Encapsulation of β-carotene in yeast: influence of culture conditions

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INTRODUCTION AND OBJECTIVE

Yeast cell was found for long times with abundant applications in food and human nutrition. This is a low cost and easy productive resource of micro-size biomaterial which can behave as the liposome to encapsulate both hydrophilic and hydrophobic compounds (Shi 2007). Beside, this wall biomaterial possesses many advantages as they are light color, flat taste which is suitable for flavor or color encapsulation. *Yarrowia lipolytica* is oleaginous yeast, which can use lipid as sole source of carbon and is found in many application in biotechnology as oil pollution treatment, single cell oil production, flavor biotransformation … and in food application as it is GRAS – Generally Recognized As Safe. Beside the classical yeast *Saccharomyces cerevisiae*, *Y. lipolytica* is now immerged as a new model for biotechnology research.

In this paper, we investigated the hydrophobic compound encapsulation capacity of two strains of *Y. lipolytica* in compare with *S. cerevisea* and influence of their culture conditions on encapsulation efficiency. The β-carotene was used as model core material due to its hydrophobicity and its facilities in extraction and quantification by spectrometry.

MATERIALS AND METHODS

Strains, media and culture conditions

Two strains of Y. lipolytica 0544 and W29 and two strains of S. cerevisea HNS.c and TNS.c were cultured in YPDA medium (peptone 20 g/l, glucose 20 g/l, yeast extract 10 g/l, agar 15 g/l) for 48 h and used to inoculate to different liquid media YPD (YPDA without agar) or YNBO (yeast nitrogen base 6.7 g/l, castor oil 5 g/l, Tween80 0.2 g/l) and grown in Erlenmayer flask (50ml and 200 ml) shaking at 150 rpm, 25 - 27°C (Ta 2010). Cells in mid-logarithm growth phase (19h for YPD, 24h for YNBO) were collected and incubated with β-carotene at room temperature for over-night. The treated cells were then harvested for β-carotene (dissolved in soybean oil) accumulated estimation.

Intracellular β-carotene extraction and analysis

Cells slurry were harvested after incubated with β-carotene (0.5 g) than extracted with 10 ml n-hexane and the amount of β-carotene was determined through absorbance at 450nm with absorption coefficient ($A^{1\%}_{1cm}$) in hexane equal to 2592 (Rodriguez-Amaya 2010).

RESULTS AND DISCUSSION

Beta-carotene accumulation depends on yeast strains and carbon sources

Two conventional strains and two oleaginous strains were tested (Tab. 1). The results showed that without promolyse treatment, oleaginous strains encapsulated more carotene than conventional strains (from 1.5 up to 10 folds). It seems that oleaginous yeast cells have a system of canal on their membrane, which are induced by presence of lipid in medium, to facilitate the intake of lipid into cytoplasm (Osumi 1975).

Table 1. Cellular β-carotene accumulation in different yeast strains

Y. lipolytica		*S. cerevisea*	
W29	0544	HNS.c	TNS.c
1.17 µg/g	6.59 µg/g	0.79 µg/g	0.66 µg/g

Similar results were observed when changed carbon source in culture media. Cells grown on lipid medium could encapsulate more beta-carotene (8.6 folds) than cells grown on glucose medium (Tab.2). In fact, a different surface structure between cells grown on lipid and cells grown on no-lipid media was reported. Cells grown on lipid have a rough surface with plural protrusions which confer to cells a high affinity with lipid droplets (Mlickova 2004).

Table 2. Cellular β-carotene accumulation of W29 strain when cultured in glucose and lipid media

Glucose (YPD)	Lipid (YNBO)
1.17 µg/g	10.14 µg/g

Beta-carotene accumulation depends on oxygen exchange rate

Agitation condition is another factor that could affect the encapsulation ability of cells because it could change cellular membrane properties due to change in oxygen exchange rate. In this study, we observed that cells grown in higher agitation rate and lower volume of culture could encapsulate more β-carotene (Tab. 3). The oxygen conditions during culture was reported to influence on the membrane sterol accumulation and homeostasis (Rosenfeld 2003). This would confer to membrane a less or more fluidity to uptake outer

lipids (Ta 2010). Membrane of cells grown on more oxygenated conditions would be more fluid which facilitate β-carotene transfer into cytoplasm. This point has to be elucidated.

Table 3. Cellular β-carotene accumulation of W29 strain cultured in glucose medium at different agitation regimes

Culture conditions			Beta-carotene accumulation (μg/g)
pH	V (ml)	Agitation (rpm)	
5	200	150	1.17
5	50	150	2.56
5	50	250	6.30

CONCLUSIONS

Encapsulation of β-carotene into yeast cells has been investigated. The preliminary results showed an influence of strains and culture conditions such as carbon source, agitation regime ... on encapsulation efficiency. Further study on membrane properties is necessary to understand the encapsulation mechanism of not only β-carotene but also other hydrophobic compounds into yeast cells.

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