ABSTRACT

Three extracellular lipase producing bacteria selected from the effluent of the Ghee Mills and were screened by using Rhodamine B agar medium containing mustard oil as a substrate at 37 °C for 72 hours. On the basis of morphological and biochemical characteristics the three strains were identified as *Pseudomonas aeruginosa, Micrococcus luteus,* and *Shewanella putrefaciens.* The 16S rRNA sequencing was implemented. Phylogenetic analysis based on results of 16S rRNA gene sequencing revealed that these three strains were close in identity to Pseudomonas *aeruginosa, Micrococcus luteus* and *Shewanella putrefaciens.* The 16S rRNA sequences were also subjected to GenBank. For all three strains temperature of 37 ⍰C, pH range of 7 - 9, 2% inoculums size, 0.6 % substrate concentration and an incubation period of 48 hours was found to be optimum for biomass and lipase production through shake flask fermentation. Under optimized conditions highest lipase activity of 8.166 U/ml was seen in case of *Micrococcus luteus,* followed by 6.66 U/ml of *Pseudomonas aeruginosa* and 6.166 of *Shewanella putrefaciens.* Characterization of partially purified enzyme by ammonium sulfate revealed that at 37 ⍰C specific activity of Pseudomonas *aeruginosa* was 63 U/mg, 61.3U/mg of *Micrococcus luteus,* and 57.7U/mg of *Shewanella putrefaciens.* In between pH range of 7 – 9 maximum specific activity of 59.3 U/mg was registered in case of *Micrococcus luteus,* 58 U/mg by *Pseudomonas aeruginosa* and 46.6U/mg by *Shewanella putrefaciens.*