Process Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Solid state fermentation for the production of γ-decalactones by *Yarrowia lipolytica*

Sophal Try[a,c,*,1], Joëlle De-Coninck[a,b], Andrée Voilley[a], Thavarith Chunhieng[c,1], Yves Waché[a,1]

[a] Univ. Bourgogne Franche-Comté, AgroSup Dijon, PAM UMR A 02.102, F-21000 Dijon, France
[b] Welience-Plateform for Development in Biotechnology, SATT GRAND EST, 17 rue Sully, 21065 Dijon, France
[c] Department of Chemical Engineering and Food Technology, Institute of Technology of Cambodia, Russian Federation Blvd., P.O. Box 86, Phnom Penh, Cambodia

ARTICLE INFO

Keywords:
Solid state fermentation
Yarrowia lipolytica
Aroma production
β-Oxidation
Luffa sponge
Castor oil
γ-Lactones
Forced aeration

ABSTRACT

The production of γ-decalactones as aroma compounds is highly dependent on the access of the biocatalyst to substrate and co-substrate (oxygen). In this work, the potential of solid state fermentation (SSF) is investigated for this production with *Y. lipolytica* W29. Luffa sponge was used as an inert support and the investigation focused on the impact of aeration on metabolites. In that goal, experiments were carried out in three different SSF reactor types, wide-mouth Erlenmeyer flask (static aeration), forced aeration mini-reactor, and small-headspace bottle (without aeration). Four lactones were detected by GC–MS during the degradation of ricinoleic acid from castor oil by *Y. lipolytica* W29: 3-hydroxy-γ-decalactone, which reached the high concentration of 5 g/L (in wide-mouth Erlenmeyer flask), γ-decalactone, dec-2-en-4-olide and dec-3-en-4-olide. In this study, some yeast cells changed their morphological properties from the yeast-like shape to pseudo-mycelium and mycelium. These cells may undergo a metabolic shift resulting in the high production of 3-hydroxy-γ-decalactone. The yield of lactone in the small-headspace bottle was very low suggesting insufficient oxygen availability. For their part, forced-aeration conditions in mini-reactors resulted in the stripping of lactone compounds. From the present work, an alternative process is proposed as a novel model for lactone production.

1. Introduction

Lactones are aroma compounds present naturally in many fruits and fermented foods. Their biotechnological synthesis comes from C18-hydroxylated fatty acids. Five lactones may be detected during the degradation of ricinoleic acid, by yeast cells: 5-hydroxy-ε-dodecalactone, γ-decalactone, 3-hydroxy-γ-decalactone, dec-2-en-4-olide and dec-3-en-4-olide [1–3] (the latter decalactones are shown in Fig. 1). During the peroxisomal β-oxidation of hydroxylated fatty acids by the yeast *Y. lipolytica*, which is considered as a model organism for the metabolism of hydrophobic compounds [38], the four γ-decalactones encountered above have been detected [2–5].

The production of lactones in submerged cultures has been widely investigated, including the metabolic pathway and the improvement of the β-oxidation flux which has been studied on genetically modified yeast strains [6–10]. Some authors have focused on the effect of environmental condition changes by modifying pH, substrate concentration, inoculum density, or aeration to improve the production of active compounds [11–14]. From their studies, oxygen availability appears to be a key parameter driving the extent of oxidation [11–15]. Interestingly, an airlift biofilm reactor explored by Escamilla-García et al. [13] led to the formation of adhering biofilms of *Y. lipolytica* and to the concomitant stimulation of the production of 3-hydroxy-γ-decalactone with a ten-fold concentration increase. These results drove us to get interested in a solid state fermentation (SSF) process which would allow the microorganisms to keep in tight contact with the substrate to be in a direct contact with the air oxygen. *Y. lipolytica* has been widely used in submerged cultures in large applications such as the production of lipases, organic acids and lactones. Only few research works have investigated SSF for production by this species and these papers were limited to the production of lipase [22–25]. In recent years, SSF has greatly expanded its application to the production of various metabolites [16,39–44]. It was found that SSF has various advantages compared to submerged fermentation [17–21]. In our aim to produce lactone in SSF, by taking into account the oxygen availability as the main factor involving peroxisomal β-oxidation in lactone production, different aeration conditions will be studied. When the substrate structure does not enable microorganisms to grow in SSF, inert supports have to be used. In our study, different inert supports and substrate were selected for the first time to study the growth of *Y. lipolytica* and the

* Corresponding author. Present address: AgroSup Dijon/University of Burgundy – Franche-Comté, 1 Esplanade Erasme, 21000 Dijon, France.
E-mail address: try@itc.edu.kh (S. Try).
[1] Network Tropical Fermentation.

http://dx.doi.org/10.1016/j.procbio.2017.10.004
Received 3 July 2017; Received in revised form 7 September 2017; Accepted 8 October 2017

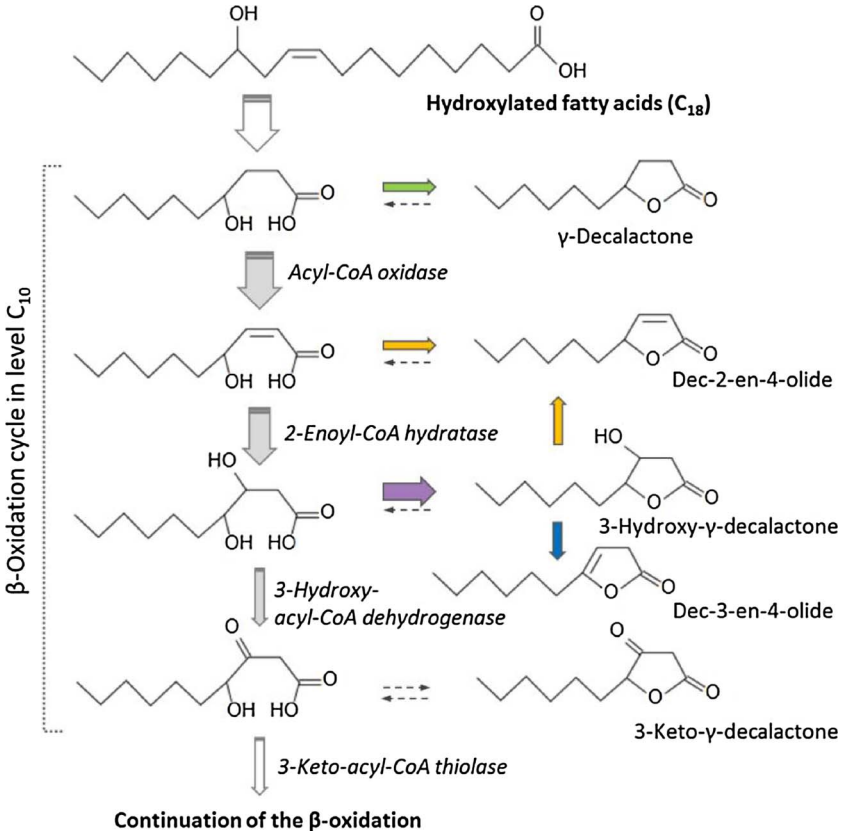

Fig. 1. Degradation pathway of hydroxylated fatty acids (in the form of esters of coenzyme A) by the biofilm of yeast *Y. lipolytica* at the C10-β-oxidation cycle level. The metabolic shift is represented by the size of the arrows that refers to the activity of enzymes and subsequent lactones production.

production of γ-decalactones in SSF. Inert supports were used after impregnation with the liquid biotransformation medium containing castor oil as the substrate. The most efficient inert support or substrate for lactone production was selected for the study of the importance of oxygen in SSF. Experiments with three different conditions in SSF reactor types, wide-mouth Erlenmeyer flask (static aeration), forced aeration mini-reactor and small-headspace bottle (without aeration) have been carried out. An alternative system for lactone production by *Y. lipolytica* is proposed for the first time in this study.

2. Materials and methods

2.1. Solid supports

Corncob, luffa sponge and cellulose sponge were used as the solid supports and castor seed was used as the substrate in this study. Corncob (Grits®, France) was used without any pretreatment. Luffa sponge was obtained from the dried fruit of Luffa free of seeds (Fig. 2) (origin Cambodia). It was cut into rectangular prism shape (approximately 1 cm by 1 cm by 0.5 cm). The pretreatment procedure was modified from Pazzetto et al. [26]: The rectangular prisms of luffa sponge were washed thoroughly with tap water, boiled using distilled water for 30 min, and left for 3 day in distilled water and changed three times. They were then dried at room temperature in thin layer (approximately 2 cm) for two days. Cellulose sponge (Spontex®, France) was cut into a cubic shape (approximately 0.5 cm^3). The pretreatment procedure was the same as the procedure used for luffa sponge, except that it was then oven-dried at 105 °C for 3–4 h. Castor seeds were grinded to the diameter sizes of approximately 0.1 cm–0.4 cm before use.

2.2. Strain and inoculum preparation

The strain used in this study was *Y. lipolytica* W29 (ATCC 20460). It

Fig. 2. Luffa sponge support.

was cultured at 27 °C for 48 h on YPDA (Yeast Peptone Dextrose Agar: 20 g/L of glucose, 20 g/L of tryptone pancreatic digest of casein, 10 g/L of yeast extract and 15 g/L of agar). Cells were used to inoculate a 500 mL baffled Erlenmeyer flask containing 200 mL of YPD medium to give inoculum concentration of 6×10^6 cells/mL. Flasks were shaken for 18–19 h at 140 rpm until the cultures reached the late logarithmic growth phase. Cells from the pre-culture media were harvested by centrifugation for 5 min at 4000*g*, washed twice with sterile saline solution (0.9% of NaCl (w/v)) and then they were recovered in biotransformation medium in order to impregnate the solid support. An inoculation concentration of 2×10^7 cells/mL (or approximately 7×10^7 cells/g of dried weight for luffa sponge and cellulose sponge, or approximately 1.6×10^7 cells/g of dried weight for corncob) of biotransformation medium was carried out in this study. Only distilled water inoculated with yeast cells was used for impregnating the solid