

SUMMARY OF THE THESIS

About eighty two marine bacteria were isolated from marine water sample, sponge sample and sea sediments sample collected from Bay of Bengal, India at 30 meter depth. Bacterial strains (~22) isolated from marine water sample were subjected to 16S rRNA gene sequencing. The 16S rRNA gene sequencing analysis of 22 marine isolates showed that only one strain MW 10<sup>T</sup> exhibits less than 97% similarity, indicating this is a novel bacterium. Therefore, strain MW 10<sup>T</sup> was selected for further characterization by polyphasic approach of taxonomy. Strain MW 10<sup>T</sup> was Gram-positive, rod shaped, yellow coloured, non-motile, non spore forming, aerobic bacterial strain. The 16S rRNA gene sequence revealed that strain MW 10<sup>T</sup> was showed highest similarity with *Psychrobacillus psychrodurans* (96.15%) and *Psychrobacillus psychrotolerans* (96.01%) and also showed less than 96% similarity with members of genus *Paenisporosarcina*, *Planococcus*, *Sporosarcina* and *Planomicrobium*. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain MW 10<sup>T</sup> formed a separate clade from members of all closely related genera. The morphological, physiological and chemotaxonomical characteristics of strain MW 10<sup>T</sup> did not match to any closely related genera. The major fatty acid in strain MW 10<sup>T</sup> was iso-C<sub>15:0</sub> whereas menaquinones present in this strain were MK-7 (48.4%), MK-8 (32.3%), MK-7H<sub>2</sub> (13.7%) and MK-6 (5.6%). The major polar lipids were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), a phospholipid (PL), an unknown lipid (UL) and a glycolipid (GL). The cell wall peptidoglycan type was L-Lysine-D-Asp. The DNA G+C content (53.4 mol %) of strain MW 10<sup>T</sup> was found to be significantly different from members of all closely related genera. On the basis of morphological, physiological and chemotaxonomical characteristics as well as G+C contents and phylogenetic analysis, we conclude that strain MW 10<sup>T</sup> should be considered as a member of a novel genus and species, for which the name *Chryseomicrobium imtechense* gen. nov. sp. nov., is proposed. The type strain of *Chryseomicrobium imtechense* is MW 10<sup>T</sup> (=MTCC10098<sup>T</sup> = JCM 16573<sup>T</sup>).

Bacteria strains isolated from marine sample were screened for their lipolytic activity on different substrates including tributyrin, olive oil, tween 20, tween 40, tween 60, and tween 80 at 10°C, 20°C and 30°C. One strain designated as SS-33 hydrolyzed all

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lipolytic substrates at low temperature as well as mesophilic temperature. Therefore, this strain is suitable candidate for bioremediation of the oil contaminated cold site. Lipase from strain SS-33 was partially purified by acetone precipitation. The molecular weight of lipase protein was determined 67 kDa by SDS-PAGE. Zymography was also performed to check the activity of lipase band in Native-PAGE. The optimum pH of lipase protein was pH 7.0. Calcium ions increased twofold activity of lipase protein. The enzyme assay showed that optimum temperature of lipase protein was 30°C. The lipase activity was found significantly decrease above the temperature 30°C. However, at 25°C lipase showed almost similar activity as observed in 30 °C. At 20°C and 15°C, 95% lipase activity was observed. Interestingly, lipase from strain SS-33 showed 90% activity of its optimum temperature at 10°C. Furthermore, lipase became more stable at 10°C as compare to 30°C. The lipase activity and stability at low temperature has wide range applications in various industrial processes. Therefore, cold adapted lipase from strain SS-33 may be used for industrial applications. Cold adapted lipase producing strain SS-33 was subjected to polyphasic approach of taxonomy to determine its taxonomic position. 16S rRNA gene sequencing and phylogenetic analysis showed that strain SS-33 is a member of the genus *Stahylococcus*. Strain SS-33 has all characteristics features of genus *Stahylococcus*. Cells of strain SS-33 were gram positive, cocci shaped, non-spore forming, non-motile, catalase positive and oxidase negative. The major fatty acid detected in strain SS-33 was 15:0 ANTEISO and menaquinone was MK-7. The genomic DNA G+C content was 33 mol%. On the basis of polyphasic approach, strain SS-33 was identified as *Staphylococcus* sp.

2-Chloro-4-nitrophenol (2C4NP) is considered as recalcitrant to microbial degradation due to electron withdrawing properties of chloro and nitro groups. Two 2C4NP utilizing bacteria designated as strain SJCon and strain RKJ 800 were isolated from pesticide contaminated soil of Punjab by enrichment method. Strain SJCon and strain RKJ 800 were identified as *Arthrobacter* sp. and *Burkholderia* sp. respectively on the basis of 16S rRNA gene sequencing analysis. Both of the strains utilized 2C4NP as sole source of carbon, nitrogen and energy. The degradation of 2C4NP by strain SJCon and strain RKJ 300 was initiated with oxidative removal of nitro group. The stoichiometric amounts of nitrite and chloride ions were detected during the

degradation of 2C4NP by strain SJCon and strain RKJ 800. The metabolite(s) of the degradation pathway of 2C4NP were identified on the basis of TLC, HPLC and GC-MS. Inhibition study was also carried out to elucidate the degradation pathway of 2C4NP. Enzyme activity was also observed in the crude extract of 2C4NP induced cells of strain SJCon and strain RKJ 800. Strain SJCon degraded 2C4NP via chlorohydroquinone (CHQ) that further cleaved to maleylacetate by CHQ dioxygenase activity whereas strain RKJ 800 degraded 2C4NP via hydroquinone that further cleaved to  $\gamma$ -hydroxymuconic semialdehyde by manganese dependent hydroquinone dioxygenase activity. This study clearly showed that *Arthrobacter* sp. strain SJCon and *Burkholderia* sp. RKJ 800 degraded 2-chloro-4-nitrophenol via two different pathways. Furthermore, *Arthrobacter* sp. strain SJCon degraded 2C4NP via a novel pathway.

*Bacillus safensis* strain MW-1 decolourized and detoxify 4-chloro-2-nitrophenol (4C2NP) only in the presence of additional carbon source. On the basis of TLC, HPLC and GC-MS, 4-chloro-2-aminophenol, 4-chloro-2-acetaaminophenol and 5-chloro-2-methylbenzoxazole were identified as metabolites. Resting cells depleted 4C2NP with stoichiometric formation of 5-chloro-2-methyl benzoxazole. This is the first report of the formation of 5-chloro-2-methylbenzoxazole from 4C2NP by any bacterial strain.

Oxygenases belong to the oxidoreductive group of enzymes (E.C. Class 1), which oxidize the substrates by transferring oxygen from molecular oxygen (O<sub>2</sub>) and utilize FAD/NADH/NADPH as the co-substrate. Oxygenases can further be grouped into two categories i.e., monooxygenases and dioxygenases on the basis of number of oxygen atoms used for oxidation. They play a key role in the metabolism of organic compounds by increasing their reactivity or water solubility or bringing about cleavage of the aromatic ring. A database of biodegradative oxygenases (OxDBase) was developed which provides a compilation of the oxygenase data as sourced from primary literature in the form of web accessible database. There are two separate search engines for searching into the database i.e. mono and dioxygenases database respectively. Each enzyme entry contains its common name and synonym, reaction in which enzyme is involved, family and subfamily, structure and gene link and

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literature citation. The entries are also linked to several external database including BRENDA, KEGG, ENZYME and UM-BBD providing wide background information. At present the database contains information of over 235 oxygenases including both dioxygenases and monooxygenases. This database is freely available online at <http://www.imtech.res.in/raghava/oxdbase>. OxDBase is the first database that is dedicated only to oxygenases and provides comprehensive information about them. Due to the importance of the oxygenases in chemical synthesis of drug intermediates and oxidation of xenobiotic compounds, OxDBase database would be very useful tool in the field of synthetic chemistry as well as bioremediation.