44%	1.00E-13	25%	XP_755001.1	MULTISPECIES: lactaldehyde dehydrog	236	236	98%	2.00E-73	31%	WP_001155443.1	PP_3961	hda	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	121	121	98%	2.00E-13	29%	XP_755425.1	MULTISPECIES: enoyl-CoA hydratase [Pu	270	270	82%	1.00E-91	56%	WP_003250095.1	PP_2217	hcb	
			enoyl-CoA hydratase [Aspergillus fumigatus AF293]	103	103	97%	2.00E-16	30%	XP_753538.1	MULTISPECIES: enoyl-CoA hydratase [Pu	139	139	91%	1.00E-10	32%	WP_001292533.1	PP_2217	hcb	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	92.4	92.4	93%	1.00E-12	27%	XP_747085.1	MULTISPECIES: enoyl-CoA hydratase [Pu	183	183	89%	4.00E-58	43%	WP_003250095.1	PP_2217	hcb	
enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	92	92	98%	2.00E-12	28%	XP_748661.1	MULTISPECIES: enoyl-CoA hydratase [Pu	137	137	90%	3.00E-40	33%	WP_003250095.1	PP_2217	hcb				
mitochondrial methylglutacetyl-CoA hydratase [Auh] [Aspergillus fumigatus AF293]	78.6	78.6	95%	2.00E-17	26%	XP_754771.1	MULTISPECIES: enoyl-CoA hydratase [Pu	106	106	84%	7.00E-28	30%	WP_003250095.1	PP_2217	hcb				
mitochondrial 3-hydroxyisobutyryl-CoA hydratase [Aspergillus fumigatus AF293]	76.6	76.6	73%	3.00E-16	28%	XP_753741.1	MULTISPECIES: crotonase [Pseudomonas	220	220	58%	3.00E-67	37%	WP_010954541.1	PP_3481	hcb				
enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	67	67	77%	2.00E-13	29%	XP_748455.1	MULTISPECIES: enoyl-CoA hydratase [Pu	86.3	86.3	70%	1.00E-20	35%	WP_010954561.1	PP_3481	hcb				
16	PP_3276	b1394	enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	106	106	96%	1.00E-17	31%	XP_755425.1	MULTISPECIES: enoyl-CoA hydratase [Pu	270	270	82%	1.00E-91	56%	WP_003250095.1	PP_2217	hcb	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	105	105	95%	1.00E-17	31%	XP_748661.1	MULTISPECIES: enoyl-CoA hydratase [Pu	137	137	90%	3.00E-40	33%	WP_003250095.1	PP_2217	hcb	
			enoyl-CoA hydratase/isomerase family protein [Aspergillus fumigatus AF293]	102															