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Modification of the method for cryo-preservation of rumen anaerobic fungi

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Anaerobic *Chytridiomycete* fungi penetrate and degrade efficiently plant cell walls in the alimentary tract of herbivores. They possess a wide range of polysaccharide-degrading enzymes and play an important role in animal nutrition (Fonty and Joblin, 1991, in Physiological aspects of Digestion and Metabolism in Ruminants, T Tsuda *et al* eds, 655-680). Increasing interest to these unusual microorganisms during last decade is explained by their production of highly active cellulases and xylanases (Wilson and Wood, 1992, Enzyme Microbial Technol, 14, 258-264 ; Mountfort and Asher, 1989, Appl Env Microbiol, 55, 1016-1022). However preservation of rumen *Chytridiomycetes* is a serious problem.

So far the only reliable technique reported was cryo-preservation of the fungi by deep freezing in liquid nitrogen with 5 % (v/v) dimethyl sulfoxide (DMSO) as a protecting agent (Yarlett *et al*, 1986, Lett Appl Microbiol, 3, 1-3). The procedure includes 24-h pre-freezing of fungal cultures at -70°C following with transfer of the material into liquid nitrogen. Although the technique provides good results, it is time consuming, demands special equipment and constant supply of liquid nitrogen. Modification of the method, which allows to preserve the anaerobic fungi in a freezer at -80°C for at least 4 months is reported here.

Strains *Neocallimastix sp.* TU1 from ovine and *Neocallimastix sp.* TU2, *Piromyces sp.* TU3, unidentified polycentric fungus TU4 and *Caecomyces sp.* TU5 from bovine rumen were isolated and subcultured by the method described earlier (Kostyukovsky *et al*, 1991, J General Microbiol, 137, 1759-1764). Cultures were grown 2 d in 5 ml glucose (0.2 %, w/v) medium supplemented with agar, 0.05 % (w/v), in a Pyrex culture tubes (105 x 11 mm, "Iwaki Glass", Japan) with butyl rubber stoppers ("Terumo", Japan). DMSO (250 µl) was injected into the cultures to make the final concentration 5 % (v/v). The tubes were centrifuged for 5 min at 3,000 rpm (20°C), 4.5 ml supernatant was removed by syringe, and the tubes were placed at -80°C in a freezer.

For viability check the fungi were thawed in a water bath at 37°C immediately after retracting from a freezer, and inoculated into fresh glucose medium. Since DMSO inhibited growth of anaerobic fungi at the concentration higher than 1 % (v/v) (unpublished observations), it is necessary to reduce time between injection of the agent and freezing, and between thawing and inoculation.

All 5 strains tested remained viable after storage at -80°C for at least 4 months. Successful preservation of *Caecomyces sp.* strain at these conditions is reported for the first time. The reason for this positive results is probably in fewer steps and the absence of contacts of the fungi with the oxygen. It is also possible that addition of 0.5 % (w/v) agar to culture medium not just facilitated fungal growth, but probably play some role in protecting the fungi during freezing. Further investigation in possible influence of the procedure on fungal morphology and fermentation characteristics is necessary.