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University of Calcutta, Kolkata 700 019, India

In-vitro pectinolytic enzyme activity of some fungi associated with ripe banana

INDRANI BANDYOPADHYAY AND S. CHAUDHURI
Plant Pathology Laboratory, Department of Botany, Kalyani University, Kalyani 741235

Four fungal isolates belonging to *Penicillium* and *Aspergillus* spp. from ripe apparently blemish-free banana exhibited significant *in vitro* pectinolytic enzyme activity. The isolate BA1 showed high endo-PMG and both endo-and exo-PG activity. In isolates BA2 and BA4, exo-PG activity was recorded whereas BA3 showed very low exo-PG activity. *In vitro* PMTE activity was found in culture filtrates of BA2 and BA3. However, none of the isolates showed and PGTE activity. The presence of these enzymes may have a role to play in softening and rotting of ripe banana pulp under storage condition.

Key words : *Penicillium* spp., *Aspergillus* spp., banana, pectinstylic activity

INTRODUCTION

The potential for significant post harvest losses of fruits and vegetables from decay and degradation caused by microorganisms is greater than is often realized. Any produce lost in storage or reduction of its market value, economically worths more than an equal amount lost in the field. It is, therefore, imperative to understand the importance of post harvest infection and the associated microorganisms in the continuous strive to reduce such losses. Role of pectinolytic enzymes have been implicated in degradation and rotting of fruits caused by several fungi (Wheeler, 1975; Thind *et al.* 1976). In the present work, the possible production of pectinolytic enzymes by four fungal species isolated from the pulp of ripe banana was studied.

MATERIALS AND METHODS

Four fungal isolates, two each of *Penicillium* and *Aspergillus* spp. (BA1, BA2, BA3 and BA4) from the pulp of ripe, bruish and blemish-free banana fruits (Singapuri var Kabuli) were used in the present investigation. The fungi were grown for 10 days at 28±1°C in broth medium containing 0.5% dextrose, 0.4% ammonium oxalate, 0.1% potassium dihydrogen phosphate and 0.05% magnesium sulphate and supplemented with either 1% washed pectin [for polymethylgalacturonase (PMG) and polymethyl transeliminase (PMTE)] or sodium polypectate [for polygalacturonase (PG) and polygalacturonase transeleminase (PGTE)]. At the end of incubation period the vacuum filtered filtrate was dialysed in cellulose tubing (Sigma) against distilled water at 0°-4°C for 24 hrs. The clear supernatants were collected, a few drops of toluene added to avoid contamination and assayed for enzyme activity.

Enzyme Assay method

Pectinolytic enzyme activity was detected by loss in viscosity of 1% buffered pectin or sodium polypectate in Ostwalds' viscometer (Mahadevan and Sridhar, 1982). PG and PMG activity was measured at pH 5.5 and that of PGTE and PMTE at pH 8.5, 0.1 ml of 0.01 M $CaCl_2$ was added to the reaction mixture containing lyase enzyme. Unit of enzyme activity was experssed as relative enzyme activity (REA) which is reciprocal of the time in minutes for 50% loss in viscosity of the reaction mixture by 1000. Non hydrolytic nature of cleavage by pectin transeliminase (PMTE) was determined by thiobarbituric acid (TBA) test (Sherwood, 1966). Reducing group liberated during PG enzyme action on sodium polypectate was quantified by the

dinitrosalicylic acid (DNS) test (Hancock, 1968).

RESULTS AND DISCUSSION

Among the four isolates, BA1 exhibited high PMG and PG activity of 233 and 177 units respectively and there was very rapid decrease in substrate viscosity. In isolates BA2 and BA4, the PG activity was low as indicated by a very slow reduction in viscosity (Fig. 1). Reduction of viscosity of sodium polypectate and pectin was negligible at pH 8.5 by BA1 and BA4, indicating the absence of transeliminases in their culture filtrates. Non hydrolytic nature of cleavage of pectin by enzyme preparations of isolates BA2 and BA3 were confirmed by the presence of absorption peak only at 550 nm in TBA test (Fig. 2).

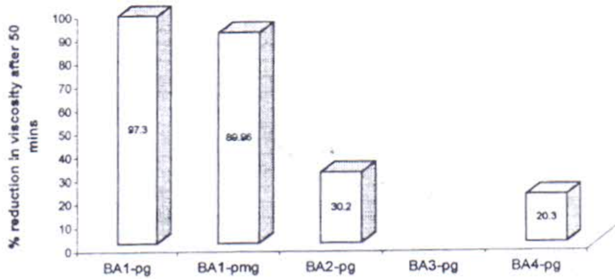

Fig. 1 : Relative enzyme activity of PG and PMG of different banana isolates

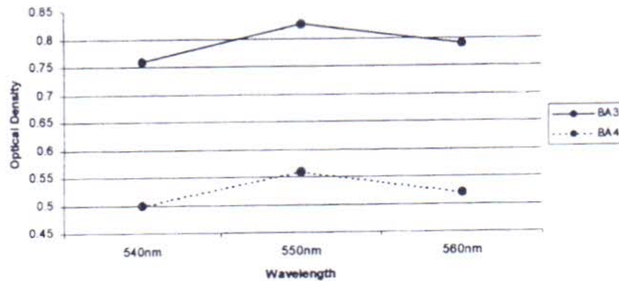

Fig. 2 : PMTE activity of BA3 and BA4 isolates

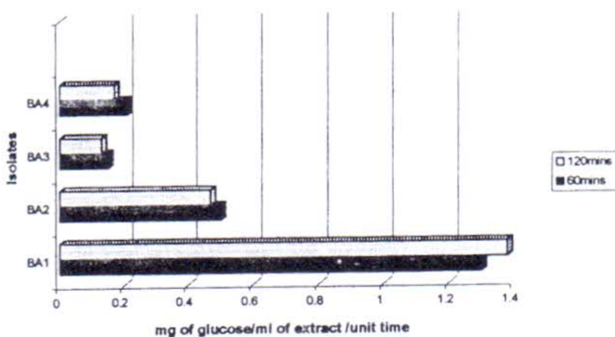

Fig. 3 : Release of reducing sugar by different banana isolates by DNS test

Release of reducing sugars as estimated by DNS acid test by BA1 was 1.3 mg of glucose/ml of enzyme extract/unit time, whereas the rates of release by BA2, BA3 and BA4 were 0.5 mg/ml, 0.19mg/ml and 0.21 mg/ml respectively (Fig. 3).

Since the enzyme extract of BA1 brought about rapid decrease in visocity in both pectin and sodium polypectate, it indicated the presence of both endo-PG and endo-PMG. Rapid release of reducing sugar by BA1, when sodium polypectate was the substrate, also suggested the presence of exo-PG. In isolates BA2 and BA4, there was slow decrease of viscosity but rapid release of reducing sugar indicating a terminal attack on substrate, hence the presence of exo-PG. Though there was negligible reduction of viscocity by BA3, liberation of reducing sugar as quantified by DNS test, indicated the presence of small amounts of exo-PG. No transeliminase enzyme activity was found in culture filtrates of BA1 and BA4. However, in BA2 and BA3 there was considerable PMTE activity. Thus, the results indicated that the four isolates from ripe banana which caused post-harvest softening of the pulp and subsequent rotting of the fruits exhibited *in vitro* production of considerable amounts of hydrolase and transeliminase enzymes, which may have an important role in causing the symptoms when produced *in vitro*.

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