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**Title of the Ph.D. thesis: "Studies on microbial bioconversion of Catechol in Catechu extract "**

**ABSTRACT OF Ph.D. THESIS**

Chewing of betel quid (BQ; Pan) and pan masala (comprising areca nut, lime, catechu & tobacco) is an ancient custom in South-East Asian countries, South Pacific islands and Taiwan, and this habit causes oral, pharyngeal and oesophageal cancer. Apart from the medical repercussions, recalcitrance of the constituent compounds of BQ and pan masala to the environment and soil is a matter of grave concern. Soils infested with BQ litter are largely constituted by catecholates which are highly recalcitrant in nature. Keeping Catechol's hazardous effects in view, soil infested with catechol must be targeted for the detoxification purpose in order to restore the environmental hygiene and it's a matter of prime concern among scientific community. Hence, the present study aims for microbial bioconversion studies of catechol using catechu extract.

Bacterial cultures isolated from chewed BQ litter and pan masala infested soils were subjected to phenotypic characterization and tested for hydrolytic enzyme production potential for catechol degradation by screening for *meta* and *ortho*-cleavage dioxygenases. Isolates R-3, R-32, R-33 and R-34 were found positive, in which only R-32 could catalyze catechol degradation via *meta*-pathway, whereas *ortho*-cleavage dioxygenase was detected in R-3, R-33 and R-34 cultures. Positive cultures were directed to mutagenic strain improvement and resulted into less active strains as compared to wild type. Positive isolates were then subjected to 16S rDNA sequencing, compared with sequences available in NCBI's GenBank database and submitted there under Accession No. (Acc.): FJ462637, FJ462638, FJ462639 and EU579530. Isolates namely, R-3, R-32, R-33 and R-34, were identified as members of *Pseudomonas sp.*, *Pseudomonas pseudoalcaligenes, Cupriavidus sp*. and *Pseudomonas pseudoalcaligenes*.

Antibiotic resistance pattern for positive isolates showed different levels of resistance. PCR based characterization of *xylE* (catechol 1,2- dioxygenase) and *catA* (2,3-dioxygenase) responsible for catechol degradation showed that amplicons of *catA* from R-3, R-33 and R-34 were of ~915, ~924 and ~930 bp size, respectively, whereas amplicons of *xylE* from R-32 were of ~919 bp in size. Amplicons of *xylE* and *catA* were cloned separately into pGEM-T vector and sequenced. Full length putative *catA* gene showed that the amplified products of *catA* genes were of 915 bp for R-3, 924 bp for R-33 and 930 bp for R-34, respectively. Similarly for *xylE* gene 919 bp size was noticed. Sequence alignment studies showed that *catA* nucleotide sequences from 3 different cultures showed ~94% homology with each other. Global alignment for *catA* proved that nucleotide sequences of *catA* for R-3 was ~92% similar to *Pseudomonas sp.* B-3 (Acc. FJ237621.1), R-33 was ~95% homologous to *Pseudomonas putida* (Acc. U12557.1) and R-34 was ~94% similar to *Pseudomonas putida* (Acc. AF36324). Sequence alignment of R-32's *xylE* with standard *P. putida* MTCC 2445 showed ~92% homology. Global alignment of *xylE* showed 95% homology with already characterized *xylE* from *P. putida* mt2 (Acc. V001161.1).

This is very first time catechu extract has been used as a source of catechol to study the degradative capability of catechol oxygenases. Also, this is very first report mentioning the member of *Cupriavidus sp.* producing the catechol oxygenases.