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Isolation of transposon mutant of Escherichia coli with altered biofilm formation

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The investigation of biofilm formation is critical to reducing infection rates as more than 60% of the clinically infectious diseases are associated with biofilm formation. Here, we used a plasmid pRL27, having an R6Kγ origin of replication, to generate transposon mutants altered in biofilm development in Escherichia coli 2443 (K-12 strain). The R6Kγ origin of replication requires the pir gene encoding the π protein, which binds at a 22-bp recognition sequence to initiate DNA replication. As the Escherichia coli 2443 is a pir negative strain, the plasmid pRL27 cannot replicate in it and this makes the plasmid an effective suicide vector for genome wide transposon mutagenesis. The plasmid was maintained in MFDpir strain, which is a diaminopimelate (DAP) auxotrophic mutant (dap–) of Escherichia coli and produces the π protein. The plasmid pRL27 was conjugatively transferred from MFDpir to Escherichia coli 2443 on minimal medium plates. Transconjugants were selected by plating the mating mixtures onto LB plates supplemented with dap (300 µg/ml) and kanamycin (50 µg/ml). The transposon insertion mutants obtained were screened for altered biofilm formation using crystal violet staining. The biofilm formation was normalized to planktonic growth, thus obtaining the biofilm formation index (BFI). Based on the results two mutants RM 56 and RM 69, showing significantly lower and higher BFI respectively, were selected. These mutants will be characterized further to identify the site of the transposon insertions and the underlying mechanism altering biofilm formation. This may be of benefit to develop a better understanding of biofilm formation.

Biography

Akash Kumar is pursuing PhD in Department of Biotechnology, Indian Institute of Technology Kharagpur. He has published one research paper in Research in Microbiology.

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Transformation of cholesterol by two novel isolates of Streptomyces sp

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Cholesterol oxidase (COD) is a bacterial FAD-containing flavooxidase that catalyzes the oxidation of cholesterol (cholest-5-en-3β-ol) using oxygen as an electron acceptor to form 4-cholesten-3-one (CEO) and hydrogen peroxide. Microbial cod is an enzyme of great commercial value, widely employed by laboratories routinely for the determination of cholesterol concentrations in serum and other clinical samples as a diagnostic enzyme. In addition, the enzyme has potential applications as a key enzyme in the biosynthesis of the polyene macrolide pimaricin antifungal antibiotic, used in biocatalysis for the production of number of steroids such as 4-androstene-3,17-dione and 1,4-androstadiene-3,17-dione, in the production of precursors for chemical synthesis of steroid hormones, as an insecticidal protein against boll weevil (Anthonomus grandis grandis Boheman) larvae. We are reporing production of extracellular COD by two novel Streptomyces sp. and transformation of cholesterol into CEO.

Extracellular COD production by the two Streptomyces sp. isolates was compared with the known COD producing culture of Streptomyces violascens NRRL-B2700. Maximum extracellular enzyme production was 10.5 U/ml at 48 hr and 8.3 U/ml at 64 hr respectively at 30°C and 180 rpm. Cholesterol was used as inducer at various concentrations from 0.1%-0.5%. Approximately 80% of cholesterol at 1g/l concentration was converted within 96 hr to CEO. The detection of cholesterol degradation products was ascertained by thin layer chromatography and quantified by HPLC. The maximal UV absorbance of cholesterol degradation product was at 240nm. Its mass spectra revealed an ion peak at m/z ratio 385.3 and the product was chemically characterized as CEO.

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