DISCUSSION

The present work deals with the studies on the fermentative production of pectinases by fungi. For this purpose, a systematic investigation has been made on (i) the screening of natural sources for pectinase producing fungi, (ii) the selection of the most potent strain among the isolates, (iii) characterization of the most active isolate (identified as a strain of Aspergillus niger), (iv) standardization of the conditions of fermentation, (v) nutritional requirements of the organisms for the production of pectinases, (vi) effect of some growth factors and metabolic inhibitors on the production of pectinases and (vii) the metabolic changes during the production of pectinases.

In course of the screening of 15 soil samples and 12 rotten fruits for the isolation of pectinase-producing fungi, 60 fungal cultures have been found to show varying degrees of enzyme activity. Studies on the production of pectinases by fungal isolates show that high-yielding strains are rare in the natural sources examined. Amongst 60 fungal cultures, only 2 strains have been found to show considerable pectolytic activity. These two strains were further studied for final selection. The fungal isolate (A_{45}) has been tipped as the most potent organism out of these isolates.

Studies on the morphological and cultural characteristics of the isolate (A_{45}) indicate its similarity with

Aspergillus niger. So the isolate has been identified as a strain of Aspergillus niger. However, since this culture (A_{45}) was isolated from a soil sample collected from the campus of Jadavpur University it is designated as Aspergillus niger JU. The production of pectolytic enzymes by Aspergillus species viz. Aspergillus niger, Aspergillus wentii, Aspergillus oryzae, Aspergillus aureus, Aspergillus fumigatus and Aspergillus parasiticus has been reported by a number of workers (9,28,46,47a,47b,50,51,52,53,54,64,68).

The selected strain of A. niger JU has been studied to standardize the conditions of fermentation. It appears from these studies that optimum conditions for the production of pectinases are pH 4.5, period of incubation-7 days, temperature - 30°C and volume of inoculum 0.5 ml suspension containing 5 x 10^3 spores per 30 ml medium. The shake flask process is superior to stationary culture process in giving higher yield of the enzymes in a shorter period of time indicating that adequate aeration is essential for optimal yield of the enzyme. It is of interest to note that there is no direct relation between the growth of the organism and production of pectinases, although sufficinent growth is necessary for maximum formation of the enzymes. This may be due to difference in nature of metabolic process involved for the production of pectolytic enzymes and those for cell synthesis.

During studies on the requirements of carbon and nitrogen compounds by the organism for the production of pectinases in a basal synthetic medium, it has been observed that pectin gives maximum yield of the pectolytic enzymes in the fermentation broth while maltose, glucose and starch are also suitable carbon sources. Among nitrogen sources, peptone, casein, wheat bran and gelatin are superior to other nitrogen sources tested in giving higher yield of the enzymes. Among inorganic nitrogen sources, NH_4NO_3 is superior to others for the production of pectinases. The maximal yield of the enzymes is obtained with organic nitrogen sources on the 6th day of fermentation and with inorganic nitrogen sources on the 7th day. Peptone, however, favours the maximum production of pectin degrading enzymes. The extensive elaboration of pectolytic enzymes by A. niger JU takes place in the medium with the C/N ratio at 10 whether peptone or ammonium nitrate is used as the nitrogen source. It appears from these studies that the pectin degrading enzymes of Aspergillus niger JU are adaptive in nature, since the production of the enzymes was increased remarkably in the presence of pectin. This observation confirms the results concerning the endopolygalacturonase production with Aspergillus niger as reported by Tuttobello et al (64) and Saito et al (47). Increased activity of pectolytic enzymes may also be partly due to the induced synthesis of exopolygalacturonase as reported by Saito et al (47). The

culture A. niger JU shows a difference in the biochemical characteristics from the culture of Tuttobello and Mill (64) as the former gives maximal yield of the enzyme in the presence of pectin as the carbon source while the later giving optimal yield in the presence of a mixture of sucrose and pectin. Both the strains, however, give maximum production of the enzymes in the presence of organic nitrogenous compounds. On the contrary, organic nitrogenous sources were found to repress the production of endopolygalacturonase by Aspergillus saitoi as reported by Makari et al (66) and Aspergillus aureus reported by Sreekantiah et al (65). The present investigation also shows that it is possible to increase the yield of pectolytic enzymes appreciably by maintaining a proper carbon-nitrogen balance in the medium. However, the utilization of one kind of nutrient is often conditioned by the presence of other available nutrients. Thus, the utilization of a particular source of carbon by a pectinase-producing organism may vary with different sources of nitrogen and vice versa.

In course of studies on the mineral requirements of the strain A. niger JU for optimum production of pectinases it has been observed that the salts K_2HPO_4, $MgSO_4 \cdot 7H_2O$, KCl, Na_2SO_4 and $CaCl_2 \cdot 2H_2O$ are required in concentrations of 0.20%, 0.025%, 0.05%, 0.05% and 0.022% respectively while trace elements like Fe, Zn, Mo and Cu are required in amounts

(µg/ml) of 1.0, 0.8, 4.0 and 1.0 respectively. But other trace elements like Co and Mn are found to have no effect on the production of pectolytic enzymes. It is further observed that the optimum concentrations of inorganic salts for growth of the organism and enzyme production are different.

During the studies on the effect of some growth factors and metabolic inhibitors on the production of pectinases by A. niger JU it has been observed that amongst the growth factors studied, yeast extract (0.75%) has got stimulatory effect on both the production of the enzymes and cell synthesis while of the metabolic inhibitors studied, methylene blue (0.025 mM) strongly activates the synthesis of pectinases.

The present investigation has resulted in the selection of a suitable medium consisting of Pectin - 4%, Peptone - 1.5%, Yeast extract - 0.75%, K_2HPO_4 - 0.2%, $MgSO_4.7H_2O$ - 0.025%, KCl - 0.05%, Na_2SO_4 - 0.05%, $CaCl_2.2H_2O$ - - 0.022%, $FeSO_4.7H_2O$ - 0.0005%, $ZnSO_4.7H_2O$ - 0.0004%, $Na_2MoO_4.2H_2O$ - 0.001%, $CuSO_4.5H_2O$ - 0.0004% and Methylene blue - 0.025 mM (pH 4.5). This medium was further investigated to determine the course of metabolic changes during the biosynthesis of pectinases. In practice, the changes in the composition of fermentation broth were noted in relation

to cellular growth, pH, utilization of galacturonic acid (free and combined) and different forms of nitrogen, production of organic acids and pectinases. It is observed that the rates of production of pectinases, utilization of galacturonic acid, acid production and cellular growth are greatly accelerated between the period of 48-144 hrs. As the utilization of carbon and nitrogen sources (pectin and peptone) is a prerequisite for high pectinase - yielding fermentation, the maximum rate of formation of the enzyme between the period of 48-144 hrs. can be explained on that basis.