1. ABSTRACT

Lichens grow under extreme environmental conditions and hence the characteristics of amyloglucosidase from the fungus in the lichen niche collected from mango tree were studied. Due to the difficulties in isolating the fungus from the lichen, the fungus in lichen niche was isolated by growing on potato agar plate. It didn't sporulate under our culture conditions. Hence the mycelia and not spores were used as inoculum in our studies. The fungus was identified as Botryodiplodia theobromae and it attained the stationary phase in 60 h.

For amyloglucosidase production the fungus was grown in a submerged culture containing potato starch 2% (W/V) under aeration at room temperature at pH 6. The amyloglucosidase activity was 32 nmol ml^{-1} min^{-1} at 26 h. However when grown under agitation in an orbital shaker (160 rpm) the amyloglucosidase activity increased to 324 nmol ml^{-1} min^{-1} in 42 h.

The effect of different sources of starch from potato, corn and manioc on enzyme production was investigated. When the starch provided was 2% (W/V), the amyloglucosidase produced in the medium containing corn starch was 20% more than that produced in the medium containing manioc starch. However manioc starch was selected as a source of carbon for further studies since the production of manioc in Northern part of Sri Lanka is higher than that of corn.

When the manioc starch medium (2% W/V) was supplemented with $(NH_4)_3PO_4$ 0.2% (W/V) and peptone 0.5% (W/V), the enzyme activity dropped to 138 nmol ml^{-1} min^{-1} and the pH of the medium decreased from 6 to 1.8 . The decrease in activity could be due to the denaturation of enzyme at low pH. However when the pH of the medium was maintained at 6 by the addition of K_3PO_4 0.25% (W/V) and $CaCO_3$ 0.36% (W/V), the enzyme activity increased to 900 nmol ml^{-1} min^{-1} at 46 h incubation. The enzyme production further increased to 1700 nmol ml^{-1} min^{-1} on the addition of soya bean powder 2% (W/V) to the medium at pH 5. But under the same conditions, amyloglucosidase production increased by 14% (1950 nmol ml^{-1} min^{-1}) at pH 6. When the medium was supplemented with 0.3%(W/V) yeast extract, the amyloglucosidase production increased marginally. Tween 80 at different concentrations (0.001, 0.01 and 0.1% W/V) had no influence on enzyme production or its release into the medium. When the medium was supplemented with different sources of nitrogen ($(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl) instead of $(NH_4)_3PO_4$, no improvement on enzyme production was observed.

For the continuous batchwise production, contamination of fungus with bacteria is a problem. The contamination is normally avoided by growing the fungus at low pH. Hence the fungus was grown at pH 5. Continuous batchwise production of amyloglucosidase was carried out at pH 5 over a period of 17 days. The amyloglucosidase production reached a peak in 48h. Four batches of enzyme were

produced in 16.5 days. The enzyme activity decreased in the 5th cycle on the 17th day. With continuous culturing, the medium colour also changed from yellow to pink.

This crude enzyme showed zero order kinetics for 1 h at $52^{o}C$ in 0.02 M citrate-phosphate buffer (pH 5.1). The optimum temperature and pH of this enzyme were found to be $52^{o}C$ and 5.1 respectively. Although the temperature optimum was $52^{o}C$, the enzyme lost 51% of the activity in 5 h and 90% of the activity in 49 h at $52^{o}C$. The enzyme lost 4% of the activity in 24 h at $40^{o}C$. It retained 100% activity for 49 h at $21^{o}C$ and $29^{o}C$.

Amyloglucosidase in the culture medium purified by DEAE - Cellulose gave a 4.3 fold purification with 100% recovery. The culture medium had very little α - amylase activity (0.7%) which was confirmed by Phadebas amylase test. The inhibition of amyloglucosidase by glucose was studied. The extent of inhibition increased with increasing concentration of glucose and was fully (100%) inhibited at the glucose concentration of 12 g l^{-1}.