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Analysis of the *Staphylococcus epidermidis* Genes *epiF*, *-E*, and *-G* Involved in Epidermin Immunity

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The lantibiotic epidermin is produced by *Staphylococcus epidermidis* Tü3298. The known genes involved in epidermin biosynthesis and regulation are organized as operons (*epiABCD* and *epiQP*) that are encoded on the 54-kb plasmid pTü32. Here we describe the characterization of a DNA region that mediates immunity and increased epidermin production, located upstream of the structural gene *epiA*. The sequence of a 2.6-kb DNA fragment revealed three open reading frames, *epiF*, *-E*, and *-G*, which may form an operon. In the cloning host *Staphylococcus carnosus*, the three genes mediated an increased tolerance to epidermin, and the highest level of immunity (sevenfold) was achieved with *S. carnosus* carrying *epiFEG* and *epiQ*. The promoter of the first gene, *epiF*, responded to the activator protein EpiQ and contained a palindromic sequence similar to the EpiQ binding site of the *epiA* promoter, which is also activated by EpiQ. Inactivation of *epiF*, *-E*, or *-G* resulted in the complete loss of the immunity phenotype. An epidermin-sensitive *S. epidermidis* Tü3298 mutant was complemented by a DNA fragment containing all three genes. When the *epiFEG* genes were cloned together with plasmid pTepi14, containing the biosynthetic genes *epiABCDQP*, the level of epidermin production was approximately fivefold higher. The proteins EpiF, -E, and -G are similar in deduced sequence and proposed structure to the components of various ABC transporter systems. EpiF is a hydrophilic protein with conserved ATP-binding sites, while EpiE and -G have six alternating hydrophobic regions and very likely constitute the integral membrane domains. When EpiF was overproduced in *S. carnosus*, it was at least partially associated with the cytoplasmic membrane. A potential mechanism for how EpiFEG mediates immunity is discussed.

Epidermin (1) is a well-characterized member of the lantibiotics, a group of peptide antibiotics that are distinguished by the presence of the rare amino acid lanthionine (for reviews, see references 16 and 31). The bactericidal action is mainly caused by pore formation in the cytoplasmic membrane (30). Epidermin, like all other lantibiotics found so far, is ribosomally synthesized. The structural gene for the epidermin precursor peptide, *epiA*, is encoded on the 54-kb plasmid pTü32 of *Staphylococcus epidermidis* Tü3298 (34). A 14-kb DNA fragment, sufficient for low-level epidermin production, has been subcloned in *Staphylococcus carnosus* TM300 (4). The nucleotide sequence revealed the presence of seven genes that are organized in three transcriptional units (33). *epiA* is cotranscribed with *epiB*, *-C*, and *-D*, which are involved in the posttranslational modification of epidermin. The flavin mononucleotide-containing enzyme EpiD, which is unique to the epidermin system, has been recently shown to catalyze the oxidative decarboxylation of the C terminus of pre-epidermin (21–23). EpiB and EpiC are proposed to catalyze the dehydration of serine and threonine residues and the formation of thioether bonds. Genes related to *epiB* and *-C* are involved in the biosynthesis of the lantibiotics nisin (8, 20) and subtilin (19). The genes *epiP* and *epiQ* are transcribed in the opposite direction. EpiP has homology with serine proteases and is, therefore, proposed to be involved in epidermin leader peptide processing. A similar function has been demonstrated for the homologous protease NisP (38). EpiQ, a transcriptional activator of the *epiABCD* unit (27), binds to a palindromic sequence in the *epiA* promoter region. Upstream of *epiA*, the third transcriptional unit comprises only one gene, *epiT*, whose function remains unclear since it is interrupted by a frameshift deletion (33). The EpiT sequence is similar to that of ABC transporter proteins, such as HlyB (9) and the related proteins SpaT and NisT (4, 19, 20).

Here we report the cloning, sequencing, characterization, and regulation of the genes *epiF*, *-E*, and *-G*, which are organized in an operon-like structure. These genes confer epidermin immunity and mediate increased epidermin production.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *S. epidermidis* Tü3298 is the epidermin-producing wild-type strain (1). The mutant strain *S. epidermidis* Tü3298 EMS13 is defective in *epiB*, leading to the loss of epidermin production (Epi$^-$ phenotype) (4). Moreover, it bears an additional mutation that leads to increased epidermin sensitivity (Epi^s phenotype; see below). *S. carnosus* TM300 (13) was used for heterologous expression of epidermin genes, as a test organism in the bioassay for epidermin, and as a cloning host. DNA fragments were also cloned in *Escherichia coli* JM83 (39) by using pUC18 (42) or the *E. coli-Staphylococcus* shuttle vector pRB473, a derivative of plasmid pRB373 (5) in which the pUB110 kanamycin resistance gene is replaced by the chloramphenicol acetyltransferase (*cat*) gene. pT181mcs is a staphylococcal cloning vector conferring tetracycline resistance (4). pPS44 (27) is a staphylococcal promoter-screening vector containing the promoterless lipase gene of *Staphylococcus hyicus* (12). This lipase gene is silent unless a DNA fragment conferring promoter activity is introduced into the single *Bam*HI site, located 32 bp upstream from the lipase start codon. pTX15, a xylose-inducible staphylococcal expression vector conferring tetracycline resistance, was derived from plasmid pCX15 (41) by exchanging the pC194 replicon for the pT181 replicon (25). pTX15 contains the *xylA* promoter from the *xyl* operon of *Staphylococcus xylosus* and *xylR*, which encodes a repressor for the *xylA* promoter (37). Insertion of a gene at the single *Bam*HI site (located downstream of the *xylA* promoter) allows xylose-inducible expression of the gene. Full induction was achieved by the addition of 0.5% xylose to the medium. In plasmid pTX16, the lipase gene is deleted by *Bam*HI and *Nar*I digestion and blunt-end religation; this plasmid was used as a negative control in the *epiF* expression studies. Plasmids pTepi14 and pTepiQ10 carry portions of the previously described *epi* region (4, 27). *E. coli* cells were grown in Luria-Bertani (LB) medium containing 1% tryptone (Difco, Detroit, Mich.), 0.5% yeast extract (Gibco BRL, Gaithersburg, Md.), and 0.5% NaCl. *S. epidermidis* was grown in BM medium (LB medium supplemented with 0.1% K_2HPO_4 and

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0.1% glucose), and *S. carnosus* was grown in LB or BM medium. All bacteria were incubated at 37°C.

Plasmid preparation and DNA transformation and sequencing. Staphylococcal plasmid DNA was prepared by using Nucleobond AX 100 columns (Macherey-Nagel GmbH, Düren, Federal Republic of Germany). The manufacturer's instructions were followed with the exception that the cells were incubated for 15 min in 4 ml of buffer S1 containing 25 μg of lysostaphin (Sigma, St. Louis, Mo.) per ml before buffer S2 was added. Transformation and cloning procedures for *S. carnosus* have been described (3, 13). *E. coli*-specific recombinant DNA techniques were performed as described by Sambrook et al. (32). Enzymes for molecular cloning were obtained from Boehringer Mannheim (Mannheim, Federal Republic of Germany), Gibco BRL, and Pharmacia (Freiburg, Federal Republic of Germany); assay conditions were as recommended by the suppliers.

DNA sequence analysis (6) was carried out on the Pharmacia A.L.F. DNA sequencer. Both strands were sequenced with sets of nested deletions prepared by exonuclease III digestion. Oligonucleotides, used as primers in sequencing reactions, were synthesized on a Pharmacia gene assembler. Computer analyses of DNA and amino acid sequence data were performed with the programs MicroGenie (Beckman, Palo Alto, Calif.) and MacDNASIS Pro (Hitachi Software Engineering, San Bruno, Calif.). Amino acid sequence homology searches were performed with the nonredundant protein database and the BLAST program of the NCBI (Bethesda, Md.) (2).

Epidermin immunity assay. Precultures (5 ml in BM broth) were grown to mid-exponential phase, diluted with fresh medium to an optical density of 0.3 at 600 nm, and shaken for 5 h. In a serial dilution test, 5 ml of BM broth containing increasing concentrations of purified epidermin was inoculated with 50 μl of the preculture and cultivated for 20 h. The degree of immunity was deduced from the relationship between growth and the epidermin concentration. Six independent tests were carried out for each bacterial strain. Purified epidermin was kindly provided by H.-P. Fiedler, Universität Tübingen.

Lipase assay. Cultures (5 ml) of the strains to be tested were grown as described for the epidermin immunity assay (see above), except that LB medium was used and the final cultures were incubated for 16 h. The supernatants were checked for lipase activity by monitoring hydrolysis of *p*-nitrophenylcaprylate (Sigma) at 405 nm as described previously (29).

Analysis of epidermin production. Since the amounts of epidermin produced by *S. carnosus* strains were too low for high-performance liquid chromatography analysis, we chose a more sensitive bioassay. The chloramphenicol- and tetracycline-resistant strain *S. carnosus*(pRB473/pT181mcs) was used as an epidermin-sensitive indicator organism. A 30-μl aliquot of a late-stationary-phase culture (optical density of 10 at 600 nm) of the indicator strain was added to 40 ml of molten BM agar and poured into an agarose gel tray (11 by 14 cm). For the preparation of samples, 5-ml cultures (in BM broth) of the various epidermin-producing strains were grown as described for the epidermin immunity assay (see above), except that the final cultures were incubated for 24 h. The supernatants (10 μl; *S. carnosus* supernatants were concentrated 25-fold by lyophilization) were loaded into agar slots formed by combs. Three samples of one culture were loaded onto one agar plate, together with several samples containing increasing amounts of purified epidermin. The concentration of epidermin in the supernatants was deduced from the size of the inhibition zones, which were compared with the inhibition zones mediated by epidermin standards. Six independent tests were carried out for each bacterial strain.

Overproduction and detection of EpiF. LB medium (500 ml) containing 0.5% xylose was inoculated with 5 ml of overnight cultures of *S. carnosus* strains bearing plasmid pTXepiF or pTX16 and cultivated for 16 h at 37°C. Cells were harvested by centrifugation, washed in buffer (20 mM Tris-HCl, pH 8.0), and disrupted by glass beads, as described recently (37). Intact cells were removed by centrifugation at 2,000 × *g* for 20 min. Soluble and insoluble proteins (membrane fraction) were separated by ultracentrifugation at 200,000 × *g* for 90 min. The supernatant (soluble fraction) was saved. The viscous pellet (membrane fraction) was washed with 2 ml of buffer and ultracentrifuged again; the pellet was resuspended in the same buffer. Appropriate amounts of protein from the soluble and membrane fractions were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 12% Tris-glycine gels (24) and stained with Coomassie brilliant blue G 250.

Nucleotide sequence accession number. The GenBank accession number for the nucleotide sequence of the epidermidin immunity determinant (see Fig. 2) is U29130.

RESULTS

Cloning and sequencing of the epidermin immunity determinant. Previously, it has been shown that the 14-kb DNA fragment of plasmid pTü32, carrying the *epiA*, *-B*, *-C*, *-D*, *-Q*, and *-P* genes (pTepi14), is sufficient to mediate weak epidermin production in the heterologous host *S. carnosus* TM300 (33). However, the fragment does not confer immunity to epidermin (Table 1). We cloned adjacent DNA fragments into the shuttle vector pRB473 and tested the resulting *S. carnosus* clones for alterations in epidermin sensitivity. Plasmid pRBepi7, containing a 7-kb *Xho*I-*Bgl*II fragment located downstream of *epiT* (Fig. 1), conferred moderate immunity to epidermin. The 7-kb insert of pRBepi7 was digested with exonuclease III on both ends, and the resulting sets of nested deletions were assayed for loss of the immunity phenotype (data not shown). A 2.6-kb region, 1.4 kb from the previously published sequence (33), was necessary for epidermin immunity (Fig. 1). The nucleotide sequence of this region revealed three open reading frames in the same orientation (Fig. 2). The coding regions of *epiF* and *-E* are separated only by a stop codon, and those of *epiE* and *-G* overlap by three codons. All three genes are preceded by regions with reasonable similarity to the Shine-Dalgarno consensus sequence. Downstream of *epiG*, there is a rho-independent-like transcription termination sequence.

TABLE 1. Influence of *epi* genes on immunity and epidermin production

Strain and plasmid(s)	MIC (ng/ml)	Epidermin production (μg/g of cells [dry wt])
S. carnosus TM300		
pRB473/pT181mcs[a]	57	0
pRB473/pTepi14	59	11
pRB473/pTepiQ10	54	ND[b]
pRBepiFEG/pT181mcs	170	ND
pRBepiFEG/pTepi14	250	57
pRBepiFEG/pTepiQ10	410	ND
pRBepiEG/pT181mcs	50	ND
pRBepiEG/pTepi14	46	16
pRBepiEG/pTepiQ10	65	ND
pRBepiFG/pT181mcs	48	ND
pRBepiFG/pTepi14	55	29
pRBepiFG/pTepiQ10	64	ND
pRBepiFE/pT181mcs	51	ND
pRBepiFE/pTepi14	49	26
pRBepiFE/pTepiQ10	54	ND
S. epidermidis Tü3298		
pTü32	1,800	2,100
pTü32/pRB473	1,800	ND
S. epidermidis Tü3298 EMS13		
pTü32-13/pRB473[c]	610	0
pTü32-13/pRBepiFEG	2,100	ND
pTü32-13/pRBepiEG	490	ND
pTü32-13/pRBepiFG	490	ND
pTü32-13/pRBepiFE	530	ND

[a] Control.
[b] ND, not determined.
[c] The mutated epidermin plasmid of *S. epidermidis* Tü3298 EMS13 was named pTü32-13.

Homology of EpiF, -E, and -G to ABC transporter proteins. EpiF is a hydrophilic protein of 231 residues with a molecular mass of 25,901 Da. The amino acid sequence shows sequence identities with bacterial ABC transporter proteins, such as MalK (28%) (35) and HisP (26.5%) (10). Furthermore, EpiF is homologous to McbF, SpaF, and NisF, which are involved in export and/or immunity of the bacteriocins microcin B17 (11), subtilin (18), and nisin (36), respectively. The highest percent identity was found in the N-terminal region of SpaF (49%).

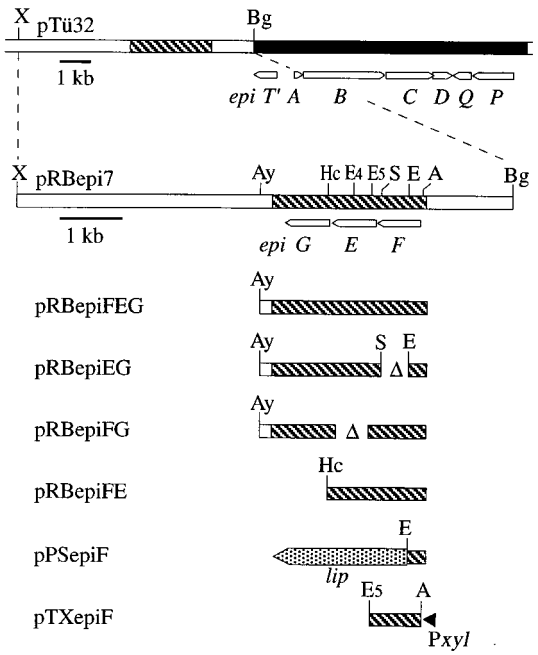

FIG. 1. Organization of the epidermin gene cluster and plasmids used in this study. The black box represents the known *epi* sequence; the striped box indicates the region with the *epiFEG* genes (shown enlarged). The *epi*-containing plasmids used in this work are presented below the maps. The various plasmids were constructed by ligating the indicated DNA fragments to suitable restriction sites of the respective cloning vector (plasmid names starting with pRB, pPS, or pTX refer to the cloning vectors pRB473, pPS44, and pTX15, respectively). The right ends of the inserts in pRBepiFEG, pRBepiEG, pRBepiFG, pRBepiFE, and pPSepiF resulted from exonuclease III digestion. pRBepiEG was derived from pRBepiFEG by digestion with *Spe*I and *Eco*RV, Klenow enzyme treatment, and religation, resulting in the deletion of a 357-bp fragment. For the construction of pRBepiFG, plasmid pRBepiFEG was linearized by *Eco*47III digestion, treated with nuclease *Bal* 31, and recircularized. DNA sequence analysis revealed the loss of 623 bp of the *epiF* sequence. The deletions of the genes *epiF*, *-E*, and *-G* (open triangles) did not affect adjacent genes or their ribosomal binding sequences. The lipase reporter gene of pPSepiF (*lip*) is indicated as a stippled arrow. The *xylA* promoter sequence (P*xyl*) that expresses *epiF* in plasmid pTX epiF is indicated as a black triangle. Abbreviations: A, *Acc*I; Ay, *Acy*I; Bg, *Bgl*II; E, *Eco*RV; E4, *Eco*47III; E5, *Eco*57I; Hc, *Hin*cII; S, *Spe*I; X, *Xho*I.

The genes *epiE* and *epiG* encode proteins of 255 amino acids (28,966 Da) and 230 amino acids (26,773 Da), respectively. Both proteins are predicted to be very hydrophobic. EpiE has a large number of amino acids which are identical to those in the C-terminal part of SpaF (26%). EpiG is less similar to SpaG (19%). SpaG and SpaF are involved in immunity to subtilin (18).

Involvement of *epiF*, *-E*, *-G*, and *-Q* in epidermin immunity. *S. carnosus* clones containing various combinations of *epi* genes were tested for their sensitivity to epidermin in liquid culture. *S. carnosus*(pRBepiFEG) tolerated threefold-higher concentrations of epidermin than did the control strain (Table 1). This immunity effect was abolished when any one of the three genes was deleted (Fig. 1; Table 1). *S. carnosus* clones harboring pRBepiFEG and the compatible plasmid pTepi14, which contains the *epiA*, *-B*, *-C*, *-D*, *-Q*, and *-P* genes, tolerated an even higher concentration of epidermin than did clones containing pRBepiFEG alone. The highest level of immunity (sevenfold) was achieved with *S. carnosus*(pRBepiFEG/pTepiQ10), suggesting that EpiQ, the only intact epidermin protein encoded by plasmid pTepiQ10, activates the *epiFEG* gene cluster in addition to the *epiA*, *-B*, *-C*, and *-D* genes.

The *epiB* mutant EMS13 of *S. epidermidis* Tü3298 has an Epi$^-$ phenotype (4) and a second mutation (Epi^s) that results in increased sensitivity to epidermin (Table 1). The Epi^s mutation was complemented by transformation of strain EMS13 with plasmid pRBepiFEG; the epidermin tolerance of the resulting strain exceeded the wild-type immunity level (Table 1). None of the plasmids containing only two of the immunity genes restored the immunity phenotype.

Influence of EpiQ on *epiF* promoter activity. In order to obtain more information on the regulation of *epiF* promoter activity by EpiQ, we inserted a 455-bp *Eco*RV-*Hin*dIII fragment likely to contain the *epiF* promoter into the promoter probe vector pPS44, yielding plasmid pPSepiF (Fig. 1). The influence of EpiQ on the *epiF* promoter was determined by comparing the extracellular lipase activities of *S. carnosus* (pPSepiF/pT181mcs) and *S. carnosus*(pPSepiF/pTepiQ10). By itself, the *epiF* promoter mediated a moderate expression of the lipase gene (3.9 U/g of cells [dry weight], compared with 0.2 U/g obtained by using pPS44). When *epiQ* was present on the compatible plasmid pTepiQ10, lipase expression increased approximately 15-fold (57 U/g of cells [dry weight]), indicating that EpiQ positively controls the *epiF* promoter. The proposed *epiF* promoter region contains a palindromic sequence 75 nucleotides upstream of the *epiF* start codon (Fig. 2) which is very similar to the EpiQ-binding site of the *epiA* promoter (27).

Influence of *epiF*, *-E*, and *-G* on epidermin production. In order to study the influence of the *epiFEG* gene cluster on epidermin production, we transformed *S. carnosus*(pTepi14), bearing the epidermin biosynthetic genes, with the compatible plasmid pRBepiFEG. The presence of pRBepiFEG led to a fivefold increase in epidermin production (Table 1). Inactivation of *epiF* resulted in an almost complete reduction of this effect, while in the absence of *epiE* or *epiG*, the level of epidermin production was still two- to threefold higher.

Detection of the *epiF* gene product. In order to verify the existence of the *epiF* gene product, we cloned *epiF* in the staphylococcal expression vector pTX15 to construct the plasmid pTXepiF (Fig. 1). Cell extracts (soluble and insoluble fractions) of *S. carnosus* clones bearing pTXepiF or the control plasmid pTX16, which is identical to pTXepiF except for the *epiF* gene, were analyzed by SDS-PAGE (Fig. 3). A protein band with an apparent molecular mass of 28 kDa appeared in cell extracts of *S. carnosus* strains expressing EpiF (calculated EpiF mass is 25.9 kDa). Considerable amounts of EpiF were present in both the soluble and insoluble fractions of *S. carnosus*(pTXepiF). The band was not detectable in extracts of uninduced cells (data not shown). Overproduction of the proposed membrane proteins EpiE and EpiG was unsuccessful.

DISCUSSION

The *epiFEG* genes were shown to be tightly clustered; the *epiE* and *-G* genes even overlap by three codons. This arrangement and the absence of any transcriptional terminator-like structure between the genes suggest that the *epiFEG* gene cluster forms an operon. This supposition is supported by the finding that *epiF* promoter activity is sensitive to the regulator EpiQ. In our promoter test system, in which the *epiF* promoter was fused with the lipase indicator gene, we showed that in the presence of *epiQ* (located on a compatible plasmid), lipase activity increased 15-fold. Previously, we have shown that EpiQ activates transcription of the *epiABCD* operon from the *epiA* promoter. Gel mobility shift experiments indicate that EpiQ binds to a palindromic sequence upstream of the −35 region of the *epiA* promoter (27). A quite similar palindromic sequence precedes the first gene, *epiF*. On the basis of these two palin-

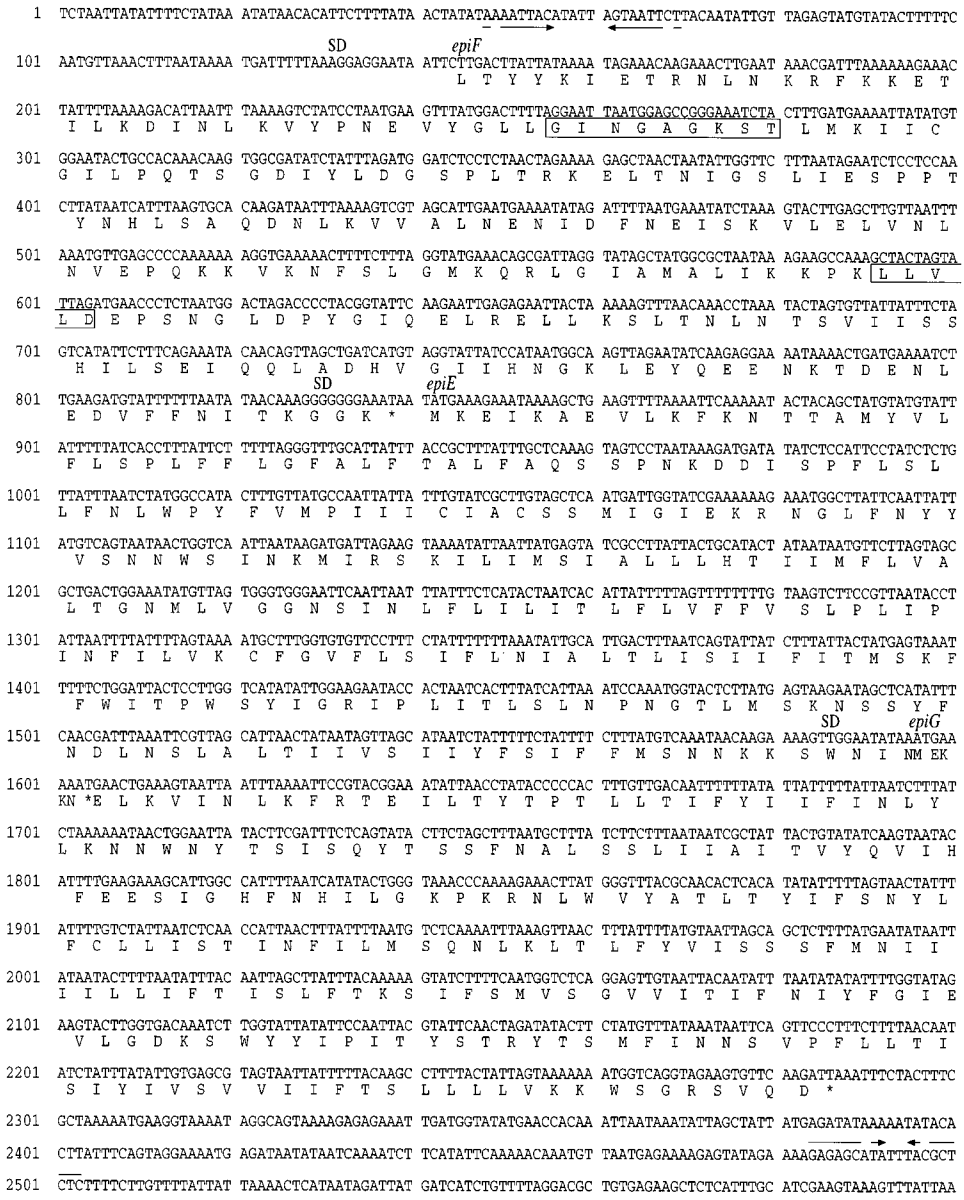

FIG. 2. Nucleotide sequence of the epidermin immunity determinant and deduced amino acid sequences of the *epiF*, *epiE*, and *epiG* products. The amino acid sequences are given below and the proposed translational start sites and Shine-Dalgarno sequences (SD) are indicated above the DNA sequence. Stop codons are designated with asterisks. The proposed EpiQ binding site preceding *epiF* and the terminator-like structure downstream of *epiG* are indicated by arrows. Boxed amino acids represent the conserved ATP-binding motifs (40) within the EpiF sequence.

dromic sequences, the tentative consensus motif of the EpiQ binding site is ANAATTACNNNNNNGTAATTNT.

The first conspicuous phenotype mediated by the *epiFEG* genes was the increased tolerance of *S. carnosus* to epidermin. *S. carnosus*(pRBepiFEG) tolerated a threefold-higher epidermin concentration than did the control strain, and in the presence of *epiQ*, there was a sevenfold increase (Table 1). This is not a high resistance level, but, nevertheless, it indicates that a certain defense mechanism is involved. For the expression of immunity, all three genes (*epiF*, *-E*, and *-G*) were necessary; inactivation of any one of them affected the immunity function. Plasmid pRBepiFEG complemented the Epi^s mutation of *S. epidermidis* Tü3298 EMS13, which provides further evidence for a role of EpiFEG as an epidermin immunity system. All three genes were necessary to restore the immunity phenotype, suggesting that all three genes are affected by the Epi^s mutation.

EpiF shows homology to the ATPase subunits of many membrane permease complexes, such as MalK (35) and HisP (10), which belong to the so-called ABC transporters (14). These systems mediate the ATP-dependent import or export of substances across the cytoplasmic membrane of bacteria and eukaryotes. Extensive investigations of various ABC transporter systems have shown that a complete transport complex consists of four domains: two hydrophobic membrane components (each comprising six *trans*-membrane α-helices) and two ATPase subunits (each containing one ATP binding site) (14). The four components can be formed by one, two, three, or four protein molecules, and the corresponding genes are always close to each other. In contrast to the ATP-binding subunits,

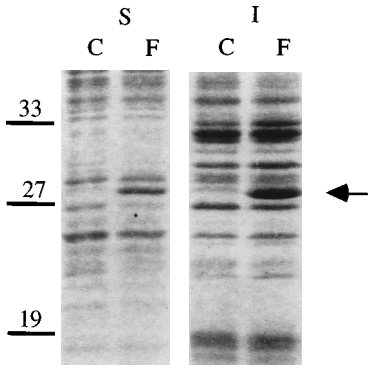

FIG. 3. Detection of the *epiF* gene product. Soluble (S) and insoluble (I) proteins (40 μg) from various *S. carnosus* strains were subjected to SDS-PAGE on a 12% Tris-glycine gel and stained with Coomassie brilliant blue G 250. Lanes C and F contain fractions derived from *S. carnosus* strains bearing the plasmid pTX16 (control) or pTXepiF, respectively. Sizes of standard proteins in kilodaltons are indicated on the left. The arrow indicates the proposed EpiF band (25.9 kDa).

the integral membrane domains reveal little overall sequence similarity but show a very similar distribution of hydrophilic and hydrophobic regions (14). EpiE and EpiG are similar in length and hydrophobicity profiles to ABC transporter membrane components such as the well-characterized MalG (7) (data not shown).

Because of the similarities in sequence and structure, we assume that EpiF, -E, and -G form an ABC transporter resembling the *E. coli* $MalFGK_2$ and $HisMQP_2$ transporters (17, 26). The two membrane domains would be formed by the proteins EpiE and EpiG, while two molecules of EpiF (with the ATP-binding sites) would be mainly located in the cytosol and contribute to energizing the complex. Immunity would thus be mediated by active transport of epidermin molecules, as outlined in the model presented in Fig. 4. This hypothesis is in agreement with our finding that EpiF is loosely associated with the cytoplasmic membrane.

The EpiFEG proteins also have similarities to the SpaFG, NisFEG, and McbFE proteins, which are involved in immunity against subtilin (18), nisin (36), and microcin B17 (11), respectively. The proteins with an ATP-binding motif are fairly homologous. The proposed integral membrane proteins show few or no sequence identities; however, they have similar hydrophobicity profiles, with six possible membrane-spanning regions (data not shown). It is, therefore, very likely that the four systems have the same mode of action.

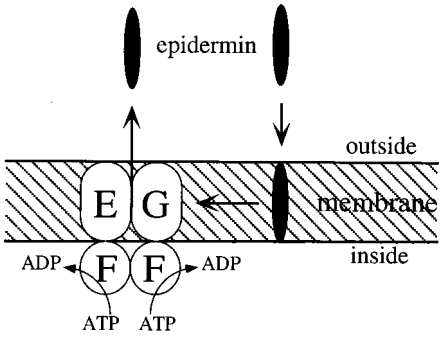

FIG. 4. Proposed location and function of the proteins EpiF, -E, and -G. An ABC transporter complex formed by EpiF, -E, and -G is proposed to mediate immunity by active extrusion of epidermin molecules (black ellipses) from the cytoplasmic membrane.

Immunity could be mediated by active extrusion or by uptake and intracellular degradation of the respective peptide. In the latter case, reduced production of epidermin would be expected when the epidermin-producing strain *S. carnosus* TM300 (pTepi14) is transformed with the *epiFEG* genes. However, the opposite was the case, as the level of epidermin production was approximately fivefold higher in the presence of plasmid pRBepiFEG. This result favors an export function of the proposed EpiFEG transporter. It is tempting to speculate that the membrane-damaging peptides are bound directly within the cytoplasmic membrane, as was proposed for substrate binding of the human multidrug resistance protein (15). The immunity system would thus keep the lantibiotic concentration in the cytoplasmic membrane below a critical level (Fig. 4). This hypothesis, however, remains to be verified.

The EpiFEG proteins have no similarities to the LanI proteins, which are involved in immunity to the lantibiotics Pep5, nisin, and subtilin (18, 20, 28). The mode of action of these proteins, which are attached to the outside of the cytoplasmic membrane by an N-terminal protein anchor sequence (PepI) or a lipid anchor (NisI or SpaI), remains unclear. No *epiI* gene has yet been found.

It is possible that the proposed EpiFEG transporter also plays a role in epidermin secretion, which would explain the increased epidermin production in the strain containing the *epiFEG* genes. However, the maximal level of epidermin production achieved in *S. carnosus* is much lower than the level of production in the natural epidermin producer, suggesting that additional factors for epidermin biosynthesis are still missing.

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