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New biotransformation process for the production of the fragrance γ-dodecalactone from 10-hydroxystearate by permeabilized *Waltomyces lipofer* cells

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γ-DODECALACTONE PRODUCTION BY *WALTOMYCES LIPOFER* CELLS

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ABSTRACT

A new biotransformation process for the production of the flavor lactone was developed by permeabilized *Waltomyces lipofer*, which was selected as an efficient γ-dodecalactone-producing yeast among 10 oleaginous yeast strains. The optimal reaction conditions for γ-dodecalactone production by permeabilized *W. lipofer* cells were pH 6.5, 35°C, 200 rpm, 0.7 M Tris, 60 g/liter 10-hydroxystearic acid, and 30 g/liter of cells. Under these conditions, non-permeabilized cells produced 12 g/liter γ-dodecalactone after 30 h, with a conversion yield of 21% (w/w) and a productivity of 0.4 g/liter/h, whereas permeabilized cells obtained after sequential treatments with 50% ethanol and 0.5% Triton X-100 produced 46 g/liter γ-dodecalactone after 30 h, with a conversion yield of 76% (w/w) and a productivity of 1.5 g/liter/h. These values were 3.7- and 3.8-fold higher than those obtained using non-permeabilized cells. This is the highest reported concentration, conversion yield, and productivity for the production of the bioflavor lactone.

INTRODUCTION

γ-Lactones are industrially important flavor compounds that are widely distributed in foods, fruits, and beverages and used in many fruity aromatic foods and cosmetics (1, 2). γ-Dodecalactone is a flavor compound that exists in apricot, peach, strawberry (3), pineapple (4), mango (5), plum (6), acerola (7), and milk (8). γ-Dodecalactone has been used as an aroma or taste component of consumable materials such as foodstuffs, chewing gums, toothpastes, cosmetic powders, hair preparations, medicinal products, smoking tobaccos, detergents, perfume compositions, and perfumed articles (9).

Many synthetic γ-lactones have been utilized as artificial flavors. However, the consumer perception that natural is good has led to the increased demand for natural flavors. Natural lactones, such as γ-decalactone and γ-dodecalactone, have been produced from free fatty acids, hydroxy fatty acids, or oils through several enzymatic steps in the β-oxidation system of yeast. A microbial process for producing γ-lactones exhibits higher conversion yield than a natural process. However, microbial production has a critical problem such as a low conversion yield that results from the barrier effect of the cell wall or membrane (10). Cell permeabilization improves the transfer of the reaction substrate and product across the cell membrane, and thus increases the production of metabolites (11-14).

10-Hydroxystearic acid is metabolized to 4-hydroxydodecanoic acid and acetic acid through β-oxidation cycle. 4-Hydroxydodecanoic acid is converted to γ-dodecalactone by lactonization and acetic acid is used to the synthesis of oleic acid by the several reactions of acetyl-CoA synthase, acetyl-CoA carboxylase, fatty acid synthetase, fatty

acid elongase, and fatty acid desaturase in the yeast strains *Rhodosporidium toruloides* (15), *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* (16). Oleic acid is converted to 10-hydroxystearic acid by baker's yeast (17) and it is converted to γ-dodecalactone by *Sporobolomyces odorus* (18). Thus, the metabolic pathway from 10-hydroxystearic acid to γ-dodecalactone by yeast could be proposed (Fig. 1).

In this study, to increase γ-dodecalactone production by effectively transferring the substrate and product into cells, permeabilization was attempted for *Waltomyces lipofer*, which was selected as an efficient γ-dodecalactone-producing yeast among 10 oleaginous yeast strains. The reaction conditions were optimized for the whole permeabilized cells, and a new biotransformation process for the production of γ-dodecalactone from 10-hydroxystearic acid was developed under the optimized conditions.

MATERIALS AND METHODS

Microorganisms, media, culture conditions, and reaction conditions. *Candida oleophila* KTCT 7652, *Candida palmioleophila* KTCT 17452, *Cryptococcus curvatus* KTCT 7225, *Lipomyces spencermartinsiae* KTCT 17184, *Myxozyma lipomycoides* KTCT 7899, *Rhodotorula aurantiaca* KTCT 7776, *Rhodotorula glutinis* KTCT 7948, *Rhodosporidium toruloides* KTCT 7130, *Waltomyces lipofer* KTCT 17657, and *Yarrowia lipolytica* KTCT 17170 were used as γ-dodecalactone-producing yeasts. A single colony was inoculated into 10 ml of yeast malt (YM) broth, which consisted of 3.0 g/liter yeast extract, 3.0 g/liter malt extract, 5.0 g/liter peptone, and 10.0 g/liter

dextrose, and cultivated at 27°C with agitation at 200 rpm for 18 h. The seed was then transferred into a 2-liter baffled flask containing 500 ml of YM broth and cultivated at 27°C with agitation at 200 rpm for 18 h. The cells were harvested from the culture broth by centrifugation at 13,000 × *g* for 20 min at 4°C and then washed twice with 50 mM Tris-HCl buffer (pH 6.5) to prepare a concentrated cell suspension, which was then used for the production of γ-dodecalactone from 10-hydroxystearic acid. Unless otherwise stated, the reaction was performed in 0.7 M Tris, 10 g/liter 10-hydroxystearic acid, 5 g/liter whole cells, and 0.05% (w/v) Tween 80 at pH 6.5, 35°C, and 200 rpm for 10 h in a 500-ml baffled flask containing 50 ml of reaction medium.

Preparation of 10-hydroxystearic acid and the reaction product obtained from 10-hydroxystearic acid using permeabilized *W. lipofer* cells. 10-Hydroxystearic acid, as a precursor substrate of γ-dodecalactone (19), was produced from oleic acid by a recombinant *Escherichia coli* containing oleate hydratase from *Stenotrophomonas maltophilia* (20). An equal volume of ethyl acetate was added to the reaction solution containing oleic acid and 10-hydroxystearic acid, and the solvent was removed from the solution using a rotary evaporator. To prepare 10-hydroxystearic acid, a mixture of 30% acetonitrile and 70% acetone was added to the extract solution at room temperature. The solution was cooled in an ultra-low temperature freezer for 24 h at −80°C. After cooling, the liquid fraction of oleic acid was removed at room temperature, and the solvent was removed from the solid fraction of 10-hydroxystearic acid using a rotary evaporator. This fractionization procedure was repeated 3 times. As a result, 10-hydroxystearic acid was obtained with high purity (>99%) and used as a substrate in subsequent

experiments.

To purify the reaction product, an equal volume of mineral oil was added to the reaction solution, which was obtained from 10-hydroxystearic acid by the reaction of permeabilized *W. lipofer* cells. The product in the mixture was purified using vacuum distillation in a silicon oil bath held below 130°C and then the purified product (>99%) was obtained.

Cell permeabilization by detergent and/or solvent treatment for increased production of γ-dodecalactone. To prepare permeabilized whole *W. lipofer* cells, the harvested cells were resuspended in 0.1% (w/v) detergent solutions, where the detergents were sodium dodecyl sulfate (SDS), Triton X-100, and Tween 80, and in 50% solvent solutions, where the solvents were ethanol, methanol, and toluene. The solutions were incubated at 4°C for 15 min and washed twice with distilled water, and the cells were used for γ-dodecalactone production. The effects of treatments of ethanol and Triton X-100 at several concentrations were investigated by varying the concentrations from 0 to 90% and from 0 to 1.0%, respectively. To obtain the combined effect of cell permeabilization, the harvested cells were treated sequentially with 50% ethanol and 0.5% Triton X-100.

Optimization of reaction conditions for γ-dodecalactone production. The effect of nitrogen source on γ-dodecalactone production by permeabilized *W. lipofer* cells was evaluated. Various nitrogen sources added to the reaction media with an equivalent amount of 0.1 g/liter of nitrogen. The nitrogen sources were yeast nitrogen base, yeast

extract, malt extract, beef extract, peptone, polypeptone, casitone peptone, proteose peptone, soytone, and tryptone as organic nitrogen sources; and ammonium chloride, ammonium sulfate, ammonium acetate, ammonium citrate, ammonium phosphate, calcium nitrate, Tris (2-amino-2-hydroxymethyl-propane-1,3-diol), MES (2-(*N*-morpholino)ethanesulfonic acid), PIPES (1,4-piperazinediethanesulfonic acid), and urea as inorganic nitrogen sources. The effect of nitrogen concentration in Tris was investigated by varying it from 0 to 20 g/liter. To examine the effects of pH, temperature, and agitation speed on γ-dodecalactone production by permeabilized *W. lipofer* cells, The pH, temperature, and agitation speed were varied from pH 5.5 to pH 7.5, from 25 to 45°C, and from 0 to 250 rpm, respectively.

To determine the optimal concentrations of the permeabilized cells and substrate for maximum γ-dodecalactone production, the concentration of permeabilized cells was varied from 10 to 50 g/liter in the presence of 50 g/liter 10-hydroxystearic acid, and the substrate concentration was varied from 10 to 100 g/liter in the presence of 30 g/liter permeabilized cells. The time course reactions of γ-dodecalactone production by non-permeabilized and permeabilized *W. lipofer* cells were investigated with 30 g/liter permeabilized cells and 60 g/liter 10-hydroxystearic acid.

Analytical methods. The cell mass was determined using a calibration curve that related optical density at 600 nm to the dry cell weight. The reaction solution was acidified at 100°C for 30 min by adjusting it to pH 2.0 with addition of 6 M HCl and then extracted with an equal volume of ethyl acetate. The solvent was removed from the extract using a rotary evaporator. The obtained sample containing 10-hydroxystearic

acid was silylated with a 2:1 mixture of pyridine and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (20). γ-Dodecalactone, silylated oleic acid, and silylated 10-hydroxystearic acid in the organic phase were analyzed using a gas chromatograph (GC) (Agilent 6890N) equipped with a flame ionization detector and a Supelco SPB-1 capillary column and the standard γ-dodecalactone (Sigma-Aldrich). The column temperature was increased from 150 to 210°C at 4°C/min and maintained at 210°C. The injector and detector were maintained at 260 and 250°C, respectively. The column temperature was increased from 150 to 210°C at 4°C/min and maintained at 210°C. The injector and detector were maintained at 260 and 250°C, respectively. The purified product (>99%) was identified by GC/mass spectrometry (MS) (Agilent 5973N) with an electron impact ionization source. The ion source was operated at 70 eV and held at 230°C. Acetic acid were analyzed using a high-performance liquid chromatography (HPLC) system (Agilent 1100) equipped with a UV detector at 210 nm and an ODS-AQ column (YMC, Kyoto, Japan). The column was eluted with 20 mM NaH_2PO_4-H_3PO_4 at a temperature of 30°C and a flow rate of 1.0 ml/min.

RESULTS AND DISCUSSION

Identification of γ-dodecalactone and selection of an efficient γ-dodecalactone-producing strain. A mass spectrum of GC/MS was observed for the product obtained from 10-hydroxystearic acid by the action of *W. lipofer* cells (see Fig. S1 in the supplementary material). A peak at *m/z* 85 resulted from the loss of C_8H_{17} and $C_4H_5O_2$

for the product peak at *m/z* 198, respectively, arising from the cleavage between the C4 and C5 positions. These fragment peaks identified the product as a γ-dodecalactone. The main fragment peak, a peak for the pentagonal ring of γ-dodecalactone, was reported as a peak at *m/z* 85 (21).

As a typical yeast, *Y. lipolytica* has been used for the production of γ-decalactone and γ-dodecalactone; however, its production, productivity, and conversion yield are not high (9, 22, 23). To improve γ-lactone production, a new type of oleaginous yeast is required. To select an effective γ-dodecalactone-producing strain, γ-dodecalactone production was performed with 10 oleaginous yeast strains in the reaction medium containing 10-hydroxystearic acid. The γ-dodecalactone-producing activity of 10 oleaginous yeast strains followed the order *W. lipofer* > *C. palmioleophila* > *L. spencermartinsiae* > *Y. lipolytica* > *C. oleophila* > *R. aurantiaca* > *M. lipomycoides* > *R. glutinis* > *R. toruloides* > *C. curvatus* (see Fig. S2 in the supplementary material). The activity of *W. lipofer* was the highest among the 10 oleaginous yeast strains, and was especially higher than that of the typical γ-lactone-producing yeast *Y. lipolytica*. Thus, *W. lipofer* was selected as an efficient γ-dodecalactone-producing yeast and was used in all subsequent experiments for γ-dodecalactone production. *W. lipofer* belongs to the genus *Lipomycetaceae* and can synthesize various fatty acids, including palmitic, palmitoleic, stearic, oleic, linoleic, α-linolenic, γ-linolenic, dihomo-γ-linolenic, and arachidonic acids (24). The yeast accumulates up to 60–70% of storage lipids in the lipid droplets or lipid particles, which promote β-oxidation of long-chain fatty acids (25). *W. lipofer* (Synonym: *Lipomyces lipofer*) used in the present study does not belong to GRAS microorganisms. γ-Lactone has been used not only food but also industrial aromatic

cosmetics. Thus, the application of the strain to cosmetics causes no problem.

Permeabilization of *W. lipofer* by detergent and/or solvent treatment for increased production of γ-dodecalactone from 10-hydroxystearic acid. In the present study, permeabilized cells were first applied to the production of the flavor lactone. γ-Dodecalactone production by permeabilized *W. lipofer* cells after treatment of solvent or detergent followed the order ethanol > Triton X-100 > SDS > methanol > Tween 80 > non-treated > toluene (Fig. 2). γ-Dodecalactone production by cells treated with ethanol or Triton X-100 was 1.6- or 1.5-fold higher than that by non-treated cells, respectively. Treatment with ethanol or Triton X-100 was tested at concentrations ranging from 0 to 90% and from 0 to 1%, respectively. The maximum production of γ-dodecalactone was observed at concentrations of 50% ethanol or 0.5% Triton X-100. To obtain the combined effect for cell permeabilization, 50% ethanol and 0.5% Triton X-100 were sequentially treated. The sequential treatments provided the highest γ-dodecalactone production (Fig. 2). Thus, sequential treatments with 50% ethanol and 0.5% Triton X-100 were chosen as the cell permeabilization method for γ-dodecalactone production. Although the compounds used for permeabilization have been known to decrease the viability of permeabilized yeast cells, the cells as whole-cell biocatalysts are effective for increasing the activities of enzymes (10, 26). The combined effect for cell permeabilization may be due to the different mechanism of action of alcohol and Triton X-100. Water-alcohol mixture damages not cell wall but cell membrane (27), whereas Triton X-100 damages both cell wall and cell membrane (28).

Optimization of reaction conditions for γ-dodecalactone production by permeabilized *W. lipofer* cells. The pH, dissolved oxygen, agitation and aeration rates for the microbial production of γ-lactone were optimized. However, γ-lactone production is still low yield (29, 30). The reaction conditions for γ-dodecalactone production, including nitrogen source, pH, temperature, agitation speed, and the concentrations of the substrate and cells, were optimized as follows: Generally, yeast nitrogen base has been used as the nitrogen source for γ-lactone production (31, 32). However, γ-dodecalactone production using Tris was 1.5-fold higher than that using yeast nitrogen base and was higher than that using other nitrogen sources (Fig. 3), and the optimal nitrogen concentration was 10 g/liter. Thus, 10 g/liter nitrogen in Tris, which corresponded to 0.7 M, was used for γ-dodecalactone production.

The effects of pH, temperature, and agitation speed on γ-dodecalactone production by permeabilized *W. lipofer* cells were investigated. The maximal activity for γ-dodecalactone production was observed at pH 6.5, 35°C, and 200 rpm in a 250-ml flask (see Fig. S3 and S4 in the supplementary material). γ-Dodecalactone production by baker's yeast (33), *Sporidiobolus salmonicolor* (34), and *Sporobolomyces odorus* (18) was performed at pH 7.0, 25°C, and 130 rpm in a 500-ml flask; pH 6.0, 25°C, and 250 rpm in a 500-ml flask; and 22°C and 80 rpm (pH was not described) in a 1-liter flask, respectively.

The optimal cell concentration for γ-dodecalactone production was investigated using 50 g/liter 10-hydroxystearic acid as a substrate by varying the concentration of permeabilized cells from 0 to 50 g/liter after 10 h (see Fig. S5A in the supplementary material). At concentrations less than 30 g g/liter permeabilized cells, γ-dodecalactone

246 production increased as the concentration of the permeabilized cells increased; however,
247 at concentrations higher than 30 g of permeabilized cells per liter, γ-dodecalactone
248 production reached a plateau. Therefore, the optimal cell concentration was determined
249 to be 30 g/liter. The production of γ-dodecalactone from 10-hydroxystearic acid was
250 assessed in 30 g of g/liter permeabilized cells by varying the concentration of 10-
251 hydroxystearic acid from 0 to 100 g/liter after 10 h (see Fig. S5A in the supplementary
252 material). Within 60 g/liter 10-hydroxystearic acid, increases in the substrate
253 concentration resulted in proportional increases in the production of γ-dodecalactone.
254 However, the production of γ-dodecalactone reached a plateau at concentrations higher
255 than 60 g/liter; the optimal substrate concentration was 60 g/liter. Thus, the production
256 of γ-dodecalactone from 10-hydroxystearic acid was optimal at pH 6.5, 35°C, 200 rpm,
257 0.7 M Tris, 0.05% (w/v) Tween 80, 60 g/liter 10-hydroxystearic acid, and 30 g/liter
258 permeabilized cells.
259
260 **γ-Dodecalactone production by non-permeabilized and permeabilized *W. lipofer***
261 **cells under the optimized conditions.** Under the optimized conditions, time-course
262 reactions for γ-dodecalactone production were performed using non-permeabilized and
263 permeabilized whole *W. lipofer* cells (Fig. 4). Permeabilized cells of *W. lipofer* produced
264 46 g/liter γ-dodecalactone (232 mM) from 60 g/liter 10-hydroxystearic acid (200 mM)
265 after 30 h, with a molar conversion yield of 116% (76%, w/w), and a volumetric
266 productivity of 1.5 g/liter/h, and a specific productivity of 0.05 g/g (dry weight) of
267 cells/h, whereas non-permeabilized cells produced 12 g g/liter γ-dodecalactone after 30
268 h, with a conversion yield of 21% (w/w), a volumetric productivity of 0.4 g/liter/h, and

a specific productivity of 0.01 g/g (dry weight) of cells/h. The conversion yield and volumetric and specific productivities of the permeabilized cells were 56%, 3.8-fold, and 5.0-fold higher than those of non-permeabilized cells, respectively, which indicates that cell permeabilization was an efficient method for increasing γ-dodecalactone production.

The maximal possible amount of γ-dodecalactone produced from 60 g/liter 10-hydroxystearic acid based on the molar yield of 1 was 40 g/liter. However, permeabilized whole cells of *W. lipofer* produced 46 g/liter γ-dodecalactone, showing a molar yield of > 1 with the small amounts of the by-products acetic acid and oleic acid (Fig. 4A). To explain the higher yield, the transformations of acetic acid and oleic acid were investigated. After 20 h, the cells metabolized 15 mM acetic acid to 8 mM oleic acid with a molar conversion yield of 53% (Fig. 4B) and they converted 15 mM oleic acid to 11 mM 10-hydroxystearic acid with a molar conversion yield of 73% (Fig. 4C). Thus, acetic acid formed through β-oxidation cycle in *W. lipofer* cells was reused to the synthesis of 10-hydroxystearic acid, indicating that fatty acid synthesis occurred in the same time during β-oxidation and the molar conversion yield of 10-hydroxystearic acid to γ-dodecanolactone could be more than 100%.

γ-Dodecalactone production from 10-hydroxystearic acid or fatty acid by yeast strains is summarized in Table 1. *Mortierella isabellina* produced 4.1 g/liter γ-dodecalactone from 19.2 g/liter dodecanoic acid after 24 h, which was previously the highest observed concentration of γ-dodecalactone (35). *Y. lipolytica* produced 3.5 g/liter γ-dodecalactone from 14.4 g/liter 10-hydroxystearic acid after 18 h, with a conversion yield of 24.3% and a productivity of 194 mg/liter/h, which were previously the highest observed

conversion yield and productivity for γ-dodecalactone (9). The concentration, yield, and productivity achieved in the present study using permeabilized cells of *W. lipofer* were 11.1-fold, 52%, and 7.9-fold higher than the highest previously observed values for γ-dodecalactone production. Recently, γ-decalactone productivity for batch cultures of *Y. lipolytica* was observed 168 mg/liter/h using 30 g/liter methyl ricinoleate (36). *Y. lipolytica* produced 12.3 g/liter γ-decalactone from 60 g/liter castor oil with a conversion yield of 21% and a productivity of 240 mg/liter/h (37), which was previously the highest reported concentration and productivity in the production of flavor lactones. The concentration and productivity of γ-lactone obtained in the present study were 3.7- and 6.3-fold higher than those obtained using *Y. lipolytica*, respectively, which indicates that γ-dodecalactone production by permeabilized *W. lipofer* cells is the highest ever reported.

In the present study, a new biotransformation process for the production of the natural flavor lactone was developed using permeabilized cells. γ-Dodecalactone production by the new process using permeabilized *W. lipofer* cells was significantly higher than that using non-permeabilized cells and these cells displayed the highest concentration, yield, and productivity observed to date in the microbial production of the flavor lactone. These results will contribute to the industrial microbial production of γ-lactones.

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417
418

Figure Legends

FIG. 1. Proposed metabolic pathway from 10-hydroxystearic acid to γ-dodecalactone by yeast.

FIG. 2. Effect of detergent and/or solvent treatment on permeabilization of *W. lipofer* for γ-dodecalactone production from 10-hydroxystearic acid. The reactions were the performed in 0.7 M Tris, 10 g/liter 10-hydroxystearic acid, 5 g/liter permeabilized cells, and 0.05% (w/v) Tween 80 at pH 6.5, 35 °C, and 200 rpm for 10 h. Data represent the means of three separate experiments and error bars represent the standard deviation.

FIG. 3. Effect of nitrogen source on γ-dodecalactone production from 10-hydroxystearic acid by permeabilized cells of *W. lipofer*. The reactions were performed in 0.1 g/liter nitrogen, 10 g/liter 10-hydroxystearic acid, 5 g/liter permeabilized cells, and 0.05% (w/v) Tween 80 at pH 6.5, 35 °C, and 200 rpm for 10 h. Data represent the means of three separate experiments and error bars represent the standard deviation.

FIG. 4. Time-course reactions of for γ-dodecalactone production from 10-hydroxystearic acid, acetic acid, and oleic acid using permeabilized *W. lipofer* cells under optimal conditions. (A) Acetic acid, oleic acid and γ-dodecalactone production from 10-hydroxystearic acid. γ-Dodecalactone production (●) from 10-hydroxystearic acid (▲) by permeabilized cells with the byproducts acetic acid (□) and oleic acid (■); and γ-dodecalactone production (○) from 10-hydroxystearic acid (△) by non-

permeabilized cells The reactions were performed in 0.7 M Tris, 60 g/liter 10-hydroxystearic acid, 30 g/liter cells, and 0.05% (w/v) Tween 80 at pH 6.5, 35°C, and 200 rpm. (B) Transformation reaction of acetic acid to oleic acid. The reactions were the performed in 0.7 M Tris, 15 mM acetic acid, 30 g/liter cells, and 0.05% (w/v) Tween 80 at pH 6.5, 35°C, and 200 rpm. Acetic acid (□) and oleic acid (■). (C) Transformation reaction of oleic acid to 10-hydroxystearic acid. The reactions were performed in 0.7 M Tris, 15 mM oleic acid, 30 g/liter cells, and 0.05% (w/v) Tween 80 at pH 6.5, 35°C, and 200 rpm. 10-Hydroxystearic acid (▲) and oleic acid (■). Data represent the means of three separate experiments and error bars represent the standard deviation.

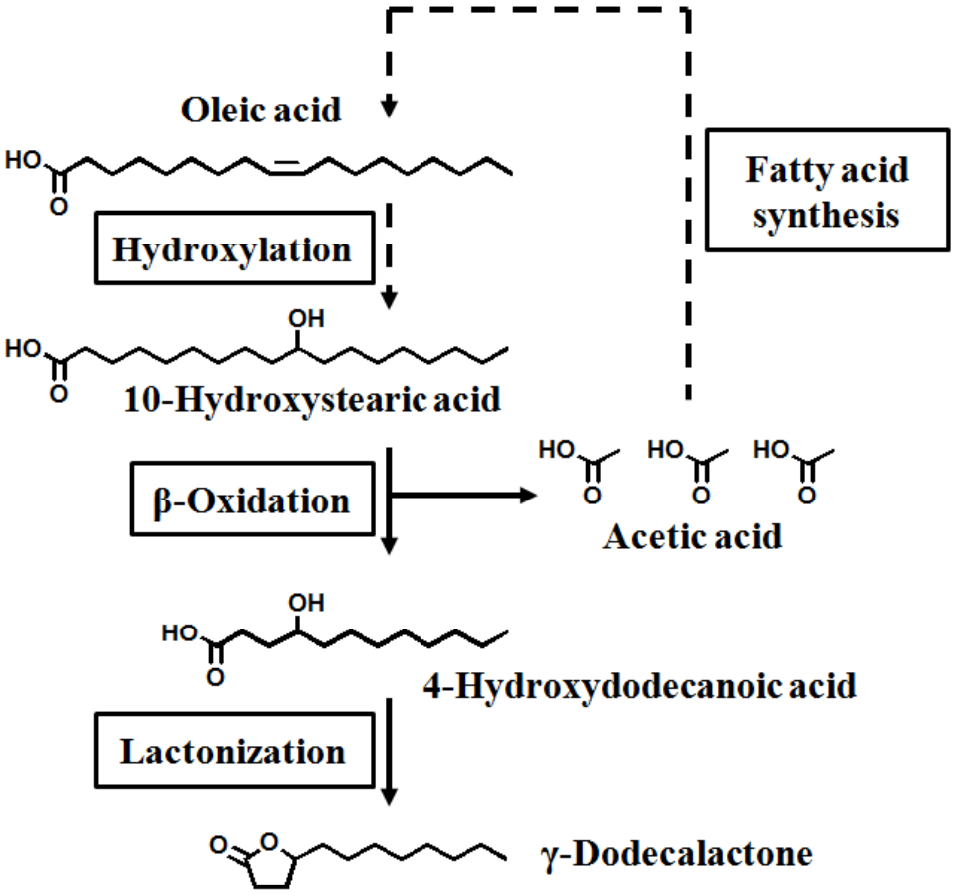

FIG. 1

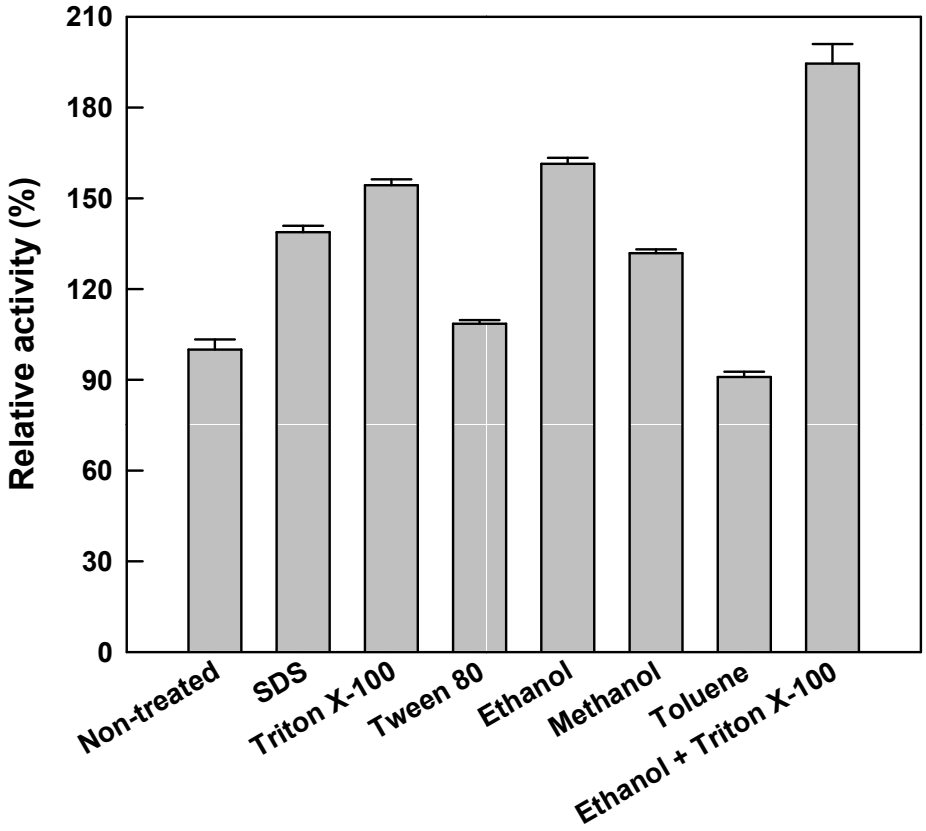

FIG. 2

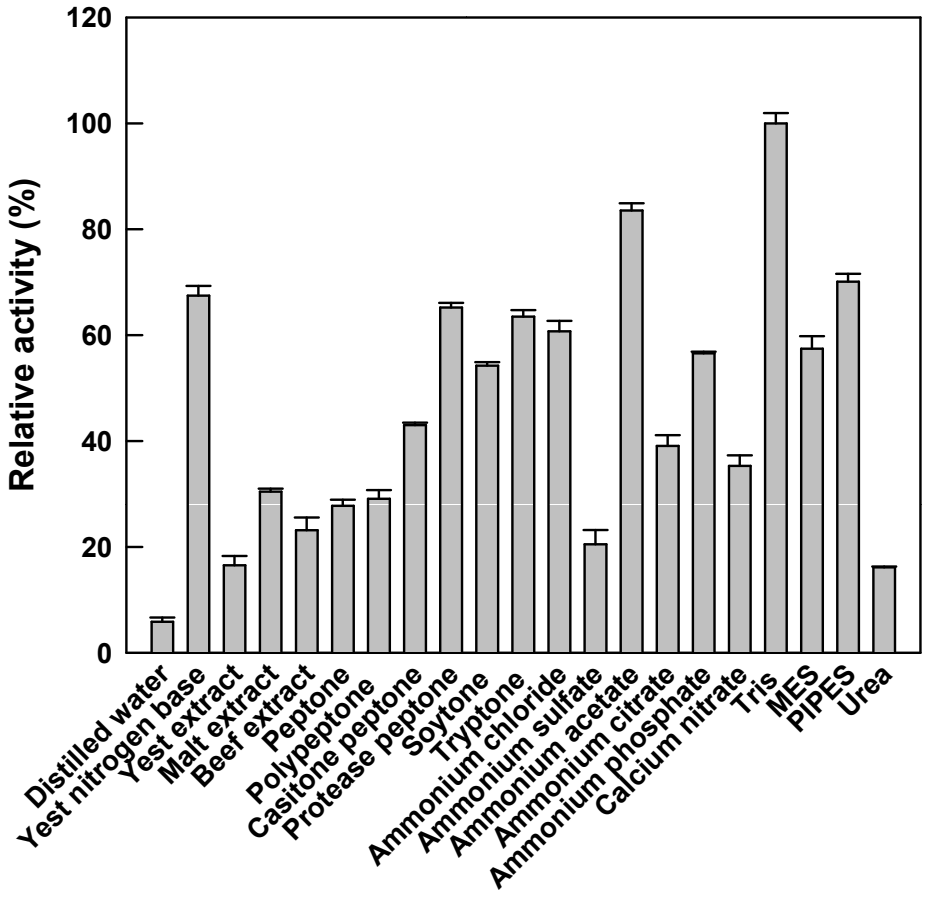

FIG. 3

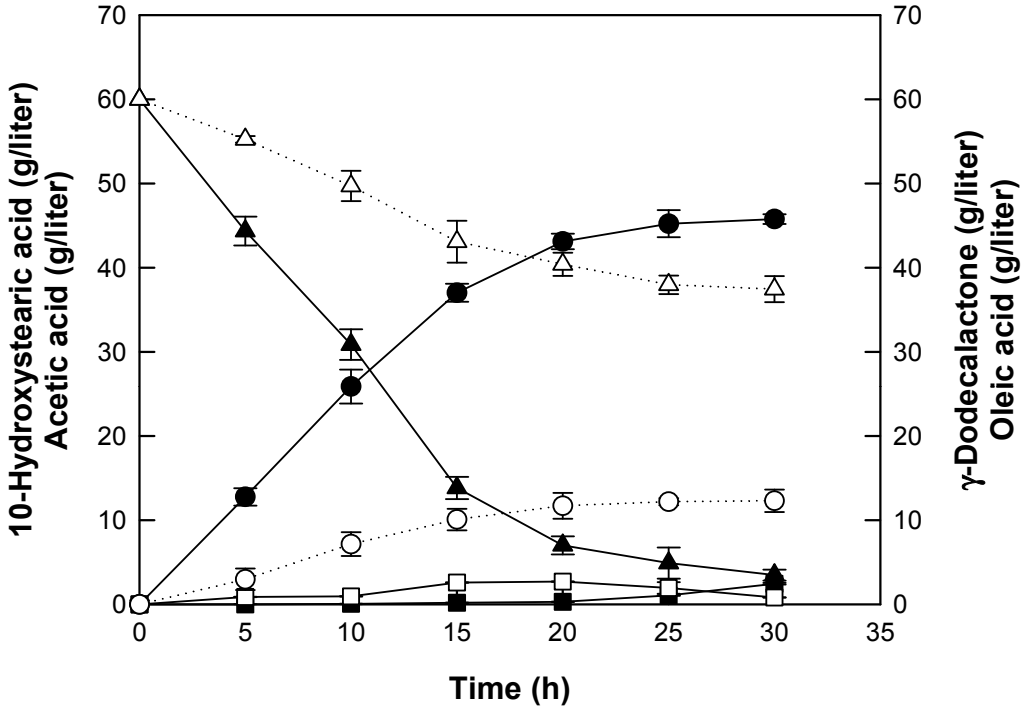

FIG. 4A

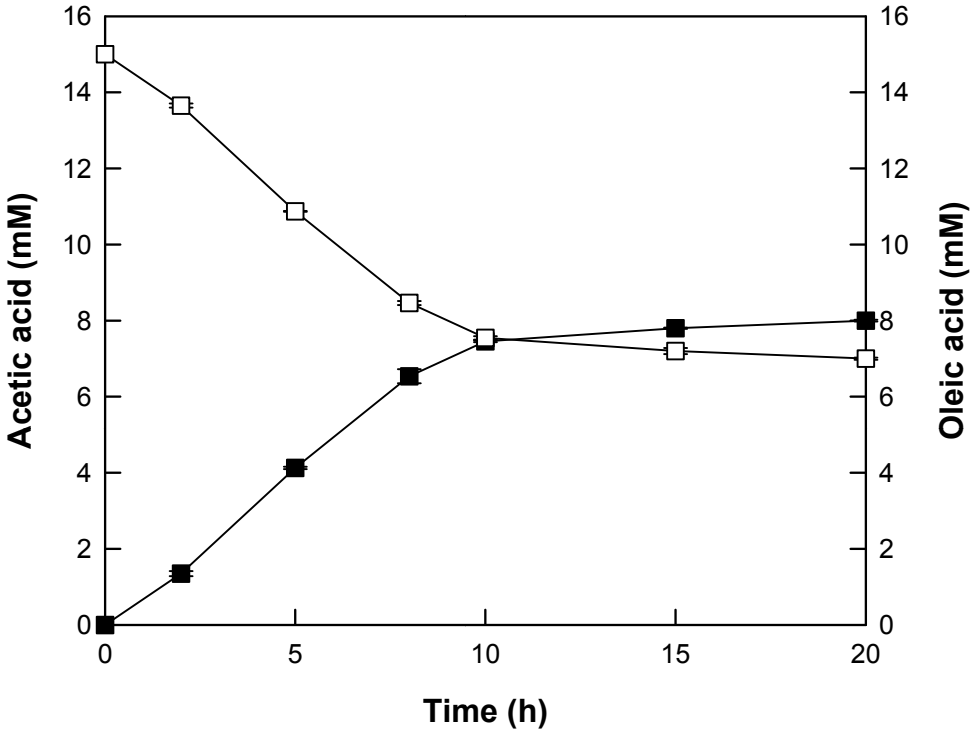

FIG. 4B

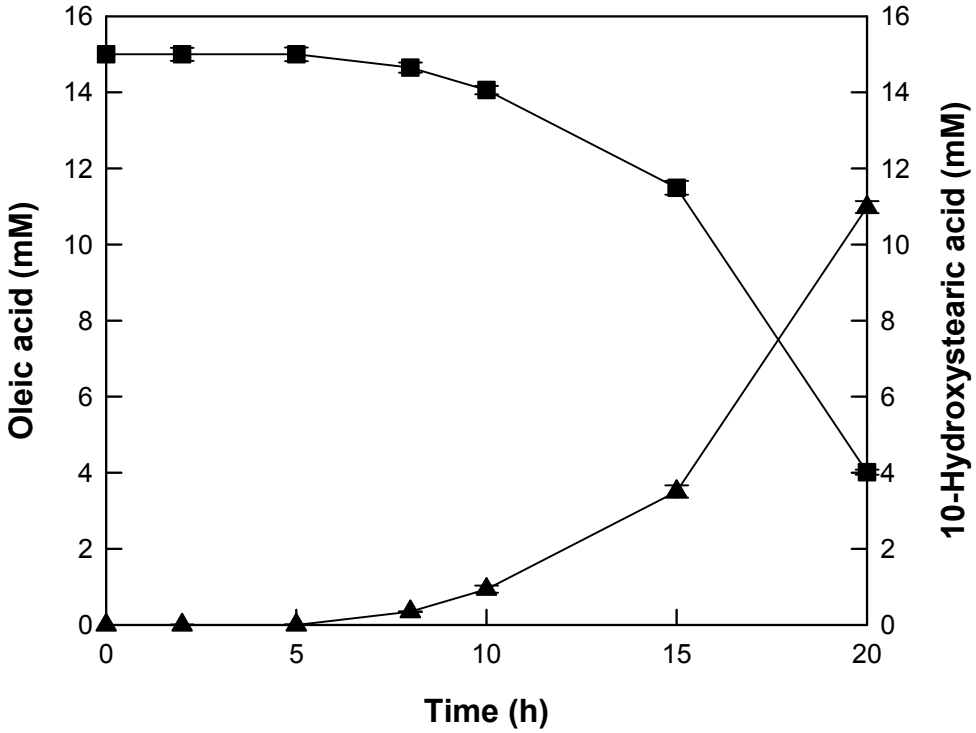

FIG. 4C

TABLE 1. γ-Dodecalatone production by fermentation and whole cell conversion of yeast strains

Biocatalyst	Source	Substrate (g/liter)	γ-Dodecalatone (g/liter)	Productivity (mg/liter/h)	Conversion yield (%, g/g)	Reference
Fermentation	*Yarrowia lipolytica*	10-Hydroxystearic acid (14.4)	3.5	194	24.3	(9)
	Mortierella isabellina	Dodecanoic acid (19.2) [a]	4.1	171	21.4	(35)
	Sporobolomyces odorus	Oleic acid (0.25)	< 0.035	NR	< 14.0	(18)
	Sporidiobolus salmonicolor	Culture medium (0)	< 0.0006	NR	NR	(34)
Whole cells	Baker's yeast	10-Hydroxystearic acid (0.5) and oleic acid	NR	NR	22.5	(33)
Permeabilized cell	*Waltomyces lipofer*	10-Hydroxystearic acid (60)	45.7	1,523	76.2	This study

NR: Not reported.

[a] Dodecanoic acid at 0.8 g/liter was fed at a period of 24 h

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