Regeneration of Yeast Protoplasts prepared by Snail Enzyme

It has previously been shown[1,2] that yeast protoplasts prepared by autolysis of the cell wall may regenerate. The rate of regenerating protoplasts grown on the surface of solid media was very low (1–2 per cent). The cultivation of protoplasts embedded into high-percentage gelatine led to almost quantitative regeneration[3].

The regeneration ability of yeast protoplasts liberated by digestion of the cell wall with enzymes of the snail *Helix pomatia* has either not been demonstrated at all[4], or has been observed occasionally only[5,6]. We have been successful in achieving a high rate of regeneration of yeast protoplasts prepared by snail enzyme by embedding the protoplasts in nutrient media containing gelatine.

Cells of *Saccharomyces cerevisiae* Hansen, strain No. 7 (VULKP, Prague), were used. The protoplasts were prepared by the modified procedure of Eddy and Williamson[7]. Log phase cells were washed with distilled water and then incubated in the concentration of 10^7–10^8 with the following medium: citrate phosphate buffer (*p*H 5·4), 0·8 M mannitol and dried gut juice of *Helix pomatia* 10 mg/ml. After about 1 h of incubation at 28° C, 95–98 per cent of cells were converted into protoplasts. The enzyme was removed by washing the protoplasts with mineral nutrient medium. The protoplasts were then partly grown on the surface of agar films in moist microscopic chambers and partly embedded in mineral nutrient medium containing 30 per cent gelatine. The procedure of embedding has been described previously[3].

The character of growth of protoplasts prepared by snail enzyme on the surface of solid media was the same as that of those prepared by autolysis of the cell wall. Plasmatic formations that have already been described in both types of protoplasts arise[1,4]. The majority of these plasmatic formations died within 48 h and only a small proportion (about 1 per cent) regenerate into new yeast cells after 5 days of incubation. Both morphology and time lapse of regeneration is the same with protoplasts prepared by autolysis of the cell wall.

The protoplasts embedded into high percentage gelatine grow (their diameter increases 2–3 times), maintaining the spherical shape. After 10–14 h of incubation (at room temperature) the formations begin to bud on one or several sites simultaneously (Fig. 1). After 20–24 h, microcolonies of regenerated yeast cells can be found in the preparations. The rate of regenerating protoplasts may reach 80 per cent under optimal conditions. The morphology of the regeneration process of protoplasts prepared by snail enzyme is entirely the same as in that prepared by autolysis. The time lapse, however, or the appearance of morphological changes, respectively, is substantially shorter (by 10–15 h). This is based probably on the physiological state of protoplasts, as the preparation of protoplasts by autolysis uses the cells of stationary cultures.

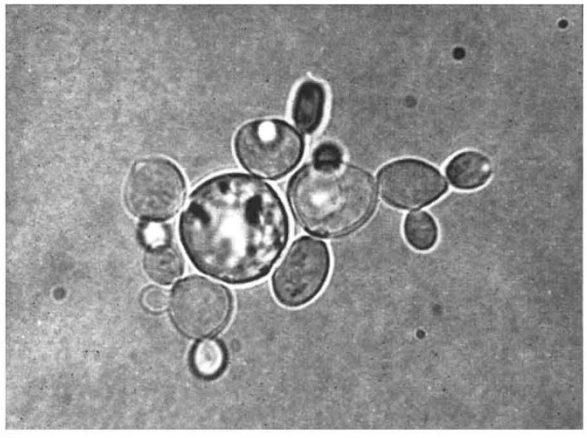

Fig. 1. Regenerating protoplast of *S. cerevisiae* after 24 h incubation in 30 per cent gelatine (× *c.* 1,133)

Formation of the cell wall also takes place in the same way in both the types of protoplasts. The fibrillar network representing the groundwork of the new cell wall[8] is formed first and then the cement substances of the matrix. Just before the regeneration, the formation of a structurally and functionally complete cell wall is accomplished. The whole process of formation of the cell wall in protoplasts prepared by snail enzyme accordingly takes place faster.

In the course of further experiments, the protoplasts prepared by autolysis of the cell wall were treated with snail enzyme under the same conditions as the protoplasts prepared by snail enzyme. When embedding in gelatine medium, the regeneration rate as well as the morphology and time lapse of regeneration of these protoplasts was the same as those of controls.

The identity of protoplasts prepared by autolysis of the cell wall or by snail enzyme from the point of view of the regeneration ability has thus been confirmed. More detailed cytological aspects of the possible differences between these protoplasts prepared in different ways are under investigation at present.

A. Svoboda
O. Nečas

Department of Biology,
Medical Faculty,
J. E. Purkyně University,
Brno, Czechoslovakia.

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An Arthropod Intermediate Host of a Pentastomid

The life cycles of pentastomids, or tongue worms, are little known although the biology of these creatures has been the subject of investigations since the pioneer studies of Rudolf Leuckart[1] in 1860. In the adult stage, pentastomids are almost exclusively parasites of reptiles, exceptions being species of *Linguatula* from carnivores and *Reighardia sternae* from gulls and terns. The immature stages of these parasites have hitherto been found only in vertebrate animals, ranging from fish to mammals. In every case in which life cycles have been either partially or totally elucidated the intermediate host has always proved to be a vertebrate. As Baer[2] says, "It is particularly interesting to record that no larval pentastomid has ever been found in an invertebrate and that, on the other hand, many species of warm-blooded animals can act as intermediate hosts of reptilian parasites". Our discovery recently of larval pentastomids in cockroaches in Singapore is therefore of unusual significance since it appears to be the first record of the finding of tongue worms in invertebrates.

During routine dissections of the American cockroach, *Periplaneta americana*, taken from a house in a well-to-do area of Singapore, a few cockroaches were discovered harbouring hundreds of pentastomid larvae. Subsequently, systematic examinations of *P. americana* from the same and from neighbouring localities revealed a high percentage of parasitized insects. 108 cockroaches were dissected and, of these, eleven contained larvae. Most of the infestations were light, numbering not more than twelve larvae per insect, but in the case of two cockroaches, infection was so heavy that the haemocoelic cavity gave the impression of being a writhing mass. In addition to