Molecular analysis of an esterase-encoding gene from a lipolytic psychrotrophic pseudomonad

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An esterase gene (estA) from a lipolytic psychrotroph (Pseudomonas sp. LS107d2), was cloned in Escherichia coli and its nucleotide sequence was determined, revealing an ORF encoding a polypeptide of 389 amino acid residues, with a molecular mass of 42276 Da. Labelling of plasmid-encoded proteins with [35S]methionine, using the maxicell procedure, gave a single polypeptide of molecular mass 42 kDa, consistent with that calculated from the ORF. Colonies of E. coli cells containing estA produced a clear halo when grown on solid media containing tributyrin; no clearance was produced when cells were grown on media containing triolein. Extracts of cells containing estA also hydrolysed water-soluble nitrophenol esters, but were unable to cleave water-insoluble substrates. The preference for water-soluble substrates indicates that the gene product is an esterase.

Introduction

The significance of psychrotrophic organisms in the spoilage of milk is widely recognized (Cousin, 1989). Milk spoilage can occur because of the accumulation of free fatty acids produced from the hydrolysis of triacylglycerides by lipases. Organisms of the genus Pseudomonas constitute the most common psychrotroph isolated from milk (Muir et al., 1979; Cousin, 1982; Deeth & Fitzgerald, 1983). In particular, psychrotrophic pseudomonads dominate the lipolytic microflora of raw bovine milk samples which show lipolytic defects, and caprine milk samples which have been refrigerated for several days (Shelley et al., 1986; Cox & MacRae, 1989). Pseudomonas fluorescens and Pseudomonas fragi have been identified as the most predominant lipolytic psychrotrophic species; the relative importance of these two species in lipolytic spoilage may depend on other factors such as initial cell numbers, growth rates and, in regard to products derived from heat-treated milk, the thermostability of relevant enzymes (Griffiths, 1981; Shelley et al., 1986; Cox & MacRae, 1989). Lipolytic spoilage is mainly due to the production of even-carbon-number short-to-medium chain (C4 to C12) fatty acids from triacylglycerides (Cousin, 1989). Because nearly all triacylglycerides are completely insoluble in water, the enzymes responsible for initial hydrolysis are, by definition, lipases since they act at an oil-water interface. It is unclear, however, whether esterases, which act only on soluble substrates, also play a part in lipolytic spoilage by hydrolysing soluble mono- and diacylglycerides. As part of a study on the individual enzymes involved in lipolytic spoilage, and the genes which encode them, we have initiated a molecular genetic analysis of strains of psychrotrophic lipolytic pseudomonads isolated from caprine milk. We describe the isolation and characterization of a gene which encodes an esterase active on tributyrin, and on artificial substrates (nitrophenol esters) with acyl chain lengths up to C10:

Methods

Bacterial strains, plasmids and media. The Pseudomonas strain used in this study, LS107d2, was one of a number of strains isolated in a survey of proteolytic and lipolytic psychrotrophs in caprine milk (Cox & MacRae, 1989). It derives from phenon G of this study, and resembles...
*Pseudomonas fluorescens*. *E. coli* strain LE392 (supE44 supF supR58 hsdR514 galK2 galT22 metB1 trpR35 lacY1, Sambrook et al., 1989) was the host strain used for cosmid bank construction and TG1 (supE hsdS trbC6) cosmid library constructions. pHP34 (Prentki & Krisch, 1982) and pSK (BLUESCRIPT M13+) were employed as cloning vectors. For general strain maintenance and recombinant DNA manipulation, LB medium (Sambrook et al., 1989) was used, either as broth, or solidified with 1.5% (w/v) bacteriological agar. Butterfat agar (BFA), containing 0.1% butterfat, was made according to Shelley et al. (1987). Tributylin agar (TBA) and triolein agar (TOA) were made in the same way as BFA, except that 1% (v/v) tributyrin or 0.1% triolein were added in place of butterfat. Where appropriate, ampicillin and tetracycline were added to final concentrations of 50 μg ml⁻¹ and 20 μg ml⁻¹, respectively.

**Cosmid library construction and screening.** Chromosomal DNA was isolated from LS107d2 and purified on a caesium chloride gradient as described by Sambrook et al. (1989). The DNA was partially digested with Sau3A to yield high-molecular-mass fragments, and then ligated with BamHI-digested pHC79 without prior fractionation. The ligation mix was packaged, using 'Packagene' extract (Promega), transfected into LE392, and plated onto LB plates containing ampicillin. To screen for hydrolytic activity, individual recombinants were patched onto BFA or TBA plates and incubated at 30 °C for 5–10 d; hydrolytic activity is indicated by zones of clearing around the colonies.

**Recombinant DNA techniques.** Unless described in detail, all recombinant DNA methods were essentially as described by Sambrook et al. (1989).

**Creating overlapping unidirectional deletions using exonuclease III and mung bean nuclease.** The method was essentially as described by Henikoff (1984). The fragment to be analysed was cloned into pSK and plasmid DNA was prepared. The plasmid DNA was digested with two enzymes, one leaving a 3' single stranded overhang and the other a blunt end or 5' overhang. The digested DNA was then incubated at 37 °C in a reaction mixture containing (per time point) 1 μg DNA, 2·5 μl 2·0 exonuclease III buffer (100 mM-Tris/HCl, pH 8·0, 10 mM-MgCl₂, 20 μg ml⁻¹ tRNA), 0·5 μl β-mercaptoethanol, 0·2 μl exonuclease III (Promega, 25 units μl⁻¹), and a final volume of 5 μl. Reaction mixture (5 μl) was removed at 60 s intervals to tubes containing 4 μl of 10× buffer (300 mM-NaOAc, pH 5·0, 500 mM-NaCl, 10 mM-ZnCl₂, 50% (v/v) glycerol) and 31 μl sterile water, and placed in liquid nitrogen. The exonuclease III was then inactivated by incubation at 68 °C for 15 min, 1 μl of mung bean nuclease (Promega, 3 units μl⁻¹) was added and the mixture was incubated at 30 °C for 30 min. After the incubation, 1·5 μl 10% (w/v) SDS, 2 μl 1 M-Tris/HCl pH 8·6, 4 μl 8 M-LiCl and 50 μl phenol/chloroform (saturated with 10 mM-Tris/1 mM EDTA, pH 8·0) were added, the mixture vortexed, and then centrifuged for 3 min. The aqueous phase was extracted once with chloroform, and then ethanoll-precipitated, washed, dried and resuspended in sterile distilled water. The deleted DNA samples were then ready for analysis on agarose gels, prior to ligation. The extent of deletion was determined by nucleotide sequence analysis.

**Nucleotide sequence analysis.** Suitable fragments were either subcloned into M13mp18 or M13mp19 (Messing, 1983) or sequenced directly from recombinant plasmids using supercoiled plasmid templates. Supercoiled template preparation was essentially as described by Kraft et al. (1988), and templates were denatured as described by Lim & Pen (1988). Nucleotide sequence analysis was performed by the dye-terminator chain termination method using a modified T7 DNA polymerase (Tarbor & Richardson, 1987). Sequencing reactions were electrophoresed on 6% (w/v) denaturing polyacrylamide gels, which were cast as wedge gels with a thickness gradient of 0·2 mm (top) to 0·6 mm (bottom) (Olson et al., 1984).

**Detection of plasmid encoded protein products by maxicell analysis.** Proteins encoded by cloned DNA fragments were specifically labelled with [³⁵S]methionine using 'maxicells', and radiolabelled proteins were analysed by denaturing PAGE (Calhoun & Grey, 1981).

**Preparation of cell-free extracts and enzyme assay.** Cells containing the appropriate plasmid were grown to stationary phase in LB medium (typically 500 ml) containing ampicillin. Following growth, cells were harvested by centrifuging at 6000 g for 10 min and resuspending the pellet in 200 ml 50 mM-Tris/HCl buffer, pH 8·0. The cells were again collected by centrifugation at 6000 g for 10 min and the pellet was resuspended in a small volume (5–7 ml) of ice-cold 50 mM-Tris/HCl buffer pH 8·0. Each sample of washed cells was then disrupted by ultrasonication on ice, and the sonicates were centrifuged at 4 °C for 20 min at 10000 g. The supernatants were assayed for enzyme activity using a method based on that of Winkler & Stuckmann (1979). Assays were carried out by adding 100 μl cell-free extract, 25 μl substrate solution (9 mM in isopropanol) and 975 μl assay buffer (50 mM-NaHPO₄, 5·75 mM-sodium deoxycholate and 0·11% gum arabic, pH 8·0) prewarmed to 37 °C. Rates of hydrolysis of substrates by cell-free extracts were measured on an Hitachi recording spectrophotometer at 37 °C. In all cases, rates of hydrolysis of substrates by the control extracts were subtracted from sample rates; p-nitrophenyl-butyrate and p-nitrophenylacetate were found to spontaneously hydrolyse at a significant rate in aqueous solution (0·005 nmol min⁻¹). Specific activity was expressed as nmol substrate hydrolysed per minute per mg of protein present in the cell-free extract. Protein was estimated using a bicinchoninic assay reagent kit (Pierce).

**Results and Discussion**

**Isolation of the estA gene**

DNA from LS107d2 was used to construct a cosmid library, from which 2000 colonies were screened on BFA and TBA plates. After 10 d incubation, nine positive colonies were detected on TBA and none on BFA. Cosmid DNA was isolated from the most active TBA clone, pLGB1, for further analysis. The cosmid was subcloned by partial digestion with Sau3A followed by BamHI-digested pH34. Recombinants were then patched on media containing TBA to detect active subclones. Two positive subclones, pLGB11 and pLGB12 (see Fig. 1a), were selected for further
estA gene from a lipolytic Pseudomonad

(a) BamHI-digested pHCl9

Sau3A partial digest of
LS107d2

Ligate
Package
Transduction into
LE392

Screening resulted in 9 positive clones.
One of these, pLGB1, was subcloned:

BamHI-digested pH34

Ligate
Transform
Screen on TBA

Two positive clones, pLGB11 and
pLGB12, chosen for analysis.

(b)

Plasmid Phenotype on TBA

pLGBd  ++
pLGBe  +
pLGBe274  +
pLGBe271  +
pLGBe120  −
pLGBeCA  −
pLGBeSS  −
704  

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1  GTAAAGCCACCTGCGGCCTGGGACCAACTGGGCAAGGCGAGGCGAAAGCCAAAGGCCGCAAGGCCTGTCG

2  CGTGAAGGTTAGGGCTGAAGGCGGTAAGTCCTGGGCTAGGCGGAAAAGCCCGAGCGGCGGCGGGATGGCGTG

3  SD

143  GCACCTGCCAAGCTGCTCTGATGCTCGGGCTGACGGCAGCTGGTACAAACCTGCAAGGGCTGGCAACAGCTG

214  CCACTTCTACATGGTCTTTCTCGAGCGCTACAGCTGAGGTGATGATGATGACGATCATTACATGCTGG

16  Met Val Phe Glu Ala Ala Tyr Val Gin Gin Gly His Tyr

AsuI

268  GAA CTG CAG TTT GAA GCG ATG GTA GAA GCG TTT GCC GCC GCG TTT GAT GAT GAC CC

16  Glu Leu Gln Phe Glu Ala Ile Arg Glu Ala Ala Leu Phe Asp Asp Pro

322  CAG GAG GTT GGG GCC GGG CTT TTC GAG GCG GCT TAC GTG CAG ATT CAG GGT CAT TAC

1b Met Phe Phe Glu Ala Ala Tyr Val Gln Ile Gln Gly His Tyr

AsulI

413  GCACTCCGCCAACGGTCATTGAGATCGCTGACTTTCCACCTACAGTCAACATGTC

214  CCACTTTCA ATG GTG TTT TTC GAG GCG GCT TAC GTG CAG ATT CAG GGT CAT TAC

1b Met Phe Phe Glu Ala Ala Tyr Val Gln Ile Gln Gly His Tyr

AsulI

506  GAA CTC CAG TTC GAA GCG ATG GTA GAA GCG TTT GCC GCC GCG TTT GAT GAT GAC CC

16  Glu Leu Gln Phe Glu Ala Ile Arg Glu Ala Ala Leu Phe Asp Asp Pro

560  CGG GAG TTC GAA GCG ATA CGT GAA GCG TTT GCC GCG CTG TTC GAT GAC CCA

16b Glu Leu Ala Glu Gln Phe Glu Ala Ile Arg Glu Ala Phe Phe Asp Asp Pro

646  TGG GAA CTC CAG TTC GAA GCG ATG GTA GAA GCG TTT GCC GCC GCG TTT GAT GAT GAC CC

16  Glu Leu Gln Phe Glu Ala Ile Arg Glu Ala Ala Leu Phe Asp Asp Pro

730  CTG TGG GCC GGC ACC GCT GAC ATG CTG CCG GCC GAA GCA CCC TGG TGG ACG CCG GGC

178  Arg Arg Ala Asp Gly Arg Gly Pro Gly Glu Ser Ile Val Ala Arg Val Leu Cys

824  CAG GCG GGT TTG CCG GCG ATC CGC GAG ATG CTG CCC ACC GAG GCC CTG TAT GAC

124  Gln Ala Gly Leu Pro Ala Ile Arg Glu Met Pro Thr Glu Ala Leu Tyr

SacI

918  TGG GCG GCT TTT GCC GCC GCG GGT AAG GCC ATC ACG CTG CGC CAG TTG CTC TGC CAT

16  Leu Val Ala Glu Gly Leu Leu Leu Asp Pro Val Val Lys Tyr Thr Pro

1012 GAA TTT GCC GCT GCC GGT AAG GCC ATC ACG CTG CGC CAG TTG CTC TGC CAT

16  Glu Leu Ala Glu Gly Leu Leu Leu Asp Pro Val Val Lys Tyr Thr Pro

1106 CCG GCC GGG GGG CTT TTC TCC TGC ACC AAG ACA TTT ACT GCC GTC ACG GCC CTG CAA

34b Gln Glu Arg Arg Gly Ala Thr Leu Lys Thr Phe Thr Ala Val Val Gly

1200 ATC GTC AAT CTT TTC TCC TGC ACC AAG ACA TTT ACT GCC GTC ACG GCC CTG CAA

7ob Ile Val Asn Leu Ser Pro Thr Val Phe Ser Cys Thr Lys Thr Leu Val Val Ala

SacI

1304 TTG GCG GCT TTT GCC GCC GCG GGT AAG GCC ATC ACG CTG CGC CAG TTG CTC TGC CAT

16  Leu Val Ala Glu Gly Leu Leu Leu Asp Pro Val Val Lys Tyr Thr Pro

1408 CAA GCC CAT GGT TAC GAG GCG ATC ACC TAC GCC TGG CTG GTC GCC GAA CTG CTG

16  Gin Gly Glu Phe Glu Ala Glu Ile Thr Thr Thr Gln Thr Ala Val Leu

1492 GCC CCG CCG GAT GCC CCG GCT AAT GGT CAC GGT AAT GCG CGT AGC CTG GCG GGT TTT TAT AGT GGT

214  Pro Leu Gln Leu Thr Leu Thr Leu Pro Thr Glu Ala Tyr Ser

SacI

1586 CCT TGG GCC CTG GAC TTT CAT GTG GCC CTG GCG GCC GGT AAG GCA CCC TGG TGG ACG CCG GCC

16  Glu Leu Ala Glu Gly Leu Leu Leu Asp Pro Val Val Lys Tyr Thr Pro

1680 CGG CCG GGG GGG CTT TTC TCC TGC ACC AAG ACA TTT ACT GCC GTC ACG GCC CTG CAA

34b Gln Glu Arg Arg Gly Ala Thr Leu Lys Thr Phe Thr Ala Val Val Gly

1774 ATC GTC AAT CTT TTC TCC TGC ACC AAG ACA TTT ACT GCC GTC ACG GCC CTG CAA

7ob Ile Val Asn Leu Ser Pro Thr Val Phe Ser Cys Thr Lys Thr Leu Val Val Ala

SacI

1868 TTG GCC TCT ATG GTA ATG ATG CGT GAA CCC ACC GCC ATG ACC ACA CGG GCA TTT GCC AAT

232  Leu Glu Val Met Arg Glu Thr Ala Met Thr Thr Arg Ala Phe Asn

SacII

1962 GAA TTT GCC GCT GCC GGT AAG GCC ATC ACG CTG CGC CAG TTG CTC TGC CAT

16  Leu Val Ala Glu Gly Leu Leu Leu Asp Pro Val Val Lys Tyr Thr Pro

2056 GCC CAT GAT GCC CGC AGC AAA GCC AAT ATG GCC GAT GAA GCT GCG CAA CTG TTA

214  Ala His Ala Arg Ser Lys Gin Met Asp Glu Ala Glu Gin Arg Leu

2150 CTG CAA GAA ATG AGT GCT GAA CCA ACC GCC ATG ACC ACA CGG GCA TTT GCC AAT

232  Leu Glu Val Met Arg Glu Thr Ala Met Thr Thr Arg Ala Phe Asn

SacII

2244 CCA CGG TCT ATT TGG ACC AGT ACT AAT AAA CCT GAA TGG CGA CCC ATG CAG CAG

250  Pro Pro Ser Ile Thr Leu Thr Leu Thr Thr Thr Leu Ala Ala Ala Leu

SacII

2338 CCC CGG GCT AAT GGT CAC GCT GAT GCC GGT TTT TAT AGT GGT

268  Pro Ala Ala Asn Gly His Gly Asn Ala Arg Ser Leu Ala Ala Tyr Ser Gly

2432 TGG TTT GAC GGT ATG TTT GAA GCC GAC ATG CTA GAA GAG TGG ACC GGT

286  Leu Leu Asp Gly Ser Leu Leu Glu Ala Asp Met Leu Leu Gly Thr Arg Gly

2526 CAC ATG ATC GGG CGG GAT AAA ACA TTA TTG ACA ACA ACT CGG TTT GCC TGG GCC

304  His Ser Ile Gly Pro Asp Lys Thr Leu Leu Thr Arg Phe Gly Leu Gly

2620 TGC ATG TTC GAT CCG CAG ATG CCT CGT GCC CTT GCC CCC CCG CTG

322  Cys Met Leu Asp Gin Pro Gin Met Pro Asn Ala Thr Phe Gly Leu Gly Pro Arg

2714 GCT TTC GGG CAT CCT GGT CCA GGT TGA TTT GCC GCC CCC GAA CAC

340  Ala Phe Gly His Pro Gly Ala Gly Gly Ser Val Phe Ala Asp Pro Gly His
**estA gene from a lipolytic Pseudomonad**

The nucleotide sequence of the **estA** gene and its flanking regions was determined entirely on both strands using templates generated by one of three methods: (1) When sequencing regions were not contained within pLGBe or pLGBd, fragments from pLGB11 were cloned into M13 vectors; (2) pLGBd and pLGBe were digested with restriction enzymes and self-ligated; or (3) overlapping deletions of pLGBe were created using exonuclease III and mungbean nuclease.

The nucleotide sequence of the **estA** coding region is shown in Fig. 2. An ORF encoding a polypeptide of 389 amino acid residues with a calculated molecular mass of 42276 Da initiates with a methionine codon at nucleotide position 224, and terminates with a TAA codon at nucleotide position 1393. A notably weak Shine–Dalgarno (SD) sequence, comprising only two bases, is present, appropriately spaced, 9 bp upstream from the initiating methionine residue (see Gold et al., 1981). Codon usage analysis of **estA** (data not shown) reveals a preference for synonymous codons with C or G residues in the third position, which is particularly evident in the case of codons for alan, leu and cys. This is consistent with the high genomic G+C content in pseudomonads (West & Iglewski, 1988). Comparison of **estA** with sequences in GenBank did not reveal any significant similarities at the nucleotide or amino acid levels. Also, no significant similarity is evident between the deduced amino acid sequence of the **estA** gene product and that of an esterase from an isolate of *Pseudomonas fluorescens* (Choi et al., 1990).

**Definition of the coding region of the estA gene**

Because of the convenient orientation of the polylinker in pLGBe, deletions of this plasmid (initially constructed for sequencing) were used to define the coding region of the **estA** gene. TG1 was transformed with either pLGBe or deletions of pLGBe, plated onto TBA and incubated at 30 °C for 4–8 d. Activity was scored as a clear halo around individual colonies. The results of these deletions on activity are presented in Fig. 1(b). Deletions outside the coding region near the 3' end of the ORF (pLGBe274 and pLGBe271) do not result in any loss of activity compared to the undeleted plasmid (pLGBe). Cells containing pLGBe230 are **EstA**-, as are cells containing pLGBeSS, formed by deleting from the SacII site in the ORF to the SacII site in the polylinker. Similarly, a deletion in the 5' region of the ORF, from *AsuII* to the ClaI site in the polylinker (to produce pLGBeCA), also results in a total loss of activity. These deletions are

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The nucleotide sequence of the **estA** coding region is shown in Fig. 2. Nucleotide and derived amino acid sequences are numbered on the left. The proposed Shine–Dalgarno sequence (SD) is underlined. Relevant restriction sites are marked above the sequence.

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therefore consistent with the ORF derived from the nucleotide sequencing data.

Further evidence on the identification of the ORF was obtained by selectively labelling plasmid-encoded proteins with \[^{35}\text{S}\]methionine using the maxicell procedure (Fig. 3). A single polypeptide was observed with a molecular mass of 42 kDa, consistent with the molecular mass of 42276 Da calculated from the ORF.

Fig. 3. SDS-PAGE analysis of plasmid-encoded polypeptides labelled by the incorporation of \[^{35}\text{S}\]methionine using maxicells. Samples were electrophoresed through a 12% (w/v) polyacrylamide gel which was then dried and fluorographed. The exposure time was 20 h. Bars on the left side of the figure represent the position of \(^{125}\text{I}\)-labelled molecular mass standards. Top arrow indicates the position of the estA gene product, molecular weight 42 kDa (calculated from mobilities of labelled standards). Bottom arrow indicates the position of \(\beta\)-lactamase, molecular weight 30 kDa. Lanes: 1, \(^{125}\text{I}\)-labelled standards; 2, extract of maxicells containing pLGBd, containing estA (see Fig. 1b); 3, extract of maxicells containing a control plasmid (pSK containing an insert of non-bacterial origin).

Fig. 4. Effect of solubility on nitrophenol ester hydrolysis by either previously characterized enzyme preparations, or E. coli extracts containing estA or lipA. Hydrolysis was measured by nitrophenol release and specific activities were calculated as described in the text. Previously characterized enzyme preparations were suspended in 50% (v/v) glycerol/phosphate buffer, pH 7.0, and stored at \(-20^\circ\text{C}\); cell-free extracts (prepared as described in Methods) were derived from cells grown at 25\(^\circ\text{C}\). (a) Lipase from Pseudomonas sp. (Boehringer); (b) esterase from rabbit liver (Sigma); (c) cell-free extract from E. coli (lipA); (d) cell-free extract from E. coli (estA). Cell-free extracts from E. coli containing a control recombinant plasmid gave a small level of activity in the nitrophenol substrates used (corresponding to between 0.003 and 0.08 nmol min\(^{-1}\) (mg protein\(^{-1}\)) and were subtracted from sample rates. Data are representative of at least three independent experiments, activities are the means of triplicate determinations. Abbreviations: P-ac, p-nitrophenylacetate; P-but, p-nitrophenylbutyrate; O-but, o-nitrophenylbutyrate; P-myr, p-nitrophenylmyristate; P-pal, p-nitrophenylpalmitate; P-ste, p-nitrophenylstearate.
Characterization of the estA gene product

In this investigation, 1% (v/v) tributyrin plates were used to screen for enzymes that may be responsible for the production of short-chain fatty acids which cause rancidity in milk. Since tributyrin is partially soluble (0.01%, Brockerhoff, 1969) in water, the enzyme responsible for clearing tributyrin in solid media (see Methods) could be either an esterase (acting on the soluble component of the substrate) or a lipase (acting on the insoluble portion). To further characterise the estA gene product, cells containing pLGBd were grown on media containing 0.1% triolein (a completely insoluble compound) together with cells containing a plasmid which contains a gene (lipA) which encodes a lipase (unpublished results) derived from the same cosmid library as pLGBd. Only those cells containing lipA gave noticeable clearance of triolein after 3–4 d at 30°C (data not presented). Since the lipA gene product was able to act on an insoluble substrate, the enzyme responsible can therefore be identified as a lipase. However, the inability of the estA gene product to hydrolyse triolein may be due to reasons other than the solubility of the substrate. We have therefore compared the activity in cell-free extracts using soluble and insoluble substrates. For comparison, we have also examined a previously characterized esterase and lipase, obtained from commercial sources. Fig. 4(a) shows that water-insoluble compounds (p-nitrophenylmyristate, p-nitrophenylpalmitate and p-nitrophenylstearate) are all substrates for a lipase derived from a species of Pseudomonas, whereas only one water-soluble compound (p-nitrophenylbutyrate) is a substrate for this enzyme; this result is consistent with the enzyme being a lipase, since other lipases have been shown to hydrolyse a few water-miscible esters (Brockerhoff, 1969). In contrast, esterase from rabbit liver only gave significant rates of hydrolysis with the water-soluble substrates p-nitrophenylbutyrate and o-nitrophenylbutyrate (Fig. 4b). Consistent with observations that cells containing lipA can hydrolyse triolein (a water-insoluble substrate), Fig. 4(c) shows that extracts of cells containing lipA hydrolyse the water-insoluble esters of nitrophenol in preference to the water-soluble substrates. In marked contrast, extracts of cells containing estA only gave significant rates of hydrolysis with the water-soluble substrates (p-nitrophenylbutyrate, p-nitrophenylacetate and o-nitrophenylbutyrate), and not with the insoluble substrates; this result suggests that the estA gene codes for an esterase (Fig. 4c).

The characterization of estA will allow a determination of its possible role in the generation of fatty acids from milk triglycerides. For example, estA mutants of Pseudomonas LS107d2 may be constructed by site-directed mutagenesis using the cloned gene. Also, studies on the specificity of the estA gene-product will be aided by expression in E. coli.

References


