Identification of a second lipase gene, *gehD*, in *Staphylococcus epidermidis*: comparison of sequence with those of other staphylococcal lipases

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The identification and molecular characterization of a previously unidentified lipase, *gehD*, from the human cutaneous commensal *Staphylococcus epidermidis* is reported. A lipase-GehC-deficient but otherwise isogenic mutant of *S. epidermidis* 9 was constructed by allele replacement. However, the mutant was found to retain 50% of the wild-type lipase activity in liquid culture. Rescreening of a genomic library revealed the presence of a second lipase gene, *gehD*, which was subsequently mapped and sequenced. In common with other staphylococcal lipases, GehD appeared to be translated as a 650–700 amino acid precursor which is processed post-translationally to an extracellular mature lipase of 360 amino acids with a size of approximately 45 kDa. Comparison of the amino acid sequence of GehD with those of other staphylococcal lipases revealed a high level of conservation between the mature lipase domains of different species. By hybridization studies, both *gehC* and *gehD* genes were found to be present in *S. epidermidis* isolates from both clinical and non-clinical backgrounds, but neither hybridized to DNA isolated from other staphylococcal strains. Construction of a phylogenetic tree and calculation of amino acid sequence homologies between mature lipases, however, suggested that the lipases of *S. epidermidis* may be more closely related to those of *Staphylococcus aureus* than to each other.

**Keywords:** *Staphylococcus epidermidis*, lipase, *gehC*, *gehD*, skin

**INTRODUCTION**

*Staphylococcus epidermidis* forms a major part of the resident cutaneous microflora of human skin where it is found predominantly on the lipid-rich regions of the upper body and in the axillae. Until relatively recently, coagulase-negative staphylococci were considered to be avirulent commensals; however, with advances in medical technology, cutaneous staphylococci have emerged as important opportunistic pathogens associated with infections of synthetic medical devices, including prosthetic heart valves and intravenous catheters (Huebner & Goldmann, 1999).

Lipids are found ubiquitously on the surface of human skin, and are largely composed of sebum-derived triacylglycerides (Nicolaides, 1974). Understandably therefore, most organisms colonizing human skin possess some lipolytic activity, and this is believed to be responsible for the hydrolysis of sebaceous lipids, liberating free fatty acids onto the cutaneous surface (Marples *et al*., 1971).

Lipases have been implicated as possible virulence determinants in the pathogenesis of a number of localized infections such as boils or abscesses (Hedström, 1975; Hedström & Nilsson-Ehle, 1983; Rollof *et al*., 1987), and recent experiments utilizing *in vitro* expression technology (IVET) have also indicated that lipases are produced during infection in a murine abscess model (Lowe *et al*., 1998). The contribution of these enzymes to virulence, however, is not clearly understood, although it has been suggested that lipases may be important for the colonization and persistence of resident organisms on the skin, possibly in terms of...
nutrition or by the release of free fatty acids which may promote adherence (Gribon et al., 1993).

Previous studies aiming to identify extracellular colonization factors important for the persistence of cutaneous bacteria on the skin resulted in the identification and subsequent cloning of the lipase gene gebC from S. epidermidis strain 9 (Farrell et al., 1993). GebC was found to show homology with other staphylococcal lipases, notably those of Staphylococcus aureus, and was proposed to be exported from the cell as a 97 kDa precursor protein which is subsequently processed extracellularly to a 43 kDa mature lipase form.

Physiological studies comparing lipase-deficient mutants with otherwise isogenic strains could provide insight into the importance of lipases in colonization of the skin by S. epidermidis. This study describes the inactivation of gebC by allele-replacement mutagenesis, the subsequent identification of a second lipase gene (gehD) in S. epidermidis, and the characterization of the nucleotide and amino acid sequences of gebC and gehD in comparison with those of other staphylococcal lipases.

**METHODS**

**Bacterial strains, plasmids and media.** All bacterial strains and plasmids used in this study are listed in Table 1. For cloning and DNA manipulations in Escherichia coli, strain XL-1 Blue (Stratagene) was used. To enable transfer of recombinant DNA to S. epidermidis from E. coli, plasmids were passaged through S. aureus RN4220, a mutant strain derived from S. aureus 8325-4 which is capable of accepting DNA from E. coli (Kreiswirth et al., 1983). Staphylococci were routinely cultivated in BHI broth (Oxoid) containing 10 µg erythromycin ml⁻¹ when appropriate. E. coli was grown in nutrient broth (Oxoid) with 100 µg ampicillin ml⁻¹ selection when necessary. Strains were stored in 40% (v/v) glycerol in PBS (150 mM NaCl, 2 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, pH 7.4) at −70 °C. All chemicals were purchased from Sigma unless otherwise stated.

**DNA manipulation.** All molecular biological and recombinant DNA techniques were carried out using standard procedures as described by Sambrook et al. (1989). Construction and screening of the genomic library of S. epidermidis 9 in the replacement vector lpA7.1 was achieved as previously described (Farrell et al., 1993). Nucleotide sequencing was achieved by the dideoxynucleotide method (Sanger et al., 1977) using [³²P]dATP and T7 DNA polymerase.

**Transformation of staphylococci.** DNA was electroporated into S. aureus RN4220 according to the protocol of Augustin & Gotz (1990). Transfer of recombinant DNA into S. epidermidis was achieved by sphaeroplast transformation. Sphaeroplasts of S. epidermidis were prepared and transformed according to the method of Chang & Cohen (1979) with the following modifications. Antibiotic Medium no. 3 (Oxoid) was used in place of Penassay Broth and supplemented with 0.5% (w/v) glycine. SMM buffer comprised 0.7 M sucrose, 0.02 M sodium maleate, 0.02 M MgCl₂ (pH 6.5). Cells were sphaeroplasted with 100 µg lysostaphin ml⁻¹ at 37 °C for 1.5 h. Sphaeroplasts were regenerated on DM3 agar containing sucrose at a final concentration of 0.8 M. The antibiotics used for selection were 5 µg chloramphenicol ml⁻¹, 5 µg erythromycin ml⁻¹ or 5 µg tetracycline ml⁻¹. For transformations 3–30 µg DNA was used.

**Enzyme assays.** The presence of lipase activity in liquid cultures was determined by a titrimetric assay measuring the release of oleic acid from triolein (Ingham et al., 1981). Assays were performed in duplicate and units of lipase activity were expressed as mean µmol oleic acid produced min⁻¹. For selection of lipase-producing recombinants on solid media, cultures were initially grown on tributyrin agar plates; positive colonies were then subcultured onto plates containing the lipase-specific substrate, triolein (Farrell et al., 1993). Lipolytic activity was indicated by a zone of clearing or a zone of blue precipitation around colonies on tributyrin and triolein plates, respectively. Assays for protease and phosphatase activity in cell cultures during growth were performed as described by Farrell et al. (1993).

**In vitro disruption of the gebC gene.** The gebC gene was inactivated by the in vitro insertion of an erythromycin resistance gene. The 2.85 kb ClaI fragment from pUL500 containing gebC was cloned into pBluescript KS(+) forming pUL5010. The erythromycin resistance gene cassette (ermC) excised from pEE194 with TagI was inserted into pUL5010 at the intragenic HinIII site to form pUL5011. Lipase-negative E. coli colonies containing pUL5011 (gehC::ermC) were identified on tributyrin agar with erythromycin selection. The insertion of ermC into the ORF of gebC was confirmed by restriction with HinII. To allow replication of the plasmid in staphylococci, a shuttle vector, pUL5012, was constructed by ligation of the HinIII fragment of pCW59, containing a staphylococcal origin of replication and chloramphenicol resistance marker, into the EcoRI site of pUL5011.

**In vitro disruption of the gehD gene.** The inactivation of gehD was achieved using the same cloning strategy as described for gebC. Briefly, the gehD ORF was disrupted by insertion of the SalI–XhoI fragment of pEE194 containing the ermC cassette into the SalI site of pUL5041. The resultant vector, pUL5046, was then ligated to the HindIII fragment of pCW59 to yield the shuttle vector pUL5047, which was capable of replication in staphylococcal cells.

**Inactivation of chromosomal lipase genes by allele replacement.** To construct mutants of S. epidermidis 9 isogenic for either gebC or gehD but which were otherwise identical to the wild-type strain, with minimal introduction of foreign genetic material such as transposons, mutants were constructed by allele replacement mutagenesis (Foster, 1998). Shuttle vectors containing disrupted lipase genes (Cam⁶⁺, Ey⁸⁺) were transformed into S. epidermidis 9 sphaeroplasts. In addition, strains were co-transformed with pTS01 (O’Reilly et al., 1986), a plasmid harbouring a resistance marker (Tet⁶⁺) different to those carried on the shuttle vector containing the disrupted allele. Transformed bacteria were initially cultured in media containing tetracycline and chloramphenicol to enrich for transformants harbouring both plasmids. In order that the chromosomal genes were replaced by the disrupted alleles, transformants were subcultured into media containing tetracycline only. Under this selection, vectors containing the disrupted lipase gene could not persist; however, in some cells homologous recombination took place, resulting in the replacement of the chromosomal lipase gene with the mutant allele. Bacteria in which this occurred were identified by screening for a lipase-negative phenotype in colonies that demonstrated resistance to erythromycin and tetracycline but that were sensitive to chloramphenicol. In addition, replacement of the wild-type gene by the disrupted allele was verified...
**Table 1. Bacterial strains and plasmids used**

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype/phenotype/description</th>
<th>Reference/source</th>
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<tbody>
<tr>
<td><strong>Strains</strong></td>
<td></td>
<td></td>
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<tr>
<td>E. coli C600</td>
<td>lac thr leu thi tonA hsdR hsdM</td>
<td>Appleyard (1954)</td>
</tr>
<tr>
<td>E. coli XL-1 Blue</td>
<td>recA1 lac endA gyrA96 thi hsdR17 supE44 relA1 F'</td>
<td>Stratagene</td>
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<td></td>
<td>[proAB lacI</td>
<td></td>
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<td></td>
<td>lacZAM15 Tn10 (tetR)]</td>
<td></td>
</tr>
<tr>
<td>S. aureus RN4220</td>
<td>Restriction-system-deficient strain ((\tau_{e}) (m_{a}))</td>
<td>Kreiswirth et al. (1983)</td>
</tr>
<tr>
<td>S. epidermidis 9</td>
<td>Wild-type isolated from volar forearm; (\text{gehC}^{+}) (\text{gehD}^{+})</td>
<td>Farrell et al. (1993)</td>
</tr>
<tr>
<td>S. epidermidis 2J24</td>
<td>Mutant of S. epidermidis 9; (\text{gehC}^{+}) (\text{gehD}^{+}); \text{ermC}</td>
<td>This study</td>
</tr>
<tr>
<td>S. epidermidis KIC82</td>
<td>Mutant of S. epidermidis 9; (\text{gehC}^{+}) (\text{gehD}^{+}); \text{ermC}</td>
<td>This study</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
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<tr>
<td>pUL5000</td>
<td>Derivative of pBR322 containing (\text{gehC}); (\text{Ap}^{R})</td>
<td>Farrell et al. (1993)</td>
</tr>
<tr>
<td>pUL5010</td>
<td>2.85 kb ClaI fragment from pUL5000 spanning (\text{gehC}) gene ligated into pBluescript II KS(+); (\text{Ap}^{R})</td>
<td>Farrell et al. (1993)</td>
</tr>
<tr>
<td>pUL5011</td>
<td>1.4 kb TaqI fragment from pE194 containing erythromycin resistance cassette (\text{ermC}), ligated into pUL5010 at HinII site disrupting (\text{gehC}); (\text{Ap}^{R}) (\text{Ey}^{R})</td>
<td>This study</td>
</tr>
<tr>
<td>pUL5012</td>
<td>Shuttle vector derived from ligation of pUL5011 and HindIII fragment of pCW59 allowing replication in E. coli and staphylococci; (\text{Ap}^{R}) (E. coli), (\text{Ey}^{R}) (staphylococci)</td>
<td>This study</td>
</tr>
<tr>
<td>pUL5040</td>
<td>11 kb EcoRI fragment from (\text{gehD}) cloned into pBluescript II KS(+); (\text{Ap}^{R})</td>
<td>This study</td>
</tr>
<tr>
<td>pUL5041</td>
<td>3.3 kb EcoRI–HindIII fragment from pUL5040 spanning (\text{gehD}) gene ligated into pBluescript II KS(+); (\text{Ap}^{R})</td>
<td>This study</td>
</tr>
<tr>
<td>pUL5046</td>
<td>1.4 kb SalI–XhoI fragment of pE194 containing (\text{ermC}) ligated into pUL5041 at SalI site disrupting (\text{gehD}); (\text{Ap}^{R}) (\text{Ey}^{R})</td>
<td>This study</td>
</tr>
<tr>
<td>pUL5047</td>
<td>Shuttle vector derived from ligation of pUL5046 and HindIII fragment of pCW59 allowing replication in E. coli and staphylococci; (\text{Ap}^{R}) (E. coli), (\text{Ey}^{R}) (staphylococci)</td>
<td>This study</td>
</tr>
<tr>
<td>pCW59</td>
<td>Staphylococcal cloning vector; (\text{Cm}^{R}) (\text{Tet}^{R})</td>
<td>Wilson et al. (1981)</td>
</tr>
<tr>
<td>pTS01</td>
<td>(\text{Cm}^{R}) derivative of pCW59; (\text{Tet}^{R})</td>
<td>O’Reilly et al. (1986)</td>
</tr>
<tr>
<td>pE194</td>
<td>Staphylococcal cloning vector carrying (\text{ermC}); (\text{Ey}^{R})</td>
<td>Gryczan et al. (1982)</td>
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by Southern blot hybridization of genomic DNA digests using the pE194 TaqI fragment to confirm the presence of the erythromycin resistance marker, \(\text{ermC}\).

**Southern blotting of genomic DNA from staphylococcal isolates.** Isolates of S. epidermidis \((n = 100)\) were recovered by the facial scrub method (Williamson & Kligman, 1965) from the faces of acne patients attending the Leeds General Infirmary. Clinical isolates of S. epidermidis were obtained from venous catheter tips \((n = 3)\), paediatric central nervous system catheter blood cultures \((n = 4)\) and renal catheters \((n = 2)\). The identities of all coagulase-negative isolates were confirmed by growth and negative acid production on mannitol and trehalose plates. In addition, laboratory isolates of S. aureus, Staphylococcus capitis, Staphylococcus cohnii, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus saprophyticus, Staphylococcus simulans, Staphylococcus warneri and Staphylococcus xylosus were also probed for hybridization to \(\text{gehC}\) and \(\text{gehD}\). Staphylococcal genomic DNA was electrophoresed and transferred to Hybond-N+ membrane. For Southern blot hybridization analyses, digoxigenin-labelled DNA probes for \(\text{gehC}\) and \(\text{gehD}\) were prepared from the 1.2 kb HindIII fragment of pUL5000 and the 2.3 kb HinII fragment of pUL5041, respectively.

**Partial purification of extracellular lipases from isogenic mutants.** Extracellular lipases GehC and GehD were partially purified from the supernatants of isogenic mutants KIC82 and 2J24, respectively, grown in tryptone soya broth. At the post-exponential phase of growth, crude supernatant was separated from bacterial cells by centrifugation \((10000 \, \text{g}, 15 \, \text{min})\) and filtration \((0.45 \, \mu\text{m} \, \text{pore size})\). Subsequent steps were performed at 4°C. Protein containing the lipase fraction was precipitated by addition of ammonium sulphate to 50% saturation. The saturated solution was maintained at 4°C with slow stirring for 24 h before being centrifuged for 30 min at 8000 g. The pellet of precipitated protein was redissolved in PBS (pH 8) and desalted overnight by membrane dialysis in the same buffer. Following dialysis, samples were concentrated by lyophilization and redissolved in 1/10 volume PBS (pH 8).

**Detection of lipase activity following SDS-PAGE by zymography.** Protein samples were separated on 7.5% (w/v) SDS-PAGE gels under non-reducing conditions. Following electrophoresis, SDS was removed from the gels by sequential washes in 0.1% (v/v) Triton X-100 in water, 0.1% (v/v) Triton X-100 in PBS and PBS only, for 20 min each. The gels were then placed on nutrient agar plates containing 1% (w/v) tributyrin and overlaid with 10 ml molton tributyrin agar (at 40°C). Plates were incubated at 37°C for 24-48 h and bands exhibiting lipase activity defined by zones of clearing.

**N-terminal sequencing of GehD.** Partially purified proteins were separated by SDS-PAGE under reducing conditions and transferred to nylon membrane using dry-blot apparatus.
according to the instructions of the manufacturer (LKB). Polypeptides were visualized by staining with 0.1% (w/v) Coomassie brilliant blue in 50% (v/v) methanol and the appropriate polypeptide band excised. N-terminal sequence analysis was performed by A. Moir, University of Sheffield.

**Amino acid sequence analysis.** The translated amino acid sequences of GehC and GehD were predicted from the available sequence data using the software package DNASIS (Hitachi). Sequence homologies were calculated using the BLAST2 program (Tatusova & Madden, 1999; http://www.ncbi.nlm.nih.gov/gorf/bl2.html). Amino acid sequences of other microbial lipases were obtained from the GenBank database (http://www.ncbi.nlm.nih.gov/). The partial genome sequences of S. aureus COL (http://www.tigr.org/tdb/mdb/mdb.html) and 8325 (http://www.genome.ou.edu/staph.html) were accessed through their respective websites. Primary amino acid alignment of the lipases GehC and GehD with other bacterial lipases was performed using the clustalw program (Thompson et al., 1994; http://www2.ebi.ac.uk/clustalw/). A phylogenetic tree was constructed from the aligned sequences by the neighbour-joining method based on p-distances using clustalw. The tree presented in Fig. 5 is a bootstrap consensus after 1000 repetitions and was displayed by TreeView (Page, 1996).

**RESULTS AND DISCUSSION**

**Construction of a lipase-deficient mutant (gehC::ermC)**

A mutant of S. epidermidis, deficient for the production of lipase GehC, was constructed by allele replacement mutagenesis. The gehC ORF was disrupted in vitro by insertion of the 1.4 kb ermC gene, conferring erythromycin resistance, adjacent to the predicted lipase active site motif. The shuttle vector pUL5012, containing the disrupted lipase gene, and an incompatible plasmid, pTS01, were co-transformed into S. epidermidis 9 and cultures enriched for cells harbouring pTS01. Only cells in which the wild-type gehC had been replaced by the disrupted gehC::ermC showed resistance to tetracycline and erythromycin. To verify loss of lipase activity, mutants were screened on tributyrin and triolein agar. In addition, genomic DNA digests from mutant strains were probed for the presence of the ermC marker. Wild-type and mutant strains also showed similar levels of protease and phosphatase, indicating that decreased lipase activity was probably not due to a spontaneous regulatory mutation. One isogenic mutant, 2J24, was chosen for further studies. Although the gehC::ermC mutant exhibited no lipase activity on tributyrin plates (Fig. 1a), 50% of the wild-type lipase activity was observed during growth in BHI at 37°C when measured with a quantitative lipase assay using triolein as substrate (Fig. 1b). This result suggested that S. epidermidis 9 may produce more than one lipase during growth.

**Identification of a second lipase gene (gehD) in S. epidermidis 9**

To determine whether the lipase activity observed in liquid assays was a result of a second lipase gene, five lipase-expressing recombinants from the genomic li-

![Fig. 1. Growth and lipase activity of wild-type Staphylococcus epidermidis 9 and its GehC mutant 2J24. (a) Growth on tributyrin agar plates. (b) Titrimetric assay of culture supernatant. ○, Growth of wild-type; □, growth of mutant; ●, lipase activity of wild-type; ■, lipase activity of mutant. Lipase assays were performed in duplicate and mean values are plotted.](image)

brary constructed in λL47.1 were screened by hybridization to a gehC-specific probe. Two of the recombinants did not hybridize, indicating the presence of a second lipase gene in S. epidermidis 9 that was not homologous to gehC. An 11 kb EcoRI fragment was subcloned from one of the recombinant phages into pBluescript to give pUL5040, which was then transformed into E. coli XL-1 Blue. Lipase expression was detected from transformants by observation of a zone of clearing in the tributyrin emulsion surrounding E. coli colonies. As tributyrin hydrolysis can also be attributed to the presence of esterases, lipase activity was confirmed in positive clones by subculture onto triolein agar (a lipase-specific substrate).

**Deletion analysis of pUL5040**

To localize the region of DNA responsible for the production of lipase activity, restriction digestion of pUL5040 was used to produce a series of subclones of differing sizes. The resultant plasmids were then transformed into E. coli and screened for lipase activity. The minimal region of DNA required for lipase activity was localized to a 33 kb EcoRI–HindIII fragment from pUL5041. The nucleotide sequence of this region was...
A second lipase in Staphylococcus epidermidis

**Fig. 2.** Structural organization of staphylococcal lipases as preproenzyme precursors. sepi GehC, S. epidermidis 9 GehC; sepi GehD, S. epidermidis 9 GehD; sau Geh, S. aureus PS54 Geh; sau Sal-2, S. aureus NCTC 8530 Sal-2; shy Lip, S. hyicus subsp. hyicus DSM 20459 Lip; shaem Lip, S. haemolyticus Lip. The signal peptide (SP), propeptide (PP) and mature lipase (ML) domains are indicated, with their lengths in amino acid residues below. The amino acids proposed to form the catalytic active site triad are indicated and are numbered relative to the start of the mature lipase domain.

**Fig. 3.** SDS-PAGE of partially purified GehD under non-reducing conditions. Proteins were purified from culture supernatant of the gehC mutant S. epidermidis 2J24 by precipitation with ammonium sulphate, dialysis and lyophilization prior to electrophoresis. Lanes: M, molecular mass markers; D, partially purified GehD; Z, overlaid tributyrin agar zymogram of SDS-PAGE gel. The zone of lipid hydrolysis is denoted with an arrow.

determined from both DNA strands and was found to contain a single 2·0 kb ORF (gehD) encoding a polypeptide of 643 amino acids with a predicted molecular mass of 72 kDa (GenBank accession no. AF090142). From analysis of the nucleotide sequence data, the translation initiation codon was predicted to be TTG which, although unusual, has been reported for other staphylococcal proteins including SirR, protein A and β-lactamase (Hill et al., 1998; Löfdahl et al., 1983; McLaughlin et al., 1981). The initiation codon was found to be preceded by a potential ribosome-binding site, AGAGGTG, identical to that found upstream of gehC. Analysis of the region upstream of the gehD translation initiation codon revealed potential σ^70-like promoter motifs with homology to those described for gehC (Farrell et al., 1993). In addition, the region of DNA downstream of the predicted translational stop codon contained a palindromic sequence capable of forming a hairpin-loop structure which could possibly act as a transcription terminator.

**Distribution of gehC and gehD in S. epidermidis isolated from clinical and non-clinical sites**

To investigate whether both lipase genes were present in other isolates of S. epidermidis, digoxigenin-labelled DNA probes were made that were specific for internal regions of gehC or gehD. For Southern blot hybridization, 100 non-clinical isolates of S. epidermidis were obtained and the genomic DNA prepared from 10 randomly selected isolates. In addition, genomic DNA was prepared from 9 clinical S. epidermidis isolates recovered from catheter-related infections and a number of other staphylococcal strains. Genomic DNA from all S. epidermidis isolates, including those from clinical and non-clinical sites, was positive for hybridization to both gehC and gehD probes, whilst neither probe hybridized to genomic DNA from other staphylococci, including closely related strains from the same staphylococcal species group such as S. capitis or S. haemolyticus. Although only a relatively small number of S. epidermidis strains have been screened to date, these preliminary results would suggest that both gehC and gehD are widely distributed within the S. epidermidis population, with the apparent lack of difference between
Fig. 4. Amino acid sequence alignment of the mature domains of *S. epidermidis* lipases GehC and GehD with those from other staphylococcal lipases. segehd, *S. epidermidis* 9 GehD (GenBank accession no. AF090142); segehc, *S. epidermidis* 9 GehC (M95577); saugeh, *S. aureus* PS54 Geh (M12715); sausal2, *S. aureus* NCTC 8530 Sal-2 (AF086783); shylip, *S. hyicus* subsp. *hyicus* DSM 20459 Lip (X02844); shaelip, *S. haemolyticus* Lip (AF096928). Identical amino acid residues are indicated in bold type with asterisks below. The conserved serine, aspartic acid and histidine residues, predicted to be important in the lipase active site, are indicated by crosses above the sequences. Amino acid numbers are shown on the right.
clinical and non-clinical strains possibly being due to the inherently opportunistic nature of *S. epidermidis* infections.

**Molecular characterization of *S. epidermidis* lipases**

Comparison of the predicted amino acid sequence of GehD with other bacterial proteins indicated a high level of similarity with other bacterial lipases, particularly those identified from other staphylococci. The hydropathy profile of GehD indicated that the first 37 amino acid residues of the translation product contained adjacent regions of high hydrophilicity or hydrophobicity typical of a signal peptide. Furthermore, following this domain there is an Ala-Glu-Ala [motif, which has been proposed as the cleavage site for a signal peptidase (Perlman & Halvorson, 1983).

One structural character which so far appears to be common to all staphylococcal lipases is their synthesis as preproenzymes. The translated precursor consists of three major domains: signal peptide, propeptide and mature lipase. The signal peptide is essential for secretion and is removed during export of the proprotein. The propeptide domain (207–270 residues) has been found to be important for efficient translocation and proteolytic stability during secretion (Liebl & Gotz, 1986) and following export is sequentially degraded from the N-terminus by extracellular proteases to leave the mature lipase enzyme. Sequence data suggests that GehD is similarly translated as a preproenzyme (Fig. 2). Partially purified GehD from the isogenic mutant 2J24 indicated that the size of the active extracellular lipase form of GehD is approximately 45 kDa (Fig. 3). This is in contrast to the 72 kDa translation product predicted from the nucleotide sequences. N-terminal sequencing of the 45 kDa GehD polypeptide revealed the first 12 residues to be AQAQYKNQYPVV, which is similar to that of the GehC mature lipase (Farrell et al., 1993) and corresponds to the region of GehD homologous to the start of the processed mature domain of other staphylococcal lipases.

Multiple alignment of the amino acid sequences of GehC and GehD with those of lipases from *S. aureus*, *S. haemolyticus* and *Staphylococcus hyicus* showed that the translation products consisted of three distinct domains which corresponded well with the predicted locations of the signal peptide, propeptide and mature lipase shown in Fig. 2. The mature lipase regions in particular showed a high degree of conservation between all staphylococcal lipase sequences (Fig. 4). The propeptide region, in contrast, was found to be highly variable and this may suggest a degree of specificity in the secretion or stability of their respective lipases. Although microbial lipases are a structurally diverse...
group of enzymes, the secondary and tertiary structures do contain some common elements. A common structural feature of lipases is a catalytic active site triad similar to that found in serine proteases. This commonly consists of Ser-His-Asp residues, with the active site serine being present in the highly conserved pentapeptide motif Gly-X-Ser-X-Gly, where X is most often histidine or tyrosine, and X₂ is methionine, leucine or glutamine (Brady et al., 1990). As no X-ray crystallographic model exists for a staphylococcal lipase, the precise location of the enzyme active site or any catalytic residues cannot be determined directly. However, by alignment of highly conserved regions of the staphylococcal mature lipases it was possible to predict which residues may be catalytically important in the active sites of GehC and GehD. The deduced positions of the catalytic triad residues and surrounding sequences were highly conserved between all five staphylococcal lipases with those from S. epidermidis 9, predicted to be Ser¹¹⁶-, Asp⁰⁸⁷-, His²¹⁶ in GehC and Ser¹¹⁶-, Asp⁰⁸⁷-, His²¹⁹ in GehD (Fig. 4).

Multiple alignment also allowed the construction of a phylogenetic tree based on a comparison of the staphylococcal mature lipases and the well-characterized lipase of Burkholderia glumae (Noble et al., 1994) (Fig. 5). The mature lipase domain was selected as this region is more highly conserved between lipases. Both GehC and GehD were more closely related to lipases from other staphylococci than to the lipase of B. glumae. However, although they are related to each other, within the staphylococcal lipase family the two lipases of S. epidermidis are grouped into separately branched clusters.

The phylogenetic analysis showed that GehC is closely related to lipase Sal-2 from S. aureus NCTC 8530 (84% identity), whilst GehD has greater homologies to the S. aureus PS54 lipase, Geh (58% identity), and the lipase of S. haemolyticus, Lip (70% identity). In addition, GehC and GehD showed 51% identity to each other (Table 2).

As GehC and GehD show homologies to two separate lipases, Geh and Sal-2, which have been isolated from different S. aureus strains, the partially completed genome databases for S. aureus strains COL and 8325 were searched for genes with homology to geh and sal-2. Regions of DNA encoding homologues with 100% identity to both previously described S. aureus lipases were found on the databases for both COL and 8325. This suggests that a number of S. aureus isolates may also produce two lipases and therefore, the identification of different lipases in different strains may be a result of one lipase masking the presence of the other.

Work is in progress to determine whether there are any differences in the regulation or substrate specificity of GehC and GehD which could explain why S. epidermidis should encode two functionally similar enzymes. However, the production of two lipolytic enzymes by S. epidermidis and possibly S. aureus may be indicative that lipases are important to the organisms for colonization and growth within the lip-id-rich environment of the skin.

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Received 29 November 1999; revised 1 March 2000; accepted 7 March 2000.