the eight formulations, the formulation F_2 was considered best because, it showed delayed release and the drug release in pH 7.2 buffer was found to be almost complete (98.06%) and sustained.

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REFERENCES

1. Insel, A.P. In; Hardman, G.J. Ed; Goodman & Gilman's The Pharmacological basis of Therapeutics, 9th Edn., Pergaman Press, New York, 1966, 638.
2. Reynolds, E.F., Ed; MARTINDALE: The Extra Pharmacopoeia, 29th Edn., The Pharmaceutical Press, London, 1989, 18.
3. Bolton, S. In; Pharmaceutical Statistics, Marcel Dekker Inc., New York, 1984, 266.
4. Grass IV, G.M., In; Banker G.S. and Rhodes, C.T., Eds; Modern Pharmaceutics, 2nd Edn., Marcel Dekker Inc., New York, 1990, 654.

Synergestic Action of Maltose and Dextran on Extracellular Dextranase Production

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Maltose along with dextran provides a synergestic action on dextranase production by *Penicillium lilacinum*. Compared to dextran alone, dextran with maltose did not only improve the productivity and check the autolysis of cells but also cut down the period of maximum production of dextranase by two days.

Dextranase, the specific enzyme for degrading the α-1,6-glucosidic linkages in dextran (a microbiologicallly produced high molecular weight polysaccharide) is useful in medicine[1,2] and in industry[3]. In view of the potential uses of dextranase in medicine as a means for degrading dextran to molecular size-range suitable for use as synthetic blood volume expander and other useful purposes, studies on dextranase producing organisms and other parameters including different carbon sources had been performed and reported[4]. Of all organisms, *P. lilacinum* gave the maximum dextranase production in shaking condition on the 5th day at 26±2°, while maximum growth was observed on the 3rd day after which autolysis of cells would start. Among all carbon sources, only maltose other than dextran, produced dextranase in very low yield. Keeping in mind the lysis of cells and maltose-induced dextranse production, the present work was undertaken to investigate the synergestic action of maltose and dextran on dextranase production and other related aspects.

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The *Penicillium lilacinum* (NRRL-895) strain was obtained from Common Wealth Mycological Institute, Kew, England. Dextran was a gift from Tata Fison Industries, Calcutta, India as 6% (w/v) dextran in saline. O-Toluidine was obtained from S.D. Fine Chemicals Pvt. Ltd., Mumbai, India. Maltose was procured from Merck, West Germany. All other chemicals used were standard laboratory reagents of analytical grade.

One millilitre of spore suspension (in sterile distilled water containing approximately $7x10^7$ spores/ml) from a seven day old potato corrot-agar slants was used to inoculate 50 ml of the media (0.5% dextran, 0.08% $MgSO_4$, $7H_2O$, 1.2% KH_2PO_4, 0.04% KCl, 0.05% $CaCl_2.2H_2O$,

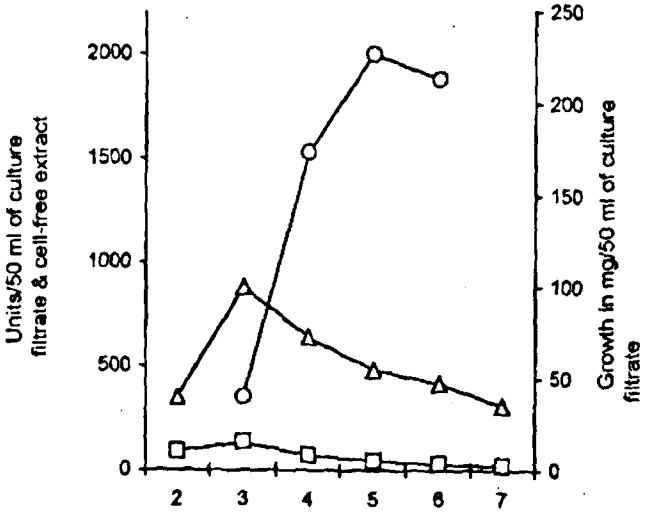

Fig. 1: Relation among extracellular dextranase activity (O–O), growth (Δ – Δ) and cell-free extract dextranase activity (□–□) using dextran as only carbon source Each point is a mean of 5 observations

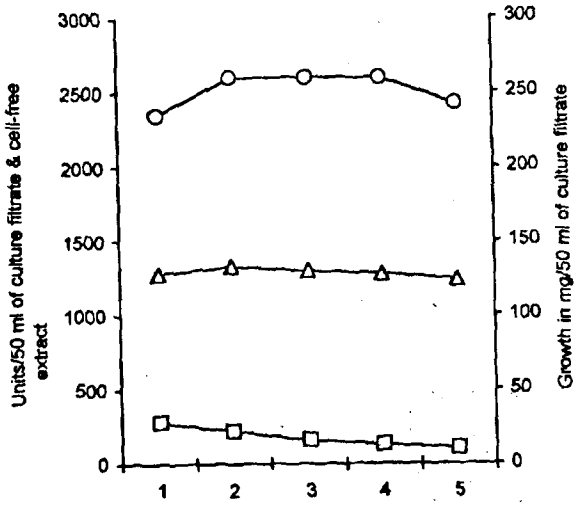

Fig. 2: Effect of Maltose-Dextran on extracellular dextranase activity (O–O), growth (Δ – Δ) and cell-free extract dextranase activity (□–□) Each point is a mean of 5 observations

0.67% NH_4NO_3; the pH being within 5.6-6.0) in a 250 ml Erlenmeyer Flask, incubated at 25±1° for five days on a rotary shaker (150 rpm). The % concentration of dextran (W/v) and maltose (W/v) were varied as required.

The dextranase activity of the culture filtrate was assayed by the method using the thiourea-borax modified O-toluidine colour regent[6-8] and sugar liberated was followed colorimetrically at 635 nm. One unit of dextranase activity was defined as the amount of enzyme required to liberate 1.0 µm of glucose per min. at 37° at pH 4.8.

For cell-free extract dextranase activity, mycelium (day 2 to 5) obtained after filtration were washed twice in 0.1 M citrate-phosphate buffer (pH - 5.0) and sonicated in ice-cold condition for 25 min, using ultrasonic disintigrator followed by centrifugation of the suspension at 11,789, g for 15 min. The supernatant obtained was used for dextranase assay.

To monitor the growth, the mycelium was filtered by suction, washed with glass distilled water and then dried to a constant weight at 85° in weighed planchets. All the results were computed by taking the mean of five different observations.

Previous study[4] revealed that dextranase production level after an initial lag phase increased with growth but attained the peak when the cell autolysis started. So it became obvious to determine whether the enzyme was truly extracellular or was just being released as the cells autolysed. The study also indicated that among all the carbon sources, only maltose (other than dextran) induced dextranase production (2.07±1.06 units/ml filtrate). In view of the lysis of the mycelia and maltose-induced production in low yield, experiments related to synergestic action of maltose and dextran on enzyme production; relation among growth, extracellular dextranase production and cell-free extract dextranase activity were performed. The results (Fig. 2) reflected that dextran along with maltose controlled the lysis of the cells and it was also observed that maltose was a better inducer of the enzyme than dextran alone. Dextran with maltose induced maximum enzyme production on day 3 (Fig. 2) while, with dextran alone, it was on day 5 (Fig. 1). The results clearly indicated that maltose with dextran did check the autolysis of the cells, enhanced productivity and cut down the period of maximum enzyme production by two days. Cell-free extract showed very little dextranase activity compared to that of the culture filtrate (Fig. 2) indicating that P. *lilacinum* was capable of producing dextranase of both types i.e. extracellular and intracellular, but the dextranase under investigation was found to be truly extracellular type. When P. *lilacinum* was grown in media containing 0.25% to 3% (w/v) dextran alone, maximum production was observed at 1.5% (W/v) dextran concentration[4]

TABLE 1 : EFFECT OF DIFFERENT CONCENTRATIONS OF MALTOSE AND DEXTRAN ON EXTRACELLULAR ENZYME PRODUCTION ON DAY 5

Dextran and maltose as carbon source (W/v)	Enzyme activity (units/ml culture filtrate)
0.5% Dextran + 0% Maltose	39.99±1.79
0.5% Dextran + 0.15% Maltose	48.72±2.12
0.5% Dextran + 0.25% Maltose	52.71±3.34
0.5% Dextran + 0.5% Maltose	49.80±2.65
0.5% Dextran + 0.75% Maltose	48.32±2.48
0.5% Dextran + 1.5% Maltose	36.72±2.60
0.5% Dextran + 2% Maltose	26.35±3.24
0% Dextran + 0.25% Maltose	2.07±1.06
0.25% Dextran + 0.25% Maltose	45.82±2.91
0.5% Dextran + 0.25% Maltose	52.71±2.34
0.75% Dextran + 0.25% Maltose	54.06±2.65
1% Dextran + 0.25% Maltose	52.06±2.21
1.5% Dextran + 0.25% Maltose	48.20±2.45
0.75% Dextran + 0.5% Maltose	52.01±2.59

Each value is a mean of 5 different shake-flask cultures with standard deviation

while along with maltose (0.25% w/v), maximum production level was attained at 0.75% (W/v) dextran concentration. Increased concentration of maltose and dextran, however, was found to have adverse effects on productivity, (Table 1); the decreased enzyme, production at higher concentration might, probably be due to the accumulation of significant amounts of rapidly metabolised sugar which favoured growth with less enzyme induction.

It is therefore, concluded that dextran and maltose has synergestic action on dextranase production and these two together may be effectively utilised as a carbon source in order to increase the productivity of extracellular dextranase, thereby reducing the cost of this potential enzyme of clinical and biotechnological importance.

REFERENCES

1. Carlson, W.V. and Carlson, W.W., **Science,** 1952, 115, 43.
2. Fukumoto, J., Tsuji, H. and Tsuru, D., **J. Biochem.,** 1971, 69, 1113.
3. Richards, G.N. and Streamer, M., **Carbohyd. Res.,** 1972, 25, 323.
4. Das, D.K. and Dutta, S.K., **Indian J. Pharm. Sci.,** 1992, 54, 145.
5. Hultman, E., **Nature,** 1959, 183, 108.
6. Dobowski, K.M., **Clin. Chem.** 1962, 8, 215.
7. Cooper, C.R. and McDeniel, V., In; Standard methods of clinical chemistry, Vol. 6, Academic Press, New York, 1970, 159.
8. Gowenlock, A.H., Eds., In: Varleys Practical Clinical Biochemistry: 6th Edn., Heinemann Medical Books, London, 1998, 320.