Formation and cell wall regeneration of protoplasts from Schizophyllum commune

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SUMMARY

Osmotically sensitive protoplasts were released from the mycelium of the basidiomycete *Schizophyllum commune* through the action of an extracellular enzyme preparation isolated from the culture filtrate of *Trichoderma viride* (recently renamed *T. harzianum*) grown on hyphal walls of the former organism. The conditions for obtaining stable protoplasts were determined. Maximum numbers of protoplasts were released from young growing mycelium by using 0.5 M $MgSO_4$ or 0.3 M KCl in the presence of 0.05 M Na-maleate buffer at pH 5.8 (osmotic potential −15.8 atm). Protoplasts emerged through ruptures in the wall, initially at the apices, but later also from older parts of the hyphae.

The Trichoderma lytic enzyme system appeared to be very effective in releasing spheroplasts from other fungi, too. In 32 out of 33 basidiomycetous species tested spheroplast release was achieved whereas snail-gut enzymes were effective in 7 cases only. Also with ascomycetes the Trichoderma enzyme system was superior to snail enzymes.

Protoplast release in *Schizophyllum commune* was accompanied by degradation of the three major wall polymers, S-glucan, R-glucan and chitin. Part of the S-glucan was resistant. The resistance of S-glucan to enzymatic degradation increased with culture age and concomitantly the yield of protoplasts was reduced. Isolated S-gluan was also partly resistant to degradation, probably due to its crystallinity. Analysis of S-glucan by the techniques of optical rotation measurement, infrared spectroscopy, periodate oxidation, methylation analysis, partial acid hydrolysis and enzymatic hydrolysis demonstrated that this glucan mainly consists of 1,3-linked α-D-glucosyl residues. In living cells S-glucan protected chitin and possibly R-glucan against degradation by external enzymes.

S-glucanase, R-glucanase, chitinase and exo-laminarinase were purified from the Trichoderma enzyme mixture. Addition of only S-glucanase and chitinase was essential to achieve protoplast release. R-glucan was degraded endogenously.

In the presence of $MgSO_4$, protoplasts containing a nucleus developed a large vacuole and could be selected due to their low buoyant density. These protoplasts were all capable of wall regeneration and about 50 percent reverted to the hyphal mode of growth in

liquid medium. The kinetics of the formation of the three main cell-wall components, and of RNA and protein synthesis were studied by using both chemical and isotope labelling techniques. S-glucan and chitin accumulation as well as RNA and protein synthesis started simultaneously after a short lag, but the formation of R-glucan only began after several hours of rapid R-glucan synthesis. Cycloheximide added at zero time regeneration in a concentration blocking protein synthesis also inhibited the formation or R-glucan and the reversion to hyphal growth but the formation of chitin and S-glucan did start and continued seemingly unimpaired for several hours. This indicates that the enzymes or enzyme systems responsible for the synthesis of these wall polymers remained intact during protoplast release. Polyoxin D not only inhibited chitin synthesis but also completely arrested the synthesis of R-glucan. S-glucan synthesis was not inhibited by polyoxin D but no reversion to hyphal growth occurred. These inhibitor studies as well as the kinetics of R-glucan formation during normal regeneration suggest that the synthesis of R-glucan is required for the initiation of hyphal growth.