JOURNAL OF BACTERIOLOGY, Mar. 1995, p. 1123–1128
0021-9193/95/$04.00+0

Vol. 177, No. 5

MINIREVIEW

Lipoproteins of Gram-Positive Bacteria

IAIN C. SUTCLIFFE* AND ROY R. B. RUSSELL

Department of Oral Biology, The Dental School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne NE2 4BW, United Kingdom

INTRODUCTION

Our view of the complexity of the Gram-positive bacterial cell envelope has altered considerably in recent years, with the recognition of the presence of a variety of proteins retained by different mechanisms. Lipoproteins, i.e., proteins containing lipid covalently linked to an N-terminal cysteine residue, have been extensively studied in gram-negative bacteria (7), but examples from gram-positive species have only quite recently been recognized (Table 1).

Despite its thickness, the peptidoglycan layer of gram-positive bacteria remains a relatively porous structure. Thus, in the absence of the retentive outer membrane, components carrying out functions within the gram-positive cell envelope must somehow be tethered in order to prevent their loss into the growth environment. The lipidated N terminus is presumed to anchor lipoproteins into the outer leaflet of the cell membrane, and lipoproteins can be compared topographically to the other major class of macroamphiphiles present in the gram-positive cell envelope, the lipoteichoic acids and lipoglycans (65).

Several approaches can be used to identify lipoproteins: (i) metabolic labelling with radiolabelled fatty acid, usually palmitic acid; (ii) interference with protein processing by the antibiotic globomycin, which inhibits lipoprotein processing by signal peptidase II; and (iii) presence of a conserved consensus sequence within prelipoprotein signal peptides, which is thought to direct processing of the prelipoprotein to form the mature acylated protein (6, 7, 71). The last has become the standard method for recognition of putative lipoproteins, but it must be stressed that relatively few lipoprotein structures have yet been the subjects of rigorous chemical analysis.

THE FUNCTIONS OF LIPOPROTEINS

Lipoproteins and antibiotic resistance. The first demonstration of a gram-positive lipoprotein was made in 1981, when three groups independently demonstrated that the extracellular penicillinase of *Bacillus licheniformis* existed in a posttranslationally lipidated, membrane-associated form and that the acyl moiety was comparable to that of Braun's lipoprotein from *Escherichia coli* (36, 48, 64). The production of membrane-associated lipoprotein forms of the β-lactamases appears to be part of a secretion pathway for the production of extracellular enzyme; the exoenzyme activity is thought to be derived by proteolytic cleavage of an N-terminal extension from the mature protein (47, 50, 51). The proportion of the enzyme that is released to the extracellular medium varies considerably (49, 72), and in some strains of *Staphylococcus aureus* almost all of the enzyme was retained in the cell-associated form (11).

* Corresponding author. Phone: 0191-222-7918. Fax: 091-222-6137. Electronic mail address: i.c.sutcliffe@newcastle.ac.uk.

Substrate-binding lipoproteins in transport systems. A substantial number of the proteins located in the gram-negative periplasm are solute-binding proteins, components of high-affinity binding protein-dependent systems responsible for the transport of a wide range of substrates (16, 67). After substrate binding, the binding proteins interact with integral membrane protein components which translocate the substrate across the membrane. The genes encoding the components of these systems are generally found organized together as an operon (16).

In contrast to the gram-negative bacteria, binding protein-dependent transport systems have only recently been identified and characterized in gram-positive bacteria. The first examples recognized were the *mal* and *ami* operons of *Streptococcus pneumoniae*, which show strong homology to maltose (*mal*) and oligopeptide (*opp*) uptake systems of enterobacteria, respectively. Gilson et al. recognized the problem of the localization of the binding protein component and proposed that these components underwent lipoprotein modification, since the genes included signal peptides with the lipoprotein-processing consensus site (20). The *ami* oligopeptide transport system has been the subject of rigorous studies by Claverys and coworkers (1, 2), and the binding protein, AmiA, has been labelled with [^{3}H]palmitate (1). It is notable that the cloning of both the *malX* and *amiA* lipoprotein genes presented considerable difficulties (39) and, since the cloning of a number of other lipoprotein genes has resulted in similar problems (21, 54, 62), it appears that high-level expression of foreign lipoproteins can have deleterious effects on *E. coli* hosts. Interestingly, AmiA can be inactivated without abolishing oligopeptide transport by *S. pneumoniae*, and this apparent dispensability of the binding protein was demonstrated to be due to the presence of two additional lipoproteins, AliA and AliB, which are not encoded by the *ami* operon (1). These two lipoproteins are both highly homologous to AmiA, and each of the three binding proteins appears to have a slightly different specificity for oligopeptides, allowing a wide range of substrates to be transported via the Ami transport system.

The *Streptococcus mutans* Msm system also transports multiple substrates, all sugars structurally related to raffinose (59, 69), and the MsmE binding protein has been shown to be a lipoprotein, as was suggested by the presence of a lipoprotein signal peptide sequence (66). In this case, transport of multiple substrates appears to involve one lipoprotein only.

A substantial number of transport systems have now been identified for which it is proposed that the solute-binding proteins are lipoproteins (Table 1). The majority of these transport systems have yet to be thoroughly characterized in terms of their biochemical properties and physiological roles; their functions have been deduced primarily from the comparative analyses of gene sequences.

Lipoproteins as adhesins. Cell surface molecules play many important roles in governing the adherence of bacteria to dif-

TABLE 1. Lipoproteins of gram-positive bacteria

Organism	Lipoprotein[a]	Function[b]	Signal[c]	Cys position[d]	Lipid labelling[e]		Reference(s)
Alicyclobacillus acidocaldarius	ORF2	Maltose transport	TVAGCG	21			29
Bacillus cereus	BlaZ	β-Lactamase	VLLSCA	24	+		49, 51
Bacillus sp. strain 170	LIPEN	Penicillinase	TLIGCS	24			27
Bacillus licheniformis	BlaP	Penicillinase	ALAGCS	21	+		36, 47, 48, 64
Bacillus subtilis	PrsA	Protein secretion	ALSACS	21	+		32, 33
	RbsB	Ribose transport	LLTACS	19			75
	FhuD	Iron-siderophore transport	ALAACG	24			62
	OppA(*Spo0KA*)	Oligopeptide transport/sporulation	VLSACG	21			55, 57
	AppA	Oligopeptide transport/sporulation	FLSACS	24			28
	DciAE	Dipeptide transport/sporulation	ALMGCT	23			40
	SpoIIIJ	Sporulation	LLAGCS	21			12
	GerBC	Germination	LLALCG	18			10
	GerAC	Germination	LLSGCW	18			78
	GerD	Germination	SVTACA	21			76
	GerM	Germination	LLSGCG	23			63
	Slp	Peptidoglycan associated	ALSGCT	19			23
	LppX/LytA	Unknown	LLSGCG	17			35, 38
	LplA	Unknown	MIAGCS	22		+	21
Clostridium sordellii	CsS	Sialidase	VLSACN	20			56
Corynebacterium diphtheriae	ORF1	Iron-siderophore transport	TLAACG	23			61
Enterococcus faecalis	TraC	Pheromone receptor	ILGACN	22			68
	PrgZ	Pheromone receptor	VLSACG	22			58
Lactococcus lactis	PrtM	Proteinase maturation	LLSGCQ	24	+		22
	OppA	Oligopeptide transport	LLSACG	20			70
	NisI	Nisin immunity	GLSGCY	20			34
Micrococcus luteus	38-kDa protein	Unknown			+		73
Mycobacterium tuberculosis	38-kDa (Ag B) protein	Phosphate binding	AAAGCG	24	+		3, 8, 77
	19-kDa protein	Unknown	GLSGCS	22	+		5, 77
Mycobacterium intracellulare	MI22	Unknown	GISGCS	22			44
	MI43	Unknown	SLSACG	29	+		45
Mycobacterium bovis	19-kDa protein	Unknown	GLSGCS	22			9
Staphylococcus aureus	BlaZ	Penicillinases Type A and C	VLSACN	17	+		11, 42, 49
	TraH	Conjugative plasmid transfer	VLAGCG	18		+	15
Streptococcus equisimilis	Has	Hyaluronate synthetase	ALMACP	28			37
Streptococcus gordonii	SarA	Adhesin	VLAACS	23	+		25
	ScaA	Adhesin	GLAACS	20			30
Streptococcus mutans	MsmE	Sugar transport	LLAACN	23	+	+	59, 66
Streptococcus parasanguis	FimA	Fimbrial adhesin	GLLACS	21			13
Streptococcus pneumoniae	MalX	Maltose transport	LLVACG	25			20
	PlpA	Oligopeptide transport	VLAACS	?	+		54
	AmiA	Oligopeptide transport	VLAACS	23	+		1, 20
	AliA	Oligopeptide transport	TLAACS	23	+		1
	AliB	Oligopeptide transport	LLSACG	25	+		1
	PsaA	Adhesin	GLLACS	21			60
Streptococcus sanguis	SsaB	Adhesin	TLFACS	20		+	17, 18
Streptomyces chrysomallus	FKBP-33	Immunophilin	SAVACG	19	+		53

[a] Includes putative lipoproteins awaiting direct biochemical evidence for lipoprotein modification.
[b] Some of the proposed functions are still speculative.
[c] The prelipoprotein signal peptide consensus sequence.
[d] Position of the cysteine residue in the prelipoprotein signal peptide sequence.
[e] + indicates that metabolic radiolabelling with palmitate has been demonstrated either in the source organism (left column) or when expressed in *E. coli* (right column).

ferent substrata, host tissues, and other bacteria. These interactions are well illustrated by the complexity of interactions that occur between oral bacteria and their microenvironment, and a number of recent studies have identified adhesins from oral bacteria that have either been directly demonstrated to be lipoproteins or are predicted to be lipoproteins from sequence analyses (26, 31).

Streptococcus sanguis produces a 34.7-kDa lipoprotein, SsaB, which has been proposed to be an adhesin interacting with a salivary receptor and possibly involved in coaggregation with *Actinomyces naeslundii* (17–19, 31). *S. sanguis* SsaB shares significant amino acid homology with a number of other streptococcal proteins which are thought to be adhesins and whose sequences also include a presumptive signal peptide containing the lipoprotein processing consensus sequence (4, 13, 52, 60).

Sequence analysis indicates that the *scaA* gene of *Streptococcus gordonii* is located within an operon which may encode a binding protein-dependent transport system and the genes for homologous proteins of other streptococci also appear to be located within operons of similar genetic organization (30, 60). These findings suggest that, in addition to their proposed roles as adhesins, these lipoproteins may also participate in transport systems; their ability to mediate coaggregation could be due to the recognition of fixed ligands on the surfaces of other bacteria.

Protein secretion. *Lactococcus* spp. produce proteinase en-

zymes which play a central role in the degradation of casein. The expression of these proteinases in an enzymatically active extracellular form has been shown to depend on a proteinase maturation protein (PrtM), and this protein has been convincingly demonstrated to be a membrane-associated lipoprotein (22).

The *B. subtilis* PrsA protein shows 30% sequence identity to the lactococcal PrtM protein and is the most abundant lipoprotein detected in *B. subtilis* (32, 33). This indispensable protein appears to act at a rate-limiting stage of the *B. subtilis* protein export pathway, most likely through affecting protein folding after protein translocation across the cell membrane (24, 32, 33).

The involvement of PrsA in an extracytoplasmic stage in protein export is directly comparable with the role of lactococcal PrtM in production of mature extracellular protease in that both proteins appear to influence the mature conformations of exported proteins and their anchoring as lipoproteins clearly reflects their extracytoplasmic functions.

Lipoproteins may be involved in sensory (signalling) systems. In gram-negative bacteria, solute-binding proteins of the periplasm are involved in chemoreception and sensory mechanisms (67), and it seems likely their gram-positive lipoprotein counterparts may perform similar functions. In *B. subtilis*, mutations at *spo0* loci are known to affect the initiation of sporulation. Studies of one of these loci, *spo0K*, demonstrated that the mutations were located within a five-gene operon encoding a binding protein-dependent oligopeptide transport system, and the *oppA* binding protein gene contained a signal sequence with a lipoprotein processing site (55, 57). Thus, the *B. subtilis opp* transport system is similar to the other binding protein-dependent transport systems as described above. However, the contribution of this operon to the sporulation process suggests a role in sensory processes rather than nutrient transport. The initiation of sporulation may be triggered by the transport of a peptide signal, such as degradation products derived from peptidoglycan (55, 57). Recently, a second oligopeptide transport system which may also play a role in the sporulation process has been characterized (28), and a dipeptide transport system is rapidly expressed following the initiation of sporulation (40). The precise contribution of these various peptide transport systems to the complex sporulation pathway awaits determination.

Oligopeptide transport systems may also influence the development of genetic competence by having a role in sensing changes in growth conditions (1, 28, 54, 55, 57).

The *B. subtilis* spore cycle involves putative lipoproteins. Analysis of the *B. subtilis spo87* locus has revealed that a putative lipoprotein, SpoIIIJ, may be involved in sporulation (12). Mutations in *spoIIIJ* block a late stage of sporulation at which the membrane-bound prespore is engulfed within the mother cell. It was suggested that a membrane location for SpoIIIJ would be consistent with a function as part of the communication pathways between the prespore and the mother cell (12).

In order to be an effective survival strategy, spore formation must be accompanied by pathways to allow germination in response to specific external stimuli. Different *ger* mutants have been described, of which the *gerA* and *gerB* loci are thought to encode receptors involved in sensing different germinant signals (43). Examination of the *gerA* and *gerB* loci has revealed that each contains an operon encoding three proteins and that the respective GerA/GerB protein pairs (e.g., GerAA-GerBA) exhibit significant homology (10, 43, 78). All three proteins in each operon appear to be membrane associated, and the GerAC-GerBC homologs are putative lipoproteins (10, 78). The prediction that these lipoproteins would be located at the spore membrane requires further study, as the GerAC protein has been tentatively localized to the more external spore integument layers, perhaps because the protein is only temporarily membrane associated (43).

Bacterial conjugation may involve lipoproteins. Lipoproteins have been proposed to be involved in the *Enterococcus faecalis* conjugation process. The pAD1 conjugative plasmid of this organism contains a gene, *traC*, encoding a 61-kDa protein with a lipoprotein signal peptide (68). The protein is thought to function as a receptor for the peptide sex pheromone cAD1, and it is therefore plausible that it should be localized to the surface by a lipoprotein anchor. Likewise, the *E. faecalis* pCF10 conjugative plasmid encodes a putative lipoprotein, PrgZ, which is highly homologous to TraC and is responsible for the binding of the cCF10 sex pheromone (58). Both TraC and PrgZ exhibit sequence homologies with oligopeptide binding proteins (see above) but are not included within operons encoding transport systems. It remains possible that these pheromone-binding proteins could interact with the components of a chromosomally encoded transport system, a relationship analogous to the interaction of the AliA and AliB lipoproteins with the *ami* transport system (1).

DNA sequencing of a 14.4-kb region from the staphylococcal conjugative plasmid pSK41 identified 15 genes likely to be involved in the conjugative transfer process (15), and one of these, TraH, is a lipoprotein. A second gene from this cluster, TraJ, encoded a product with sequence homology to the chromosomally encoded signal peptidase II of *S. aureus*. Intriguingly, the last eight amino acids of the signal peptide predicted to be released by signal peptidase II during TraH processing show significant homology with the peptide sequences of several conjugation-associated pheromones from *E. faecalis* (15), and further processing of the pro-TraH signal peptide has been shown to produce a cAD1-like pheromone activity (14).

Other functions of lipoproteins and lipoproteins of unknown function. Table 1 reveals that a number of enzyme activities such as sialidase (56) and hyaluronate synthetase (37) are associated with putative lipoproteins. A lipoprotein with binding activity for the immunosuppresant FK506 (i.e., an immunophilin), which also possessed peptidyl-prolyl *cis-trans* isomerase activity, has been identified in *Streptomyces chrysomalus* (53). The function of a putative lipoprotein expressed as part of the *B. subtilis* operon which encodes genes for this organism's major cell wall autolysin and its modifier protein (35, 38) remains unknown.

A number of gram-negative bacteria produce lipoproteins which have strong noncovalent interactions with peptidoglycan (7). These lipoproteins apparently perform essential structural functions, possibly stabilizing the interaction between the outer membrane and the peptidoglycan. In the absence of an outer membrane, it would be anticipated that related lipoproteins would not be present in gram-positive bacteria. However, a gene (*slp*) from *B. subtilis* has been sequenced which encodes a putative lipoprotein with low but significant amino acid sequence homology to the peptidoglycan-associated lipoproteins of *E. coli* and *H. influenzae* (23). Whether lipoproteins of gram-positive bacteria are involved in structural interactions with peptidoglycan warrants further study.

The pioneering work on the palmitate labelling of the β-lactamases of *B. cereus*, *B. licheniformis*, and *S. aureus* (49, 50) indicated that these lipoproteins belonged to larger sets of lipoproteins (up to 10 in the *Bacillus* species). Likewise, studies of *S. gordonii* and *S. mutans* have indicated that both these streptococci produce at least 10 lipoproteins which have yet to be attributed functions (25, 66), and several lipoproteins dis-

TABLE 2. Frequency distribution of amino acid residues in the predicted cleavage site of the gram-positive bacterial prolipoprotein signal peptide sequences shown in Table 1

No. of occurrences of amino acid at position:					
−4	−3	−2	−1	+1	+2
Leu (11)	Leu (39)	Ser (18)	Ala (26)	Cys (45)	Ser (18)
Val (9)	Val (2)	Ala (15)	Gly (17)		Gly (15)
Ala (7)	Ala (2)	Leu (3)	Leu (1)		Asn (4)
Gly (7)	Ile (2)	Ile (1)	Ser (1)		Ala (2)
Thr (5)		Met (2)			Thr (2)
Ser (3)		Thr (2)			Tyr (1)
Ile (1)		Gly (1)			Trp (1)
Phe (1)		Phe (1)			Gln (1)
Met (1)		Val (2)			Pro (1)

tinct from those involved in transport processes were identified in *S. pneumoniae* (1). Consequently, it seems likely either that novel lipoprotein functional classes will be discovered or that the above lipoproteins will be attributed functions comparable to those already described. Moreover, it should be noted that these lipoprotein sets were detected either without specific induction or under conditions in which other lipoproteins were being induced, and consequently additional lipoproteins may be inducible by more appropriate growth conditions.

CONCLUSIONS

The broad range of functions for the lipoproteins of gram-positive bacteria demonstrates that lipid modification provides a versatile method for localizing proteins to the gram-positive cell envelope (Table 1). The last five years has seen a substantial increase in the number of proteins considered to be lipoproteins (as deduced either directly from biochemical studies or indirectly from sequence data), and it seems inevitable that this trend will continue. Moreover, this minireview has not considered the lipoproteins produced by bacteria from the class *Mollicutes* (7, 74). Even though these organisms are phylogenetically related to the gram-positive bacteria, they lack a peptidoglycan cell wall; consequently, their cell surfaces have been subject to many distinctive adaptations, and their lipoproteins may have unique roles such as the generation of surface antigenic variation (74).

Most of the lipoproteins in Table 1 have only been identified by predictions from sequence analysis, matching the consensus for the lipoprotein signal peptide cleavage site, LA(G,A) ↓ C (6, 7, 71). This consensus sequence is being continually refined by the addition of novel sequences, and the data from Table 1 are summarized in Table 2. It is noteworthy that the consensus remains L(S,A)(A,G) ↓ C(S,G), the same as that for the larger set of sequences from gram-negative lipoproteins (7). While sequence analysis remains a valuable tool for the identification of putative lipoproteins, it must be strongly emphasized that evidence beyond sequence data alone is required to demonstrate convincingly that a protein is expressed as a lipoprotein in the original parent bacterium (as distinct from the cloning host).

Although this review has emphasized the role of lipoprotein modification in cell surface localization, it should also be noted that lipoproteins can be released from the cell surface in an acylated form, as illustrated by the recovery of lipoproteins from the culture supernatants of *S. mutans* (66). Other lipoproteins appear to be released from the bacterial cell depending on the phase of growth (54, 55), and lipoprotein enzymes may be released via proteolytic cleavage (50, 51, 56). Whether the release of lipoproteins from the cell is widely exploited as a mechanism for regulating their activity at the cell surface or is simply a consequence of general cell turnover and lysis is unclear.

In contrast to N-terminal acylation, the other major mechanism by which proteins are retained at the gram-positive cell envelope is dependent on the presence of well-characterized C-terminal structural motifs (46). These two distinctive anchoring mechanisms would therefore appear to complement each other. The number of proteins thought to be anchored by one or the other of these motifs is continually increasing, and previous models of the gram-positive cell envelope may have underestimated its complexity. It has been argued that it may be possible to define the compartments of bacterial cells not solely in terms of membrane-bound structures but also in terms of composition and function (41), and it seems appropriate to consider the gram-positive cell envelope as such a "functional compartment". Indeed, it has been argued previously that lipoprotein modification may provide the gram-positive bacterium with a functional equivalent to periplasmic localization (50) and that macroamphiphilic lipoteichoic acids and lipoglycans also contribute to the architecture of this compartment (65).

In conclusion, the recognition that the cell envelopes of gram-positive bacteria may contain substantial numbers of lipoproteins provides further evidence that these structures are more complex than previously thought and that lipoproteins must be incorporated into future models describing the architecture of the gram-positive envelope.

ACKNOWLEDGMENTS

The authors are grateful for the financial support of the Wellcome Trust (grant no. 038465/Z/93) and the National Institutes of Health (grant no. DE08191).

REFERENCES

1. **Alloing, G., P. de Philip, and J.-P. Claverys.** 1994. Three highly homologous membrane-bound lipoproteins participate in oligopeptide transport by the Ami system of *Streptococcus pneumoniae*. J. Mol. Biol. **241:**44–58.
2. **Alloing, G., M. C. Trombe, and J.-P. Claverys.** 1990. The *ami* locus of the Gram-positive bacterium *Streptococcus pneumoniae* is similar to binding protein-dependent transport operons of Gram-negative bacteria. Mol. Microbiol. **4:**633–644.
3. **Andersen, A. B., L. Ljungqvist, and M. Olsen.** 1990. Evidence that protein antigen b of *Mycobacterium tuberculosis* is involved in phosphate metabolism. J. Gen. Microbiol. **136:**477–480.
4. **Andersen, R. N., N. Ganeshkumar, and P. E. Kolenbrander.** 1993. Cloning of the *Streptococcus gordonii* PK488 gene, encoding an adhesin which mediates coaggregation with *Actinomyces naeslundii* PK606. Infect. Immun. **61:** 981–987.
5. **Ashbridge, K. R., R. J. Booth, J. D. Watson, and R. B. Lathigra.** 1989. Nucleotide sequence of the 19 kDa antigen gene from *Mycobacterium tuberculosis*. Nucleic Acids Res. **17:**1249.
6. **Bairoch, A.** 1993. The PROSITE dictionary of sites and patterns in proteins, its current status. Nucleic Acids Res. **21:**3097–3103.
7. **Braun, V., and H. C. Wu.** 1994. Lipoproteins, structure, function, biosynthesis and model for protein export, p. 319–341. *In* J.-M. Ghuysen and R. Hakenbeck. (ed.), New comprehensive biochemistry, vol. 27: bacterial cell wall. Elsevier Science, Amsterdam.
8. **Chang, Z. Y., A. Choudhary, R. Lathigra, and F. A. Quiocho.** 1994. The immunodominant 38-kDa lipoprotein antigen of *Mycobacterium tuberculosis* is a phosphate-binding protein. J. Biol. Chem. **269:**1956–1958.
9. **Collins, M. E., A. Patki, S. Wall, A. Nolan, J. Goodger, M. J. Woodward, and J. W. Dale.** 1990. Cloning and characterization of the gene for the '19 kDa' antigen of *Mycobacterium bovis*. J. Gen. Microbiol. **136:**1429–1436.
10. **Corfe, B. M., R. L. Sammons, D. A. Smith, and C. Mauel.** 1994. The *gerB* region of the *Bacillus subtilis* 168 chromosome encodes a homologue of the *gerA* spore germination operon. Microbiology **140:**471–478.
11. **East, A. K., and K. G. H. Dyke.** 1989. Cloning and sequence determination of 6 *Staphylococcus aureus* β-lactamases and their expression in *Escherichia coli* and *Staphylococcus aureus*. J. Gen. Microbiol. **135:**1001–1015.
12. **Errington, J., L. Appleby, R. A. Daniel, H. Goodfellow, S. R. Partridge, and M. D. Yudkin.** 1992. Structure and function of the *spoIIIJ* gene of *Bacillus*

subtilis: a vegetatively expressed gene that is essential for σ^G activity at an intermediate stage of sporulation. J. Gen. Microbiol. **138:**2609–2618.
13. **Fenno, J. C., D. J. Leblanc, and P. Fives-Taylor.** 1989. Nucleotide sequence analysis of a type 1 fimbrial gene of *Streptococcus sanguis* FW213. Infect. Immun. **57:**3527–3533.
14. **Firth, N., P. D. Fink, L. Johnson, and R. A. Skurray.** 1994. A lipoprotein signal peptide encoded by the staphylococcal conjugative plasmid pSK41 exhibits an activity resembling that of *Enterococcus faecalis* pheremone cAD1. J. Bacteriol. **176:**5871–5873.
15. **Firth, N., K. P. Ridgway, M. E. Byrne, P. D. Fink, L. Johnson, I. T. Paulsen, and R. A. Skurray.** 1993. Analysis of a transfer region from the staphylococcal conjugative plasmid pSK41. Gene **136:**13–25.
16. **Furlong, C. E.** 1987. Osmotic-shock-sensitive transport systems, p. 768–796. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
17. **Ganeshkumar, N., N. Arora, and P. E. Kolenbrander.** 1993. Saliva-binding protein (SsaB) from *Streptococcus sanguis* 12 is a lipoprotein. J. Bacteriol. **175:**572–574.
18. **Ganeshkumar, N., P. M. Hannam, P. E. Kolenbrander, and B. C. McBride.** 1991. Nucleotide sequence of a gene coding for a saliva-binding protein (SsaB) from *Streptococcus sanguis* 12 and possible role of the protein in coaggregation with actinomyces. Infect. Immun. **59:**1093–1099.
19. **Ganeshkumar, N., M. Song, and B. C. McBride.** 1988. Cloning of a *Streptococcus sanguis* adhesin which mediates binding to saliva-coated hydroxyapatite. Infect. Immun. **56:**1150–1157.
20. **Gilson, E., G. Alloing, T. Schmidt, J.-P. Claverys, R. Dudler, and M. Hofnung.** 1988. Evidence for high affinity binding-protein dependent transport systems in Gram-positive bacteria and in *Mycoplasma*. EMBO J. **7:**3971–3974.
21. **Gómez, A., D. Ramón, and P. Sanz.** 1994. The *Bacillus subtilis* lipoprotein LplA causes cell lysis when expressed in *Escherichia coli*. Microbiology **140:** 1839–1845.
22. **Haandrikman, A. J., J. Kok, and G. Venema.** 1991. Lactococcal proteinase maturation protein PrtM is a lipoprotein. J. Bacteriol. **173:**4517–4525.
23. **Hemilä, H.** 1991. Sequence of a PAL-related lipoprotein from *Bacillus subtilis*. FEMS Microbiol. Lett. **82:**37–41.
24. **Jacobs, M., J. B. Andersen, V. Kontinen, and M. Sarvas.** 1993. *Bacillus subtilis* PrsA is required in vivo as an extracytoplasmic chaperone for secretion of active enzymes synthesized either with or without pro-sequences. Mol. Microbiol. **8:**957–966.
25. **Jenkinson, H. F.** 1992. Adherence, coaggregation, and hydrophobicity of *Streptococcus gordonii* associated with the expression of cell surface lipoproteins. Infect. Immun. **60:**1225–1228.
26. **Jenkinson, H. F.** 1994. Cell surface protein receptors in oral streptococci. FEMS Microbiol. Lett. **121:**133–140.
27. **Kato, C., Y. Nakano, and K. Horikoshi.** 1989. The nucleotide sequence of the lipo-penicillinase gene of alkalophilic *Bacillus* sp. strain 170. Arch. Microbiol. **151:**91–94.
28. **Koide, A., and J. A. Hoch.** 1994. Identification of a second oligopeptide transport system in *Bacillus subtilis* and determination of its role in sporulation. Mol. Microbiol. **13:**417–426.
29. **Koivula, T. T., H. Hemilä, R. Pakkanen, M. Sibakov, and I. Palva.** 1993. Cloning and sequencing of a gene encoding acidophilic amylase from *Bacillus acidocaldarius*. J. Gen. Microbiol. **139:**2399–2407.
30. **Kolenbrander, P. E., R. N. Andersen, and N. Ganeshkumar.** 1994. Nucleotide sequence of the *Streptococcus gordonii* PK488 coaggregation adhesin gene, *scaA*, and ATP-binding cassette. Infect. Immun. **62:**4469–4480.
31. **Kolenbrander, P. E., N. Ganeshkumar, F. J. Cassels, and C. V. Hughes.** 1993. Coaggregation: specific adherence among human oral plaque bacteria. FASEB J. **7:**406–413.
32. **Kontinen, V. P., P. Saris, and M. Sarvas.** 1991. A gene (*prsA*) of *Bacillus subtilis* involved in a novel, late stage of protein export. Mol. Microbiol. **5:**1273–1283.
33. **Kontinen, V. P., and M. Sarvas.** 1993. The PrsA lipoprotein is essential for protein secretion in *Bacillus subtilis* and sets a limit for high-level secretion. Mol. Microbiol. **8:**727–737.
34. **Kuipers, O. P., M. M. Beerthuyzen, R. J. Siezen, and W. M. de Vos.** 1993. Characterization of the nisin gene cluster *nisABTCIPR* of *Lactococcus lactis*. Requirement of expression of the *nisA* and *nisI* genes for development of immunity. Eur. J. Biochem. **216:**281–291.
35. **Kuroda, A., M. H. Rashid, and J. Sekiguchi.** 1992. Molecular cloning and sequencing of the upstream region of the major *Bacillus subtilis* autolysin gene: a modifier protein exhibiting sequence homology to the major autolysin and the *spoIID* product. J. Gen. Microbiol. **138:**1067–1076.
36. **Lai, J.-S., M. Sarvas, W. J. Brammar, K. Neugebauer, and H. C. Wu.** 1981. *Bacillus licheniformis* penicillinase synthesized in *Escherichia coli* contains a covalently linked fatty acid and glyceride. Proc. Natl. Acad. Sci. USA **78:** 3506–3510.
37. **Lansing, M., S. Lellig, A. Mausolf, I. Martini, F. Crescenzi, M. O'Regan, and P. Prehm.** 1993. Hyaluronate synthase: cloning and sequencing of the gene from *Streptococcus* sp. Biochem. J. **289:**179–184.
38. **Lazarevic, V., P. Margot, B. Soldo, and D. Karamata.** 1992. Sequencing and analysis of the *Bacillus subtilis lytRABC* divergon: a regulatory unit encompassing the structural genes of the *N*-acetylmuramoyl-L-alanine amidase and its modifier. J. Gen. Microbiol. **138:**1949–1961.
39. **Martin, B., G. Alloing, C. Boucraut, and J.-P. Claverys.** 1989. The difficulty of cloning *Streptococcus pneumoniae mal* and *ami* loci in *Escherichia coli*-toxicity of *malX* and *amiA* gene products. Gene **80:**227–238.
40. **Mathiopoulos, C., J. P. Mueller, F. J. Slack, C. G. Murphy, S. Patankar, G. Bukusoglu, and A. L. Sonenshein.** 1991. A *Bacillus subtilis* dipeptide transport system expressed early during sporulation. Mol. Microbiol. **5:**1903–1913.
41. **Mayer, F.** 1993. Principles of functional and structural organization in the bacterial cell: 'compartments' and their enzymes. FEMS Microbiol. Rev. **104:**327–346.
42. **McLaughlin, J. R., C. L. Murray, and J. C. Rabinowitz.** 1981. Unique features of the ribosome binding site sequence of the Gram-positive *Staphylococcus aureus* β-lactamase gene. J. Biol. Chem. **256:**11283–11291.
43. **Moir, A., H. Kemp, C. Robinson, and B. M. Corfe.** 1994. The genetic analysis of bacterial spore germination. J. Appl. Bacteriol. Symp. **76**(Suppl.)**:**9S–16S.
44. **Nair, J., D. A. Rouse, and S. L. Morris.** 1992. Nucleotide sequence analysis and serologic characterization of the *Mycobacterium intracellulare* homologue of the *Mycobacterium tuberculosis* 19 kDa antigen. Mol. Microbiol. **6:**1431–1439.
45. **Nair, J., D. A. Rouse, and S. L. Morris.** 1993. Nucleotide sequence analysis and serologic characterization of a 27-kilodalton *Mycobacterium intracellulare* lipoprotein. Infect. Immun. **61:**1074–1081.
46. **Navarre, W. W., and O. Schneewind.** 1994. Proteolytic cleavage and cell wall anchoring at the LPXTG motif of surface proteins in Gram-positive bacteria. Mol. Microbiol. **14:**115–121.
47. **Neugebauer, K., R. Sprengel, and H. Schaller.** 1981. Penicillinase from *Bacillus licheniformis*: nucleotide sequence of the gene and implications for the biosynthesis of a secretory protein in a Gram-positive bacterium. Nucleic Acids Res. **9:**2577–2588.
48. **Nielsen, J. B. K., M. P. Caulfield, and J. O. Lampen.** 1981. Lipoprotein nature of *Bacillus licheniformis* membrane penicillinase. Proc. Natl. Acad. Sci. USA **78:**3511–3515.
49. **Nielsen, J. B. K., and J. O. Lampen.** 1982. Membrane-bound penicillinases in Gram-positive bacteria. J. Biol. Chem. **257:**4490–4495.
50. **Nielsen, J. B. K., and J. O. Lampen.** 1982. Glyceride-cysteine lipoproteins and secretion by Gram-positive bacteria. J. Bacteriol. **152:**315–322.
51. **Nielsen, J. B. K., and J. O. Lampen.** 1983. β-Lactamase-III of *Bacillus cereus* 569: membrane lipoprotein and secreted protein. Biochemistry **22:**4652–4656.
52. **Oligino, L., and P. Fives-Taylor.** 1993. Overexpression and purification of a fimbria-associated adhesin of *Streptococcus parasanguis*. Infect. Immun. **61:** 1016–1022.
53. **Pahl, A., and U. Keller.** 1994. *Streptomyces chrysomallus* FKBP-33 is a novel immunophilin consisting of two FK506 binding domains: its gene is transcriptionally coupled to the FKBP-12 gene. EMBO J. **13:**3472–3480.
54. **Pearce, B. J., A. M. Naughton, and H. R. Masure.** 1994. Peptide permeases modulate transformation in *Streptococcus pneumoniae*. Mol. Microbiol. **12:** 881–892.
55. **Perego, M., C. F. Higgins, S. R. Pearce, M. P. Gallagher, and J. A. Hoch.** 1991. The oligopeptide transport system of *Bacillus subtilis* plays a role in the initiation of sporulation. Mol. Microbiol. **5:**173–185.
56. **Rothe, B., P. Roggentin, R. Frank, H. Blöcker, and R. Schauer.** 1989. Cloning, sequencing and expression of a sialidase gene from *Clostridium sordellii* G12. J. Gen. Microbiol. **135:**3087–3096.
57. **Rudner, D. Z., J. R. Ledeaux, K. Ireton, and A. D. Grossman.** 1991. The *spo0K* locus of *Bacillus subtilis* is homologous to the oligopeptide permease locus and is required for sporulation and competence. J. Bacteriol. **173:**1388–1398.
58. **Ruhfel, R. E., D. A. Manias, and G. M. Dunny.** 1993. Cloning and characterization of a region of the *Enterococcus faecalis* conjugative plasmid, pCF10, encoding a sex pheromone-binding function. J. Bacteriol. **175:**5253–5259.
59. **Russell, R. R. B., J. Aduse-Opoku, I. C. Sutcliffe, L. Tao, and J. J. Ferretti.** 1992. A binding protein-dependent transport system in *Streptococcus mutans* responsible for multiple sugar metabolism. J. Biol. Chem. **267:**4631–4637.
60. **Sampson, J. S., S. P. O'Connor, A. R. Stinson, J. A. Tharpe, and H. Russell.** 1994. Cloning and nucleotide sequence analysis of *psaA*, the *Streptococcus pneumoniae* gene encoding a 37-kilodalton protein homologous to previously reported *Streptococcus* sp. adhesins. Infect. Immun. **62:**319–324.
61. **Schmitt, M. P., and R. K. Holmes.** 1994. Cloning, sequence, and footprint analysis of 2 promoter/operators from *Corynebacterium diphtheriae* that are regulated by the diphtheria toxin repressor (DtxR) and iron. J. Bacteriol. **176:**1141–1149.
62. **Schneider, R., and K. Hantke.** 1993. Iron-hydroxamate uptake systems in *Bacillus subtilis*: identification of a lipoprotein as part of a binding protein-dependent transport system. Mol. Microbiol. **8:**111–121.
63. **Slynn, G. M., R. L. Sammons, D. A. Smith, A. Moir, and B. M. Corfe.** 1994.

Molecular genetical and phenotypical analysis of the *gerM* spore germination gene of *Bacillus subtilis* 168. FEMS Microbiol. Lett. **121:**315–320.
64. **Smith, W. P., P. C. Tai, and B. D. Davis.** 1981. *Bacillus licheniformis* penicillinase: cleavages and attachment of lipid during cotranslational secretion. Proc. Natl. Acad. Sci. USA **78:**3501–3505.
65. **Sutcliffe, I. C.** 1994. The lipoteichoic acids and lipoglycans of Gram-positive bacteria: a chemotaxonomic perspective. Syst. Appl. Microbiol., in press.
66. **Sutcliffe, I. C., L. Tao, J. J. Ferretti, and R. R. B. Russell.** 1993. MsmE, a lipoprotein involved in sugar transport in *Streptococcus mutans*. J. Bacteriol. **175:**1853–1855.
67. **Tam, R., and M. H. Saier, Jr.** 1993. Structural, functional, and evolutionary relationships among extracellular solute-binding proteins of bacteria. Microbiol. Rev. **57:**320–346.
68. **Tanimoto, K., F. Y. An, and D. B. Clewell.** 1993. Characterization of the *traC* determinant of the *Enterococcus faecalis* hemolysin-bacteriocin plasmid pAD1: binding of sex pheromone. J. Bacteriol. **175:**5260–5264.
69. **Tao, L., I. C. Sutcliffe, R. R. B. Russell, and J. J. Ferretti.** 1993. Transport of sugars, including sucrose, by the *msm* transport system of *Streptococcus mutans*. J. Dent. Res. **72:**1386–1390.
70. **Tynkkynen, S., G. Buist, E. Kunji, J. Kok, B. Poolman, and Venema.** 1993. Genetic and biochemical characterization of the oligopeptide transport system of *Lactococcus lactis*. J. Bacteriol. **175:**7523–7532.
71. **von Heijne, G.** 1989. The structure of signal peptides from bacterial lipoproteins. Protein Eng. **2:**531–534.
72. **Wang, P. Z., and R. P. Novick.** 1987. Nucleotide sequence and expression of the β-lactamase gene from *Staphylococcus aureus* plasmid pI258 in *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. J. Bacteriol. **169:**1763–1766.
73. **Welby, M., J. De Bony, and J.-F. Tocanne.** 1988. Occurrence of acylated proteins in the membrane of the bacterium *Micrococcus luteus*. Biochim. Biophys. Acta **943:**190–198.
74. **Wise, K. S.** 1993. Adaptive surface variation in mycoplasmas. Trends Microbiol. **1:**59–63.
75. **Woodson, K., and K. M. Devine.** 1994. Analysis of a ribose transport operon from *Bacillus subtilis*. Microbiology **140:**1829–1838.
76. **Yon, J. R., R. L. Sammons, and D. A. Smith.** 1989. Cloning and sequencing of the *gerD* gene of *Bacillus subtilis*. J. Gen. Microbiol. **135:**3431–3445.
77. **Young, D. B., and T. R. Garbe.** 1991. Lipoprotein antigens of *Mycobacterium tuberculosis*. Res. Microbiol. **142:**55–65.
78. **Zuberi, A. R., A. Moir, and I. M. Feavers.** 1987. The nucleotide sequence and gene organization of the *gerA* spore germination operon of *Bacillus subtilis*. Gene **51:**1–11.