CHAPTER V

METAL IONS AND PECTOLYTIC ENZYMES (POLYGALACTURONASE, PECTIN LYASE AND PECTIC ACID LYASE) PRODUCTION

The milieu in which the organism grows is most significantly altered by its ionic composition. Effect of ions on pectolytic enzymes is decisive. Calcium has been regarded to be essential for lyases especially for those of the bacterial origin. It is indispensable for PAL production by *Erwinia carotovora* (Starr and Moran, 1962), *Clostridium multisfermentans* (Mac Millan and Vaughn, 1964) and *Fusarium solani* f. sp. *phaseoli* (Bateman, 1966), *Xanthomonas axonopodis* (Porwal and Chakravarti, 1972) and *Verticillium dahliae* (Selvaraj, 1974). However, several bacteria and fungi do produce the enzymes freely in absence of calcium. The PL production by *Aspergillus niger* was reported by Albersheim et al. (1960) to be in fact inhibited by calcium. Between these two extremeties indispensability and inhibition, lie the multitude of behaviours which are not so easy to track. The few studies that are available in this area suggest 'no effect' or varying behaviour which are conditioned by other factors most important among which is the pH of the medium. Thus, Sherwood (1966) found no stimulatory or otherwise effect of calcium on PL production by *Rhizoctonia solani*. The
modulating effect of pH has been recorded in our laboratory earlier by Dube and Bordia (1981). The concentration of Ca is another important parameter of its effects viz. stimulation, inhibition, no effect etc. Thus, Dube and Bordia (loc. cit.) observed that at pH 5, the PAL activity of Helminthosporium sacchari was enhanced at all concentrations tried ($5 \times 10^{-4} M$ to $5 \times 10^{-7} M$). Maximum stimulation occurred at the $5 \times 10^{-4} M$ concentration above which the stimulation decreased progressively. At pH 6 the enzyme was not affected at $10^{-3} M$ concentration, but at other concentrations the inhibition was recorded and it was in direct relation with the concentration.

Hydrolytic pectolytic enzymes too show varying effect of ions especially calcium. Calcium is shown by Bateman (1968) to provide resistance against pectolytic enzymes. Inhibition of PG by calcium and some other ions has been reported by Cordon et al. (1964) and Cordon (1965). Dube and Mathur (1982) reported stimulation and also inhibition of PG by calcium, depending on the concentrations used. At $5 \times 10^{-5} M$ concentration it was stimulatory but above that it proved inhibitory. So concentration must be specified while suggesting stimulation or inhibition.

In the present study the effect of some metal ions at $0.1 M$ concentration was examined on the FG and PL production
by *P. expansum* and lyases of the bacterium. Calcium, cobalt and mercury were provided as chlorides while magnesium and sodium were used as sulfate and phosphate, respectively. The culture filtrates of the fungus were assayed on 4th and those of the bacterium on 2nd day of growth.

*P. expansum* growing on Richards-pectin containing various metal ions on the 4th day showed the production of hydrolases (Fig. 5.1a) in the TBA test like the control, the peak for calcium containing filtrate was little sharper. A change in the peak was noticed in the mercury amended medium which suppressed the hydrolase synthesis and instead synthesised a lyase enzyme (PAL). This is the first observation of shift in peak by mercury. Previous works in this laboratory reported failure of fungus growth on mercury containing media (Doshi, 1982; Bordia, 1980). However Mathur and Dube (1981) reported enzyme activity (PME) in mercury-containing media supporting growth of *Verticillium albo-atrum* and *V. dahliae*, though the enzyme activity was lower than in the control. The present behaviour of mercury i.e., repression of PG and induction of PAL is noteworthy. In terms of viscosity loss, the mercury medium showed (Fig. 5.2a) poorest depolymerising activity upto 20 min but by the end of the test time i.e.,
30th min it had improved upon the activity of sodium containing medium, and the trend showed possible continued degradation. Maximum viscosity reducing activity was noticed in the cobalt containing medium while calcium which showed better activity than the control initially became inactive after 20 min and then finally its activity lay below control and magnesium containing medium. Thus, the higher relative activity of calcium-containing culture filtrate (91 against 77 of control) is misleading in comparing its activity with that of magnesium (Fig. 5.2a). Cobalt alone stimulated the PG activity of *P. expansum* both in terms of relative activity and final hydrolysis of the substrate (relative activity 166 against 77 of control and % loss 87 against 76 of control). It showed 59% stimulation of enzyme activity. In order of their decreasing enzyme inhibition the metal ions can be arranged as follows: sodium (62%) mercury (59%) calcium (12%) magnesium (2%) and cobalt nil.

Pectin degradation too was brought about by all the culture filtrates including mercury which incidently showed higher O.D. in the TBA test (Fig. 5.1) than the 'control'.

The absence of a peak in TBA test at 510 and presence of a peak at 550 nm, coupled with highest relative
Fig. 52

**Pexpansum**

Richards Pectin medium (4th day)

**Substrate** NaPP (pH 5)

- **HgCl₂**
- **NaH₂PO₄**
- **MgSO₄**
- **CaCl₂**
- **Control**
- **CoCl₂**

% viscosity loss vs. time in min

**Substrate** Pectin (pH 10)

- **NaH₂PO₄**
- **CaCl₂**
- **Control**
- **CoCl₂**
- **HgCl₂**
- **MgSO₄**
Table 5.1: Showing effect of metal ions on the production of polygalacturonase and pectin lyase by *P. expansum* in Richards pectin medium after 4 days growth.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Richards-pectin medium + test chemicals</th>
<th>PG</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% viscosity loss in 30 min</td>
<td>% inhibition</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>76</td>
<td>--</td>
</tr>
<tr>
<td>2.</td>
<td>+CaCl₂</td>
<td>67</td>
<td>12</td>
</tr>
<tr>
<td>3.</td>
<td>+CoCl₂</td>
<td>87</td>
<td>--</td>
</tr>
<tr>
<td>4.</td>
<td>+HgCl₂*</td>
<td>31</td>
<td>59</td>
</tr>
<tr>
<td>5.</td>
<td>+MgSO₄</td>
<td>72</td>
<td>2</td>
</tr>
<tr>
<td>6.</td>
<td>Na₂HPO₄</td>
<td>29</td>
<td>62</td>
</tr>
</tbody>
</table>

* showed lytic peak (detailed in text)
activity (220 against only 117 of control) clears the enzyme as an endo-PL. The role of mercury in pectolytic activity of the fungus is important. In terms of % viscosity loss the +magnesium medium showed highest activity 80%; others were much below. Sodium again showed lowest support for the enzyme activity. An interesting observation is that none of the enzyme samples could continue their activity after 15 min (Fig. 5.2b). Calcium and mercury containing culture filtrates became static after 5 min of activity. Sodium became so at 10th min while cobalt and magnesium containing media could catalyse the reaction upto 15 min. None continued activity upto the end of the test time of 30 min.

E. carotovora pv. atroseptica

The culture filtrates containing various metal ions supporting 48h growth of the bacterium showed (Table 5.2) distinct lytic activity in the TBA test with both substrates (Fig. 5.3). The +calcium medium in both cases showed lowest peaks. Rest of the media showed higher absorption than control at 550 nm in pectin containing reaction mixture. Thus, none of the ions prevented enzyme synthesis.

In terms of their activity magnesium containing medium showed higher activity in the viscosity test
Table 5.2: Showing effect of metal ions on the production of pectin lyase and pectic acid lyase by E. carotovora pv. atroseptica in Czapek-pectin medium after 48 h growth.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Czapek-pectin medium + test chemicals</th>
<th>PL</th>
<th>PAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% visco-sity loss in 30 min</td>
<td>% inhibition</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>46</td>
<td>--</td>
</tr>
<tr>
<td>2.</td>
<td>+CaCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>15</td>
<td>67</td>
</tr>
<tr>
<td>3.</td>
<td>+CoCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>26</td>
<td>44</td>
</tr>
<tr>
<td>4.</td>
<td>+HgCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>42</td>
<td>9</td>
</tr>
<tr>
<td>5.</td>
<td>+MgSO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>49</td>
<td>--</td>
</tr>
<tr>
<td>6.</td>
<td>+NaH&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>25</td>
<td>45</td>
</tr>
</tbody>
</table>
**Fig. 5-3**

**E. atroseptica**

Czapek Pectin medium (48h)
Substrate Pectin (pH 10)

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**Substrate NaPP (pH 10)**

- $\text{Na}_2\text{HPO}_4$
- $\text{HgCl}_2$
- $\text{CoCl}_2$
- Control
- $\text{MgSO}_4$
- $\text{CaCl}_2$

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**Wavelength (nm)**
(Fig. 5.4) than other ion amended media. The pectin degradation was little better than the control by the Mg-containing culture filtrate but in NaPP degradation it was much behind. In terms of the % inhibition of PAL, the ions can be arranged in the following sequence Ca Hg Co Na Mg. In PL-inhibition the ions fall in the sequence as given below:
Ca Na Co Hg.
E. atroseptica
Czapek Pectin medium (48h)
Substrate Pectin (pH10)

% viscosity loss
Time in min

Substrate NaPP (pH10)