EXOCELLULAR LIPASE PRODUCTION BY A SOIL STREPTOMYCETE

DURING a soil screening programme, an actinomycete was isolated which produced exocellular lipase in the culture medium. Taxonomically the strain was designated as *Streptomyces* sp. L_4.

Preliminary screening for the lipolytic activity of the soil isolates was done using a medium[1] containing peptone 10·0, NaCl 5·0, $CaCl_2$ 2·0, agar 20·0 g/l and 4·7 ml Tween 80 per litre (pH 7·0). The appearance of cloudy zones around the colonies in plates indicated precipitation of Ca-salts of free fatty acids. Strains with this type of zones were isolated and grown in broth. The broth contained[2] polypeptone 7·5, meat extract 7·5, soluble starch 10·0, NaCl 3·0 and $MgSO_4$, $7H_2O$ 1·0 g/l (pH 7·2). Final screening was done by cup assay method using the Tween 80 agar medium. Among the different strains isolated, *Streptomyces* sp. L_4 produced a cloudy zone of 80 mm diameter on the third day of its growth at 28° C.

The broth was filtered and the crude enzyme was precipitated[3] by 60% ammonium sulphate saturation. The precipitate was collected after centrifugation (12,000 rpm for 20 min). This was then dissolved in deionised water and dialysed against deionised water in cold for 4 days using egg membrane. The activity of the dialysed product was measured by titrimetric method using olive oil as substrate[4]. 50% pure olive oil emulsion using gum acacia and sodium benzoate as stabiliser was activated with human blood serum at 37° C for 1 hr. The "Control" tube contained 0·5 ml Mg acetate (0·01 M) + 1 ml tris buffer + 3 ml of serum activated olive oil. The "test" tube contained all the above solutions and the substrate mixed with 1 ml of the enzyme solution. All the tubes were incubated at 37° C for 1 hr after thoroughly shaking the mixture. After incubation 3 ml of 95% ethanol was added to each tube to terminate the reaction. All the tubes were shaken well and to each of them 4 drops of indicator solution (0·5 g phenophthalein and 1 g of thymolphthalein in 100 ml of 95% ethanol) was added. The contents were then titrated against 0·1 N NaOH solution in a microburette. The total protein in 1 ml of the enzyme solution was estimated by the micro-kjeldahl method. Unit of activity was defined as μ moles of fatty acids formed per hour and specific activity was calculated as μ moles of fatty acids formed/hour/mg protein. The enzyme was found to be fairly stable at low temperature and can be stored at − 5° C for a long period without much decrease in its activity. The optimum pH of activity is 8.

TABLE I

Production of lipase by Streptomyces *sp.* L_4

Steps	Activity μ/ml/hr	Protein mg/ml	Specific activity	Yield (%)
Culture broth	1250	416·6	3	100
Ammonium sulphate pptn (60% saturation)	760	122	6·2	58·6
Dialysis	360	14·5	24·13	28·8

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