INFLUENCE OF CULTIVATION CONDITIONS ON THE GROWTH AND FORMATION OF PSEUDOARTROBACTER SCLEROMAE CHOLESTEROL OXIDASE

Zhukouskaya L., Sudakova K., Semashko T.
Institute of Microbiology of the National Academy of Sciences of Belarus, Belarus
e-mail: mila_zhu@mail.ru

CZU:579.61:616.1 https://doi.org/10.52757/imb22.07

Cholesterol oxidase (ChO) (EC 1.1.3.6.) is a monomeric bifunctional flavin adenine dinucleotide (FAD)-dependent enzyme belonging to oxidoreductase family and catalyzing oxidation of 3β-hydroxysteroids and isomerization of intermediate cholest-5-en-3-one to cholest-4-en-3-one to yeld hydrogen peroxide. Cholesterol oxidase catalyzes the initial degradation of cholesterol and probably other natural sterols used as carbon sources for growth of various microorganisms.

ChO is engaged in clinical diagnostics to evaluate cholesterol level in blood, other biological fluids, foodstuffs, in fabrication of biosensors, precursors of steroid hormones, as insecticidal and antimicrobial agents.

According to literature data, the main ChO producers are strains referred to genera *Rhodococcus, Streptomyces, Brevibacterium.*

Taking into account a broad spectrum of enzyme application, the studies to seek new highly active sources of ChO are extremely relevant and vital.

Earlier we performed screening of bacterial strains synthesizing extracellular ChO using plate method based on growing bacteria on differential-diagnostic medium containing cholesterol and selection of the variants showing the maximum enzyme generation activity.

Aim of the study – investigation of the effect of cultural conditions on growth of *Pseudoarthrobacter scleromae* and ChO production.

As a result, the impact of initial pH of the nutrient medium (5,0-10,0), temperature (26-30°C), time of culture (48-96 h) was assessed.

Analysis of growth and ChO biosynthetic capacity by *P. scleromae* showed that in the course of fermentation active acidity of the nutrient medium tended to rise by 0,36-2,71 from the initial pH value 5,0-9,0 and feel by 0,55 from the starting pH 10,0, staying within the range 7,71-9,45. Protein concentration upon the end of fermentation reached 8,21-14,05 mg/ml. It should be noted that this parameter was sliding up with the increase of initial pH of the medium. As to biomass concentration, its level varied by the final day of the culture from 27,86 to 30,97 mg/ml. The top ChO generation capacity was recorded at initial pH of the medium 8,0 (0,088 U/ml). Initial pH values 5,0 and 6,0 decreased ChO productivity 2-fold (0,044 U/ml). Further pH fluctuations resulted in more drastic losses of enzyme biosynthesis.

Examination of the effect of temperature on enzyme production revealed that peak amount of ChO was generated at 28°C.

The study on correlation of enzyme productivity with time of the culture demonstrated that optimal period for ChO biosynthesis equaled 72 h. More prolonged culture lasting up to 96 h failed to augment enzyme yield.

Thus, it may be concluded that the optimal parameters for growth of *P. scleromae* and secretion of extracellular ChO are 72 h culture at temperature 28°C and initial pH of the nutrient medium 8,0.