Isolation and identification of potent naringinase-producing bacteria from different plant parts of *Citrus aurantium.*

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Introduction and Objectives: Naringinase is a versatile and fascinating study subject since it is produced by various sources in nature, resulting in variance in reaction specificity. It has numerous important applications in the food and pharmaceutical industries. This study aimed to isolate, characterize, and identify potent naringinase-producing bacteria from various parts of the *Citrus aurantium* plant.

Methods: Forty-five bacterial strains were isolated from *Citrus aurantium* fruit, leave, stem and root by spread plate method and qualitatively assessed for naringinase activity using 1% $FeCl_3$. Among these isolates, nine strains (BC1-BC9) showed naringinase activity indicated by a distinct reddish-brown colour change with $FeCl_3$. These nine strains were then selected for a quantitative test using both submerged (SMF) and solid-state (SSF) fermentation methods. The SMF medium contained naringin (2.0g/L), glucose (2.0g/L), peptone (7.0g/L), $MgSO_4.7H_2O$ (0.1g/L), KH_2PO_4 (0.5g/L), $ZnSO_4.7H_2O$ (0.07%), $CuSO_4.7H_2O$ (0.07%), $FeSO_4.7H_2O$ (0.07%) and for the SSF, **rice bran (20%)** was added to the existing SMF medium, making the mixture a solid medium rather than a liquid one. The measurement of naringinase enzyme activity relied on monitoring changes in absorbance. Best naringinase producing isolate (BC2) was identified based on the morphological,biochemical screening and molecular assay using 16S rRNA sequence analysis.

Results: All nine isolates produced significantly higher naringinase enzyme on SSF media than the SMF media. Bacterial isolate BC2 exhibited significantly higher (p value<0.05) naringinase activity (497.890U/g±1.107) of the dry weight of the substrate than the other isolates under SSF condition. The yield of the enzyme was calculated by determining the amount of reducing sugars released during the enzymatic reaction. The absorbance changes were monitored at 550 nm wavelengths using a spectrophotometer. The morphological, biochemical screening and molecular study using 16S rRNA sequence analysis revealed that isolate BC2 was *Bacillus megaterium* CP026736.

Conclusions: This study identified *Bacillus megaterium* CP026736, isolated from *Citrus aurantium* fruit, as a potent naringinase-producing bacterium. Comparative assessments showed that SSF resulted in significantly higher naringinase activity than SMF across nine strains. Among them, strain BC2 demonstrated the highest enzyme activity under SSF conditions, indicating its industrial potential. Molecular assays confirmed BC2 as *Bacillus megaterium*, providing valuable insights for future applications in the food and pharmaceutical industries.

Keywords*: Bacillus megaterium, Citrus aurantium, naringinase activity, solid-state fermentation.*

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