(A Minor Research Project was sanctioned by the UGC to Dr.D.R.Majumder,Asso. Professor, Head, Department of Microbiology, Abeda Inamdar Senior College, from 2002-2004. The following is an executive summary of the report.)

Summary of MRP Project Report

Title: 'Screening Of Psychrophiles For Pectinases With Low Temperature Optima'

Pectinases are used in numerous industrial processes like extraction and clarification of wines and fruit juices, fruit maceration, reduction of juice viscosity, ex-traction of vegetable oils, and fermentation of coffee and tea.In winemaking and fruit juice processing low temperatures are favourable for the production and retention of flavour and colour components, requiring the use of cold-active enzymes. For this reason,'psychrotolerant' microorganisms have been isolated and selected based on their ability to produce pectinolytic enzymes with satisfactory activity at low temperatures. Cold storages and domestic fridges and air samples were checked for 'psychrotolerant' microorganisms who have the ability to produce pectinolytic enzymes with satisfactory activity at low temperatures. Different mature grape varieties were sampled from cold storages from Narayangaon and Baramati and Ahmednagar region .Several pectinolytic bacterial and fungal strains were isolated from grapes and other fruits and vegetables stored in the above mentioned cold storages.

Mature grapes and other vegetables and fruits belonging to the different cold storages were exhaustively washed with 2 mL of 1 % peptone water. Yeast extract peptone dextrose (in g/L of distilled water: yeast extract 10, peptone 20, glucose 20, agar 20) agar plates were inoculated with 0.3 mL of the washing fluid (1 % peptone water) and incubated at 30 °C for 5 days and 15 $^{\circ}$ C for 3 weeks. Then colonies were taken from each plate and subcultured on a specially designed medium that contained 1 % pectin (PGA). The composition (in g/L) was

as follows: PGA 10, yeast extract 2, CSL 20 ml, peptone 10, agar 10,KH_2PO_4 0.2, $CaCl_2$ 0.05, $(NH_4)_2SO_4$ 3, $MnSO_4 \cdot 7H_2O$ 0.05,$FeSO_4 \cdot 7H_2O$ 0.015, $MgSO_4 \cdot 7H_2O$ 0.8 at pH=5. Each sample was cultured on two Petri dishes, one incubated at 15 °C for 3 weeks and the other at 30 °C for 5 days. Then the plates were stained with Lugol's solution for detection of a clearance halo formed around the colonies. The ratio between the halo diameter and the corresponding colony diameter was measured in order to obtain the most productive strain.

After the primary selection that resulted in ten bacteria (*Bacillus* sp.) and five fungal strains (Aspergillus sp.,Penicillium sp.,Mucor sp.,Rhisopus sp.and Triichoderma sp.),a secondary selection was carried out using the criterion of the highest activity at 15 °C, assessed with the reducing sugar method (3,5-dinitrosalicylic acid) method (DNS). The PGase activity was also assayed by estimating the amount of reducing sugar released under assay conditions. Polygalacturonase activity was measured by determining the amount of reducing groups released according to the method described by Nelson and modified by Somogyi.

The pectinolytic activity was measured by quantification of released reducing sugars and viscosity reduction of pectin solution. Ten bacteria (*Bacillus* sp.) and five fungal strains (Aspergillus sp.,Penicillium sp.,Mucor sp.,Rhisopus sp.and Triichoderma sp.)were selected for their good pectinase activity at low temperatures.The crude enzyme from cell free extract presented good activity at 15°C, confirming that it was a cold-active enzyme.

Out of ten bacterial isolates, Isolate 9 and Isolate 5 from fungal strains produced the highest Polygalacturonase activity.

The present study has been undertaken with the aim to isolate native microorganisms from natural sources able to produce pectinases with good activity at 15 °C or below.

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