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Isolation of small protoplasts

from Aspergillus niger

ing on the incubation time during the isolation procedure, whereas protoplasts from mycelium vary strongly in size and in number of nuclei. The protoplasts were suitable for fusion and transformation experiments (Bos et al. 1983 Experientia Suppl. 45:298-299; Wernars et al. 1985 Current Genetics 9:361-368).

Although it was possible to isolate protoplasts from conidiospores of some A. niger strains in the same way, it was less successful with some other strains. In these cases only osmotically stable spheroplasts were obtained. Therefore, we searched for improvement of the methods for isolation of protoplasts both from conidiospores as well as from young hyphae with the aim to obtain a homogenous suspension of small protoplasts.

Protoplasts from conidiospores.

With the strains which produce spheroplast in the usual way (see previous paragraph), protoplasts could be released from the conidiospores by careful manipulation of the osmotic strength of the lytic medium. Protoplasts were released by a gradual reduction of the osmotic strength to less than 1.0 osmol (0.4 M KCl). In the swollen state the protoplasts are very fragile and the osmotic strength had to be increased again slowly to about 1.5 osmol (0.7 M KCl). This treatment is critical and sometimes a considerable loss of protoplasts has been observed. Another disadvantage of this procedure is that the Oerskovia enzymes which are used for the lytic treatment are not available commercially.

Protoplasts from young hyphae

Owing to the problems in preparing conidial protoplasts of some A. niger strains, we modified the procedure of Musilkova and Fencel (1968 Folia Microbiol. 13:235-239) so that we obtained homogeneous protoplast suspensions from young hyphae. A. niger strains were grown for 16 h at 30°C after inoculation of 200 ml CP medium (Bos and Slakhorst 1981 l.c.) in 1000 ml Erlenmeyer flasks on a rotary shaker at about 100 rpm. The medium was inoculated with conidiospores from a 4 or 5 day old culture to a final concentration of 10^6 spores per ml. Resulting hyphae were harvested on Miracloth on a Buchner funnel and were resuspended in 20 ml lytic medium containing per ml 10 mg Novozym 234, 0.7 M NaCl, 0.2 M CaCl₂ (osmotic strength about 1.8 osmol). Incubation was at 30°C for 2.5 h. The high osmotic strength of this lytic medium provides a slow release of the protoplasts from the young hyphal tips. Microscopically, the protoplasts appeared quite homogenous in size and they were stable in further experiments as long as the osmotic strength was kept above 1.2 osmol (0.6 M KCl) and below 2.3 osmol.

Using this method, up to 10^8 protoplasts could be prepared. Mycelial debris was removed by filtration over a glass wool plug. Protoplasts were concentrated by centrifugation at about 1100 x g, washed with and resuspended in a medium of suitable osmotic strength (1.5 osmol, e.g., 0.7 M KCl or 1.2 M sorbitol + 50 mM CaCl₂). The protoplasts have been used successfully in fusion and in transformation experiments. The regeneration frequency was usually above 30%. - - Dept. of Genetics, Agricultural University, 53 Gen. Foulkesweg, 6703 BM, Wageningen, The Netherlands