UNVEILING MULTIPLE STRATEGIC BIOREMEDIATION POTENTIAL OF BACTERIAL ENDOPHYTES FROM MEDICINAL PLANT EMILIA SONCHIFOLIA(LINN.) DC. THROUGH METAGENOMIC DETAILING

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ABSTRACT

Plant Microbe consortium developed efficient multistrategic biodegradative mechanism to cope with different toxic pollutants. Native plants with diverse applications and limited resources for large scale cultivation can ensure eco-friendly restoration of polluted areas. Genomic information generated through high sequencing technologies like illumine Hi-sequencing removed the constraints in tackling hidden non-pathogenic microbial population inside plant tissues. Endophytic microbial associations spurred phytoremediation as plant growth promotion activity. Metagenomic screening of bacterial endophytes from Emilia sonchifolia (Linn.) DC. disclosed the phytoremediation potential of both culturable and nonculturable endophytes with specific role in bioremediation. Gene annotations indicating the production of enzymes in degradative pathways of compounds like Chlorocyclohexane and chlorobenzene, Fluorobenzoate, Furfural, Dioxin, Xylene, Toluene, Polycyclic aromatic hydrocarbons, Chloroalkane and chloroalkene, Naphthalene, Aminobenzoate, Nitrotoluene, Ethylbenzene, Styrene, Atrazine, Caprolactam clearly emphasis the role of endophytes in phytoremediation potential of host plants.

Keywords: Bioremediation; Endophyte; Metagenomic sequencing; Gene functional annotations

INTRODUCTION

Pollution and pollutants came in to limelight while analyzing the anthropogenic impact of environment and the existence of organisms. A sustainable harmless technology to reduce the toxic substances from soil, air and water will pave the way for harmonious mutual development of human and environment. Phytoremediation or bioremediation focus on a plant -microbe based cycling and degradation of pollutants. Soil and rhizospheric microbes have been investigated for their participation in biodegradation of organic nutrients and its recycling. Identification of microorganisms living inside plant tissues in harmony increased the inquisitiveness to study their role in plant growth and development. The role of these endophytic microorganisms in plant growth through the production of phytohormones and different metabolites were already reported (Tao *et al*., 2021). Bioremediation assistance

provided by endophytes offer promising stress management methods to host through degradation and sequestration.

Advancement in microbe mediated biodegradation technologies improved the management strategies of toxic pollutants. Application of specially designed microorganisms increased the degradation of pollutants but the byproduct in some cases is harmful and persistent (Khan and Doty, 2011). Direct application of microbial communities (Pal *et al*., 2020) or planting resistant varieties (Gajic *et al*., 2018) in polluted sites were methods employed as part of phytoremediation.

Plant microbe interactions at the level of endophytism involves complex molecular gene expressions and regulations. Endophytes from different plant sources were isolated through culture dependent methods and their phytoremediation potential were analyzed by inoculum-based methodologies. Advanced metagenomic approaches increased our accessibility to poor understood highly diverse endophytic communities. Screening of gene functional annotations and identification of functional genes from endophytes imparting specific roles in degradative pathways of toxic pollutants gives clearcut idea about bioremediation potential of endophytes. *Emilia sonchifolia* (Linn.)DC a widely present medicinal plant used for the treatment of many inflammation related disorders(Essien *et al*., 2009). This plant shows wide distribution and require less technical management for cultivation.

Identification of specific functional gene annotations in bacterial endophytes from *E. sonchifolia* (Linn.) DC through metagenomic analysis indicates its greater application in bioremediation perspective other than its high medicinal properties.

MATERIALS AND METHODS

For metagenomic studies surface sterilised plant material *E. sonchifolia* was used for DNA extraction. The extracted DNA was quantified, fragmented and amplified by PCR (PCR for 8 cycles using P5 and P7 primers). For loading in Illumina sequencing and for the identification of sequenced reads adaptors were ligated to the DNA fragments. These NGS library preparation were done and the indexed libraries prepared were loaded on the Illumina HiSeq instrument and the different indices were multiplexed and the sequencing done according to manufacturer's instructions (Illumia, San Diego, CA, USA) (Urumbil and Anilkumar, 2021). The original data stored in fastq format after analysed by Bcl2fastq.

The quality of the reads was analysed based on ASCII standard and Phred quality score. The quality score of the first 25 reads noted and reads with quality score (Q20) less than 20 was discarded. The pass filtered data was optimised and errors were reduced using next generation data quality software Cutadapt (v.1.9.1) before downstream analysis. Host sequences were removed with the help of BWA (v0.7.12) software which can interrupt metagenomic screening. Whole genome denovo assembly and processing performed by MEGAHIT (v 1.1.3), with different K-mer (39, 59, 79, 119) and scaffolds selected for gene prediction analysis. Prodigal (v 3.02) and CD-HIT (v4.5.6) used read analysis and reduction of redundancy of the predicted gene. Pre-processed reads were now aligned to non-redundant set of genes with the help of Soap Aligner (v2.21) which generated gene abundance or reads coverage of the genes at 95% identity and 90% coverage level.

Different databases like Nr database (non-redundant protein database), KEGG pathway database (Kyoto encyclopedia of genes and genomes database), eggNOG (evolutionary geneology of genes: Non-Supervised Orthologous Groups, Version 4.0) and CAZy database (Carbohydrate Active enZYmes Database, Diamond Version 0.8.15.77 and BLAST Version 2.2.31+) were used for the database search and alignment to predict the gene functional annotations. Gene annotation resulted from each database was used to categorise

relative abundance of different functional categories. Gene functional annotations helped for the identification of relevant genes involving phytoremediation potential of endophytes.

RESULTS AND DISCUSSION

Pollutants are usually synthetic organic compounds that when accumulate in the environment and enter in to organisms causing serious health problems. Different traditional technologies like incineration, landfills, recycling etc. were employed to manage these pollutants. During the attempts to manage these pollutants by treating the toxic contaminants with microorganisms can change them to less toxic or even non-toxic compounds and the process is termed as biodegradation or bioremediation. One of the advantages of bioremediation is that it is a natural degradation process applicable to a wide range of pollutants like polycyclic and aromatic hydrocarbons, synthetic pesticides and chlorinated organic acids etc. There have been arrays of bioremediation methods developed to tackle many of the pollutants of our environment. Recently scientists reported bacteria mediated bioremediation methods as one of the natural and effective method of removal of pollutants (Sayler and Ripp, 2000, Cheung and Gu, 2007, Sim *et al*., 2019). High end sequencing technology like Illumina HiSeq were employed to analyse the microbial communities mediating the bioremediation process of hydrocarbon contaminated soil (Siles *et al*., 2018). Xu *et al*. (2017) employed biophotocatalystic system and bioremediation for the effective degradation of C16 alkane.

Endophytic microbes usually succour host plants under stress conditions. They have the ability to degrade various toxic pollutants. Metagenome analysis of endophytes from *E. sochifolia* uncovered a large number of genes involved in the degradation pathways of compounds like Chlorocyclohexane and chlorobenzene, Fluorobenzoate, Furfural, Dioxin, Xylene, Toluene, Polycyclic aromatic hydrocarbons, Chloroalkane and chloroalkene, Naphthalene, Aminobenzoate, Nitrotoluene, Ethylbenzene, Styrene, Atrazine, Caprolactam. Some enzymes in these pathways were very advantageous to host plants as they participate in the biodegradation of different toxic compounds. Haloacetate dehalogenase (*dehH, dhaA*) were present in the degradation pathway of chlorocyclohexane, chlorobenzene, chloroalkane and chloroalkene. Benzaldehyde dehydrogenase (NAD) (*xylC*) found in the degradation pathways of both xylene and toluene(Fig 1 and Fig.2). Another enzyme salicylate hydrolase was one of the enzyme members that take part in the step wise degradation of polycyclic aromatic hydrocarbons like Naphthalene. Enzymes like NADP-dependant aldehyde dehydrogensae were also present and they showed their presence in the degradation pathways of aromatic compounds. Other major enzymes involved in the degradation pathways of other toxic pollutants were summarised in (Table 1). Metabolism of xenobiotics is very common among microbes like bacteria. The endophytic metagenome analysis also detected enzyme glutathione-s- transferase (*gst*) and its presence in the metabolism of xenobiotics by cytochrome P450 has been confirmed.

Endophytic bacteria were also screened for their bioremediation property. They were associated with plants and their bioremediation activity adds benefits to plants in terms of stress tolerance to toxic pollutants. Many endophytes with phytoremediation potential were recognised. Endophytic strains of bacteria like *Pseudomonas putida* VM1441 (pNAH7) associated with *Pisum sativum* showed naphthalene degradation potential (Germaine *et al*., 2009). Endophytic bacteria from *Pseudomonas, Bacillus, Micrococcus, Rhodococcus* and *Flavobacterium* were identified from *C. cajan* and *L. purpureus*. Plants effectively managed contamination due to polycyclic aromatic hydrocarbons due to the presence of endophytes (Riskuwa-Shehu and Ismail, 2018, Yahaya *et al*., 2019). The metagenome analysis of endophytic bacteria from *E. sonchifolia* showed distinct pathways for the degradation of

pollutants like dioxin, Naphthalene, Nitrotulene etc. Presence of enzymes like haloacetate dehalogenase (*dehH*), haloalkane dehalogenase (*dha A*) and 2-haloacid dehalogenase (E3.8.1.2) indicated the ability of these bacteria to dehalogenate some toxic halogenated pollutants coming in the categories of chlorocyclo alkene, cholorocyclohexane etc. They can be effectively used for the degradation of chlorinated organic herbicides like 2, 4-D, as indexed by the phytoremediation studies to remove 2, 4-D by Germaine *et al*. (2006). Enzyme coding genes participating in the degradation of polycyclic aromatic hydrocarbons (PAH) were detected in the present study. Naphthalene a PAH degraded by the enzyme Naphtahalene 1; 2-dioxygenase (*nahAA*) was identified in the metagenomic study. It was reported that this enzyme was more prevalent in the endophytic strains when compared with the rhizospheric microorganisms (Khan and Dotty, 2011).

Metabolism of xenobiotics by endophytic bacteria was considered as another approach for phytoremediation and it decreases the amount of toxic compounds or pollutants in the soil (Barac *et al*., 2004). Presence of Cytochrome P450 mediated metabolism of xenobiotics were also recognised in the metagenome analysis (Fig.3). Presence of Cytochrome P450 mediated xenobiotics were reported from endophytic bacteria (Pawlik *et al.,* 2017). From the whole metagenome analysis, phytoremediation ability and capacity for Cytochrome P450 mediated metabolism of xenobiotics manifested the fact that further field screening analysis were to be conducted to check the phytoremediation potential of this medicinal plant in association with the diverse endophytic microbiome it harbours.

CONCLUSION

Metagenomic analysis incorporated with screening of gene annotations pawed way for the exploration of hidden plant microbial associations. The endophytes regulate plant growth in direct and indirect interactions including the production of phytohormones. When analysing the indirect role of endophytes in enhancing the plant growth phytoremediation potential of endophytes came in to lime lights. Pollution stress management were analysed in detail in this perspective. Associated with this the endophytes shows some bioremediation capacity, which enhances the stress tolerance potential of the host plant. In the present study different catabolic pathways involving gene annotations from this endophytic genome taking part in the degradation of toxic pollutants like toluene, xylene, styrene etc. indicated the phytoremediation potential of the endophyte. High efficiency degradation capacity of endophytes was confirmed by the presence of gene annotations in degradation pathways of chlorinated compounds like chlorecycloalkane and chlorocyclohexane. Multiple strategic approach employed by endophytes in phytoremediation was unveiled by the presence of gene annotations in cytochrome P450 mediated metabolism of xenobiotic. The present study clearly emphasised the metabolic potential of bacterial endophytes from *Emilia sonchifolia* (Linn.)DC. and its application in phytoremediation.

AKNOWLEDGEMENT

The first author wishes to thank University Grants Commision (UGC), Govt. of India, for the financial assistance sanctioned under faculty development programme.

CONFLICT OF INTEREST

The authors have no conflict of interest

REFERENCES

- Barac, T., Taghavi, S., Borremans, B., Provoost, A., Oeyen, L., Colpaert, J.V., Vangronsveld, J., van der Lelie, D. 2004. Engineered endophytic bacteria improve phytoremediation of water-soluble volatile, organic pollutants. Nat Biotechnol. 22:583-588. DOI:10.1038/nbt960.

- Cheung, K.H., Gu, J-D. 2007. Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: A review. Intern. Biodeterioration Biodegra. 59(1):8-15. https://doi.org/10.1016/j.ibiod.2006.05. 002
- Essien, G., Nwidu, L., Nwafor, P.A. 2009. Anti-Inflammatory and Analgesic Potential of Methanolic Extract of *Emilia Sonchifolia* (Compositae) Leaves in Rodents. African J. Biomed. Res. 12. 199-207.
- Gajic, G., Djurdjević, L., Kostić, O., ´Jaric, S., Mitrović,M., Pavlović,P. 2018. Ecological potential of plants for phytoremediation and ecorestoration of fly ash deposits and mine wastes. Front. Environ. Sci. 6:124. doi: 10.3389/fenvs.2018.00124. https://www.frontiersin.org/article/10.3389/fenvs.2018.00124
- Germaine, K.J., Keogh, E., Ryan, D., Dowling, D. 2009. Bacterial endophyte mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiol. Lett. 296: 226-234. https://doi.org/10.1111/j.1574-6968.2009.01637.x.
- Germaine, K.J., Liu, X., Cabellos, G.G., Hogan, J.P., Ryan, D., Dowling, D.N. 2006. Bacterial endophytes enhanced phytoremediation of the organochlorine herbicide 2,4-D (dichlorophenoxyacetic acid). FEMS Microbiol. Ecol. 57(2):302-310. DOI: 10.1111/j.1574-6941.2006.00121.x.
- Khan, Z., Doty, S. 2011. Endophyte assisted phytoremediation. Curr.Top.Plt.Biol. 12:97-105
- Pal, A.K., Singh, J., Soni, R., Tripathi, P., Kamle, M., Tripathi, V., Kumar, P.2020. The role of microorganism in bioremediation for sustainable environment management. Editor(s): Vimal Chandra Pandey, Vijai Singh, Bioremediation of Pollutants, Elsevier:227-249.ISBN 9780128190258. https://doi.org/10.1016/B978-0-12-819025-8.00010-7.
- Pawlik, M., Cania, B., Thijs, S., Vangronsveld, J., Piotrowska-Seget, Z. 2017. Hydrocarbon degradation potential and plant growth promoting activity of cultivable endophytic bacteria of *Lotus corniculatus* and *Oenothera biennis* from a long-term polluted site. Environ. Sci. Pollut. Res. Int. 24(24):19640-19652. DOI: 10.1007/s11356-017-9496-1.
- Riskuwa-Shehu, M.L., Ismail, H.Y. 2018. Isolation of endophytic bacteria and phytoremediation of soil contaminated with polycyclic aromatic hydrocarbons using *Cajanus cajan* and *Lablab purperius*. BSTR. 6(1):26-30.
- Sayler, G. S., Ripp, S. 2000. Field applications of genetically engineered microorganisms for bioremediation processes. Curr.Opin.Biotech. 11(3): 286-289. https://doi.org/10.1016/ S0958 -1669(00)00097-5.
- Siles, J.A., Margesin, R. 2018. Insights into microbial communities mediating the bioremediation of hydrocarbon contaminated soil from an Alpine former military site. Appl.Microbiol. Biotech. 102:4409-4421. http://doi.org/10.1007/s00253-018-8932-6
- Sim, C.S.F., Chen, S.H., Ting, A.S.Y. 2019. Endophytes: Emerging tools for the bioremediation of pollutants. In: Bharagava R., Chowdhary P. (Eds) Emerging and eco-friendly approaches for waste management. Springer, Singapore. https://doi.org/10.1007/978-981-10-8669-4_10.
- Urumbil, S. K., Anilkumar, M. 2021. Metagenomic insights into plant growth promoting genes inherent in bacterial endophytes of *Emilia sonchifolia* (Linn)DC. Plant Science Today. Vol 8(4): 1–11.https://doi.org/10.14719/pst.2021.8.4.1357.
- Tao, L., Qiuhong, L., Fuqiang, Y., Shuhui, Z., Suohui, T., Linyuan, F. 2021. Plant growth-promoting activities of bacterial endophytes isolated from the medicinal plant

Pairs polyphylla var. yunnanensis. World J Microbiol Biotechnol.,38(1):15. doi: 10.1007/s11274-021-03194-0. PMID: 34878606.

- Xu, X., Zhai, Z., Li, H., Wang, Q., Han, X., Yu, H. 2017. Synergetic effect of biophotocatalytic hybrid system: g-C_3N_4 and *Acinetobacter* sp. JLS1 for enhanced degradation of C16 alkane. Chem. Enginee. J. 353: 520-529. http://dx.doi.org/10.1016/j.cej.2017.04.138.
- Yahaya, I. H., Lami, R-S. M., Alkali, A.I., Farouq, A.A., Abakwak, C. S. 2019. Biostimulation Potentials of *Vigna Species* (L.) in Hydrocarbon Impacted Soil. Amer. J. Biosci. Bioengineer. 7(1): 22-27. DOI: 10.11648/j.bio. 20190701.15.

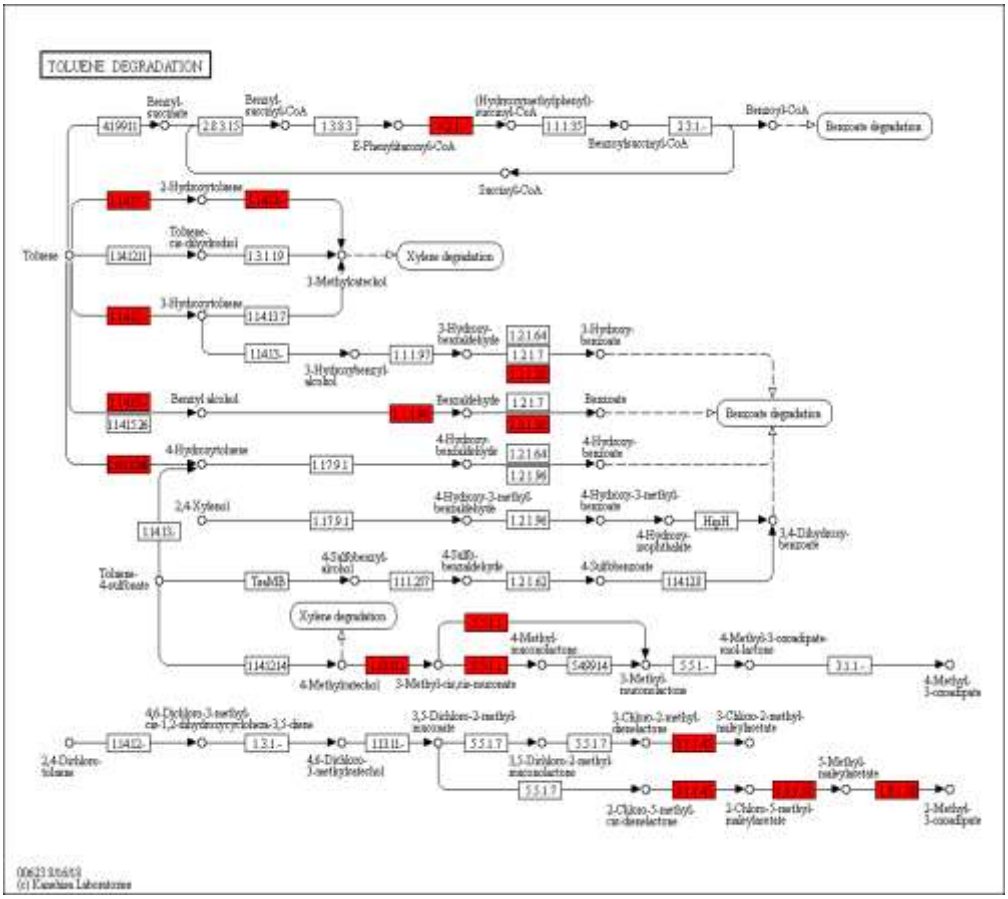

Fig:1: Toluene degradation pathway and the gene annotations from bacterial endophytes were highlighted

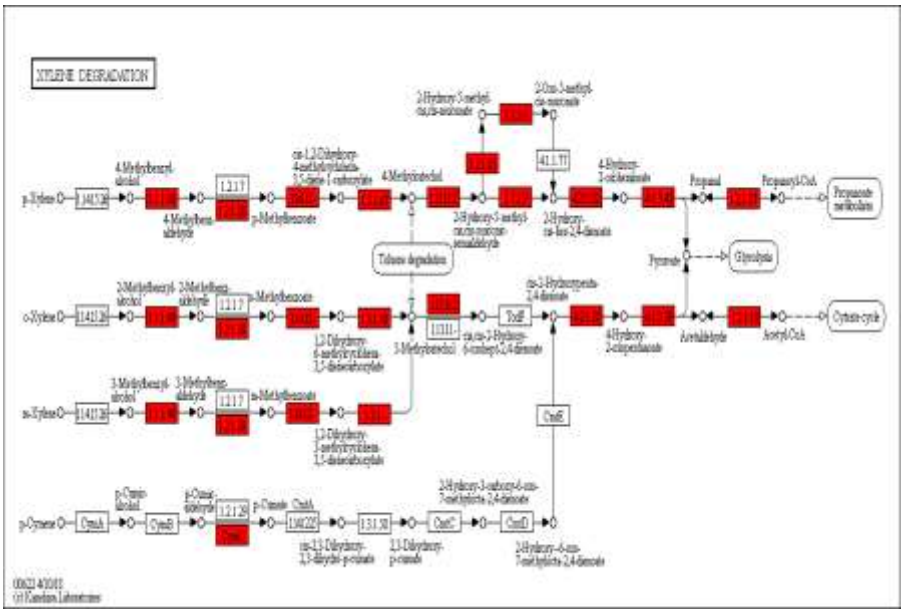

Fig:2: Xylene degradation pathway and the gene annotations from bacterial endophytes were highlighted

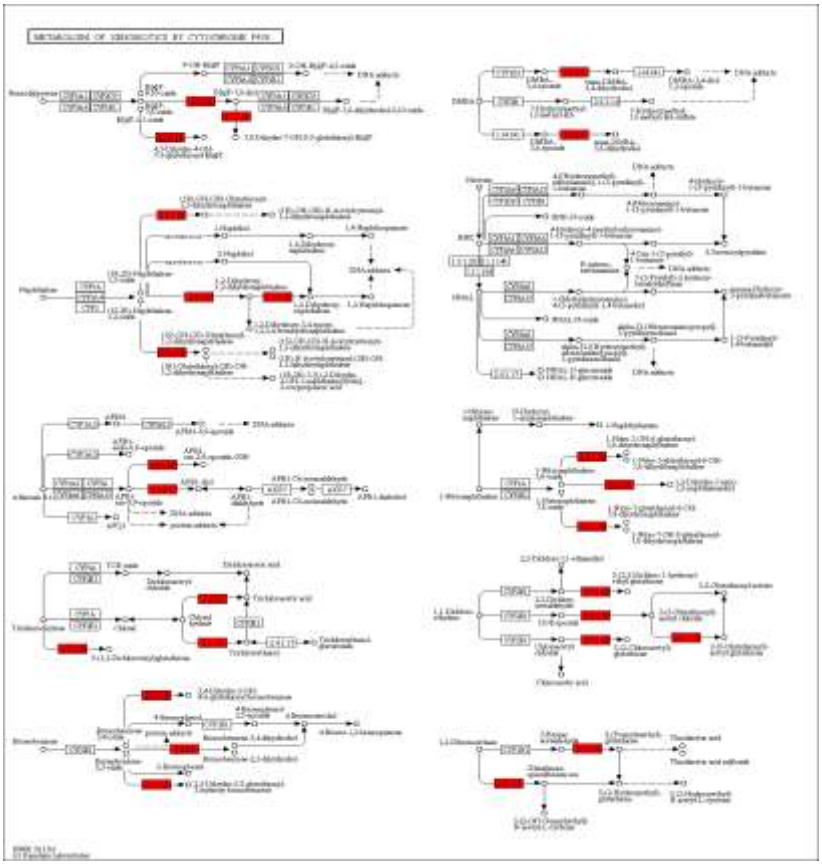

Fig 3: Pathway indicating presents of Cytochrome P450 mediated metabolism of xenobiotics and the gene annotations in the pathway

Table 1-Genes annotated in the Pathways of degradation of toxic compounds

Gene_ Name	Enzyme	EC number	P.No	Pathways involved
dehH	haloacetate dehalogenase	EC:3.8.1.3	2	ko00361 Chlorocyclohexane and Chlorobenzene degradation; ko00625 Chloroalkane and chloroalkene degradation;
dhaA	haloalkane dehalogenase	EC:3.8.1.5	2	
E3.8.1.2	2-haloacid dehalogenase	EC:3.8.1.2	2	
E3.1.1.45	Carboxymethylene butenolidase	EC:3.1.1.45	3	ko00361 Chlorocyclohexane and chlorobenzene degradation; ko00364 Fluorobenzoate degradation; ko00623 Toluene degradation;
dmpP, poxF	phenol hydroxylase P5 protein	--	4	ko00361 Chlorocyclohexane and chlorobenzene degradation;ko00623 Toluene degradation;ko01220 Degradation of aromatic compounds;
adhP	alcohol dehydrogenase, propanol-preferring	EC:1.1.1.1	9	ko00625 Chloroalkane and chloroalkene degradation; ko00626 Naphthalene degradation
benA-xylX	benzoate/toluate 1,2-dioxygenase subunit alpha	EC:1.14.12.10	4	ko00364 Fluorobenzoate degradation; ko00622 Xylene degradation; ko01220 Degradation of aromatic compounds;
xylC	benzaldehyde dehydrogenase (NAD)	EC:1.2.1.28	4	ko00622 Xylene degradation; ko00623 Toluene degradation; ko00627 Aminobenzoate degradation; ko01220 Degradation of aromatic compounds;
benD-xylL	dihydroxycyclohexadiene carboxylate dehydrogenase	EC:1.3.1.25	4	ko00364 Fluorobenzoate degradation; ko00622 Xylene degradation; ko01220 Degradation of aromatic compounds;
praC, xylH	4-oxalocrotonate tautomerase	EC:5.3.2.6	4	ko00362 Benzoate degradation; ko00621 Dioxin degradation; ko00622 Xylene degradation; ko01220 Degradation of aromatic compounds;
praC, xylH	4-oxalocrotonate tautomerase	EC:5.3.2.6	4	
hmfD	2-furoate-CoA ligase	EC:6.2.1.31	1	ko00365 Furfural degradation;
hmfH	5-(hydroxymethyl) furfural/furfural oxidase	EC:1.1.3.47	1	
E1.14.13.1	salicylate hydroxylase	EC:1.14.13.1	4	ko00621 Dioxin degradation;ko00624 Polycyclic aromatic hydrocarbon degradation;ko00626 Naphthalene degradation;ko01220 Degradation of aromatic compounds;
carAa	carbazole 1,9a-dioxygenase	EC:1.14.12.22	2	ko00621 Dioxin degradation;ko01220 Degradation of aromatic compounds;

ligB,A	protocatechuate 4,5-dioxygenase, beta chain, Alpha chain	EC:1.13.11.8	3	ko00362 Benzoate degradation;ko00624 Polycyclic aromatic hydrocarbon degradation;ko00627 Aminobenzoate degradation;
nahAa, nagAa, ndoR, nbzAa, dntAa	naphthalene 1,2-dioxygenase ferredoxin reductase component	EC:1.18.1.7	6	ko00624 Polycyclic aromatic hydrocarbon degradation;ko00626 Naphthalene degradation;ko00627 Aminobenzoate degradation;ko00633 Nitrotoluene degradation;ko00642 Ethylbenzene degradation;ko01220 Degradation of aromatic compounds;
phdE	cis-3,4-dihydro phenanthrene-3,4-diol dehydrogenase	EC:1.3.1.49	2	
pht2	phthalate 4,5-dioxygenase reductase component	EC:1.18.1.-	2	ko00624 Polycyclic aromatic hydrocarbon degradation;ko01220 Degradation of aromatic compounds;
pht5	4,5-dihydroxyphthalate decarboxylase	EC:4.1.1.55	1	ko00624 Polycyclic aromatic hydrocarbon degradation;
faaH	fumarylacetoacetate (FAA) hydrolase	EC:3.7.1.2	2	
FAH, fahA	Fumaryl acetoacetase	EC:3.7.1.2	2	ko00643 Styrene degradation;
amiE	amidase	EC:3.5.1.4	5	
feaB	phenylacetaldehyde dehydrogenase	EC:1.2.1.39	2	ko00360 Phenylalanine metabolism; ko00643 Styrene degradation;
gctA, B	glutaconate CoA-transferase, subunit A,B	EC:2.8.3.12	2	ko00643 Styrene degradation; ko00650 Butanoate metabolism;
catE	catechol 2,3-dioxygenase	EC:1.13.11.2	5	ko00361 Chlorocyclohexane and chlorobenzene degradation; ko00622 Xylene degradation; ko00643 Styrene degradation; ko01220 Degradation of aromatic compounds;
paaF, echA	enoyl-CoA hydratase	EC:4.2.1.17	14	
alkB1_2	alkane 1-monooxygenase	EC:1.14.15.3	2	ko00930 Caprolactam degradation;
aldH	NADP-dependent aldehyde dehydrogenase	EC:1.2.1.4	2	ko00930 Caprolactam degradation; ko01220 Degradation of aromatic compounds
atzF	allophanate hydrolase	EC:3.5.1.54	2	ko00791 Atrazine degradation;
ureC	urease subunit	EC:3.5.	4	

	alpha	1.5		
ureB	urease subunit beta	EC:3.5.1.5	3	
urea	urease subunit gamma	EC:3.5.1.5	3	
gst	glutathione S-transferase	EC:2.5.1.18	6	ko00980 Metabolism of xenobiotics by cytochrome P450
tehB	tellurite methyltransferase	EC:2.1.1.265	0	
tehA	tellurite resistance protein	--	0	
ter C, B,A	tellurite resistance protein TerC,B,A	--	0	