Preliminary studies on the isolation of naringinase producing fungus

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This study was aimed in isolating naringinase-producing fungus. Naringin is a bitter substance found in some fruits and food substances, which limit the utility of these substances. Treating with naringinase can eliminate this bitter taste of naringin, and the treated food substances can be used industrially. To select a narginase producing fungus, a total of hundred and fifty fungal strains were isolated from corncob (25), palmyrah fruit fibre (25), opened naringin plate (21), sugar cane (14), house garbage (19), beetroot (25) and neem fruit (21). The fungus were cultured in naringin-agar slant, which contained (gl^{-1}) naringin, 2.0; yeast extract, 1; glucose, 5.0; and 100ml mineral solution ($ZnSO_4$. $7H_2O$, 0.7; CuSO4. $5H_2O$, 0.7 and FeSO4. $7H_2O$, 0.7). One loop of 6-day-old fungus spores was transferred to 0.2 %, (v/v) Tween - 80. Then serial dilution was done up to 10^{-4}. From the dilutions, 200µl samples were spread in naringin-agar plates. Fungal spores from each colony were selected and transferred to naringin - agar slant for storage. On 6th day, spores were washed with (0.2%, v/v) Tween - 80 and 2ml was inoculated into fermentation medium. The fermentation medium contained (gl^{-1}) naringin, 2.0; glucose, 2.0; soybean, 20; peptone, 7.0; $MgSO_4$. $7H_2O$, 0.1; KH_2PO_4, 0.5 and 100ml mineral solution. From each flask, samples were taken and assayed for naringinase activity after 6th days of inoculation. The Enzyme assay was done at 50°C and pH 5.0 with 30 minutes incubation. Out of twenty-five strains from corncob, C_3^5 gave the highest activity of 1586 Unit ml^{-1} (U = nmol min^{-1}). Among the twenty-five strains from Palmyrah fruit fibre, P_2^2 strain showed the highest activity of 1656 Unit ml^{-1}. N_2^5 strain among the twenty-one strains obtains from opened naringin plate gave the highest activity of 1638 Unit ml^{-1}. Out of the fourteen strains from sugar cane K_3^1 gave the highest activity of 1780 Unit ml^{-1}. H_2^3 strain gave the highest activity of 1620 Unit ml^{-1} out of nineteen strains from house garbage. Among he twenty-five strains from beetroot, B_1^3 gave the highest activity of 2300 Unit ml^{-1}. V_2^3 gave the highest activity of 900 Unit ml^{-1}, among twenty-one strains from neem fruit. Among the 150 strains isolated, B_1^3, the strain obtained from beetroot gave the highest naringinase activity (2300 Unit ml^{-1}). Hence kinetic studies of the enzyme in the spent medium with beetroot was studied. The crude enzyme gave zero order kinetics for 10 min. Therefore for the enzyme assay, 5 min incubation time was fixed. The optimum temperature (at pH 5.0) and pH (at 50°C) for the activity were 50°C and 5.0 respectively. The Km of the crude enzyme was 1.5 gl^{-1} naringin at pH 5.0 and 50°C. Further studies are in progress to improve the strain and to purify the enzyme.