EFFECT OF LACTOSE AND OLIVE OIL ON INTRA- AND EXTRACELLULAR LIPIDS OF TORULOPSIS BOMBICOLA

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SUMMARY
Batch fermentations of Torulopsis bombicola with lactose, galactose, olive oil and cheese whey as the carbon sources were studied to develop cost-effective substrates for maximum lipids production and the simultaneous production of intra- and extracellular lipids in a single fermentation step.

INTRODUCTION
The yeast Torulopsis bombicola has been known to abundantly produce sophorose lipids under appropriate culture conditions. Sophorose lipids (SLs) which have biosurfactant properties can find numerous industrial applications particularly in humectants, foods, cosmetics, drug preparations, and environmental control. In the previous paper, we reported that optimal productivity of 135 g/L sophorose lipids occurs when both glucose and safflower oil were simultaneously present as the growth substrates (Zhou et al., 1992). However, the price of glucose is too high at the present time, considerable cost reduction might be possible by the use of other cheap or waste materials as substrates (Klekner et al., 1991).

Lactose, 4-o-β-d-galactopyranosyl-D-glucose, a disacharide composed of galactose and glucose, is the sugar present in milk and is obtained from whey as a by-product of the cheese industry at concentrations between 4.5 and 5% representing approximately 70%
of the whey solids. The disposal of cheese whey is a continuing and growing problem in the dairy industry. Over 44% of the whey produced in North America is not used as either human or animal food, but is discarded as waste. The BOD₅ value commonly around 60,000-70,000 mg/L, results mainly from the lactose present. About 115 million tons of liquid whey containing 6 million tons of lactose are produced yearly worldwide (Castillo, 1990). The rising cost of the disposal of lactose, the need to protect the environment, and cost reduction of lipids production have encouraged studies for lactose utilization as a more efficient and cost-effective substrate for lipids production.

Different studies have been carried out to improve the production of extracellular lipids by Torulopsis bombicola, however, no literature information is available on the intracellular lipids of this yeast and also no report is on using lactose or cheese whey as cheap substrate. The objective of this work is to develop cheap substrates for the simultaneous production of intra- and extracellular lipids in a single fermentation step which should be of great interest and importance both from the economic and environmental viewpoints.

MATERIALS AND METHODS

**Microorganism.** Torulopsis bombicola ATCC 22214 was maintained as previously reported (Zhou et al., 1992).

**Medium.** The nitrogen-limited medium contained 0.1% KH₂PO₄, 0.5% MgSO₄.7H₂O, 0.1% FeCl₃, 0.01% NaCl, and 0.25-0.3% yeast extract (Difco). The medium was supplemented by glucose, galactose, lactose, olive oil, safflower oil (Sigma), and cheese whey (A&P) for the study of medium composition, as described in the text.

**Culture Conditions.** Batch cultures were carried out in 500 ml Erlenmeyer flasks containing 100 ml of medium on a rotary shaker (250 rpm) and in 1-L Bellco jar glass fermenter containing 700 ml of medium with flow rate of 2 vvm air at 30°C for 10-12 days. The initial pH was about 4.5 and was not adjusted during cultivation. Medium and glassware were sterilized at 121°C for 30 minutes.

**Analysis.** Biomass, reducing sugars and extracellular lipids (sophorose lipids) were determined as previously reported (Zhou et al., 1992). The intracellular lipids were determined by extraction of biomass with chloroform:methanol (2:1) after extraction of SLs. The method for quantitative estimation of intracellular lipids was the same as that of sophorose lipids, but chromatorods were developed in petroleum ether/ether/acetic acid (90:10:1).