Regioselective lipase-catalyzed synthesis of 3-*O*-acyl-derivatives of resveratrol and study of their antioxidant properties.

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ABSTRACT

One of the approaches to increase bioavailability of resveratrol is to protect its 3-OH phenolic group. In this work, regioselective acylation of resveratrol at 3-OH was achieved by transesterification with vinyl acetate catalyzed by immobilized lipase from *Alcaligenes* sp. (lipase QLG). The maximum yield of 3-*O*-acetylresveratrol was approximately 75%, as the lipase also catalyzes its further acetylation affording the diester 3,4'-di-*O*-acetylresveratrol and finally the peracetylated derivative. Long saturated and unsaturated fatty acid vinyl esters were also effective as acyl donors with similar regioselectivity. In contrast, the lipase B from *Candida antarctica* catalyzes the acylation of the phenolic group 4'-OH with 80% yield and negligible formation of higher esters. The analysis of the antioxidant properties showed that the Trolox Equivalent Antioxidant Capability (TEAC) values for the acetyl and stearyl derivatives at 3-OH were, respectively, 40% and 25% referred to resveratrol. The addition of an acyl chain in the position 3-OH caused a higher loss of activity compared with the 4'-OH.

KEYWORDS: Antioxidants, Acylation, Lipases, Regioselectivity, Polyphenols, Vinyl esters, Trolox equivalent antioxidant activity, TEAC.

INTRODUCTION

Antioxidants protect cells against the effects of harmful free radicals and play an important role in preventing many human diseases (*e.g.*, cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration, inflammatory disorders, diabetes) and aging itself (*1-4*). The study of antioxidants is of great interest for the role they play in protecting living systems against lipid peroxidation and other anomalous molecular modifications (*5-7*). The modification of natural antioxidants to improve their chemical, oxidative or thermal stability, bioavailability and/or pharmaceutical efficacy yields a series of “semisynthetic” antioxidants (*e.g.*, tocopheryl acetate, L-ascorbyl palmitate) with great impact in the industry (*8, 9*).

Resveratrol (*trans*-3,5,4'-trihydroxystilbene) (**1**) is a polyphenolic phytochemical found in grapes and many plants (*10*) that is biosynthesized in response to pathogenic attack or stress conditions. It possesses a variety of antioxidant (*6*), anti-inflammatory (*11,12*), antitumour (*13, 14*) cardioprotective (*15*), neuroprotective (*16*) and immunomodulatory (*17*) bioactivities, as well as a delaying effect on aging (*18*). As phenolic compound, resveratrol contributes to the antioxidant potential of red wine (*19*) and may be related with the decrease in coronary heart disease observed among wine drinkers (French paradox). At present, resveratrol is under Phase-II clinical trials for the prevention of colon cancer (*4*). It has been well reported that minimal chemical modification on the stilbene nucleus may cause large variations in the biological activity and, more specifically, in the antitumour properties of resveratrol (*16, 20-22*). Lipophylic derivatives of resveratrol, and in particular esters bearing acyl chains, may be important in view of their enhanced affinity with lipophylic membrane constituents, thus increasing their bioavailability (*23*). Some acylated derivatives have shown higher cell-growth inhibition towards DU-145 human prostate cancer cells than resveratrol itself (*20*).

A reported problem with resveratrol is its limited bioavailability owing to its fast metabolism in the liver yielding the less-active 3-sulfate and 3-glucuronide derivatives (*24*). As a consequence, the circulating resveratrol has a serum half-life of 8-14 min (*25*). One of the approaches to increase bioavailability is to obtain resveratrol analogues or derivatives with comparable activity that cannot be sulfated or glucuronated. For that reason, the regioselective modification of the 3-OH is of great interest (*24*).

As the phenolic groups of resveratrol at positions 3- and 4'- exhibit very similar reactivity (the phenolic groups at 3- and 5- are chemically equivalent, as resveratrol is a symmetric molecule), the extraordinary selectivity of enzymes (26) is being exploited for its regiospecific acylation (20), oxidation (27) or glycosylation (28). Thus, acylation of resveratrol by *Candida antarctica* lipase B with vinyl acetate afforded regioselectively 4'-*O*-acetyl-resveratrol (20, 29, 30); however, reactions with longer acyl donors such as vinyl decanoate and vinyl cinnamoate were notably slower.

Regioselective derivatization of resveratrol at positions 3,5- was achieved by a chemo-enzymatic procedure based on standard chemical peracetylation followed by lipase-catalyzed alcoholysis of 4'-acyl group in organic solvents (30).

As part of our interest in regioselective biotransformations of polyhydroxylated compounds (31-34) using immobilized enzymes (35, 36), we have investigated the one-step enzymatic synthesis of acylated resveratrol by lipase-catalyzed transesterification. Our results suggest that the regioselectivity of the process can be controlled by an adequate selection of the biocatalyst (37).

MATERIALS AND METHODS

Materials

Resveratrol from *Polygonum cuspidatum* was purchased in Shanghai Seebio Biotechnology.

Immobilized lipases from *C. antarctica* B (Novozym 435), *Thermomyces lanuginosus* (Lipozyme TL IM) and *Rhizomucor miehei* (Lipozyme RM IM) were kindly donated by Novozymes A/S. Lipase from *Pseudomonas cepacia* (lipase PS IM) was purchased from Amano. Immobilized lipases from *Alcaligenes* sp. (lipases PLG and QLG) were kindly donated by Meito Sangyo Co. (Japan).

Tripelopionin was from Acros. Vinyl acetate, 2-methyl-2-butanol (*t*-amyl alcohol), α -tocopherol, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) and potassium persulfate were purchased from Sigma-Aldrich.

Vinyl stearate was from TCI-Europe. Vinyl oleate was from ABCR GmbH & Co (Germany). Solvents were dried over 3 Å molecular sieves (water content was less than 0.2% v/v by Karl-Fischer). All other reagents were of the highest available purity and used as purchased.

Determination of enzyme activity

The hydrolytic activity was measured titrimetrically at pH 8.0 and 30 °C using a pH-stat (Mettler, Model DL 50). The reaction mixture contained tripelopionin (0.3 ml, final concentration 80 mM), acetonitrile (0.7 ml) and Tris-HCl buffer (19 ml, 1 mM, pH 8.0) containing NaCl (0.1 M). The immobilized biocatalyst was then added and the pH automatically maintained at 8.0 using 0.1 N NaOH as titrant. Experiments were done in triplicate. One enzyme unit (U) was defined as that catalyzing the formation of 1 μ mol of fatty acid per min.

General procedure for enzymatic reactions on analytical scale

Resveratrol (59.2 mg, 50 μ M) and vinyl acetate (0.345 ml, 750 mM) or vinyl stearate (1.16 g, 750 mM) were incubated in 2-methyl-2-butanol (5 ml) in sealed 30 ml dark vials under nitrogen at 40 °C with 150 rpm orbital shaking (SI50, Stuart Scientific). The biocatalyst was added to a final concentration of 150 mg/ml. Aliquots (200 μ l) were withdrawn at intervals, filtered using an

Eppendorf tube containing a Durapore® 0.45µm filter and the progress of the reaction analyzed by HPLC.

Preparative-scale enzymatic reactions

The reaction mixture contained resveratrol (570 mg, 250 mM), vinyl acetate (3.45 ml, 3.75 M), lipase QLG (1.5 g) and 2-methyl-2-butanol (6.55 ml). The mixture was incubated under similar conditions to those described in the analytical scale procedure and monitored by HPLC. After 30 h, the mixture was cooled, filtered, the solvent evaporated and the crude preparation loaded onto a silica-gel column using heptane/ethyl acetate (3:1) as eluent, yielding pure 3-*O*-acetyl-resveratrol (**3**) and 3,4'-di-*O*-acetyl-resveratrol (**5**). For the acylation with vinyl stearate, the reaction mixture contained resveratrol (171 mg, 150 mM), vinyl stearate (3.5 g, 2.25 M), lipase QLG (750 mg) or Novozym 435 (1 g), and 2-methyl-2-butanol (5 ml). The mixture was incubated under similar conditions to those described in the analytical scale procedure and monitored by HPLC. After 72 h, the mixture was cooled, filtered, the solvent evaporated and the crude product purified by chromatography on a silica-gel column using heptane/ethyl acetate (2:1) as eluent, yielding 3-*O*-stearoyl-resveratrol (**7**) with lipase QLG and 4'-*O*-stearoyl-resveratrol (**8**) with Novozym 435.

HPLC analysis

HPLC analysis was performed using a ternary pump (model 9012, Varian) coupled to a thermostated (25 °C) autosampler (model L-2200, VWR International). The temperature of the column was kept constant at 45 °C (MEF-01 oven, Análisis Vínicos, Spain). Detection was performed using a photodiode array detector (ProStar, Varian) in series with an evaporative light scattering detector (ELSD, model 2000ES, Alltech), and integration was carried out using the Varian Star LC workstation 6.41. For the analysis of resveratrol acetylation, the column was a Lichrospher 100 RP8 (4.6 x 125 mm, 5 µm, Analisis Vinicos) and mobile phase was 70:30 (v/v) H₂O/methanol (H₂O contained 0.1% of acetic acid) at 1 ml/min for 5 min. Then, a gradient from this mobile phase to 50:50 (v/v) H₂O/methanol was performed in 5 min, and this eluent was maintained during 15 min. The resveratrol esters were quantified by measuring the absorbance at 310 nm, and ELSD was used to follow the

formation of free fatty acid. For resveratrol stearate synthesis, the column was a Mediterranea-C18 (4.6 x 150 mm, 5 μ m, Teknokroma, Spain). The mobile phase was 90:10 (v/v) methanol/H₂O (H₂O contained 0.1% of formic acid) at 1.5 ml/min.

HPLC/MS

Samples were analyzed by HPLC (model 1100, Agilent Technologies) coupled to a mass spectrometer (model QSTAR pulsar, Applied Biosystems). Chromatographic conditions were as described above, except for the mobile phase that contained 1% (v/v) formic acid and the flow rate was lowered to 0.6 ml/min. Ionization was performed by electrospray and the mass spectrometer had a hybrid QTOF analyzer.

Nuclear Magnetic Resonance (NMR)

NMR spectra of the different compounds were recorded on a Bruker DRX 500 spectrometer using DMSO as solvent. A temperature of 298 K was employed with concentrations around 10 mM. Chemical shifts were reported in ppm, and referenced versus the solvent signal. Vicinal proton-proton coupling constants were estimated from first order analysis of the spectra. The 2D-TOCSY experiment (60 ms mixing time) was performed using a data matrix of 256x1K to digitize a spectral width of 5000 Hz. Four scans were used per increment, with a relaxation delay of 2 s. 2D-NOESY (600 ms) and 2D-T-ROESY experiments (500 ms) used the standard sequences provided by the manufacturer, and the data matrixes described above, with 32 and 48 scans per increment, respectively. In all cases, squared cosine-bell apodization functions were applied in both dimensions. The spectral widths for the HSQC spectra were 5000 Hz and 18000 Hz for the ¹H and ¹³C-dimensions, respectively. The number of collected complex points was 1028 for the ¹H-dimension with a recycle delay of 2 s. The number of transients was 16, and 256 time increments were always recorded in the ¹³C-dimension. The J-coupling evolution delay was set to 3.2 ms. A squared cosine-bell apodization function was applied in both dimensions. Prior to Fourier transform the data matrixes were zero filled up to 1024 points in the ¹³C-dimension. The spectral widths for the HMBC spectra were 5000 Hz and 25000 Hz for the ¹H and ¹³C-dimensions, respectively. The number of collected complex points was 1028 for the ¹H-

dimension with a recycle delay of 2 s. The number of transients was 64, and 256 time increments were always recorded in the ¹³C-dimension. The J-coupling evolution delay was set to 66 ms. Prior to Fourier transform the data matrixes were zero filled up to 1024 points in the ¹³C-dimension.

TEAC assay

To measure the antioxidant activity of the new compounds we used the Trolox Equivalent Antioxidant Capability (TEAC) assay that was previously described by Re *et al.* (38), with some modifications to adapt to 96 well plates. This assay is based on the ability of antioxidants in reducing ABTS radical. Briefly, ABTS (7 mM final concentration) was added to an aqueous solution of 2.45 mM potassium persulfate and kept in the dark at room temperature for 15 h to obtain the ABTS radical, which was stable for 2 days. The ABTS^{•+} solution was diluted with ethanol to get an absorbance of 0.70 ($\pm$ 0.02) at 734 nm and equilibrated at room temperature. In each well, 20 μ l of a solution of Trolox (standard) or of the antioxidants (0.5-10 μ M) in ethanol were added to 230 μ l of adjusted ABTS^{•+} solution. The decrease of absorbance of ABTS^{•+} solution was monitored at 734 nm during 6 min using a microplate reader (model Versamax, Molecular Devices) and the decrease of absorbance ($\Delta A_{734\text{nm}}$) for each concentration was determined using the area under the curve. The concentration vs. $\Delta A_{734\text{nm}}$ curve was plotted for the different compounds and used to calculate the equivalent Trolox concentration. The TEAC value was determined as the ratio between the slopes of concentration- $\Delta A_{734\text{nm}}$ curves for the corresponding antioxidant and Trolox.

RESULTS AND DISCUSSION

Enzyme screening for acetylation of resveratrol

Several lipases and esterases were used for a preliminary HPLC screening of acetylation of resveratrol (**1**) in 2-methyl-2-butanol (2M2B) under the following conditions: 50 mM resveratrol, 750 mM vinyl acetate, 100 mg/ml biocatalyst and 40 °C. The solvent 2M2B was selected for the screening due to the notable solubility of resveratrol and vinyl acetate. Six commercial immobilized lipases were found to catalyse this process (Table 1). Two peaks (corresponding to compounds **2** and **3**) were observed in the monoester fraction of the HPLC chromatograms (Fig. 1), as well as two peaks corresponding to the diesters **4** and **5**. The ratio between the two monoesters in the HPLC chromatograms was dependent on the nature of the biocatalyst. In particular, the lipase QLG from *Alcaligenes* sp. synthesized almost exclusively the compound **3**, whereas the immobilized lipase B from *C. antarctica* (Novozym 435), which is the preferred biocatalyst for many esterification and transesterification processes including the modification of antioxidants (39-41), formed the other isomer (**2**).

We scaled-up the reactions with lipase QLG and Novozym 435 in order to isolate and characterize the different products by mass spectrometry and 2D-NMR. The position of the substitution was unequivocally deduced by using 2D-NMR experiments. A combination of homo- (NOESY, ROESY) and hetero-nuclear sequences (HSQC, HMBC, see experimental section for details) was employed for this task, assisted by NOE-type experiments. Indeed, using chemical shift analysis alone was not possible to deduce the exact nature of the substitution. The reported data for resveratrol were also employed to further support the obtained conclusions (42). The ¹H and ¹³C chemical shifts for the different compounds are gathered in the Supporting Information Section. The key sections of the HMBC spectra are also depicted in the Supporting Information, showing the correlation of the quaternary aromatic carbon resonances to the key protons in the corresponding ring.

The use of lipase QLG initially leads to one compound that has a non-symmetric spectrum in ring A (the three substituted one, Fig. 2). It has to be mentioned that resveratrol shows a symmetric spectrum for both rings. One acetate group was clearly visible in the spectra with two free hydroxyl groups, one for ring A and one for ring B. The existence of mono acetyl substitution was also confirmed by MS analysis. NOESY spectra showed contacts between the acetate group and hydrogens H2 and H4

belonging to ring A. The HMBC showed all the expected peaks for 3-*O*-acetyl-resveratrol (**3**). A new compound was formed when the concentration of compound **3** was high enough; its MS and NMR spectra showed the presence of two acetates. The spectrum also showed loss of symmetry in ring A, while the nuclei belonging to ring B showed important shifting of their resonances. The HMBC spectra confirmed the presence of one acetate moiety in each ring, thus permitting to assign this molecule as 3,4'-di-*O*-acetyl-resveratrol (**5**). The NOE data indicated close contacts between each acetyl group and one of the resveratrol skeleton rings.

We confirmed that the immobilized lipase B from *C. antarctica* (Novozym 435) was highly specific for the phenolic group at 4'-OH (**20**), which is less sterically hindered than the 3-OH (**29**), yielding 4'-*O*-acetyl-resveratrol (**2**). In fact, the only previous report for the synthesis of monoacetate at 3-OH was carried out by a multistep procedure based on the chemical peracetylation of resveratrol followed by regiospecific alcoholysis catalyzed by *P. cepacia* and *C. antarctica* lipases (**30**).

The lipases from *Thermomyces lanuginosus* (Lipozyme TL IM) and *Pseudomonas cepacia* (Lipase PS IM) afforded mixtures of 3- and 4'- regioisomers, with a major content of the monoacetate at 3-. A lipase from a different strain from *Alcaligenes* sp. (PLG) yielded a nearly equimolar mixture of both derivatives. Lipase from *Rhizomucor miehei* (Lipozyme RM IM) synthesized the 4'- regioisomer as the main product. The reaction also took place in other solvents such as tert-butanol, isopropyl ether and 2-pentanone, or even using vinyl acetate as solvent and acyl donor at the same time.

Fig. 1 shows the HPLC chromatograms with the three lipases that synthesized 3-*O*-acetyl-resveratrol as the main product. The molar ratio between the two monoesters was 18:1 for lipase QLQ, 2.5:1 for lipase PS IM and 1.5:1 for TL IM. As shown in Fig. 3, the three biocatalysts also formed the diester 3,4'-di-*O*-acetyl-resveratrol (**5**) as well as a small amount of 3,5-di-*O*-acetyl-resveratrol (**4**).

Considering that the positions of the phenolic substituents on the aromatic rings of resveratrol may play an important role in its biological activity (**43**), and that protection of 3-OH is one of the approaches to improve resveratrol bioavailability, we focused our attention towards lipase QLQ as a simple and selective alternative to obtain monoesters at 3-OH. This biocatalyst is obtained by immobilising a lipase from *Alcaligenes* sp. (MW 31,000) on granulated diatomaceous earth. We observed by scanning electron microscopy (SEM) that the particle size was quite uniform for this

biocatalyst (approx. 500 μ m) (see Supporting Information). Up to now, the main application of this immobilized lipase has been the resolution of racemic mixtures (44).

Kinetics of resveratrol acetylation

Fig. 3 shows the progress of acetylation reaction with both lipase QLG and Novozyme 435. Lipase QLG formed initially 3-*O*-acetyl-resveratrol (**3**). When the concentration of **3** was high enough, the synthesis of diester 3,4'-di-*O*-acetyl-resveratrol (**5**) was the main process. Finally, the triester 3,4',5-tri-*O*-acetyl-resveratrol (**6**) was formed. With the lipase B from *C. antarctica*, the monoester 4'- (**2**) was synthesized, whereas the formation of diester **5** was almost negligible. The maximum yield of monoester with lipase QLG reached nearly 75% (on a molar basis) in 12 h, whereas for the diester **5** the maximum yield (approx. 60%) was achieved at 160 h. With Novozym 435 a maximum yield of 80% for monoester **2** was obtained in 50 h.

Resveratrol acylation with vinyl stearate

We tested the acylation of resveratrol with saturated and unsaturated long-chain acyl-donors. The monoester selectivity was similar to that observed in the acetylation process. Fig. 4 shows the HPLC chromatograms with lipases from *Alcaligenes* sp. (lipase QLG), *P. cepacia* (lipase PS IM) and *T. lanuginosus* (Lipozyme TL IM) in the transesterification with vinyl stearate. As shown in Fig. 4, the acylation with vinyl stearate catalyzed by lipase QLG afforded one major compound (**7**). The NMR and MS spectra showed that this was a mono-substituted molecule, whose HMBC spectrum was indeed similar to that of compound **3**. The careful HMBC analysis suggested that this molecule was 3-*O*-stearoyl-resveratrol (**7**), which was further confirmed by the existence of NOE contacts and spatial proximity between the CH₂ moiety of the fatty acid chain and hydrogens H-2 and H-4 at the aromatic ring A. Thus, the nature of **7** was unequivocally confirmed. Novozym 435 also formed a mono-substituted major compound, whose NMR spectra were different to those of monostearate **7**. The spectra showed symmetry for both rings, and the HMBC and NOESY analysis (NOEs between the CH₂ moiety of the fatty acid chain and hydrogens H-3' and H-5' at the aromatic ring B) indicated that this molecule was 4'-*O*-stearoyl-resveratrol (**8**).

The highest selectivity towards the monoester 3-*O*-stearoyl-resveratrol (**7**) was obtained again with lipase QLG, whereas PS IM and TL IM yield mixtures of the two isomers with the 3-derivative as the major product. We observed that lipase QLG also afforded regioselectively derivatives at 3-OH bearing unsaturated fatty acids (*e.g.*, oleic acid, data not shown). Lipases PLG and RM IM afforded mixtures of 3- and 4'- regioisomers, with a major content in 4'-isomer. Fig. 5 shows the progress of the acylation reaction with both lipase QLG and Novozyme 435. The formation of diesters with both enzymes was almost negligible, probably due to steric hindrance. The maximum yield of monoester with lipase QLG reached 55% in 160 h, whereas with Novozym 435 a maximum yield of 35% was obtained in 160 h. It was reported that lipase B from *C. antarctica* presented a low reactivity with acyl chains longer than acetate; Teng *et al.* reported 10% yield in the acylation of resveratrol with vinyl decanoate and negligible reaction with vinyl cinnamate (29).

Antioxidant Activity

The antioxidant activity of the new derivatives was analyzed. We initially compared the antioxidant capability of resveratrol with other phenolic antioxidants such as α -tocopherol and Trolox (used as standard). Fig. 6 shows a different behavior between resveratrol and α -tocopherol. The latter was able to complete the reaction in 1 min; in contrast, resveratrol showed a further small inhibitory effect even after 7 min reaction. Resveratrol showed also a higher activity than Trolox and α -tocopherol, which may be related to the fact that resveratrol has three hydroxyl groups (hydrogen donors) whereas α -tocopherol and Trolox present only one (6).

The new acetyl derivatives showed antioxidant activity but it decreased when increasing the degree of substitution of the phenolic groups. The results of the assay are presented in Fig. 7 and the TEAC values are summarized in Table 2. We observed that the addition of an acyl chain in the position 3-OH caused a higher loss of activity compared with the 4'-OH; the TEAC value for the acetyl derivative at 3-OH was 40% that of resveratrol. Thus, 3-OH seems to play an important role in antioxidant activity. Stivala *et al.* reported that the hydroxyl group in the 4'- position is required for the antioxidant activity (45). It is noteworthy that the ethanolic solution of resveratrol and the 4'-OH derivatives developed a yellow color (typical of resveratrol oxidation) during the assay, which was not observed with the

monoesters at 3-OH; this observation may be related with the intrinsic stability of the different compounds. Regarding the stearyl derivatives (Fig. 8), the effect of chemical modification of aromatic rings on the antioxidant activity was more pronounced than in the acetylation. Again, the substitution at 3-OH gave rise to a higher decrease of activity than the corresponding at 4'-OH.

CONCLUSIONS

Immobilized lipase from *Alcaligenes* sp. (lipase QLG) is able to catalyze in one step the regioselective synthesis of resveratrol fatty acid esters in the 3-OH phenyl group. The resulting derivatives bearing different saturated or unsaturated acyl-groups are suitable for *in vitro* and *in vivo* structure-activity relationship studies. As the stability of resveratrol in serum is extremely low due to its fast metabolism in the liver resulting in the chemical modification of the 3-OH, the novel 3-*O*-acyl derivatives may exhibit improved bioavailability and pharmacological properties.

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Supporting information available

Characterization data of the new compounds are available as Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Table 1. Screened immobilized lipases that catalyzed the acetylation of resveratrol.

Microorganism	Support	Commercial name	Supplier	Hydrolytic activity (U/g) ^a	Resveratrol selectivity
<i>Candida antarctica</i> (lipase B)	Macroporous acrylic resin	Novozym 435	Novozymes A/S	1725	4'-OH
<i>Thermomyces lanuginosus</i>	Granulated silica	Lipozyme TL IM	Novozymes A/S	2490	3-OH > 4'-OH
<i>Rhizomucor miehei</i>	Macroporous anion exchange resin	Lipozyme RM IM	Novozymes A/S	119	4'-OH > 3-OH
<i>Pseudomonas cepacia</i>	Diatomaceous earth	Lipase PS IM	Amano	3965	3-OH > 4'-OH
<i>Alcaligenes</i> sp.	Granulated diatomaceous earth	Lipase PLG	Meito Sangyo	120	3-OH = 4'-OH
<i>Alcaligenes</i> sp.	Granulated diatomaceous earth	Lipase QLG	Meito Sangyo	695	3-OH

^a Measured in the hydrolysis of tripropionin.

Table 2. TEAC values of different acyl derivatives of resveratrol.

Compound	TEAC	Slope ($\Delta A_{734}/\mu\text{M}$)	R ²
Resveratrol	1.79	5.41	0.975
3- <i>O</i> -acetyl-resveratrol	0.69	2.08	0.989
4'- <i>O</i> -acetyl-resveratrol	1.64	4.96	0.962
3,4'-di- <i>O</i> -acetyl-resveratrol	0.33	1.01	0.985
3,5,4'-tri- <i>O</i> -acetyl-resveratrol	0.16	0.50	0.992
3- <i>O</i> -stearoyl-resveratrol	0.44	1.32	0.991
4'- <i>O</i> -stearoyl-resveratrol	1.02	3.07	0.990

Figure legends

Fig 1. HPLC chromatograms showing the formation of the two monoacetates (**2** and **3**) and the two diacetates (**4** and **5**) of resveratrol using as biocatalysts: (I) lipase QLG; (II) lipase PS IM; (III) lipase TL IM. Reaction conditions: 50 mM resveratrol, 750 mM vinyl acetate, 150 mg/ml biocatalyst, 2M2B, 40 °C. The chromatograms correspond to a reaction time of 216 h.

Fig. 2. Scheme of the acetylation of resveratrol catalyzed by lipase from *Alcaligenes* sp. (lipase QLG).

Fig. 3. Kinetics of resveratrol acetylation in 2M2B catalyzed by (A) lipase QLG and (B) Novozym 435. Experimental conditions: 50 mM resveratrol, 750 mM vinyl acetate, 150 mg/ml biocatalyst, 40 °C.

Fig. 4. HPLC chromatograms showing the formation of the two monostearates of resveratrol using: (I) lipase QLG; (II) lipase PS IM; (III) lipase TL IM. Screening conditions: 50 mM resveratrol, 750 mM vinyl stearate, 150 mg/ml biocatalyst, 40 °C. The chromatograms correspond to a reaction time of 138 h.

Fig. 5. Kinetics of resveratrol acylation with vinyl stearate in 2M2B catalyzed by lipase QLG and Novozym 435. Experimental conditions: 50 mM resveratrol, 750 mM vinyl stearate, 150 mg/ml biocatalyst, 150 rpm, 40 °C.

Fig. 6. Progress of the ABTS^{•+} absorbance in presence of different antioxidants (5 µM).

Fig. 7. Effect of antioxidant concentration on the decrease of ABTS^{•+} absorbance for the acetyl derivatives of resveratrol.

Fig. 8. Effect of antioxidant concentration on the decrease of ABTS^{•+} absorbance for the stearyl derivatives of resveratrol.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8