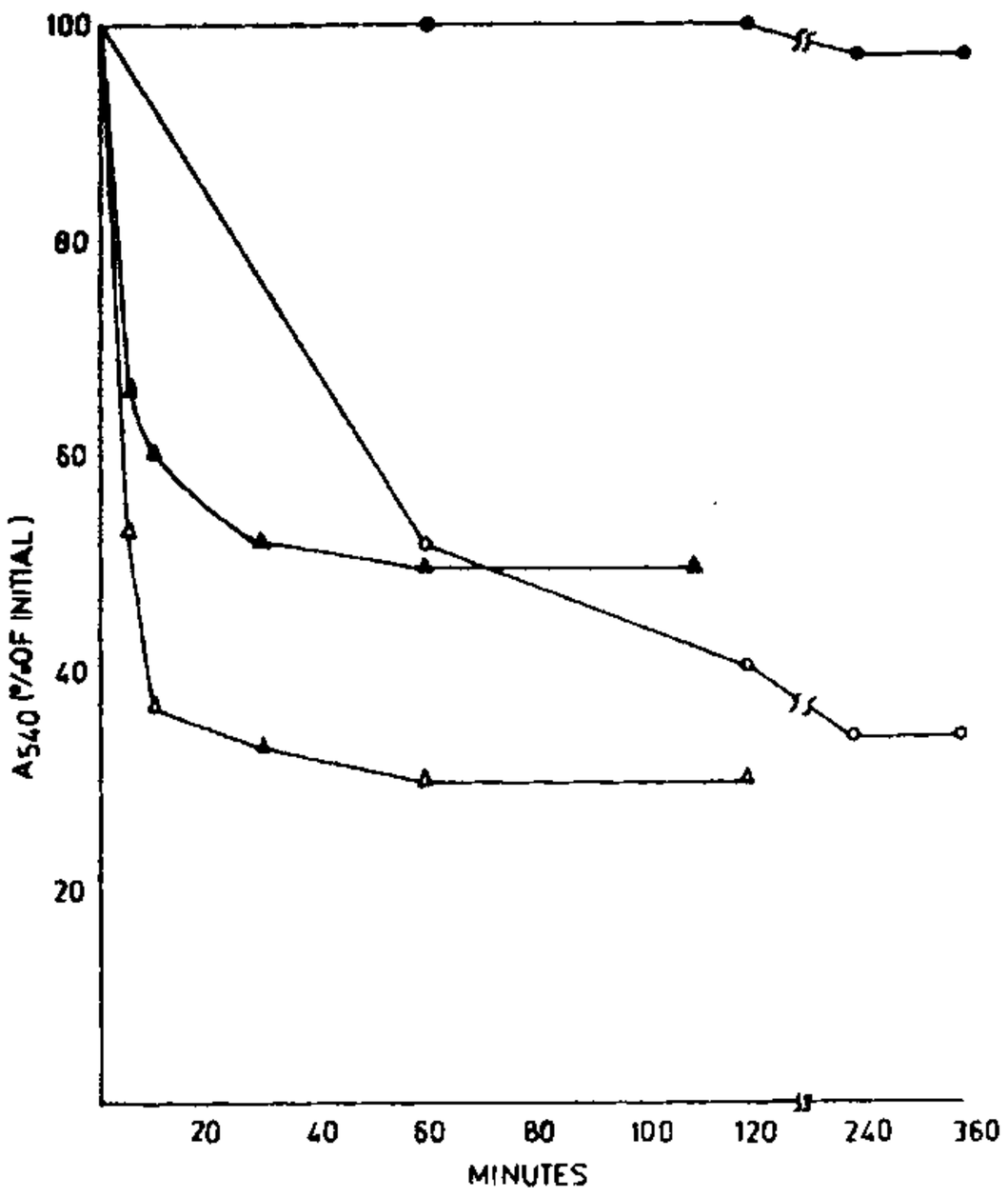

Figure 1. Relationship between treatment time of cells and drop in absorbance due to lysis of cell wall, *Saccharomyces cerevisiae* (▲—▲) and *Candida lipolytica* (Δ—Δ) treated with β-glucuronidase; *Rhodotorula glutinis* grown in glucose (●—●) or glucuronic acid (o—o) and treated with β-glucuronidase plus driselase.

2 hr, greater than 60% glucuronic acid grown cells were converted to protoplasts against less than 10% protoplast formation in glucose grown cells. The growth of *R. glutinis* in a medium containing glucuronic acid results, presumably, in cells whose cell walls contain higher than normal number of glucuronic acid moeities rendering greater sensitivity to β-glucuronidase attack. In addition to β-glucuronidase and driselase, the effect of other lytic enzymes viz zymolyase (Seikagaku Kogya), helicase (Phermindustrie), chitinase (Sigma) and lytic enzyme (Sigma) were examined. The results presented in table 1 show that combinations of β-glucuronidase, helicase and driselase cause significant cell wall lysis in *R. glutinis* grown in glucuronic acid as carbon source. Since β-glucuronidase and helicase, are both isolated from the same source, viz *Helix pomatia*, they might be similar. However, the combination of driselase with helicase was more effective than the combination of driselase with β-glucuronidase. It is likely that helicase is a crude preparation containing some lytic activities in addition to β-glucuronidase, and hence is more effective than the latter. Driselase is also a mixture of several lytic activities (cellulase, xylanase, proteinase, dextrinase etc) and has been used for preparation of protoplasts from plant cells and black yeast, *Aureobasidium pullulans*[5]. We believe that the method described in this paper may be of general use for preparing protoplasts from yeast strains belonging to genus *Rhodotorula*.

The authors are thankful to Dr K. K. G. Menon for his encouragement during this study.

12 February 1986; Revised 5 April 1986

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A METHOD FOR ISOLATION OF THERMOPHILIC ACTINOMYCETES

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SINCE thermophilic actinomycetes have been considered to be potential producers of novel antibiotics and a good source of thermostable enzymes, the isolation of this group of microorganisms has gained importance in recent years[1].

For the isolation of thermophilic actinomycetes, several agar media formulations have been recommended[2], but they offer marginal advantages. Incorporation of antifungal antibiotics or bacterial inhibitors has its own disadvantages. For the isolation of mesophilic actinomycetes, a few methods have been suggested, which do not incorporate any extraneous chemical agents. Two of these methods are differential centrifugation (DC) method[3] and the high temperature pre-incubation (HTPI) method[4]. Both these methods do not proclaim their use only for isolation of

thermophilic actinomycetes. In this report, we describe a modified combination of these methods for preferential isolation of thermophilic actinomycetes.

Forty soil samples from eight localities were collected from the vicinity of Pune and surrounding area. The samples were uniformly suspended in sterile physiological saline (10 g in 90 ml) and were subjected to three different methods of isolation, namely the DC[3], HTPI[4] and a combination of both these methods, modified as detailed below:

The soil suspension was centrifuged for 20 min in a REMI T23 centrifuge at 3000 r.p.m. (RCF 604 × G at the surface and 1610 × G at the bottom of the cuvette). One ml aliquot of the centrifuged suspension was pour-plated in triplicate using glucose yeast extract agar medium of pH 6.5. The plates were subjected to pre-incubation at 110°C for 10 min. After the pre-incubation, the plates were incubated at 55°C in a humidified incubator for 48 hr. The morphology of colonies developing on the isolation plates of all the three methods was visually examined and ascertained microscopically. The colony counts were noted as belonging to bacteria and actinomycetes. None of the methods tried yielded mold growth. The thermophilic actinomycetes isolated belonged to *Streptomyces*, *Thermoactinomyces*, *Thermomonospora*, *Micromonospora* and *Micropolyspora* species. The mean actinomycete counts (MAC) and the mean bacterial counts (MBC) obtained by the three methods are shown in table 1. In each of the methods, the MAC differed significantly from the MBC as obtained by comparing the two sample means at 1 % level of significance. This was confirmed by using the statistical formula:

$$|\bar{x} - \bar{y}|/\{[S_1^2/(n_1 - 1)] + [S_2^2/(n_2 - 1)]\}^{1/2}$$

where $\bar{x}$, $\bar{y}$ are the mean of the first and second series of counts, respectively n_1 and n_2 are number of observations in the first and second series respectively, $S_1 = \Sigma(x_1 - \bar{x})^2/n_1$ and $S_2 = \Sigma(y_1 - \bar{y})^2/n_2$. It was evident from the results that the DC + HTPI combination method was superior to the other two methods so far as the isolation of thermophilic actinomycetes was concerned. The reason for such a preferential isolation may be that the differential centrifugation helped the separation of actinomycetes spores and high temperature pre-incubation favoured the growth of thermophilic actinomycetes, at the same time preventing bacterial growth.

Table 1 *Mean thermophilic actinomycetes and bacterial counts*

Mean counts per gram of soil	DC method	HTPI method	DC + HTPI method
Mean thermophilic actinomycete count (MAC)	3.125×10^2	8.750×10^2	11.250×10^2
Mean bacterial count (MBC)	13.625×10^2	1.940×10^2	1.000×10^2

24 February 1986; Revised 25 April 1986

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INFLUENCE OF DESICCATION STRESS IN A XEROPHILIC THERMOPHILE *HUMICOLA* SP

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It has long been recognized that a fundamental difference exists between the water economy of higher and lower plants. The terms 'homoiohydrous' and 'poikilohydrous' have been used to describe this condition in higher and lower plants respectively. Unlike the former, poikilohydrous plants also absorb water from vapours present in the atmosphere. Whereas considerable work has been done regarding the response of water stress in higher plants, algae, bryophytes and lichens, relatively little is known about the biochemical changes due to desiccation stress in fungi[2,3]. It is known that in higher plants sugar and proline content increases while phospholipids decrease with increasing water stress[4]. However, no such studies exist with fungal systems. Since thermophilic moulds