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In silico analysis for prediction of degradative capacity of *Pseudomonas putida* SF1

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ABSTRACT

The study employs draft genome sequence data to explore *p*-nitrophenol (PNP) degradation activity of *Pseudomonas putida* strain SF-1 at a genomic scale. Annotation analysis proposes that the strain SF1 not only possesses the gene cluster for PNP utilization but also for the utilization of benzoate, catechol, hydroxybenzoate, protocatechuate, and homogentisate. Further, the analysis was carried out to understand more details of PNP 4-monooxygenase and its regulator. A comparative analysis of PNP 4-monooxygenase from SF1 was carried out for prediction of its tertiary structure; and also its binding affinity with PNP, FAD, NADH and NADPH using FlexX docking. The tertiary structure of regulator was also predicted along with its conserved DNA binding residues. Regulator binding site (RBS) and promoter region were mapped for the PNP degradation gene cluster. Based on genome sequence analysis, the study unveiled the genomic attributes for a versatile catabolic potential of *Pseudomonas putida* strain SF-1 for different aromatic compounds.

1. Introduction

In silico based genome analysis can be used as a proxy for assigning functional attributes to microbes (Overbeek et al., 2014). Owing to the efficacy of genome data analysis to characterize microbial features there has been an avalanche of microbial genome data in recent times. Among the various bacteria reported for PNP degradation in an environment, *Pseudomonas* being the dominant genera. Although, there exist enough genome data for *Pseudomonas*, but an intensive genome mining has not been carried out to a substantial level for PNP catabolic machinery. Moreover, the tertiary structure for protein PNP 4-monooxygenase (PnpA) has not yet been elucidated till now. This gap further extends to the quaternary protein structure of regulator of corresponding gene and determination of consensus region of Pnp gene cluster.

The microbial aerobic degradation pathway of PNP has been reported to have two different routes. In one of utilization pathway, PNP with the removal of nitrate gets converted to hydroquinone as intermediate, so it is known as hydroquinone pathway (HQ) whereas the alternative pathway results to benzenetriol as an intermediate. BT pathway is preferentially found in gram-positive bacteria such as *Rhodococcus* spp. (Kramer et al., 1999; Kitagawa et al., 2004), *Arthrobacter* spp. (Kadiyala and Spain, 1998) and *Bacillus* spp. (Jain et al., 1994) whereas HQ pathway is a feature found in many gram-negative bacteria such as *Pseudomonas* spp., *Moraxella*, and *Burkholderia* spp. (Spain et al., 2000). Moreover, a simultaneous presence of both the pathways has been reported in *Pseudomonas* strains WBC-3 and NyZ402 (Wei et al., 2010b; Zhang et al., 2009) for PNP degradation. Approximately 25 PNP degrading bacteria have been reported till date and studied for their potential to bioremediate PNP but very few have been deployed for genome sequence analysis which confers an edge over conventional microbiological approach.

In the present study, the genome of *Pseudomonas putida* SF1 strain was sequenced and analysed for the genetic features responsible for its catabolic potential. This study includes the deduction of the tertiary protein structure of PnpA which is the key enzyme in PNP degradation pathway. The enzymatic behavior of PnpA was also validated through protein-ligand docking. Additionally, the study on promoter and regulator binding region of operons $PnpC_2C_1DEC$, PnpB and PnpA stretches the data analysis in characterizing the PNP degradation gene cluster.

2. Results

2.1. Comparative genomics of Pseudomonas putida SF1 genome

The 5,805,879 bp draft genome of SF1 was assembled into 292 contigs that have been annotated by NCBI PGAAP into 5286 coding sequences (CDSs) as shown in Table S1. RAST analysis classified SF1 genome into 523 metabolic subsystems including various aromatic compounds utilization pathways. The phylogenetic tree of 16S rRNA gene

Abbreviation: PNP, para-nitrophenol; 4NC, 4-nitrocatechol; RBS, Regulator Binding Site; FAD, Flavin Adenine Dinucleotide; NADH, Nicotinamide Adenine Dinucleotide; NADPH, Nicotinamide Adenine Dinucleotide Phosphate; HQ, Hydroquinone; BT, Benzenetriol; LB, Luria Bertani; CDS, Coding Sequences; RAST, Rapid Annotations using Subsystems Technology; PGAAP, Prokaryotic Genomes Automatic Annotation Pipeline.

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of SF1 showed a very close taxonomic relationship with *Pseudomonas putida* DLL-E4 (PNP degraders) (Fig. S1). SF1 was also been found close to other PNP degrading *Pseudomonas*. Phylogenetic comparison of PnpA genes from selected PNP degrading bacteria placed the *Pseudomonas putida* SF1 PNP 4-monooxygenase in close proximity with DLL-E4, NyZ402, WBC-3 and sp.1–7; of which DLL-E4 shows 100% similarity (Fig. S2). *Burkholderia* spp. monooxygenases were also found in close similarity with SF1 but for further study and comparison, we selected only *Pseudomonas* spp. Only *Pseudomonas nitroreducens* strain PS-2 and *Pseudomonas* sp. 1–7 (2) were found to be phylogenetically distant from other PNP degrading *Pseudomonas*. PNP degrading Arthrobacter have separated far away from *Pseudomonas* which justify the presence of a different mechanism of PNP degradation in that strain.

2.2. Mining of aromatic compound degradation pathways in SF1 draft genome

The *in silico* analysis of the SF1 genome revealed the genes coding for enzymes involved in the metabolic pathways which are biochemically characterized earlier from SF1 and their arrangement on the genome was compared with previously established gene arrangement.

2.2.1. Para-nitrophenol degradation pathway

Annotation from RAST and local BLAST analysis revealed that SF1 has all the enzymatic genes responsible for degradation of PNP *via* HQ pathway. In addition, it also has one of the enzymes responsible for degradation of PNP *via* BT pathway *i.e.* 1,2,4 benzenetriol dioxygenase. The degradation of 4NC by SF1 during degradation study suggests that degradation of PNP can also follow BT pathway. PnpA may be responsible for the conversion of PNP to 4NC and subsequent conversion of 4NC to 1,2,4 benzenetriol, as reported in WBC-3 (Zhang et al., 2009). All the target genes were located in contig 16; *viz.*, PnpA (*p*-nitrophenol monooxygenase), PnpB (*p*-benzoquinone reductase), $PnpC_1$ (hydroquinone dioxygenase large subunit), $PnpC_2$ (hydroquinone dioxygenase small subunit), PnpD (4-hydroxymuconic semialdehyde dehydrogenase), PnpE (maleyl acetate reductase) and PnpC (benzenetriol dioxygenase). The LysR type of transcriptional regulator found to be necessary for regulation of complete degradation of PNP to adipate is also associated with this gene cluster. The arrangement of all the genes associated with PNP degradation was noticed to be in a pattern similar to those found in the strain DLL-E4. Even the two hypothetical proteins (X_1, X_2) present between PnpC and PnpB were reported in SF1. BLAST analysis carried out between individual genes of SF1 and other PNP degraders, DLL-E4, NyZ402 WBC-3, and sp.1–7 demonstrated a very high sequence homology; with strain DLL-E4 showing 100% homology for Pnp 4-monooxygenase, Malelylacetate reductase and Hydroquinone dioxygenase (a and b-subunit) (Table S2 and Fig. S3). Fig. 1 shows that the physical organization of Pnp genes of SF1 is similar to *Pseudomonas* Nyz402, WBC-3 and sp.1–7, but it is different from those of *Burkholderia* SJ98 (Vikram et al., 2013) where Pnp genes are in two different contigs.

2.2.2. Benzoate degradation pathway

The benzoate degradation in SF1 is initiated by benzoate dioxygenase (BenABC) to yield catechol which undergoes ortho-cleavage and *via* β-ketoadipate pathway enters the central carbon metabolism. A complete benzoate utilization system is present in SF1 including the benzoate transcriptional regulator (BenR) and benzoate transporter and porin protein (BenE, BenF, and BenK). All the benzoate utilization genes were located on contig 4. The arrangement of ben genes were quite similar to other *Pseudomonas* benzoate gene cluster (*Accession no.* KC189953) as shown in Fig. 2a. The supportive catechol pathway genes were found to be located on contig 206 (Fig. 2b). It also harbors transcriptional regulator CatR with it.

2.2.3. P-hydroxybenzoate pathway

p-hydroxybenzoate hydroxylase (PobA) responsible for converting p-hydroxybenzoate to 3.4 di-dihydroxybenzoate (protocatechuate) is present in contig 92 of SF1 genome (Fig. 2d). A pobA regulator (PobR) is also found adjacent to this gene. The downstream protocatechuate pathway genes are present in 4 different contigs *i.e.* contig 19, 57, 163 and 179 (Fig. 2c). The β-ketoadipate from PNP degradation pathway and mucono lactone from benzoate-catechol pathway converges at two points in the protocatechuate pathway suggesting an interlinked signal communication between the regulators of this pathway. The final products of all the three pathways are acetyl-CoA and succinyl

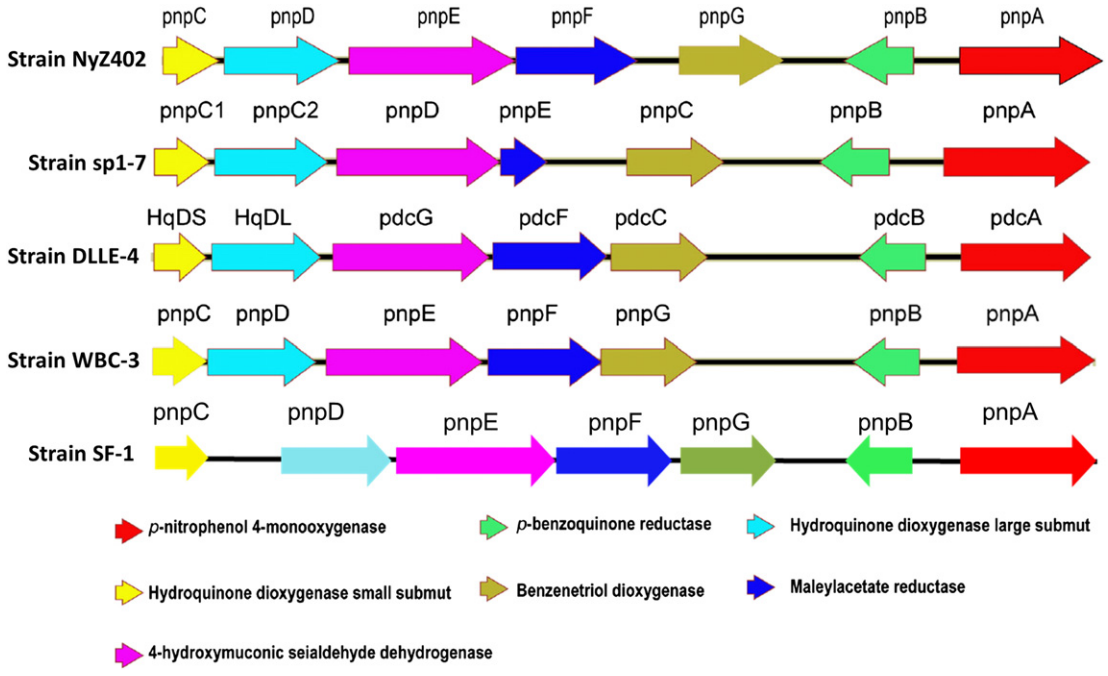

Fig. 1. Comparison of *Pseudomonas putida* SF-1 p-nitrophenol (PNP) degradation gene cluster with other *Pseudomonas* PNP gene cluster. Arrangement of all the genes responsible for PNP degradation were found to be in similar fashion in all the *Pseudomonas* strains under study.