PP-376

Selection and Characterization of the Yeast Strain Producing Polysaccharides

Leanida SAPUNOVA, Sviatlana KULISH, Iryna TAMKOVICH, Liudmila YARKHOVA
Institute of Microbiology of the National Academy of Sciences of Belarus, Republic of Belarus
leonida@mbio.bas-net.by

Aim of the study: In recent years microbial polysaccharides, including constituents of yeast cell walls turned out to be of growing demand in production of fodder and feed additives. This is determined by their antigenic, immunomodulating, antiviral, hypolipidemic, enterosorbing (detoxifying), antioxidant, cryoprotective and other properties. Earlier we isolated from soil yeast strain (identified as *Cryptococcus flavescens*) capable to produce intracellular beta-galactosidase and catalyze *in vivo* synthesisof probiotic galactooligasaccharides. In contrast to other yeast-like fungi, including representatives of genus *Cryptococcus*, strain *C. flavescens* also produced small amount of polysaccharides.This study was aimed at selection and characterization of a new yeast strain with enhanced level of extracellular polysaccharide production.
Materials and Methods: Multistage adaptive selection of yeast *Cryptococcus flavescens* 1 was carried out on Sabouraud agar medium containing (g/l): peptone – 10.0; agar-agar – 20.0; pH 7.2±0.2. Glucose and lactose were added into the medium as carbon sources and adaptation agents in steadily growing concentrations (5.0→7.0→10.0%). At the first selection stage rapidly growing yeast colonies of viscous consistency were sorted out of each 10th generation grown sequentially on the afore-mentioned media (26–28 ^{o}C, 48 ч). At the second stage the selected cultures were characterized via polysaccharide generation capacity during submerged fermentation (200 rpm; 26-28 ^{o}C, 72 h) in the media of the following composition (g/l): glucose or lactose - 3.0 (carbon content), peptone – 10.0, yeast extract – 5.0, K_2HPO_4 – 3.0, $MgSO_4x7\ H_2O$ – 0.5; initial pH – 6.8.Upon fermentation yeast cells were separated by centrifuging (7000-8000 rpm, 20 min), washed twice with distilled water and assayed for enzyme activity by generally established methods. Enzyme amount sufficient to catalyze substrate hydrolysis and to yield 1 μM of the product in 1 min was assumed as one unit of activity.Polysaccharides were isolated from cell – free cultural filtrate by ethyl alcohol fractionation (1:2 v/v) in the cold, washed with ethanol, dried at 50 ^{o}C to constant weight and estimated gravimetrically.
Results: Adaptation of yeast strain *C. flavescens* 1 to elevated concentrations of glucose and lactose conducted on agar media enabled to select 6 fast – growing colonies of viscous consistency, 3 isolates from each selective medium. Their comparative evaluation in submerged culture singled out new strain *C. flavescens* 1-AG-3 showing 1.5 – times higher level of extracellular polysaccharide production as compared to the parent culture.Strain *C. flavescens* 1-AG-3 belongs to the group of asporogenic, encapsulated yeast not capable to form pseudomycelium. The cells are oval – shaped, singular or in short chains (2-3 links in each). The culture produces on agar media with glucose round, convex, regular, even-edged, smooth, opaque, bright, viscid colonies of cream – like color acquiring with age pink tint, 3–5 mm in diameter. The yeast will not generate pigments or release exudate. It does not require artificial light. The organism is strict aerobe with growth optimas pH 6.5 and temperature 25-27 ^{o}C. Chemoorganotroph. It assimilates lactose, cellulose, sucrose, melibiose, maltose, glucose, fructose, galactose, mannitol, glycerol, utilizes peptone, urea, ammonium and nitrate nitrogen as N sources, liquefies gelatin, fails to peptonize milk. The culture is capable to synthesize beta – galactosidase, lipase, protease. The strain may find use as producer of oligo – and polysaccharides constituting the basis of synbiotic feed supplements.

Keywords: Yeast, *Cryptococcus*, selection, polysaccharides, production