Sutida Tuntigumton 2007: Squalene Production by Yeast. Master of Science (Microbiology), Major Field: Microbiology, Department of Microbiology. Thesis Advisor: Associate Professor Savitree Limtong, Dr.Eng. 111 pages.

A total of 248 yeast isolates were obtained from 75 samples of soils, fruits, exudates, flowers, mushrooms and mosses. Among these isolates, thirty isolates were found to accumulate squalene within cell. All 30 isolates together with a wild type strain RV51 and a mutant strain RV51-UV2-NTG2, which were previously reported to accumulate high squalene content (Uthaiporn, 2004), were subjected to quantitative determination of squalene accumulation when cultivation in yeast extract peptone dextrose broth. The results indicated that the wild type strain RV51 and the mutant strain RV51-UV2-NTG2 were able to accumulate much more squalene than the other 30 isolates. Therefore, both strains were selected for determination of squalene accumulation when cultivation in molasses medium containing 2% sugar. The results revealed that the amount of squalene accumulated by both strains was not different. Both strains were cultivated in molasses medium containing 2, 3 and 4% sugar and they could produce the highest concentration of squalene in molasses medium containing 3% sugar. However, the mutant strain RV51-UV2-NTG2 produced much higher squalene than did the wild type strain RV51. Therefore, only the mutant strain RV51-UV2-NTG2 was studied on its optimal medium compositions and cultivation conditions for squalene production in molasses medium by shaking cultivation. Molasses medium containing 3% sugar, 0.05% $(NH_4)_2SO_4$, 0.05% K_2HPO_4, and pH 5.0, with 100 ml of medium, initial cell concentration as optical density 1.5 at 550 nm and orbital shaking at 100 rpm, 28 °C were optimal for squalene accumulation and production. The maximal squalene accumulation and production obtained were 360.6 µg/g dry cell weight and 1,784.9 µg/l, respectively at 12 hours. Addition of 2.5 - 7.5 µg/ml terbinafine to cultivation medium resulted in increasing of squalene accumulation by 5 - 10 times and squalene production by 5 times. Freezing of culture broth at -20 °C immediately after sampling for 6 hours, resulted in increasing squalene production by 3 - 4 times. Cultivation of the mutant RV51-UV2-NTG2 in 5 liter jar-fermentor with 3 liter working volume using molasses medium containing the optimal medium compositions and optimal cultivation conditions obtained in shaking flask cultivation with addition of 2.5 µg/ml terbinafine and freezing culture broth at -20 °C immediately for 6 hours, resulted in squalene production at 19,700.6 µg/l at 15 hours, while only 3,741.4 µg/l was obtained at 12 hours in medium without addition of terbinafine.

sutida Tuntigumton — Student's signature

Savitree Limtong — Thesis Advisor's signature

29 / March / 2007