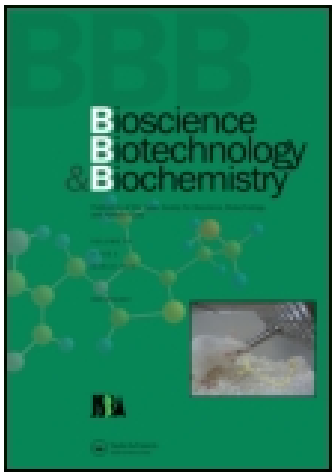

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Esterase Activities of *Brevibacterium* sp. R312 and *Brevibacterium linens* 62

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Esterase activity of *Brevibacterium linens* 62 and *Brevibacterium* sp. R312 was detected. Each strain had esterase activities that hydrolyzed *p*-nitrophenyl acetate and α-naphthyl acetate. Biosynthesis and optimum pH and temperature of the two esterase activities showed that the latter were caused by different esterases. The influence of the culture medium and the growth substrate on biosynthesis of the esterase systems were studied. Hydrolysis of methylthioacetate and phenethyl acetate by cell extracts of the two strains was done. No enzymatic ester synthesis reaction was observed. However, transfer reactions by cell extracts of the two strains were done.

Esterases catalyse the hydrolysis of various short chain fatty acid esters. They are arbitrarily classified as enzymes hydrolyzing substrates in solution, but lipases hydrolyze substrates in emulsions.[1] The use of esterases for biotechnology has been suggested frequently, and this has been reinforced by the increasing interest in specific enantioselective hydrolysis and synthesis of esters.[2-4] Esterase reactions are frequently easily reversible, depending on the concentration of reactants or availability of water.

Esterase are found widely in animals, plants and microorganisms.[5-7] Although limited investigations have been made on microbial esterases compared with those of mammalian sources, esterases have been isolated from several microorganisms such as *Bacillus stearothermophilus*,[8] baker's yeast,[9] and *Aspergillus niger*.[10]

Information is limited on the esterases of coryneform bacteria.[11,12] Thus, the objectives of this study were to detect esterase activity of two coryneform bacteria, to identify the influences of the culture medium and of the growth substrate on synthesis of the esterase system of the two strains, and to test for hydrolysis and synthesis of esters.

Materials and Methods

Microorganisms and culture conditions. Brevibacterium sp. R312 isolated by Arnaud *et al.*[13] and *Brevibacterium linens* 62 isolated from a cheese (kindly supplied by Besnier, dairy industry, Laval, France) were used.

TSB (Trypcase Soya Broth, Difco) and LP medium were used. The LP medium had the following composition per liter: $(NH_4)_2SO_4$, 2 g; $(NH_4)H_2PO_4$, 6 g; $MgSO_4$, 0.5 g; $CaCl_2$, 0.01 g; NaCl, 0.1 g; KCl, 0.5 g; H_3BO_3, 500 μg; KI, 100 μg; $FeCl_3$, 2000 μg; Ca–panthothenate 2000 μg; Thiamine, 2000 μg; Inositol, 2000 μg; Pyridoxine, 2000 μg; Nicotinic acid, 500 μg, and Biotin, 20 μg.

Different synthetic media, formulated using he LP medium supplemented with vitamins, trace elements, amino acids, or NaCl were used. The various batches of substances were made up as follows: *trace elements* (TE, $\mu g \cdot l^{-1}$): $CuSO_4$, 40; KI, 100; $MnSO_4$, 400; Na_2MoO_4, 200; and $ZnCl_2$, 400; *Vitamins* (Vit, $\mu g \cdot l^{-1}$): choline, 9; cyanocobalamin, 8; folic acid, 14; nicotinic acid, 235; riboflavin, 110; and PABA, 20; *Amino acids* (AA, $g \cdot l^{-1}$): arginine, 0.6; aspartic acid, 1; cysteine, 0.07; glycine, 0.4; glutamic acid, 3.2; histidine, 0.46; isoleucine, 0.93; leucine, 1.3; lysine, 1; methionine, 0.43; phenylalanine, 0.75; proline, 2; threonine, 0.65; tryptophan, 0.17; tyrosine, 0.45; and valine, 1.

Carbon substrate was added to the medium. Vitamins, trace elements, amino acids, and $FeCl_3$ were sterilized by filtration through an HA 0.45 μm Millipore membrane. Mineral salts were autoclaved at 120°C for 30 min.

The cultures grown on these media were buffered to pH 7 using an Na_2HPO_4–KH_2PO_4 buffer (100 mM). Aerobic cultures were grown using a shaker (80 oscillations per min, amplitude 7 cm) at 28°C in Erlenmeyer flasks filled to 1/10 of their capacity.

Cell biomass. Cell growth was monitored by measuring the absorbance at 420 nm using a Uvicon 930 (Kontron) spectrophotometer.

Preparation of cell extracts. Cells were harvested by centrifugation at 10,000 × *g* for 15 min at 4°C, the supernatant was removed and kept at 4°C. The pellet was washed twice with buffer A (Na_2HPO_4–KH_2PO_4, 20 mM, pH 6.3); it was then suspended in the same buffer and sonicated at 70 W (Branson sonifier 250) for 2 min under cooling. Cell debris was removed by centrifugation at 4°C (7000 × *g*, 15 min). The resulting supernatant, named the crushing supernatant, was ultracentrifuged at 105,000 × *g* for 1 h at 4°C. The final supernatant was used as the enzyme extract for biosynthesis study.

Assay of esterase activity. Two chromogenic substrates (*p*-nitrophenyl acetate, pNPA, and α-naphthyl acetate, α-NA) are used classically for the detection of esterase activities.[14,15]

Hydrolysis of pNPA. A reaction mixture of 0.1 ml of enzyme extract, 3.5 ml of 50 mM phosphate buffer (pH 7), and 0.4 ml of pNPA (10 mM dissolved in ethanol) was prepared. The progress of *p*-nitrophenol release was measured directly at 25°C by its absorption at 400 nm. A control reaction not containing enzyme extract was run in parallel. The molar absorption coefficient of *p*-nitrophenol at pH 7 was $10145\ l \cdot mol^{-1} \cdot cm^{-1}$.

Hydrolysis of αNA. A reaction mixture of 0.1 ml of enzyme extract, 2.5 ml of 50 mM phosphate buffer (pH 7), 0.1 ml of Fast Blue RR Salt (24 mM), and 0.4 ml of α-NA (10 mM) was prepared. α-NA and Fast Blue RR Salt were dissolved in ethanol. The Fast Blue RR Salt solution was filtered through an GVWP 0.22 μm Millipore membrane before use. The progress of α-naphthol release was measured directly at 25°C at 500 nm. A control reaction not containing enzyme extract was run in parallel. The molar absorption coefficient of α-naphthol at pH 7 in the presence of Fast Blue RR Salt was $16970\ l \cdot mol^{-1} \cdot cm^{-1}$.

The kinetics of esterase activities for pH > 6 were investigated at 25°C as described above. As staining of the *p*-nitrophenol and the bonding reactions between α-naphthol and Fast Blue RR Salt (30) were pH-dependent, the molar absorption coefficients were measured at 400 and 500 nm respectively for all the pHs values greater than 6. For pH < 6, the reactions were stopped at regular intervals by the addition of 7 volumes of phosphate buffer (7 v/v) (pH 7, 100 mM) containing 0.5 mM $HgCl_2$ per volume of reaction mixture. The Hg^{2+} ions totally inhibited the esterase activity of the two strains.

Hydrolysis of methylthioacetate. Kinetics of methanethiol formation at 30°C was monitored by direct spectrophotometric measurement at 412 nm

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Abbreviations: pNPA, *p*-nitrophenyl acetate; α-NA, α-naphthyl acetate; TE, trace elements; Vit, vitamines; AA, amino acids.

using the method described by Ferchichi *et al.*[16] The reaction mixture consisted of 1 ml of phosphate buffer (pH 7, 50 mM), 50 µl DTNB (5,5'-dithio-bis-2-nitrobenzoic acid, 5 mM dissolved in absolute ethanol), 0.1 ml enzyme extract, and 0.1 ml methylthioacetate (12.5 mM dissolved in absolute ethanol). The molar absorption coefficient of methanethiol at 412 nm was 4545 $l \cdot mol^{-1} \cdot cm^{-1}$.

One unit of esterase activity is defined as the amount of enzyme releasing 1 µmol of alcohol per minute under the conditions specified.

Hydrolysis, synthesis, and ester transfer assays. These reactions were done at 25°C with the crushing supernatant as the enzyme extract. At specific times, 0.5 µl of the reaction mixture was injected in the chromatograph column to analyse the amounts of esters and alcohols. 1-Butanol and 3-pentanol were used as internal standards.

Conditions of hydrolysis of phenethyl acetate. The reaction mixture consisted of 7 ml of phosphate buffer (50 mM, pH 7) containing either 11.5 mM or 57 mM of phenethyl acetate and 1 to 2 ml enzyme extract.

Ester synthesis conditions. The reaction mixture consisted of 4 ml of sodium phosphate buffer (pH 7, 100 mM) containing different alcohol and carboxylic acid concentrations and 0.5 ml enzyme extract.

Transfer conditions. The reaction mixture consisted of 4 ml of sodium phosphate buffer (50 mM, pH 7) with different ester and alcohol concentrations and 0.5 ml enzyme extract. In the presence of phenethyl alcohol (1 M), an emulsion was prepared by sonication in the presence of 2% polyvinylic alcohol.

Gas chromatography (GC) analysis. The corresponding esters and alcohols were analyzed by gas chromatography (Varian 3300 gas chromatograph, Varian Associates, California, U.S.A.) using a DB1 (J and W Scientific, California, U.S.A.) silica column (15 m × 0.22 mm i.d. × 0.25 µm film thickness). Helium flow was set at 0.5 $ml \cdot min^{-1}$. The injector was at 200°C and the flame ionization detector at 250°C. After 1 min at 40°C, the oven temperature was increased to 150°C at a rate of 5°C $\cdot min^{-1}$ or 10°C $\cdot min^{-1}$ according to the substance to be analysed. Final temperature was maintained for 1 min.

Protein measurement. Protein was assayed by the method of Bradford[17] using bovine serum albumin as a standard.

Electrophoresis. A precast acrylamide concentration gradient gel (4 to 15%) (Touzard and Matignon, France) was used under non-denaturing conditions. Migration was done at constant current (30 mA per gel) and bromophenol blue was used as a migration tracer.

Detection of esterase activity was done as described by Baillargeon and Sonnet[18] using α-NA and Fast blue RR Salt.

Results

Study of esterase activities of the two strains during growth in TSB medium

Biosynthesis. No esterase activity was detected in the culture supernatant of either strain. Esterase activity was measured in the crushing supernatant. Esterase activities (Fig. 1) peaked after 24 h of growth. The esterase activities of *Brevibacterium* sp. R312 were three to six times as great as those of *Brevibacterium linens* 62.

The biosynthesis curve of esterase activity assayed in the presence of α-NA was different to that assayed with pNPA for both strains, and especially for *Brevibacterium* sp. R312. The esterase activities assayed in the presence of both substrates may correspond to different enzyme systems.

Electrophoretic pattern. Seven bands of esterase activity were shown in the presence of α-NA in the crushing supernatant of *Brevibacterium* sp. R312. Five bands of esterase activity were found for *Brevibacterium linens* 62. It is stressed that the esterases with activity in the presence of pNPA may not be seen on electrophoresis in the presence of α-NA.

Influence of the culture medium on the synthesis of esterase systems

The enzymatic activities of the two strains were compared after growth on synthetic medium (glucose, 10 $g \cdot l^{-1}$) and complex TSB medium. The influence of the various groups of substances (vitamins, amino acids, trace elements, NaCl) in TSB medium was studied (Table I). Measurements were done on cell extracts at the end of the exponential growth phase.

Brevibacterium sp. R312. pNPA esterase activity was about 53 $U \cdot g^{-1}$ when the strain was grown on LP medium or LP + Vit or LP + Vit + TE. The addition of vitamins or trace elements did not affect synthesis of the pNPA esterase system. Addition of amino acids to the culture medium increased pNPA esterase activity of the strain. Addition of NaCl had a distinct positive effect on synthesis of this

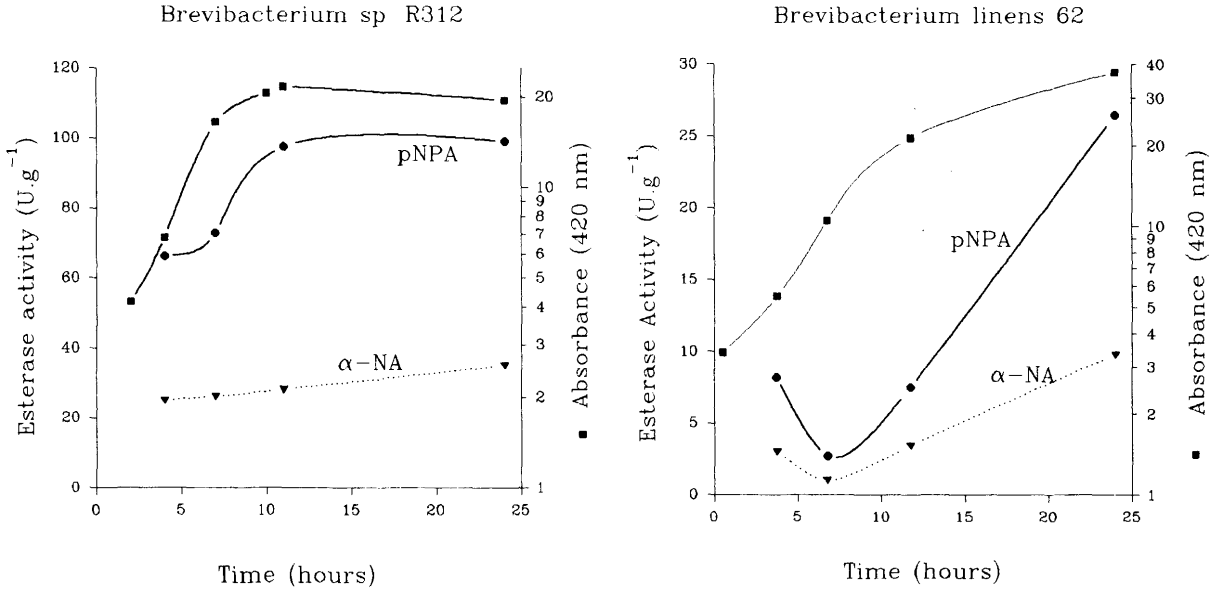

Fig. 1. Biosynthesis of Esterase Activity of *Brevibacterium* sp. R312 and *Brevibacterium linens* 62 in TSB Medium in the Presence of α-NA (▼) and pNPA (●).

Activities (µmol $\cdot min^{-1} \cdot$ (g cell protein)$^{-1}$) were measured in the crushing supernatant.

Table I. Specific *p*-Nitrophenyl Acetate (pNPA) Esterase and α-Naphthyl Acetate (α-NA) Esterase Activity ($U \cdot g^{-1}$) of *Brevibacterium* sp. R312 and *Brevibacterium linens* 62 on Different Culture Media

	Culture media						
	LP	LP1	LP2	LP+AA	LP3	LP4	TSB
Brevibacterium sp. R312							
pNPA	53	56	53	73	61	110	78
α-NA	55	29	30	18	15	24	21
Brevibacterium linens 62							
pNPA				28	33	26	16
α-NA				4.6	8.3	4.3	4.2

LP1, LP+Vit; LP2, LP+Vit+TE; LP3, LP+AA+Vit+TE, LP4, LP+AA+Vit+TE+NaCl.

No growth of *Brevibacterium linens* 62 was observed on medium without amino acids.

esterase system. The pNPA esterase activity obtained after growth on (LP+Vit+TE+AA+NaCl) medium was comparable to that achieved on TSB complex medium.

Maximum α-NA esterase activity appeared after growth of *Brevibacterium* sp. R312 on LP medium ($55\ U \cdot g^{-1}$). The various batches of substances tested decreased the activity by a factor of 2 to 3.

Brevibacterium linens 62. The minimum synthetic medium for this strain consisted of (LP+AA) medium because amino acids were essential for growth.

pNPA esterase activity was about $29\ U \cdot g^{-1}$ after growth on synthetic medium. On TSB complex medium, pNPA activity was half as strong.

Addition of vitamins and trace elements to (LP+AA) medium increased synthesis of α-NA esterase activity by a factor of about 2. Addition of NaCl to the (LP+AA)+Vit+TE medium decreased synthesis of this activity.

Electrophoretic pattern. No differences between the rela-

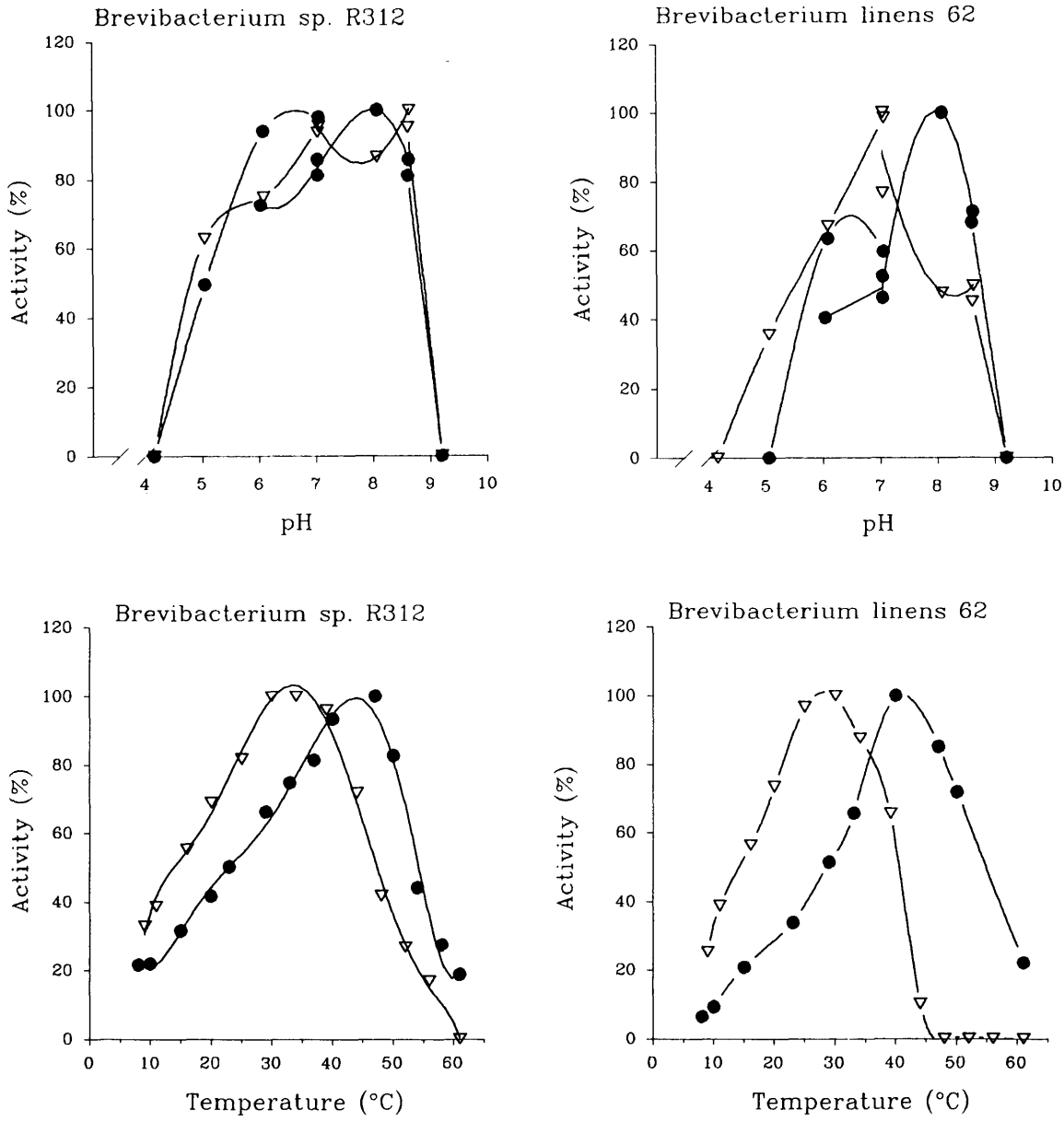

Fig. 2. Influence of the pH and Temperature on Esterase Activity Measured in the Presence of pNPA (●) and α-NA (▼) of *Brevibacterium* sp. R312 and *Brevibacterium linens* 62.

The following buffers were used to study esterase activity at different pHs: 50 mM citrate–phosphate buffer from pH 4.0 to 7, 50 mM phosphate buffer from pH 6 to 8, 50 mM Tris–maleate buffer from pH 7 to 8.5 and 50 mM glycine–NaOH buffer from pH 8.5 to 9.0.

tive proportions of the seven bands of esterase activity of *Brevibacterium* sp. R312 were found, whatever the culture medium. In the case of *Brevibacterium linens* 62, three or four new bands of esterase activity different to those obtained after growth on TSB medium were observed.

Influence of the growth substrate on synthesis of the esterase system of the two strains

Brevibacterium sp. R312 was grown on LP medium and *Brevibacterium linens* 62 on synthetic (LP + Vit + TE + AA + NaCl) medium, the composition of which similar to that of complex TSB medium. The five following carbon substrates were tested: glucose ($10\,g\cdot l^{-1}$), ethyl acetate ($5\,g\cdot l^{-1}$), Tween 80 ($3\,g\cdot l^{-1}$), tributyrate ($2\,g\cdot l^{-1}$) + Tween 40 ($1\,g\cdot l^{-1}$), colza oil ($2\,g\cdot l^{-1}$) + Tween 40 ($1\,g\cdot l^{-1}$).

Brevibacterium sp. R312. Esterase activities are comparable after growth in the presence of tributyrate, colza, oil or glucose (approximately $55\,U\cdot g^{-1}$). Ethylacetate and Tween 80 increased synthesis of pNPA esterase by a factor of 3 and the α-NA system by a factor respectively of 2.5 and 8.

Brevibacterium linens 62. Ethylacetate, tributyrate, and

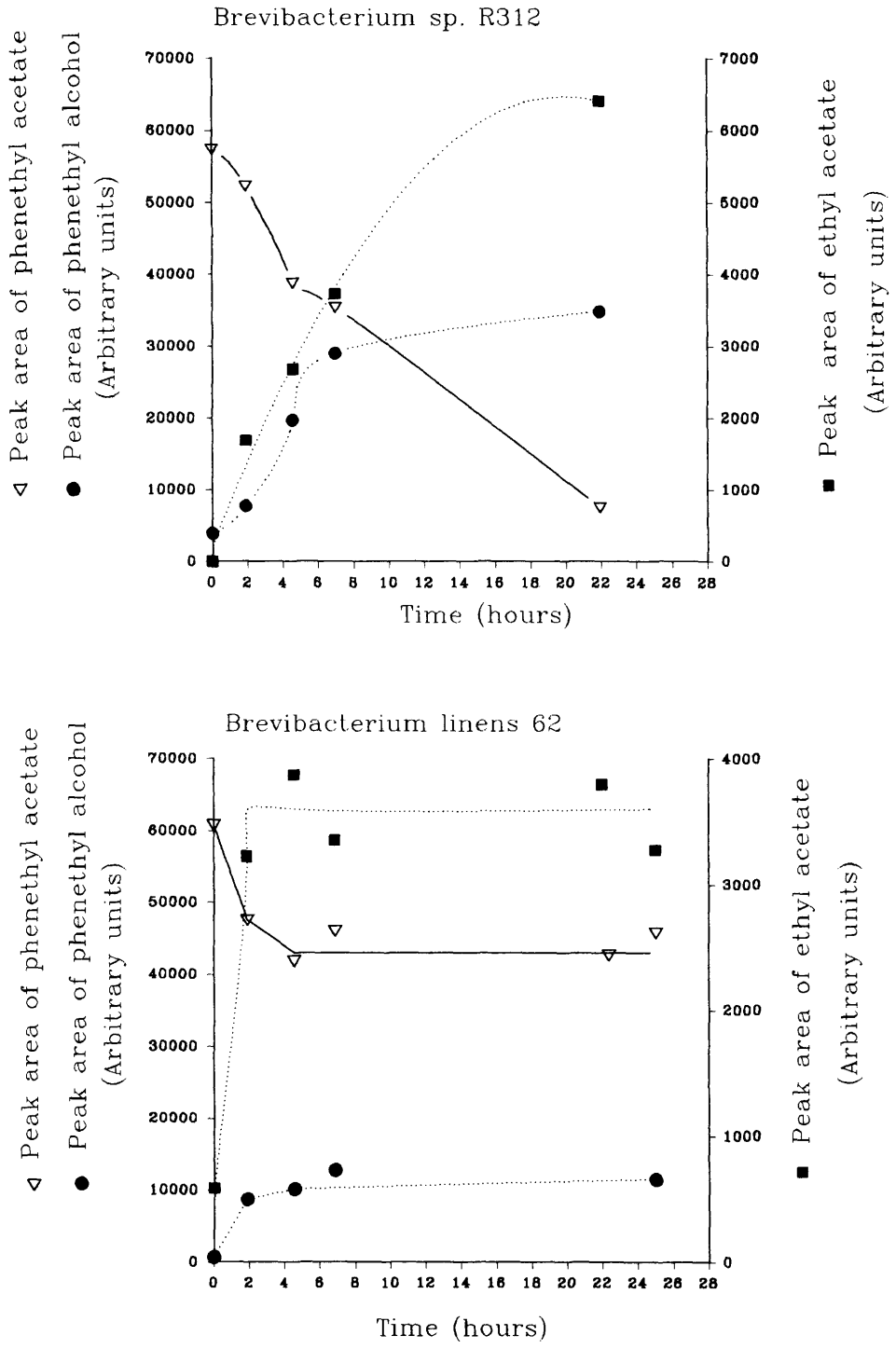

Fig. 3. Kinetics of Hydrolysis of Phenethyl Acetate and of the Appearance of Phenethyl Alcohol and Ethylacetate by Crushing Supernatant of *Brevibacterium* sp. R312 and *Brevibacterium linens* 62.

Reaction conditions were 10 mM phenethyl acetate and 1 M ethanol.

Tween 80 induced the esterase systems. pNPA and α-NA esterase activities increased by factors of 6 and 4 respectively in comparison with the activities obtained in the presence of glucose ($26\,U \cdot g^{-1}$ and $4\,U \cdot g^{-1}$, respectively). Induction of ethylacetate was more marked during the stationary phase: pNPA activity increased some 15-fold and α-NA activity increased about 12-fold.

Electrophoretic pattern. After growth in presence of ethylacetate or Tween 80, the relative proportions of the different bands of esterase activity of *Brevibacterium* sp. R312 were modified. The esterase bands of R_f 0.89 and 0.94 were more obvious when the cells were grown in the presence of ethylacetate or Tween 80, respectively. Electrophoretic patterns were identical for *Brevibacterium linens* 62 whatever the growth substrate.

Influence of pH and temperature on esterase activities

The nature of the buffer did not affect enzyme activity. The esterase activities of *Brevibacterium linens* 62 were more sensitive (Fig. 2) to pH variations than those of *Brevibacterium* sp. R312. pNPA and α-NA esterase activities of *Brevibacterium* sp. R312 were at maximum at pHs

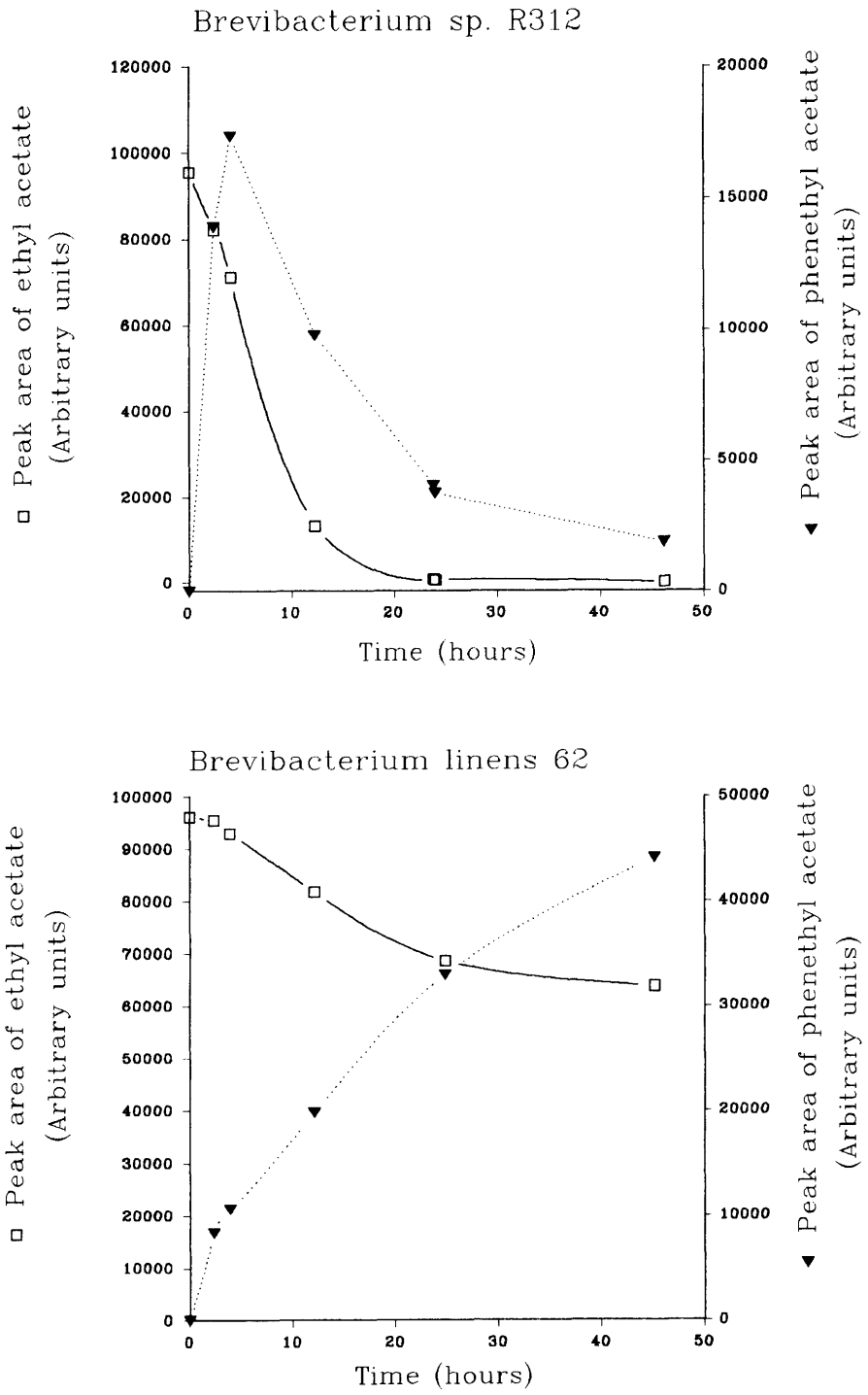

Fig. 4. Kinetics of Hydrolysis of Ethylacetate and Appearance of Phenethyl Acetate by Crushing Supernatant of *Brevibacterium* sp. R312 and *Brevibacterium linens* 62.

Reactions conditions were 10 mM ethylacetate and 1 M phenethyl alcohol in emulsion with polyvinylic alcohol 2% (P/V).

between 6 and 8 and between 7 and 8.6 respectively. Those of *Brevibacterium linens* 62 were maximum at pH 8 and pH 7, respectively.

Esterase activities measured at pH 7 in the presence of pNPA and α-NA peaked at 47°C and 33°C respectively for *Brevibacterium* sp. R312 and at 40°C and 29°C respectively for *Brevibacterium linens* 62 (Fig. 2). The optimal temperature for assaying esterase activity with pNPA was higher than that with α-NA.

Assays of hydrolysis and synthesis of esters

Ethyl and methyl esters, methanethiol, and its esters and phenethyl alcohol and its esters contribute to the formation of food flavors.[19-21]

Hydrolysis of methylthioacetate and phenethyl acetate. Specific hydrolysis methylthioacetate by *Brevibacterium* sp. R312 ($2\,U\cdot g^{-1}$) was five times as great as that by *Brevibacterium linens* 62.

Hydrolysis of phenethyl acetate was two to three times as rapid by *Brevibacterium* sp. R312 ($9.2\,U\cdot g^{-1}$) as by *Brevibacterium linens* 62. For *Brevibacterium* sp. R312, the rate of hydrolysis increased with the initial phenethyl acetate concentration ($9.2\,U\cdot g^{-1}$ in the presence of 10 mM phenethyl acetate against $16.5\,U\cdot g^{-1}$ for 50 mM). The rate of hydrolysis was steady for *Brevibacterium linens* 62 ($4.3\,U\cdot g^{-1}$) for both the phenethyl acetate concentrations tested. This seems to indicate that the esterase system of *Brevibacterium* sp. R312 has less affinity for phenethyl acetate than that of *Brevibacterium linens* 62.

Synthesis reactions. Synthesis of esters consists of the formation of an ester from acid and alcohol. Different reaction conditions favorable for the synthesis reaction were tested: high acid concentrations (10 to 500 mM) and/or high alcohol concentrations (10 to 1000 mM), or addition of glycerol ($3.32\,g\cdot l^{-1}$) to the reaction medium to lower water activity to 0.94.[22] Different pHs (7, 6.6, 5.9, 4.8, and 4) were also tested using either acetic acid or sodium acetate.

Two synthesis reactions were studied: the formation of phenethyl acetate from phenethyl alcohol and acetic acid and synthesis of ethylacetate from acetic acid and ethanol. No synthesis by the enzymatic systems of crushing supernatants of the two strains was observed after 24 h of reaction under any of the reaction conditions tested for both synthesis reactions studied.

Transesterification reaction. The transfer reaction is expressed by:

$$R_1\text{–}\underset{\underset{O}{\|}}{C}\text{–}OR_2 + R_3\text{–}OH \longrightarrow R_1\text{–}\underset{\underset{O}{\|}}{C}\text{–}OR_3 + R_2\text{–}OH$$

– Reaction in the presence of phenethyl acetate (10 mM) and ethanol (1 M). In the case of *Brevibacterium* sp. R312 (Fig. 3), 90% of the phenethyl acetate disappeared and phenethyl alcohol and ethylacetate were formed. With *Brevibacterium linens* 62, equilibrium was reached after 4 h of reaction and only 30% of the phenethyl acetate was hydrolyzed.

– Reaction in the presence of ethylacetate (10 mM) and phenethyl alcohol (1 M). With *Brevibacterium* sp. R312 (Fig. 4), ethylacetate disappeared totally after 16 h of enzymatic reaction. Phenethyl acetate was formed during the first 4 h of the reaction and was then hydrolyzed.

With *Brevibacterium linens* 62, hydrolysis of ethylacetate and simultaneous appearance of phenetylacetate were observed. No hydrolysis of the ester formed was detected. Equilibrium was reached after two days of reaction.

– Reaction in the presence of ethylbutyrate (20 mM) and methanol (1 M). Total, rapid disappearance of ethylbutyrate was observed with *Brevibacterium* sp. R312 (Fig. 5). Appearance of methylbutyrate was observed during the first 40 min followed by hydrolysis of this ester.

With *Brevibacterium linens* 62, total hydrolysis of the ethylbutyrate was observed after 18 h of enzymatic reaction, with simultaneous appearance of methylbutyrate. No hydrolysis of this ester was observed.

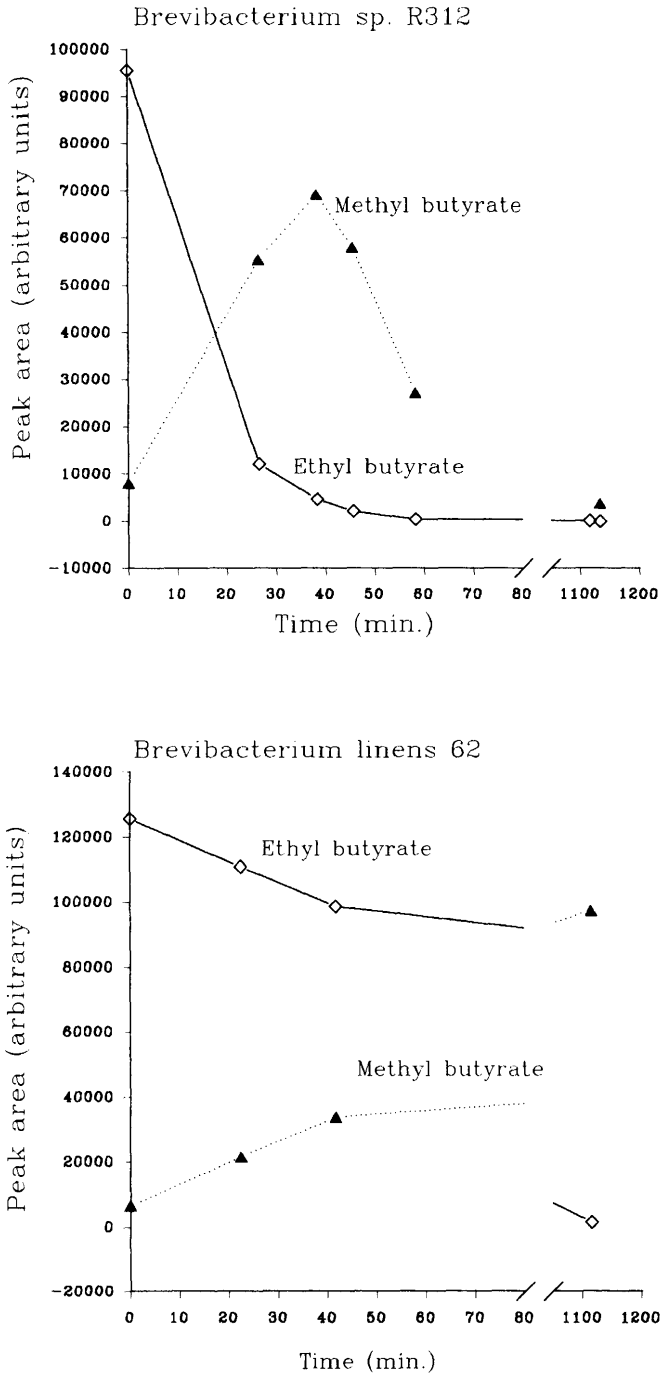

Fig. 5. Hydrolysis Kinetics of Ethylbutyrate and Appearance of Methylbutyrate by Crushing Supernatant of *Brevibacterium* sp. R312 and of *Brevibacterium linens* 62.

Reaction conditions were 20 mM ethylbutyrate and 1 M methanol.

Discussion

Esterase systems have already been described in many bacterial species.[9,23] Only a few studies on esterases of *Brevibacterium* species can be found in literature.[12,13,24] Esterase activities were detected both in intracellular fractions[25] and with whole-cell preparations.[13]

The electrophoretic patterns showed the complexity of the esterase system of *Brevibacterium linens* 62 and *Brevibacterium* sp. R312 as has frequently been observed in many other species and *Brevibacterium linens* strains.[24]

Specific esterase activities of the two strains studied were maximum during the stationary growth phase. A similar observation was reported for the esterase pNPA activity of *Lactobacillus helveticus* CNRZ 32.[26]

Comparison of specific esterase activities of *Brevibacterium* sp. R312 and *Brevibacterium linens* 62 with those of other strains is difficult because of the variability of assay conditions, substrates, and enzyme units. Hydrolysis specificity according to the length of the fatty acid carbon chain varies considerably from one strain to another. In addition, the esterase system of a strain frequently consists of several esterases with different specificities.[9,27,28] In the case of *Brevibacterium linens* 62 and *Brevibacterium* sp. R312, it is probable that the esterase activity assayed in the presence of pNPA and that assayed in the presence of α-NA concern different esterases. Their biosynthesis and optimum pH and temperature are different.

Few studies have been done on the influence of the culture medium or growth substrate on synthesis of the esterase system.[29,30] The influence of the culture medium composition on the synthesis of esterase systems was different for the two strains studied. For *Brevibacterium* sp. R312, the composition of the culture medium mainly affected overall synthesis of the esterase system of the strain. In contrast, for *Brevibacterium linens* 62, culture medium composition affected the number of enzymes forming the esterase system of the strain.

Induction of both esterase systems in the presence of substrates such as ethylacetate and Tween 80 was demonstrated in both strains. Tween 80 is in fact an ester including a fatty acid with 18 carbon atoms. Weaker induction in the presence of tributyrate + Tween 40 was also shown. The type of carbon substrate probably has a greater or lesser effect on one or the other of the esterases making up the esterase system of the strain. The electrophoretic patterns of the strain *Brevibacterium* sp. R312 confirmed this hypothesis.

The production of ethyl esters by esterases of lactic bacteria has been shown by Hosono *et al.*[31] Likewise, Cuer *et al.*[32] showed the ability of *Brevibacterium linens* to produce *S*-methylthioacetate. A transferase activity has been shown here for the two strains studied. However, two types of reaction were observed:

– disappearance of ester A and simultaneous formation of ester B. This phenomenon was observed in both strains;

– disappearance of ester A and formation of ester B followed by hydrolysis of the latter. This pattern was observed for *Brevibacterium* sp. R312 in the presence of ethylacetate or ethylbutyrate.

Rates of transfer of the various esters were similar for both strains (Table II). Activities measured in the presence of ethylbutyrate and methylbutyrate were 20 to 100-fold greater than for the other esters. It is possible that the transfer rate may have been underestimated when the transfer reaction was followed by hydrolysis because the formation and hydrolysis of the ester may be simultaneous.

Table II. Comparison of Hydrolysis and Transfer Reactions for *Brevibacterium* sp. R312 and *Brevibacterium linens* 62. Activities are Expressed in $\mu mol \cdot min^{-1} \cdot g^{-1}$

	B. sp. R312	*B. linens* 62
Phenethyl acetate (10 mM) + ethanol (1 M)		
Disappearance of phenethyl acetate	3.5	1
Formation of ethylacetate	1.9	1.2
Hydrolysis of ethylacetate	0	0
Ethylacetate (10 mM) + phenethyl alcohol (1 M)		
Disappearance of ethylacetate	13	6
Formation of phenethyl acetate	1.9	1.3
Hydrolysis of phenetyl acetate	0.3	0
Ethylbutyrate (20 mM) + methanol (1 M)		
Disappearance of ethylbutyrate	316	153
Formation of methylbutyrate	186	195
Hydrolysis of methylbutyrate	245	0

It is nevertheless difficult to find where the transfer reactions and hydrolysis are caused by the same enzyme systems. The enzyme extract used contained most of the soluble enzymes in the cells. More detailed investigation of transesterification reactions (assay conditions, substrate concentrations) should make it possible to shift reaction equilibria towards the hydrolysis reaction or the transfer reaction.

More detailed study of the different esterases making up the esterase system would be interesting for better characterization of the esterase activities of coryneform bacteria and for identification of their influence on the development of certain aromas.

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