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The use of castor oil in the production of γ-decalactone by *Yarrowia lipolytica* KKP 379

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The study deals with the ability of the *Yarrowia lipolytica* KKP 379 strain to gamma-decalactone production by biotransformation of castor oil. The content of gamma-decalactone was verified in the water and lipid phase within 7 days of reaction. On the basis of granulometric analysis, the characteristic of the emulsion was presented. The results confirmed the ability of the *Y. lipolytica* 379 KKP strain to produce gamma-decalactone in the amount of about 1.68 g/l. The granulometric analysis revealed two populations of fatty droplets in 4.5 μm and 580 μm size.

Key words: γ-decalactone, castor oil, biotransformation, *Yarrowia lipolytica*

Introduction

The biotechnological production of flavour and fragrance compounds is an excellent alternative to both the extraction of plant materials and a typical chemical synthesis. In the recent years, a growing interest to the biosynthesis of fragrances can be observed.

A popular aroma compound used both in food and cosmetic industry is the γ-decalactone which is characterized by a peach smell.

This compound can be obtained by biotransformation of ricinoleic acid (12-hydroxyoctadec-9-enoic acid) [1–2] which is a major component (about 80 %) of castor oil, a natural and non-toxic oil, biodegradable and a renewable resource obtained from seeds of the castor plant *Ricinus communis* [3]. The process involves substrate biodegradation through peroximal β-oxidation, leading to the formation of 4-hydroxydecanoic acid, which cyclizes into γ-decalactone (Fig. 1) [4–5].

So far, in the biotechnological production of γ-decalactone following yeast genera have been used: *Sporidiobolus* [2], *Pichia* [3], *Rhodotorula* [7], *Candida*, and *Yarrowia*. Nevertheless, especially high prospects are associated with the latter microorganisms, namely non-conventional yeast *Yarrowia lipolytica*, considered as a non-pathogenic and as GRAS by the American Food and Drug Administration.

The aim of this study was to evaluate the ability of the yeast *Yarrowia lipolytica* KKP 379 to produce of γ-decalactone. An oil-in-water emulsion, stabilized by the nonionic surfactant Tween 80, was used in biotransformation reactions. In this type of culture, the contact surface of fat droplets with cells of propagated microorganisms and the surface between two liquid phases are the determining factors in the degradation of a hydrophobic substrate, and thus in the cell growth and aroma production by these cells. The emulsion was characterized by determining the oil droplet size distribution by the laser granulometry technique.

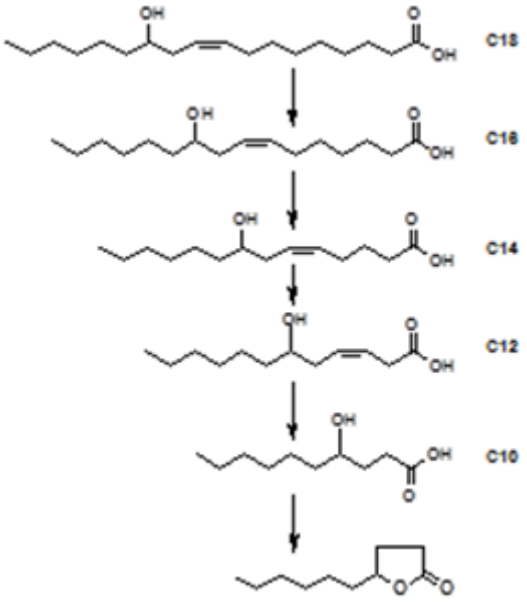

Fig. 1. Fatty acids observed as intermediates in the biotransformation of ricinoleic acid (18 carbons) into γ-decalactone (10 carbons) [6]

Materials and methods

Microorganism, media, and culture conditions

Yarrowia lipolytica KKP 379 was obtained from the microbial culture collection of the Agricultural and Food Biotechnology Institute of Warsaw (Poland). The yeast was cultured for 48 h on the YPDA medium (agar 20 g/l, glucose 20 g/l, peptone 20 g/l, yeast extract 10 g/l) at 27 °C and used to inoculate (to reach OD_{600} of about 0.25) 500 ml baffled Erlenmeyer flasks containing 200 ml of the

YPD medium (glucose 20 g/l, peptone 20 g/l, yeast extract 10 g/l). Flasks were shaken at 140 rpm and 27 °C for 19 h until the cultures reached the late-logarithmic growth phase. There suspensions were used to inoculate the biotransformation media.

Biotransformation of castor oil into γ-decalactone

For γ-decalactone production, the cells in late growth-phase (19 h) were transferred to the biotransformation medium at the initial concentration of 10^8 cells/ml. This medium was composed of castor oil (Sigma-Aldrich) at 100 g/l, peptone 20 g/l, and Tween 80 (4–5 droplets per 100 ml). The biotransformation was then conducted in 250 ml baffled Erlenmeyer flasks (27 °C, 140 rpm for 7 days).

γ-Decalactone extraction and quantification

For the quantification of γ-decalactone, 1.5 ml samples were collected regularly from the culture medium for 7 days. In order to stop the metabolism and achieve the overall lactonization of 4-hydroxydecanoic acids, 10 ml of HCl (6 N) were added to the samples. Then γ-undecalactone was introduced as an internal standard. The samples were extracted with 1.5 ml of diethyl ether. After 5 min, the ether phase was separated and analyzed by GC (Varian 3800 instrument) with a TR-WAX capillary column and with He as the carrier gas. The temperatures of the split injector and the detector were set at 250 °C and 300 °C, respectively. The oven temperature was programmed to increase from 75 °C to 135 °C at a rate of 6 °C min^{-1}, next to 180 °C at a rate of 3 °C min^{-1} and then to 250 °C at a rate of 5 °C min^{-1}. All assays were performed in triplicate.

Laser granulometry

The size of the yeast cells and of the fatty acid droplets in the medium was evaluated by Malvern Mastersizer 2000 laser granulometry measurements.

Results and discussion

In assessing the capacity of the yeast *Yarrowia lipolytica* KKP 379 for the biotransformation of ricinoleic acid, the content of γ-decalactone was verified within seven days of culture, both in the lipid and aqueous phases. As shown in Fig. 2, the strain *Yarrowia lipolytica* KKP 379 is capable of producing γ-decalactone, even in the total amount of about 1.68 g/l.

The maximum reaction efficiency falls on the 5th day of the culture when the content of γ-decalactone reaches the approximate level (about 0.8 g/l) both in the aqueous and lipid phases. In the early days of culture, γ-decalactone is mainly accumulated in the aqueous phase. During this period, its level in this phase is up to three times higher than in the lipid phase. In the final times of culture, γ-decalactone prevails in the lipid phase (the level is higher by about 30 %), but its content with the increasing cultivation time over five days is decreasing. It is assumed that this is related to the influence of pH of the medium. Waché et al. [5] indicate that the pH value can have a significant impact on γ-decalactone reconsumption. The decline of γ-decalactone is associated with the penetration of this compound into the yeast cells and its hydrolysis, which can occur at a low pH (pH 2–4) observed in the final stage of the culture.

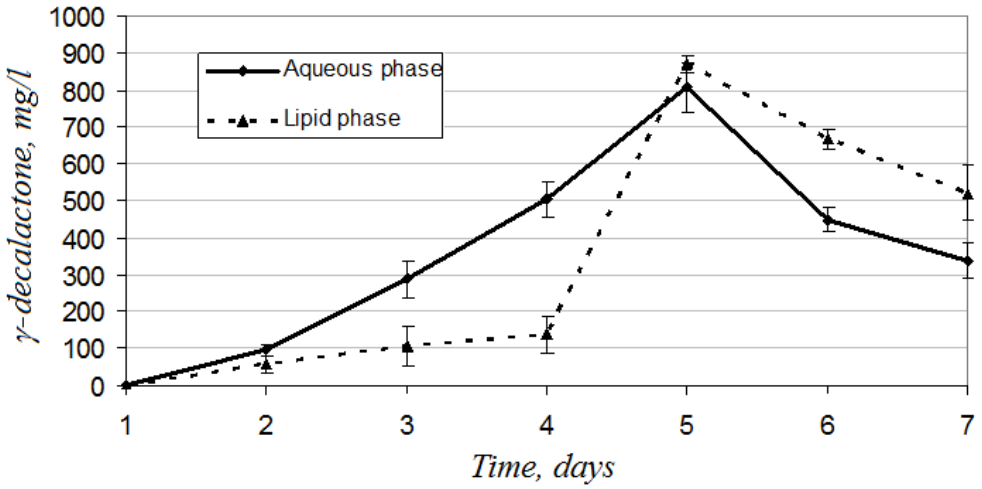

Fig. 2. Accumulation of γ-decalactone in aqueous (♦) and lipid (▲) phase during the growth of *Yarrowia lipolytica* KKP 379 strain on 100 ml medium with castor oil (100 g/l)

In assessing the ability of the *Yarrowia lipolytica* KKP 379 strain to the production of γ-decalactone by biotransformation of castor oil, the emulsion building the medium was also characterized.

The granulometric analysis of the emulsified castor oil medium with cells indicated the existence of two distinct droplet populations (Fig. 3).

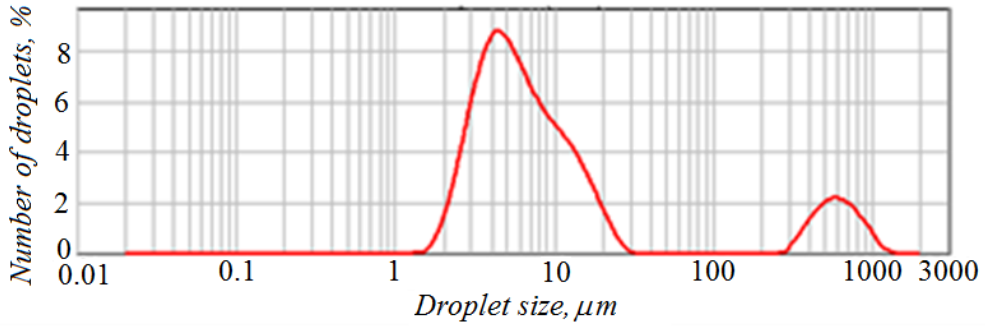

Fig. 3. Castor oil droplet size distribution (in µm) in emulsion with cells, related to the number of particles (in %). Data are the mean of three independent experiments

In a larger population, the droplet mean values of 4.5 µm were detected. To estimate the size of yeast cells, the granulometric analysis was also made after their suspension in distilled water. In this measurement, the population with the mean value of 5.3 µm was observed. These results are consistent with the data on cell size, reported by Gomes et al. [8] using a granulometric laser. It is assumed that in the area of the larger size population also the smallest oil droplets are present, which can be obscured by a large number of yeast cells. It is probable that some of the fat globules undergo adhesion to the cell surface. Another population presented in the graph (Fig. 3) corresponds to a fairly large fat droplet size – within about 580 µm.

The size of fat droplets in the medium depends on the physical and physicochemical properties of the medium (pH, ionic strength, presence of surfactants), the amount of the inoculum introduced into the culture medium, and the surface properties of propagated cells [9].

In order to confirm that during the biotransformation of ricinoleic acid by microorganism cells, because of direct contact with the hydrophobic substrate, some cells undergo adhesion to the surface of the lipid substrate, and some of the oil droplets are adsorbed on the surface of the microorganism cells. Therefore, the microscopic observations of yeast were made. The figure on the left is a real picture of the yeast cells (Fig. 4a), and the figure on the right shows the cells colored with calcofluor-white (Fig. 4b). In the picture, it is clearly visible that fat droplets are adsorbed to the surface of the cells which appear both in the form of yeast and mycelia. In the case of yeast *Yarrowia lipolytica* KKP 379, one can see that the cells undergo a strong aggregation and have a tendency to agglomerate around the fat droplets.

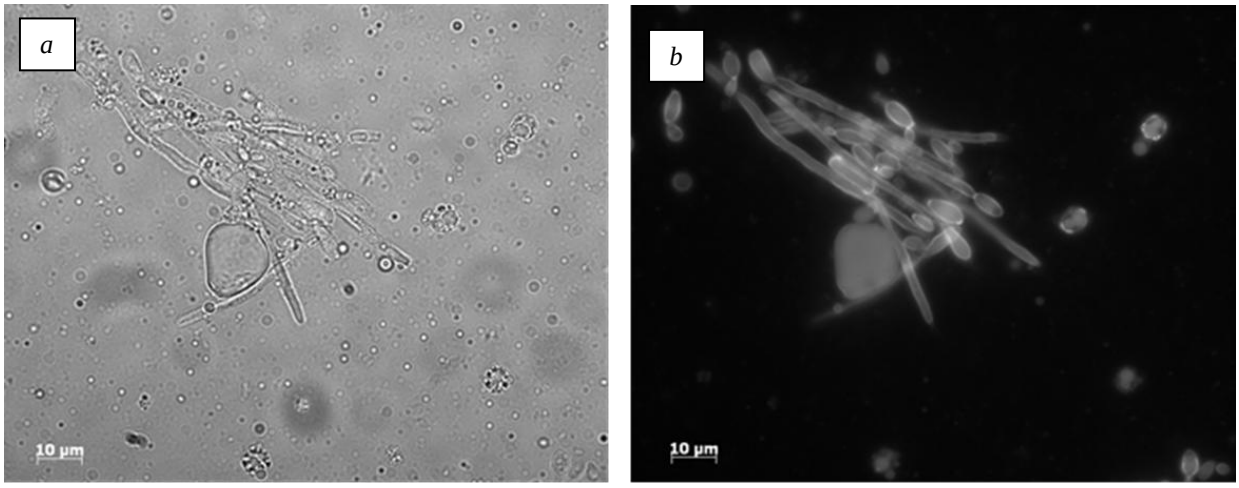

Fig. 4. Microscopy observation of *Yarrowia lipolytica* KKP 379 after 7 days of culture on castor oil:
a – real picture of the yeast cells; *b* – cells coloured with calcofluor-white

Conclusions

The *Yarrowia lipolytica* KKP 379 strain is able to produce γ-decalactone by castor oil biotransformation. The synthesis of the aroma is possible within five days of reaction. During this period, gamma-decalactone is accumulated mainly in the water phase. The granulomeric analysis is a convenient tool for better understanding the assimilation of hydrophobic substrates by the yeast *Yarrowia lipolytica* KKP 379.

Acknowledgements

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RICINOS ALIEJAUS PANAUDOJIMAS γ-DEKALAKTONO GAMYBAI *YARROWIA LIPOLYTICA* KKP 379

S a n t r a u k a

Buvo nagrinėta *Yarrowia lipolytica* KKP 379 geba biotransformuojant ricinmedžio aliejų γ-dekalaktonui pagaminti. Jo susidarymas vandens ir riebalų fazėse tirtas procesui vykstant 7 paras. Didžiausia reakcijos išeiga stebima penktą proceso parą, γ-dekalaktono kiekis abiejose fazėse tuo metu apytiksliai yra po 0,8 g/l. Pirmosiomis dienomis γ-dekalaktonas daugiausiai kaupiasi vandens fazėje, vėliau jo kiekis didėja riebalų fazėje. Po 5 parų bendrasis produkto kiekis pradeda mažėti.

Granuliometrinė biotransformuoto ricinos aliejaus emulsijos analizė parodė, kad susidaro dvi riebalų lašelių grupės, kurių dydis 4,5 ir 580 μm.