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Review

Transport capabilities of eleven gram-positive bacteria: Comparative genomic analyses

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Abstract

The genomes of eleven Gram-positive bacteria that are important for human health and the food industry, nine low G+C lactic acid bacteria and two high G+C Gram-positive organisms, were analyzed for their complement of genes encoding transport proteins. Thirteen to 18% of their genes encode transport proteins, larger percentages than observed for most other bacteria. All of these bacteria possess channel proteins, some of which probably function to relieve osmotic stress. Amino acid uptake systems predominate over sugar and peptide cation symporters, and of the sugar uptake porters, those specific for oligosaccharides and glycosides often outnumber those for free sugars. About 10% of the total transport proteins are constituents of putative multidrug efflux pumps with Major Facilitator Superfamily (MFS)-type pumps (55%) being more prevalent than ATP-binding cassette (ABC)-type pumps (33%), which, however, usually greatly outnumber all other types. An exception to this generalization is *Streptococcus thermophilus* with 54% of its drug efflux pumps belonging to the ABC superfamily and 23% belonging each to the Multidrug/Oligosaccharide/Polysaccharide (MOP) superfamily and the MFS. These bacteria also display peptide efflux pumps that may function in intercellular signalling, and macromolecular efflux pumps, many of predictable specificities. Most of the bacteria analyzed have no pmf-coupled or transmembrane flow electron carriers. The one exception is *Brevibacterium linens*, which in addition to these carriers, also has transporters of several families not represented in the other ten bacteria examined. Comparisons with the genomes of organisms from other bacterial kingdoms revealed that lactic acid bacteria possess distinctive proportions of recognized transporter types (e.g., more porters specific for glycosides than reducing sugars). Some homologues of transporters identified had previously been identified only in Gram-negative bacteria or in eukaryotes. Our studies reveal unique characteristics of the lactic acid bacteria such as the universal presence of genes encoding mechanosensitive channels, competence systems and large numbers of sugar transporters of the phosphotransferase system. The analyses lead to important physiological predictions regarding the preferred signalling and metabolic activities of these industrially important bacteria.

Keywords: Lactic acid bacteria; Transport proteins; Genomic analyses; Energetics

Contents

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1. Introduction

Membrane transport systems catalyze the uptake of essential nutrients, ions and metabolites, as well as the expulsion of toxic compounds, cell envelope macromolecules, secondary metabolites and the end products of metabolism. Transporters also participate in energy generation and interconversion, and they allow communication between cells and their environments. Transport is therefore essential to virtually every aspect of life.

Many Gram-positive bacteria are important in the food industry (see Table 1). These bacteria are used for the fermentation of vegetables and fruits, for the conversion of dairy products into cheeses and yogurt, and for the preparation of beer and wine. A key feature of lactic acid bacteria (LABs) when grown in laboratory growth media that lack hematin and related compounds is the inability to synthesize porphyrin (e.g., heme). Consequently, LABs grown under these conditions are devoid of "true" catalase activity and cytochromes. They lack electron transfer chains and rely on fermentation (i.e., substrate level phosphorylation) for the generation of energy. However if hematin is added to the growth medium, catalase and cytochromes may be formed, in some cases resulting in respiration [1–3].

Some LABs are both useful and harmful. For example, *Lactobacillus brevis* promotes both the preparation and the spoilage of beer [4]. Other Gram-positive bacteria, such as *Lactobacillus casei*, *Lactobacillus gasseri*, *Lactobacillus acidophilus*, and *Bifidobacterium longum*, in addition to being useful in the food industry, are natural inhabitants of the human gastrointestinal (GI) tract where they synthesize probiotic-like substances. These compounds stimulate the immune system, provide key nutrients and promote the development of a healthy organism [5].

The Lactic Acid Bacterial Genomics Consortium has recently carried out genome sequencing of eleven Gram-positive bacterial species [8,9]. The initial shotgun sequencing was conducted at the Joint Genome Institute in Walnut Creek, CA, and genome closure was achieved subsequently [9]. Nine of the sequenced bacteria are low G+C Gram-positive lactic acid bacteria (LABs) (*Pediococcus pentosaceus, L. brevis, L. casei, L. delbruekii, L. gasseri, Lactococcus lactis*, subspecies *cremoris, S. thermophilus*, *Leuconostoc mesenteroides* and *Oenococcus oeni*) while two of them (*B. longum* and *Brevibacterium linens*) are high G+C Gram-positive bacteria. These bacteria exhibit considerable diversity with respect to their ecological habitats, being found in milk, meat, plants, the GI tracts of humans and other animals, and elsewhere (see Table 1). They also differ with respect to their carbohydrate metabolic pathways (Table 2). Little is known about the transport capabilities of these important organisms.

In this review we report comprehensive analyses of the transport capabilities encoded within the eleven Gram-positive

Table 1
Characteristics of eleven Gram-positive bacteria important to the food and health industries

Bacterium	Strain	Abb [a]	Size [b] (Mb)	GC [c] content	No. of genes [d]	% TP [e]	Morphology	Habitat
Pediococcus pentosaceous	ATCC 25745	Ppe	1.8	37	1788	16.1	cocci	Plant materials and bacterial ripened cheeses
Lactobacillus brevis	ATCC 367	Lbr	2.3	46	2307	14.9	rod	Silage, sourdough and lactic acid fermented types of beer
L. casei	ATCC 334	Lca	2.8	46	2900	15.1	rod	Raw and fermented dairy products, fresh and fermented plants products, and the reproductive and intestinal tracts of humans and other animals
Lactobacillus delbruekii	ATCC BAA-365	Lde	1.8	49	2067	14.1	rod	Fermented dairy products: yogurt, Swiss and Italian types of cheese
Lactobacillus gasseri	ATCC33323	Lga	1.9	35	1874	16.0	rod	Human intestinal tract
Lactococcus cremoris	SK11	Lcr	2.4	35	2649	12.8	cocci	Green plants, fermented foods
Streptococcus thermophilus	LMD-9	Sth	1.8	39	2075	13.7	cocci	Fermented dairy products: yogurt and mozzarella cheese
Leuconostoc mesenteroides	ATCC 8293	Lme	2.0	37	2043	15.9	cocci	Widespread in natural environments: crop plants, fruits and vegetables, dairy products and bread dough
Oenococcus oeni	PSU-1	Ooe	1.7	37	1916	18.0	cocci	Fruit mashes and related habitats; employed in wineries to carry out malolactic conversion
Bifidobacterium longum	DJ010A	Blo	2.3	60	2015	15.1	branched rod	Intestines of humans and other animals including insects; female vagina
Brevibacterium linens	BL2	Bli	4.4	62	4040	13.4	rod	Milk and cheese crust

The order of the organisms in this table and throughout this report is based on their phylogenetic grouping starting with Ppe and following clockwise around the tree as shown in Fig. 1.

[a] The 3-letter abbreviation of the organism used throughout this report.

[b] Approximate size of the genome in Megabase pairs, based on the closed genome sequences, but including natural plasmids.

[c] Average genome G+C content.

[d] Number of genes identified in each genome including genes encoded on natural plasmids present in some of these organisms.

[e] Percent of genes encoding recognizable transport protein homologues.

bacterial genomes noted above. Identification of all homologues of recognized transport proteins included in the transporter classification database (TCDB) [6,7] and topological prediction were achieved using established methods [10]. The identified transporters were grouped according to topology, class, and function. The most important conclusions are summarized in this report.

2. Computer methods

Genomes of the eleven Gram-positive bacteria were screened for homologues of all proteins contained in TCDB, a membrane transport protein classification database (http://tcdb.ucsd.edu) [6,11]. FASTA-formatted protein sequences of the completed genomes were used. Each putative open reading

Table 2
Carbohydrate utilization and key properties of the eleven Gram-positive bacteria included in this study

Abbreviation [a]	Mode of fermentation [b]	Hexose fermentation pathway	Other information
Ppe	Homofermentative	Glycolysis	Pentoses fermented through 6-PG/PK. Hydrolyze arginine.
Lbr	Group III (obligately heterofermentative)	6-phosphogluconate/ phosphoketolase pathway (6-PG/PK)	Resistant to hop bittering substances like isohumulone. Produce biogenic amines.
Lca	Group II (facultatively heterofermentative)	Glycolysis	Pentoses fermented through the 6-PG/PK pathway.
Lde	Group I (obligately homofermentative)	Glycolysis	Do not ferment pentoses.
Lga	Group I (obligately homofermentative)	Glycolysis	Produce bacteriocins; associated with probiotic activities.
Lcr	Homofermentative	Glycolysis	Produce unique compounds via amino acid catabolism.
Sth	Homofermentative	Glycolysis	Produce exopolysaccharides; utilize proteins and peptides.
Lme	Heterofermentative	6-PG/PK	Ferment citrate to diacetyl and acetoin; produce dextrans and levans.
Ooe	Heterofermentative	6-PG/PK	Highly resistant to ethanol.
Blo	Heterofermentative	Fructose-6-phosphate phosphoketolase (F6PPK)	Resistant to methyl quinolones, heterocyclic amines, nitrosamines and azomethane.
Bli	Strict aerobe	Do not ferment sugars	Produce base, high levels of sulfur compounds, bacteriocins and carotenoids; degrade pesticides (DTT and DDE); metabolize amino acids to plant growth hormones.

[a] See Table 1 for organismal abbreviations.

[b] The physiological basis for the division of lactobacilli in three categories (Groups I–III) is the presence or absence of the key enzymes of homo- and heterofermentative sugar metabolism, fructose-1,6-bisphosphate aldolase and phosphoketolase, respectively [135–137].

frame was used as a query in the BLAST software [12,13] to search for homologues of proteins in the TCDB sequence library. In addition, each ORF was scanned with HMMTOP [14] to predict the number of putative transmembrane segments as reported in Table 3.

All of the potential transport proteins thus identified were subsequently examined in greater detail. Each transport protein identified was classified on the basis of sequence similarity into families and subfamilies of homologous transporters based on the classification system presented in TCDB. Each family/subfamily was noted with its standard abbreviation, its TC number and its typical substrates. The substrate specificities of particular homologues identified in the sequenced genomes have been predicted based on homology to functionally characterized genes and from their genomic context (see Table 1). Assignment to a family or subfamily within the Transporter Classification System usually allows specification of substrate type with high confidence [6,7,11]. The genome sequencing projects are described in Klaenhammer et al. [8] and Makarova et al. [9]. Phylogenetic trees (Neighbor joining) were based on multiple alignments generated with the Clustal X program [15] and drawn using the TreeView program [16].

Some readers may be interested in greater detail than provided in this paper regarding the identified transporters and the families/superfamilies to which they belong. These readers are advised to read this paper with the Transporter Classification Database (TCDB) at hand. The specificities, polarities of transport, mechanisms of transport, and transport protein and family descriptions are provided in detail therein. Novel substrates are continually being discovered for many transporters, especially drug exporters, some of which function physiologically in the absence of drugs. Moreover, some transport systems, initially characterized as narrow specificity systems, prove to exhibit broad specificities when examined in greater detail. When such substrates are known, they can be identified by searching TCDB, a database which is continually being updated as new experimental data become available. Table 5 provides a helpful guide as to where in TCDB to look for specific information. Because of the continual updating of TCDB, the interested reader who uses TCDB in conjunction with this paper will be up to date for years to come.

Thousands of published papers have described the functional characterization of transport systems and their constituent proteins. Several thousand such references can be found in TCDB. It is not possible to include them all in a review such as this one. We apologize to any researcher whose work we have mentioned without providing the reference(s) to the experimental work.

Table 3
Numbers of putative transport proteins with varying predicted topologies

TMS	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli
0	71	83	125	90	66	85	85	82	81	61	113
1	22	30	44	31	25	40	36	47	34	43	52
2	8	7	9	8	3	12	9	8	12	6	9
3	8	6	7	15	5	7	12	1	12	9	10
4	10	13	23	9	16	23	16	7	21	15	22
5	19	23	33	29	26	19	21	27	30	18	13
6	27	36	45	28	34	35	24	30	37	51	59
7	12	9	21	14	20	13	17	11	15	8	18
8	12	12	12	5	12	15	8	6	7	12	21
9	7	6	10	7	10	11	7	12	8	8	17
10	15	21	30	19	18	14	16	20	16	20	33
11	12	16	20	13	11	15	10	16	13	6	25
12	35	55	31	12	35	31	14	35	37	39	102
13	11	13	8	5	7	6	5	9	6	4	21
14	14	12	18	6	8	11	4	11	15	4	18
15	3	1	1		1	1		2			1
16			1		2	1				1	3
17											1
18	1		1								
19											2
24											1
25											1
Total	287	343	439	291	299	339	284	324	344	305	542

3. Genome statistics

Between 89 and 95% of all predicted transport proteins for the various genomes had significant matches (e-values $<10^{-3}$) in TCDB. Remaining predicted transmembrane transport proteins proved to be more distantly related to proteins included in TCDB. Some of these distantly related proteins could not be classified into the established TC families/subfamilies. They were therefore listed under the "Not Assigned" (N/A) category (see Table 4). The percentage of recognized genes that are predicted to encode transport proteins varies according to the microorganism analyzed between 12.8% (*L. lactis* spp. cremoris) and 18.0% (*O. oeni*). Percent transporters encoded within the various genomes showed no correlation with genome size (Table 1).

4. Transporter classification

Five distinct well-defined types of functionally characterized transport systems, based upon mode of transport and energy coupling mechanism, are recognized by the TC system [6,7,11]. These classes are: (1) channels, (2) secondary carriers, (3) primary active transporters, (4) group translocators of the sugar transporting phosphoenolpyruvate-dependent phosphotransferase system, and (5) transmembrane electron flow carriers. Of these primary classes, classes 1, 3 and 5 are subdivided (see Table 4). In addition, class 8 is reserved for accessory proteins involved in transport while class 9 specifies incompletely characterized transport systems. Class 9 is subdivided into classes 9A (recognized transporters of unknown mechanism) and 9B (putative transporters).

5. Organismal representation

Table 1 presents the eleven organisms included in this study. Two of these organisms, *B. longum* (Blo) and *B. linens* (Bli) are high G+C Gram-positive bacteria and therefore in a bacterial phylum different from that of the other nine organisms, which are classified as low G+C Gram-positive lactic acid bacteria. As expected, the phylogenetic tree based on the 16S rRNAs of

Table 4
Total numbers (upper) and total percent (lower) of transport proteins that belong to each TC class and subclass

Class	Subclass	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli
(1) Channels		13	9	10	5	10	14	9	14	8	8	5
	(1.A) α-Helical protein channels	10	8	10	5	8	11	8	8	8	8	5
	(1.C) Toxin channels	1	0	0	0	2	1	0	6	0	0	0
	(1.E) Holins	2	1	0	0	0	2	1	0	0	0	0
(2) Electrochemical potential-driven	(2.A) Porters	83	120	86	56	66	74	43	88	87	69	210
(3) Primary active transporters		106	154	217	173	131	176	165	155	154	171	218
	(3.A) P–P-bond hydrolysis-driven systems	103	151	213	171	131	169	163	153	148	165	204
	(3.B) Decarboxylation-driven systems	3	2	4	2	0	5	2	2	3	2	6
	(3.D) Oxidoreduction-driven systems	0	1	0	0	0	2	0	0	3	4	8
(4) Group translocators	(4.A) Phosphotransfer-driven systems	37	7	63	9	36	19	15	20	30	1	0
(5) Transmembrane electron carriers	(5.A) Two-electron transfer carriers	0	0	0	0	0	0	0	0	0	0	7
(8) Accessory factors involved in transport	(8.A) Auxiliary transport proteins	8	11	12	5	9	6	9	13	7	4	6
(9) Incompletely characterized transport systems		21	24	16	17	17	25	19	18	19	20	38
	(9.A) Recognized transporters of unknown biochemical mechanism	2	2	1	1	0	3	2	3	0	2	1
	(9.B) Putative uncharacterized transport proteins	19	22	15	16	17	22	17	15	19	18	37
N/A[1]		19	18	35	26	30	25	24	16	39	32	58
Total		287	343	439	291	299	339	284	324	344	305	542

Class	Subclass	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli
(1) Channels		4.5	2.6	2.3	1.7	3.3	4.1	3.2	4.3	2.3	2.6	0.9
	(1.A) α-Helical protein channels	3.5	2.3	2.3	1.7	2.7	3.2	2.8	2.5	2.3	2.6	0.9
	(1.C) Toxin channels	0.3	0.0	0.0	0.0	0.7	0.3	0.0	1.9	0.0	0.0	0.0
	(1.E) Holins	0.7	0.3	0.0	0.0	0.0	0.6	0.4	0.0	0.0	0.0	0.0
(2) Electrochemical potential-driven	(2.A) Porters	28.9	35.0	19.6	19.2	22.1	21.8	15.1	27.2	25.3	22.6	38.7
(3) Primary active transporters		36.9	44.9	49.4	59.5	43.8	51.9	58.1	47.8	44.8	56.1	40.2
	(3.A) P–P-bond hydrolysis-driven systems	35.9	44.0	48.5	58.8	43.8	49.9	57.4	47.2	43.0	54.1	37.6
	(3.B) Decarboxylation-driven systems	1.0	0.6	0.9	0.7	0.0	1.5	0.7	0.6	0.9	0.7	1.1
	(3.D) Oxidoreduction-driven systems	0.0	0.3	0.0	0.0	0.0	0.6	0.0	0.0	0.9	1.3	1.5
(4) Group translocators	(4.A) Phosphotransfer-driven systems	12.9	2.0	14.4	3.1	12.0	5.6	5.3	6.2	8.7	0.3	0.0
(5) Transmembrane electron carriers	(5.A) Two-electron transfer carriers	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3
(8) Accessory factors involved in transport	(8.A) Auxiliary transport proteins	2.8	3.2	2.7	1.7	3.0	1.8	3.2	4.0	2.0	1.3	1.1
(9) Incompletely characterized transport systems		7.3	7.0	3.6	5.8	5.7	7.4	6.7	5.6	5.5	6.6	7.0
	(9.A) Recognized transporters of unknown biochemical mechanism	0.7	0.6	0.2	0.3	0.0	0.9	0.7	0.9	0.0	0.7	0.2
	(9.B) Putative uncharacterized transport proteins	6.6	6.4	3.4	5.5	5.7	6.5	6.0	4.6	5.5	5.9	6.8
N/A[a]		6.6	5.2	8.0	8.9	10.0	7.4	8.5	4.9	11.3	10.5	10.7
Total		100	100	100	100	100	100	100	100	100	100	100

[a] Not assigned.

the eleven organisms (Fig. 1) shows Blo and Bli clustering loosely together, distant from all other organisms.

Two other organisms cluster together and separately from all others. These are *L. mesenteroides* (Lme) and *O. oeni* (Ooe) (Fig. 1). Moreover, situated in between this cluster and the large cluster of remaining lactic acid bacteria is a cluster including *Lactococcus lactic* spp. *cremoris* (Lcr) and *S. thermophilus* (Sth). Of the five remaining closely related organisms, *Pediococcus pentosaceous* (Ppe), *L. brevis* (Lbr), and *L. casei* (Lca) cluster together as do *L. delbruckii* (Lde) and *L. gasseri* (Lga). All tables included in this study will have the organisms presented according to their phylogenetic groupings starting with Ppe and proceeding clockwise around the tree shown in Fig. 1.

Table 1 presents the approximate genome sizes, G+C contents, numbers of identified genes, and percent transport proteins (TP) available for analysis. As can be seen from Table 1, most of the genomes sequenced are about 2 Mbp in size (1.7–2.8 Mbp), corresponding to 1788–2900 recognized genes, but that of Bli proved to be substantially larger (4.0 Mbp) with 4040 genes. Between 12.8 and 18.0% of these genes encode recognizable transport proteins. Except for the two high G+C Gram-positive bacteria, which have 60–62% G+C content, these bacteria have G+C contents ranging from 35 to 49%.

Table 2 summarizes some of the key properties of these bacteria, particularly with respect to carbohydrate utilization. This table summarizes the modes of sugar fermentation utilized

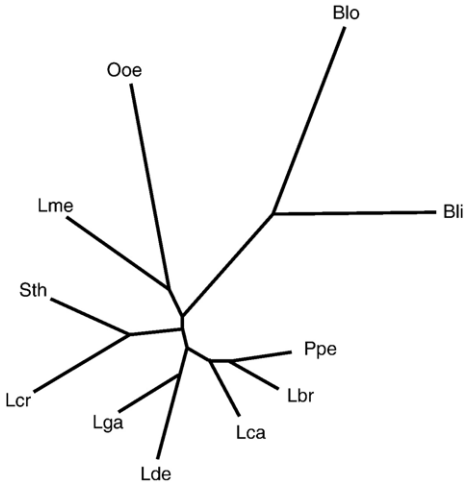

Fig. 1. Phylogenetic tree of the organisms included in this study based on their 16S ribosomal RNA sequences. The Clustal X program [15] was used to align the sequences.

and also presents other key properties, particularly those of commercial significance.

6. Topological distribution among Transport Proteins (TPs)

von Heijne and his associates have published several predictions and statistical analyses of transmembrane protein topologies [17,18] in specific organisms such as *Saccharomyces cerevisiae* [19], *Escherichia coli* [20], and over 50 000 bacterial inner membrane proteins [21]. These and other studies have revealed that for certain classes and families of transporters, topological predictions are surprisingly reliable although they are much less so for some other families of transport proteins [22]. The topological results reported here for the 11 Gram-positive bacteria examined in this report are in general agreement with previous analyses for transport proteins in other bacteria [6,23–30].

Table 3 and Fig. 2 present the predicted topologies of the transport proteins (TPs) identified on the basis of their homology to entries in TCDB. The percentages of these proteins in general decrease as the numbers of TMSs increase from 0 to 3; then they increase as the numbers of TMSs increase from 3 to 6; then they decrease again as the numbers of TMSs increase from 6 to 9 before again increasing. There are consistently more proteins with even numbers of TMSs than odd numbers of TMSs (Fig. 2). For example, there are more 10 TMS proteins than 9 or 11 TMS proteins, far more 12 TMS proteins than 11 or 13 TMS proteins, and more 14 TMS proteins that 13 or 15 TMS proteins. These facts undoubtedly reflect the mechanisms by which these proteins arose through evolutionary history, mechanisms that involved intragenic duplication [28]. Only Bli has proteins predicted to have homologues of 17 and 19–25 TMSs (see Table 3), while only Ppe and Lca have proteins with 18 putative TMSs (one such protein per organism).

The greatest variation between the organisms with respect to the numbers of TP homologues with a single predicted topology are the 5 and 12 TMS proteins. Bli has the most predicted 12 TMS TPs (18.8%) and the least 5 TMS TPs (2.4%) while Lde and Sth have the reciprocal relationship: 10.0% and 7.4% 5 TMS proteins but only 4.1% and 4.9% 12 TMS proteins, respectively. This correlates with the percentages of integral membrane proteins of MFS-type secondary active transporters versus ABC-type primary active transporters (see below). ABC uptake systems most commonly have integral membrane constituents of 5 TMSs per polypeptide chain while MFS permeases usually have 12. It is noteworthy that proportions of 5 or 12 TMS proteins in Bli and Lde relative to the other organisms do not correlate with the relative

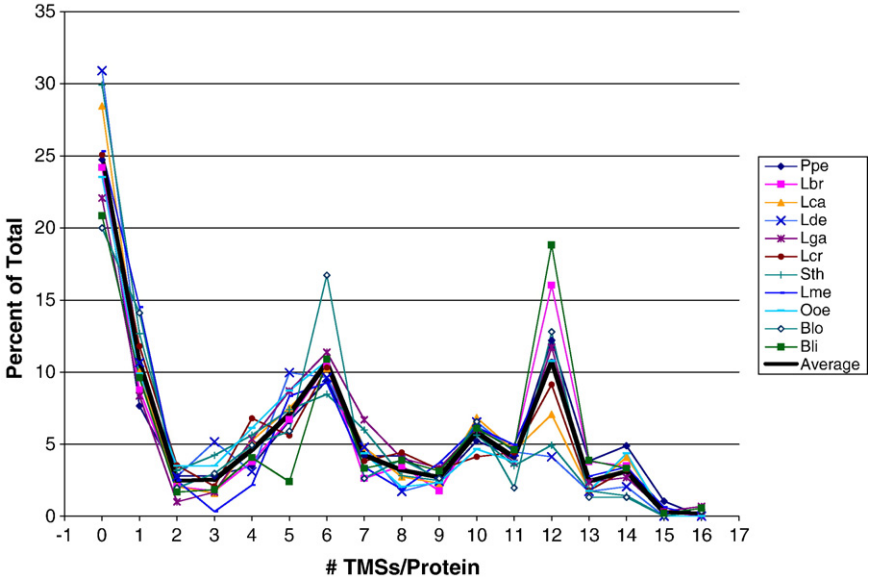

Fig. 2. Distribution of various topological types of transport proteins in eleven Gram-positive bacteria. The eleven organisms are indicated by the symbols shown to the right of the figure. The average values for all eleven organisms are shown by the thick black band.

proportions of 4 and 6 TMS proteins, or 11 and 13 TMS proteins, respectively. This fact suggests that the TMS predictions are remarkably accurate, at least for the members of the MFS and ABC superfamilies. Since Lde (with 4.1% of its TPs having 12 TMSs) clusters in Fig. 1 with Lga (with 11.7% of its TPs having 12 TMSs), it is clear that TP topological distribution does not correlate with organismal phylogeny. The percentage of 12 TMS TPs for Lga is the fifth largest of the eleven organisms examined (Table 3). Similarly, although Lbr and Lca cluster together in Fig. 1, they exhibit about a 2-fold difference in numbers of 12 TMS proteins. Based on these analyses, we conclude that transporter topology (Table 3) and mode of energy metabolism (Table 2) do not correlate well with organismal phylogeny (see below).

Three additional observations are worthy of note: (1) Blo has 16.7% 6 TMS proteins, more than any other organism examined; (2) Lde, which has the greatest percentage of 5 TMS proteins but the lowest percentage of 12 TMS proteins, also has the lowest percentage of 8 TMS proteins (Fig. 2), and (3) only Bli has proteins of greater than 18 TMSs. These facts must all be explained in terms of the types of transporters employed by these organisms.

7. Classes of TPs in the 11 organisms

Table 4 summarizes the distribution of TPs in the seven TC categories, and these values for defined classes 1–4 are shown in bar graphs in Fig. 3. Predicted transport proteins that could not be classified with certainty into specific TC families are indicated in the Not Assigned (N/A) category (Table 4). All organisms have only a small percentage of their TPs as channel proteins (5–14 proteins per organism or 1.7–4.1%). Almost all of these channel proteins are α-helical type channels of subclass 1.A. No β-type channels of class 1.B were detected, and very few channel-forming toxins (class 1.C) were identified; none of the latter was found for Lbr, Lca, Lde, Sth, Ooe, Blo and Bli. Ppe and Lcr each have 1, Lga has 2 and Lme has 6. The presence of class 1.A α-type channel proteins in these Gram-positive bacteria may relate to the need of these organisms to respond to stress such as osmotic shock [31]. Channel-forming proteins will be analyzed in greater detail below.

Secondary carriers (such as major facilitator superfamily (MFS) carriers; class 2) usually consist of single polypeptide chains, but primary active transporters (such as ABC carriers; class 3) usually consist of heterooligomeric complexes. The numbers of TPs listed in class 2 therefore probably accurately reflect the numbers of secondary carriers, but the numbers of TPs listed in class 3 are greatly in excess of the numbers of transport systems represented because of their multicomponent nature, probably by a factor of 3 or more. These facts must be taken into account when evaluating the data presented in Table 4 and Fig. 3. Bli has by far the highest class 2/3 ratio while Sth and Lde have by far the lowest, in agreement with the topological analyses discussed above. Excluding these three extreme organisms, this ratio for the remaining eight organisms studied differs by less than a factor of two.

Almost all of the primary active transport proteins are ATP hydrolysis-driven systems of class 3.A. Only one organism (Lca) has the transmembrane β-subunit of a decarboxylase-driven transporter, and consequently only Lca is predicted to have a decarboxylase-driven Na^+ pump of class 3.B. Only five organisms (Lbr, Lcr, Ooe, Blo and Bli) have subunits homologous to those of some form of oxidoreductase-driven cation transporting systems (class 3.D). However, since most of these electron transfer complexes are multisubunit, it is possible that only Bli possesses complete proton pumping electron flow systems (see Table 5).

Class 4 proteins (PTS) show tremendous variation in representation among the eleven organisms. Thus, Blo has only one recognized PTS permease, and this system is in the

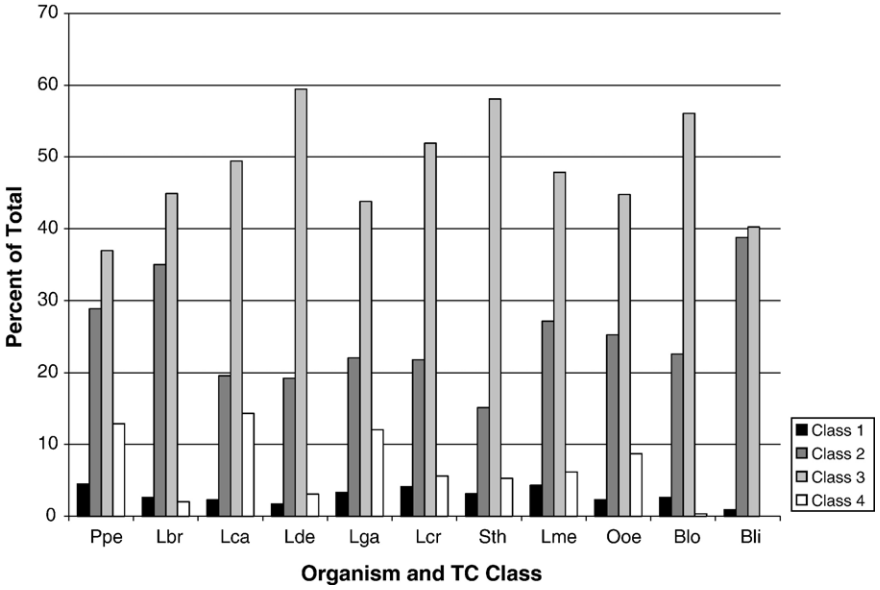

Fig. 3. Distribution of transport proteins belonging to each TC class in each of the eleven organisms analyzed. Class 1, channels; class 2, carriers (secondary active transporters); class 3, primary active transporters; class 4, PTS-type group translocators.

Table 5
Distribution of transport proteins in each superfamily/family for the eleven organisms analyzed

TC Family	Family	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli
Channels												
1.A.1	VIC	1	1			1				1	1	
1.A.8	MIP	4	3	2	1	1	4	1	2	1	2	2
1.A.11	ClC	1	1	1	1	2	2	3	2	1	2	
1.A.20	CytB								1	1		
1.A.22	MscL	1	1	1	1	1	1		1	1	1	1
1.A.23	MscS	1	1	1	1	1	1	1		1	1	1
1.A.29	UAC							1				
1.A.33	Hsp70	1	1	1	1	1	1	1	1	1	1	1
1.A.35	MIT	2	1	5	1	1	3	2	1	2	1	1
1.C.1	Colicin					2						
1.C.22	Lactococcin A						1					
1.C.24	Pediocin	1										
1.C.57	CCT								6			
1.E.11	Phi11 Holin						1					
1.E.14	LrgA Holin	1	1				1	1				
1.E.16	Cph1 Holin	1										
Carriers												
2.A.1.1	MFS-SP	2	3	3	2	1	1		3	6	2	5
2.A.1.2	MFS-DHA1	6	11	5	3	4	9	1	7	8	5	8
2.A.1.3	MFS-DHA2	8	9	15	4	5	9	1	8	11	7	14
2.A.1.4	MFS-OPA		1				1					1
2.A.1.5	MFS-OHS		1								1	
2.A.1.6	MFS-MHS			1					1		2	17
2.A.1.7	MFS-FHS		1			1				1	1	
2.A.1.8	MFS-NNP											2
2.A.1.11	MFS-OFA			1	1			1		1	1	2
2.A.1.14	MFS-ACS	1							1		1	2
2.A.1.15	MFS-AAHS	1	1	2	1	2			3	1	1	5
2.A.1.17	MFS-CP	1	2	1					1	1		2
2.A.1.18	MFS-PP										2	
2.A.1.19	MFS-OCT									1		
2.A.1.21	MFS-DHA3		1	2	1	1	1	1	1	4	2	6
2.A.1.23	MFS-BST			1	1	3						
2.A.1.24	MFS-UMF1		1								1	
2.A.1.26	MFS-UMF2											1
2.A.1.30	MFS-ADT		1	1								2
2.A.1.32	MFS-ACDE						1		1	3		
2.A.1.36	MFS-YnfM											1
2.A.1.37	MFS-AtoE	1	1			1			2	2		
2.A.1.38	MFS-EntS											1
2.A.1.40	MFS-AzgA	2	2	1	1	3	1	1	2	2	1	1
2.A.1.47	MFS-UMF6						1					
2.A.2	GPH	5	10	2	1	1	2	1	8	5	3	
2.A.3.1	APC-AAT	3	5	4	8	5	6	2	6	1	4	4
2.A.3.2	APC-APA	2	2		2		2					
2.A.3.3	APC-CAT	2	3	3		2	1	1	2	3	2	2
2.A.3.4	APC-ACT											2
2.A.3.5	APC-EAT	1										1
2.A.3.6	APC-ABT	1				2	1	1	1			4
2.A.3.7	APC-GGA	3	5	4	1	4	3			1		
2.A.3.8	APC-LAT	1	2	1	1	1				2		
2.A.3.10	APC-YAT	1			2			1				
2.A.3.11	APC-AGT	1	1	2		1						
2.A.4	CDF		2	2	1	1	2	2	2	2	1	1
2.A.6.2	RND-HAE1											2
2.A.6.4	RND-SecDF											2
2.A.6.5	RND-HAE2	1	2	1	1	1			3			3
2.A.7.1	DMT-SMR	2	2	1			1		1			1
2.A.7.3	DMT-DME	3	2	2	2	2	2	1		1	3	4
2.A.7.4	DMT-P-DME							1				
2.A.7.5	DMT-GRP	2	1	1	1	2	1	1	1	1		
2.A.7.7	DMT-RarD							1	1			2

(continued on next page)

Table 5 (*continued*)

TC Family	Family	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli
Carriers												
2.A.7.17	DMT-PE										1	
2.A.7.18	DMT-LicB-T			1								
2.A.8	GntP	1	3	1			2		2	2		2
2.A.9	Oxa1	2	2	2	2	2	2	2	2	2	1	2
2.A.10	KDGT											1
2.A.11	CitMHS			1								1
2.A.14	LctP					1						2
2.A.15	BCCT	1	1									8
2.A.17	POT	1	1	1		1	1	1				1
2.A.19	CaCA											1
2.A.20	PiT										1	3
2.A.21	SSS										1	9
2.A.22	NSS											1
2.A.23	DAACS	1	1	1		1		1				4
2.A.24	CCS			1			1		1			1
2.A.25	AGCS							1				4
2.A.26	LIVCS		3	3	1	2	1	1	1	1	1	
2.A.28	BASS								1			1
2.A.33	NhaA										1	1
2.A.35	NhaC	2	2		1	1			1		1	1
2.A.36	CPA1	2	3	2		1	1	1	2	1	1	1
2.A.37	CPA2	2	4	2	2	2	1		2	1		1
2.A.38	Trk		2				1	3	2		2	4
2.A.39	NCS1	1	2							1		2
2.A.40	NCS2	3	3	2	2	2	2	3	2	2	3	3
2.A.41	CNT	1	1						1	1		
2.A.44	FNT						1		1			
2.A.45	ArsB		1			1			1			
2.A.47	DASS	2			2	1				2		1
2.A.49	Amt		1	1	1		1	1	1	1	1	1
2.A.50	GUP									1		
2.A.51	CHR											2
2.A.52	NiCoT	1	1						1			
2.A.53	SulP						2					4
2.A.55	Nramp	3	3	3			2	2	1	3	1	2
2.A.56	TRAP-T	1	1									9
2.A.59	ACR3										2	2
2.A.61	DcuC	1										
2.A.62	NhaD			1								
2.A.63	CPA3											10
2.A.64	Tat							1				2
2.A.66.1	MOP-MATE	1	3	1			3	3	1	1	3	1
2.A.66.2	MOP-PST	1		1	7	2	1	2	3	3		1
2.A.66.4	MOP-MVF						1				1	3
2.A.67	OPT	1		1								
2.A.68	AbgT											3
2.A.69	AEC	2	2	2	1	2		1	1	5	3	1
2.A.72	KUP		1	1	1	1	2		1		2	
2.A.75	LysE											1
2.A.76	RhtB							1	1			2
2.A.77	CadD	1	3				1		1			1
2.A.78	LIV-E	1	2				1	1	1		2	1
2.A.79	ThrE											1
2.A.80	TTT			1								7
Primary Active Transporters												
3.A.1.1	ABC-CUT1		5	11	3	9	18			15	40	5
3.A.1.2	ABC-CUT2	3	9	6	3	2	8	5	8	6	12	11
3.A.1.3	ABC-PAAT	11	11	19	22	13	11	27	32	17	16	14
3.A.1.4	ABC-HAAT			5	1			5	5		5	11
3.A.1.5	ABC-PepT	6	19	17	40	8	13	23	10	12	16	38
3.A.1.6	ABC-SulT		1				1	1	1			4
3.A.1.7	ABC-PhoT	5	9	6	3	5	6	5	6	6	4	4
3.A.1.9	ABC-PhnT			4	4	5	3					3

Table 5 (*continued*)

TC Family	Family	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli
Primary Active Transporters												
3.A.1.11	ABC-POPT		5	5	4	4	4	8	4	8		4
3.A.1.12	ABC-QAT	6	6	10	2	2	4		6		1	4
3.A.1.14	ABC-FeCT		4	1			4	4	2			20
3.A.1.15	ABC-MZT	4	3	6	6	4	7	4	11	3	3	8
3.A.1.16	ABC-NitT		3		1			1				
3.A.1.17	ABC-TauT	2	1	3					3	3	1	
3.A.1.18	ABC-CoT			2					1	1		
3.A.1.19	ABC-ThiT					1						
3.A.1.20	ABC-BIT					1				1	2	
3.A.1.21	ABC-YbtPQ		1	3						2		
3.A.1.23	ABC-NiCoT	3	3	3	7	4	4	9	6	6	4	2
3.A.1.24	ABC-MUT	3	3	6	3	3	6	3	4	5	4	3
3.A.1.101	ABC-CPSE			1								
3.A.1.102	ABC-LOSE		1	2			1	3		1	1	4
3.A.1.103	ABC-LPSE					1	2	2			2	1
3.A.1.104	ABC-TAE	2	2									3
3.A.1.105	ABC-DrugE1	2	2	3	1		2			1	2	9
3.A.1.106	ABC-LipidE		3	8	11	7	1	7	1	4	5	4
3.A.1.108	ABC-GlucanE						1					
3.A.1.109	ABC-Prot1E			2		1		1	1	1		
3.A.1.110	ABC-Prot2E											1
3.A.1.111	ABC-Pep1E	1		1		2					1	
3.A.1.112	ABC-Pep2E	1	3	5	5	7	1	9	1	1		
3.A.1.114	ABC-DevE	1	1	2	1	2	2	3	1			
3.A.1.115	ABC-NatE		1	2					1	1		
3.A.1.117	ABC-DrugE2	3	3	5	5	1	7	2	2	3	1	3
3.A.1.118	ABC-McjD							1				
3.A.1.119	ABC-DrugE3											2
3.A.1.120	ABC-Drug RA1	2	3	3	1	2	2	3	3	1	3	5
3.A.1.121	ABC-Drug RA2	2	2	2	2	2	3	1		3		
3.A.1.122	ABC-MacB	2	2	6	2		1	3	3	6	6	3
3.A.1.123	ABC-Pep4E	3	2	8	4	5	4	2	4	2	1	2
3.A.1.124	ABC-Pep5E	2		9	2	3	2	1	2	3		2
3.A.1.125	ABC-LPT	1	1	3			3		1	1	3	2
3.A.1.126	ABC-βETE	1	5	2	2	2	3	1		3		2
3.A.1.128	ABC-SkfA-E			1				1	2	1		
3.A.1.201	ABC-MDR (ABCB)				1	1			2			
3.A.1.208	ABC-CT (ABCC)											1
3.A.1.210	ABC-HMT (ABCB)						2			1	1	
3.A.1.211	ABC-CPR (ABCA)									1		
3.A.2	F-ATPase	8	8	8	8	8	8	8	8	8	8	8
3.A.3	P-ATPase	6	8	12	8	13	13	7	6	4	5	8
3.A.5	Sec	11	6	6	6	6	6	6	9	6	6	7
3.A.7	IVSP	4	2	5	3	2	4		4	4	8	2
3.A.11	DNA-T	3	5	4	4	3	6	3	2	3	2	2
3.A.12	S-DNA-T	2	2	2	1	1	1	1	1	1	1	1
3.A.14	FPE	2	2	1	2	2	3	2	2	4		
3.B.1	NaT-DC[a]	3	2	4	2		5	2	2	3	2	6
3.D.2	PTH											3
3.D.3	QCR											4
3.D.4	COX										1	
3.D.6	NFO						1					
3.D.7	HHO						1					
3.D.8	FMF-DH	8	8	8	8	8	8	8	8	8	8	8
Group Translocators												
4.A.1	Glc	5		12	3	6	5	8	4	5	1	
4.A.2	Fru	2		6	1	2	2	3		1		
4.A.3	Lac	10	2	14	2	13	7	1	7	10		
4.A.4	Gut	1		4								
4.A.5	Gat		1	5		4				3		
4.A.6	Man	19	4	18	3	11	3	3	7	8		
4.A.7	L-Asc			4			2		2	4		

(continued on next page)

Table 5 (*continued*)

TC Family	Family	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli
Transport electron carriers												
5.A.1	DsbD											4
5.A.3	PMO											3
Auxiliary transport proteins												
8.A.1	MFP							1	1			
8.A.3	MPA1	2			2	2		2	3	2		1
8.A.5	KTN	3	7	5		1		1	3	1	2	5
8.A.7	EI	1	1	1	1	1	1	1	1	1	1	
8.A.8	HPr	1	1	1	1	1	1	1	1	1	1	
8.A.9	rBAT	1	2	5	1	4	4	2	4	2		
8.A.12	BEA							1				
Poorly characterized transporters												
9.A.2	MerTP				1							
9.A.4	PnuC		1				2		1			
9.A.8	FeoB			1			1	1	1			
9.A.10	OFeT	1	1									
9.A.17	PbrT							1			1	
9.A.19	MgtE	1							1			1
9.A.25	RD1										1	
9.B.3	MPE	2	3	2	2	2	2	2	2	2	2	2
9.B.10	MarC	1										
9.B.14	HEP											1
9.B.17	FAT	1	2	1	1		4	1	3	3	4	18
9.B.20	MgtC	1		1	1	1	1			1		1
9.B.22	PerM	3	5	2	3	2	2	2	3	2	1	2
9.B.24	TEGT	1	1	1	1	2	1	1	1	1	1	
9.B.25	YbbM		1	1	1	1		1	1		1	
9.B.27	YdjX-Z			2			1					1
9.B.29	YebN										1	
9.B.30	Hly III	1	1	1	1	1	1	1	1	1	2	
9.B.31	YqiH	1	1	1	2	2	1	1	1	1		
9.B.32	VGP	5	3	1		2	5	6		4	2	1
9.B.35	Transthyretin											1
9.B.37	HCC	1	1	2	2	2	1	1	1	1	2	3
9.B.44	YiaAB						1					
9.B.48	UIT1											2
9.B.50	UIT3	1	2		1	1						1
9.B.53	UIT6											1
9.B.55	UIT8		1						1	2		
9.B.57	UIT10											1
9.B.59	CstA						1				1	1
9.B.63	9-PME	1	1		1	1	1	1	1	1	1	1
Unclassified transporters												
N/A	N/A	19	18	35	26	30	25	24	16	39	32	58

[a] In the NaT-DC family (3.B.1), the presence of a homologue of the decarboxylase is not indicative of the presence of a transporter as these subunits can function in cytoplasmic decarboxylation independently of a β-subunit which transports Na^+.

Glucose (Glc) Family (4.A.1). Lbr has just 7 recognized PTS protein homologues with four Enzyme IIC permeases, two of the Lac family, one of the Gat family (just the IIC constituent), and one of the mannose (Man) family. Of these, only the Man family system is apparently a full Enzyme II complex with all requisite constituents. Lca has the most (63) PTS protein homologues with all 7 PTS families represented. This organism has between 1 and 8 intact PTS permeases per PTS family with 8 representatives in the Glc family and 1 in the Gut family. Lga and Ppe have 36 and 37 PTS proteins, respectively. These two organisms possess complete glucose (Glc), fructose (Fru), lactose (Lac) and mannose (Man) family systems, and Lga also has a galactitol (Gat)-type system. Lcr and Ooe encode in their genomes 19 and 30 PTS proteins, respectively. Both have glucose, lactose, mannose and L-ascorbate (Asc)-type systems, and Lcr also has a fructose-type system while Ooe additionally has a galactitol-type system. There is a reasonable correlation between prevalence of PTS permeases (Table 4) and organismal phylogeny (Fig. 1).

Transmembrane electron carriers that transfer electrons from one side of the cytoplasmic membrane to the other (TC class 5) are lacking in 10 of the 11 organisms. Bli is the only exception. It has 7 such proteins (1.3%) with representation in the DsbD and PMO families.

The eleven organisms have between 4 and 13 auxiliary transport proteins each (class 8). All except Bli possess Enzyme I and HPr of the phosphotransferase system (PTS). Two organisms each possess a membrane fusion protein (MFP)

family member, which in Gram-positive bacteria usually facilitates export of peptides via an ABC efflux pump [32]. Several organisms have homologues of the K^+ transport/nucleotide binding regulatory protein (KTN) family, but these homologues do not necessarily function to regulate transport and may serve an unrelated function.

Of the poorly characterized class 9 transporters, the percentages are fairly constant in the 11 organisms (3.6–7.4%). Within subclass 9A, we find putative transporters for heavy metals, including Fe^{3+} (OFeT and FeoB), Pb^{2+} (PbrT), Hg^{2+} (MerTP), and Mg^{2+} (MgtE). Three organisms also possess homologues of the nicotinamide mononucleotide (NMN) uptake permease (PnuC) family.

Within subclass 9B, several potentially important families are represented. However, the evidence that many of these function in transport is marginal or nonexistent. For example, all or most members of the FAT family of acyl CoA synthetases may not play a role in fatty acid transport [33]. The PerM, TEGT and YbbM families include 7 TMS protein members, and the YdjX-Z and YqiH families include 5 or 6 TMS members. The HCC family includes members that may be pore-forming hemolysins, and VGP family members may catalyze vectorial polymerization of polysaccharides destined to be extracellular. Of greatest interest is the Putative Bacterial Murine Precursor Exporter (MPE) family (9.B.3) which is represented in all 11 organisms, usually with 2 or 3 paralogues per organism. These putative transporters may be required for synthesis of the bacterial cell wall.

8. Channel-forming proteins

As summarized in Table 5, all of the organisms examined contain recognized α-type channel proteins of TC class 1.A. These vary in number from 5 for Lde and Bli to 11 for Lcr.

Three channel protein families (Voltage-gated Ion Channel (VIC), Large Conductance Mechanosensitive Channel (MscL) and Small Conductance Mechanosensitive Channel (MscS)) as well as some aquaporins of the MIP family (see below) may function primarily to protect the bacteria against osmotic stress [31,34–41]. All of the 11 bacteria examined have at least one such channel suggesting that relief from osmotic stress is of general importance to these bacteria as has been demonstrated for *L. lactis* [31]. However, the numbers and types of these channels vary. For example, five organisms (Ppe, Lbr, Lga, Ooe and Blo) have at least one of each of these three channel types. Lca, Lde and Lcr have two such channel proteins, one of the MscL family and one of the MscS family. In *L. lactis*, only the *mscL* gene is apparently expressed although both the MscL and MscS channel proteins from this organism are functional [31]. Lme is the only organism with just one of these channel proteins (MscL). In fact, Lme is the only bacterium that lacks an MscS channel homologue. Surprisingly, no organism has more than one member of either the VIC, MscL or MscS family. Multiple MscS family paralogues are found in many bacteria although few bacteria have more than one MscL channel [39].

The eleven organisms have from one to three chloride channel proteins of the ClC family (Table 5; Ref. [7]). Thus, Ppe, Lbr, Lca, Lde and Ooe have just one of these anion-selective channels; Lga, Lcr, Lme, Blo have 2, and Sth has three. In *E. coli*, these channels are known to promote resistance to extreme acid stress [42].

All eleven bacteria contain at least one heavy metal ion channel of the MIT (CorA) family [43,44], and five organisms have between two and five members. These divalent metal ion channels are important for the uptake of Mg^{2+}, Co^{2+} and Ni^{2+} in other bacteria in response to the membrane potential, negative inside [45]. It can be presumed that these channels provide the same function in the organisms examined here.

Glycerol facilitators and aquaporins of the MIP family allow transmembrane passage of several small neutral molecules including glycerol and other polyols, dihydroxyacetone, water, CO_2, urea and ammonia [46–48]. In prokaryotes, most MIP family members transport glycerol as their primary function. However, synthesis of aquaporins has been shown to be induced after transfer of certain bacteria to hyperosmotic media [49,50]. This fact suggests a role in osmotic shock protection.

Members of the MIP family of aquaporins and glycerol facilitators are found in all 11 bacteria examined here. Four of the LABs have a single putative glycerol facilitator, and the rest have two or more of them. Lactic acid bacteria in general cannot utilize glycerol as a sole carbon source [51], but they can often co-metabolize glycerol and glucose [52]. The universal occurrence of glycerol facilitators in these organisms may therefore reflect their abilities to use glycerol in the presence of a second carbon source. Five of the LABs (Ppe, Lbr, Lme, Blo and Bli) have putative aquaporins that may play a role in osmotic shock responses. A single bacterium (Sth) has a urea/short chain amide (UAC) family member. This protein may allow uptake of various amides as sources of nitrogen [53,54] (Table 5).

Hsp70 chaperone proteins are listed in Table 5 under class 1.A. This is because some eukaryotic Hsp70 proteins have been shown to insert into membranes to form channels [55,56]. All of the organisms examined here have such a homologue. These proteins function primarily in protein folding as part of the general stress response system [57–60]. There is no evidence for channel formation by Hsp70 homologues in prokaryotes. However, merely because of the documentation of channel formation in eukaryotic homologues, we have included these chaperones in this tabulation.

Several toxins of TC class 1.C were identified. In general, these are rare in the lactic acid bacteria examined. Six members of the large protein CCT toxin family were identified in Lme, but none was found in the other ten organisms analyzed. Members of this family are found in both Gram-negative and Gram-positive bacteria [61,62]. Surprisingly, Lga has two colicin homologues. Colicins are usually restricted to enteric bacteria. Finally, two of the organisms (Ppe and Lcr) encode peptide toxins (bacteriocins), Pediocin and Lactococcin A, respectively, which are exported to exert their toxic activities on other microorganisms [63,64].

Ppe, Lbr, Lcr and Sth each encode either one or two recognizable holin(s). These small oligomeric proteins may

promote cell death in response to stress stimuli by allowing export of autolysins that digest the bacterial cell wall [65]. These proteins might therefore play a role in programmed cell death [66–68].

9. Secondary carrier superfamilies

9.1. MFS (2.A.1)

There is tremendous variation in the numbers of secondary carriers in the different bacteria examined, and the same is true for the individual families of secondary carriers. The largest such family is the major facilitator superfamily (MFS) [69,70]. Sth has 5 such carriers while Bli has 70. All other bacteria have intermediate numbers (Table 5). Lde has 14, Ooe has 41 and all other bacteria have between 21 and 35. Of equal interest are the probable substrate specificities of these transporters. In all but two of the bacteria analyzed, Lga and Bli, drug exporters comprise the majority of all MFS permeases. This is surprising since only 3 of the 47 recognized MFS families are primarily concerned with export of hydrophobic and amphipathic substances. These three MFS families (called DHA1–3) are represented in all eleven bacteria with the exceptions of Ppe which lacks a DHA3 member. In Sth, 3 of the 5 MFS permeases are putative drug exporters; in Lde, 8 of the 14 have this function, and in Lcr, 19 of the 24 MFS homologues may export drugs. Of all the organisms analyzed, the high G+C Gram-positive bacterium, Bli, has the most non-drug-specific MFS transporters. Thus, in Bli, 28 of the 70 MFS permeases probably act on drug-like substances, and more of these systems transport anionic compounds than drugs. It should be noted that DHA1–3 exporters can act on a wide variety of compounds including hydrophilic metabolites (see TCDB). However, members of these three families always catalyze substrate efflux [69].

The sugar uptake systems of the MFS include members of the sugar porter (SP) family and the oligosaccharide:H^+ symporter (OHS) family as well as the fucose:H^+ symporter (FHS), sialate:H^+ symporter (SHS) and polyol porter (PP) families. These families are poorly represented in most of these Gram-positive bacteria. In fact, all but Lbr (5), Ooe (7), Blo (6) and Bli (5) have just 1–3 such systems.

Virtually all remaining MFS families represented (see Table 5) consist of permeases with specificity for inorganic and organic anions. With the sole exception of Bli, these anion transporting MFS permease families are poorly represented. The BST family is found only in Lga which has 3 members and in Lca and Lde which have 1 member each. Multiple paralogues of the OFA, ACS and AAHS families are found only in Bli, which has 2, 2 and 5 members of these three families, respectively, although Sth has three AAHS family paralogues. It is clear that Bli exhibits a diversity of MFS permeases that far exceeds that of the other organisms examined.

9.2. GPH (2.A.2)

In view of the paucity of MFS uptake systems for sugars, it is surprising that putative reducing oligosaccharide and non-reducing glycoside uptake systems of the GPH family (2.A.2) are represented in all of the eleven organisms except Bli, the one organism with by far the most MFS permeases. The other bacteria have between 1 and 10 GPH permeases. Of the lactic acid bacteria, only Lca, Lde, Lga and Ooe have more MFS sugar permeases than GPH permeases. All functionally characterized members of the GPH family take up oligosaccharides preferentially (hence the name Glycoside–Pentoside–Hexuronide:Cation Symporter Family (TC #2.A.2)). Some reports suggest that reducing sugars may also be substrates, but this suggestion has been disputed for some GPH members [71,72]. This suggests that most lactic acid bacteria preferentially take up oligosaccharides and non-reducing glycosides over simple sugars via secondary carriers since almost all members of the GPH family take up glycosides and oligosaccharides rather than reducing monosaccharides [71,73–75]. This observation may reflect the fact that the former compounds are common plant products. They are also generated in animals by the degradation of glycolipids and glycoproteins [76,77]. In lactic acid bacteria, simple hexoses may be taken up primarily via the PTS (see below).

9.3. APC (2.A.3)

The Amino Acid/Polyamine/Organocation superfamily, the largest superfamily of amino acid and amine uptake transporters [78], is represented in all 11 Gram-positive bacteria studied here. Six to 18 members of this superfamily are encode in each of the various genomes. Of the eleven currently recognized families within the APC superfamily, the AAT family (2.A.3.1) is most prevalent with 1–8 representatives per genome. However, ten of the eleven APC families are found in these Gram-positive bacteria, a most surprising observation since members of three of these families had not been identified in bacteria when this superfamily was last described [78]. These three families are the ACT (2.A.3.4), LAT (2.A.3.8) and YAT (2.A.3.10) families. The ACT family is represented only in Bli (2 members), and the YAT family is found only in Ppe (1 member), Lde (2) and Sth (1). However, members of the LAT family are found in six of the eleven organisms (1–2 members each).

The glutamate:γ-aminobutyrate (GABA) antiporter (GGA) family (2.A.3.7) is found with from 1 to 5 members in 7 of the 11 bacteria. All bacteria except Lde have 1–3 members of the cationic amino acid transporter (CAT) family. Two organisms (Ppe and Bli) have 1 member each of the ethanolamine transporter (EAT) family. One to 4 members of the archaeal/bacterial transporter (ABT) family are found in 6 of the 11 organisms, the exceptions being Lbr, Lca, Lde, Ooe and Blo. Four organisms (Ppe, Lbr, Lca and Lga) have 1–2 members of the aspartate/glutamate transporter (AGT) family, while four organisms (Ppe, Lbr, Lde and Lcr) have 2 members of the basic amino acid/polyamine antiporter (APA) family. It is clear that these organisms can take up a wide range of amino acids and their derivatives. The characteristics of these families and primary references describing their members can be found in TCDB [78].

9.4. RND (2.A.6)

The RND superfamily of efflux pumps is represented in 7 of the 11 organisms studied (1–7 members per organism) (Table 5). This superfamily includes eight currently recognized families. Four of these families (HME, HAE1, NFE and ORF4) predominate in Gram-negative bacteria; a fifth (SecDF) is found in both Gram-negative and Gram-positive bacteria; a sixth (HAE2) is found only in Gram-positive bacteria; a seventh (HAE3) is found primarily in archaea; and the eighth (EST) is restricted largely to eukaryotes [79].

The HAE1 family of drug and lipid exporters is represented only in Bli (2 member), as is the SecDF family of protein secretion factors (1 member). However, the HAE2 family of drug and lipid exporters is found in 7 of the 11 organisms with 1–3 members each. No other RND family is represented. It is clear that the RND superfamily is underrepresented in these bacteria.

9.5. DMT (2.A.7)

The Drug Metabolite Transporter (DMT) superfamily consists of 18 currently recognized families [80], seven of which are represented in the Gram-positive bacteria under study. In fact, all eleven organisms have DMT representation (2–7 members/organism) (Table 5).

Of the 18 families, by far the greatest representation is in the Drug/Metabolite Efflux (DME) family, the largest of the prokaryotic families found in the DMT superfamily. Ten of the eleven organisms have DME pumps, and each organism may have from 1 to 4 members. Six organisms (Ppe, Lbr, Lca, Lcr, Lme and Bli) have small multidrug resistance (SMR) export systems, and the 9 lactic acid bacteria have representation in the Glucose/Ribose Uptake Porter (GRP) family (1–2 members). These proteins probably take up glucose via a proton symport mechanism [81]. Only the two high G+C Gram-positive bacteria (Blo and Bli) lack GRP family members.

Members of other DMT families are sparsely represented. For example, Lca has 1 member of the choline uptake transporter (LicB-T) family, Sth, Lme and Bli have 1, 1 and 2 members, respectively, of the chloramphenicol-sensitivity protein (RarD) family, while Blo has a single member of the paraquat (methyl viologen) exporter (PE) family. Surprisingly, Sth has a member of the plant drug/metabolite exporter (P-DME) family, which, however, is closely related to the prokaryotic DME family.

9.6. SSS (2.A.21)

The Sodium:Solute Symporter (SSS) superfamily [82] was not represented in the low G+C Gram-positive bacteria studied but was found in the high G+C Gram-positive bacteria. Bli has nine members while Blo has one. The single SSS protein in Blo may be a proline uptake porter, and the SSS proteins in Bli most closely resemble sodium iodide (3), proline (2), sugar (2), monocarboxylate (1), and phenylacetate (1) uptake permeases of other organisms.

9.7. MOP (2.A.66)

The multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) superfamily [83] is present in all eleven bacteria under study with 2–7 members each. The vast majority of these belong to the polysaccharide transporter (PST) family within the MOP superfamily. Most organisms have 1–7 such proteins, although Lbr and Blo lack these proteins. These exporters catalyze secretion of a variety of polysaccharides and their precursors using a proton antiport mechanism. All but 2 of the organisms have at least one MATE-type multidrug resistance pump with 1–3 such systems each. Three organisms, a single low G+C Gram-positive bacterium (Lcr) and the two high G+C Gram-positive bacteria (Blo and Bli) have representation in the bacterial Mouse Virulence Factor (MVF) family. The transport substrates of members of this family are not known.

10. Smaller families of secondary carriers

10.1. Divalent cations

Monovalent and divalent cation concentrations must be strictly controlled for optimal growth. Bacteria therefore possess channels, carriers and primary active pumps that regulate these concentrations. Moreover, among the carriers, some are strict cation:cation antiporters, others are electrogenic uniporters, and still others are cation:cation symporters as documented particularly well in *E. coli* [84–87].

Five families of carriers for divalent cations are represented in these Gram-positive bacteria. The Calcium:Cation Antiporter (CaCA) family (2.A.19) is found only in Bli. These systems catalyze Ca^{2+} efflux. The Cation Diffusion Facilitator (CDF) family (2.A.4) is found in 10 of the 11 organisms. Only Ppe lacks representation. These are likely to export heavy metal divalent cations such as Zn^{2+}, Cd^{2+} and Co^{2+}. The Ni^{2+}–Co^{2+} Transporter (NiCoT) family (2.A.52) is found only in three organisms (Ppe, Lbr and Lme). All functionally characterized members of this family function in the uptake of Ni^{2+} and/or Co^{2+}. The Nramp family (2.A.55) is represented in all but two (Lde and Lga) of the eleven organisms examined, and these 9 organisms have between 1 and 3 members. Nramp carriers take up a variety of heavy metals (Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Pb^{2+}) using a cation symport mechanism. The poorly characterized CadC family (2.A.77) is also represented in Table 5. Five organisms each have 1–3 members of this family of putative Cd^{2+} efflux pumps.

10.2. Monovalent cations

Nine families of monovalent cation transporters are represented in Table 5. None of these is found in all of the eleven organisms. NhaA family (2.A.33) members are found only in the high G+C Gram-positive bacteria, Blo and Bli, each having one member. The NhaC family (2.A.35) is found in 7 of the 11 organisms with 1 or 2 members each. The CPA1 family (2.A.36) was found in all organisms except Lde while the distantly related CPA2 family (2.A.37) was lacking only in Sth

and Blo. A maximum of 4 members of each of these cation: proton antiporters is encoded in any one genome.

The Trk family (2.A.38) of monovalent cation symporters is represented in 6 of the 11 organisms, but one bacterium (Sth) has three of these systems while another (Bli) has four. A single protein of the NhaD family (2.A.62) of Na^+/H^+ antiporters is found only in Lca.

Subunits of the CPA3 family of K^+:H^+ and Na^+:H^+ antiporters (2.A.63) are found only in Bli. Normally these systems have 7 subunits, but 10 are reported for this organism. Possibly these subunits are constituents of two distinct systems. Finally, the KUP family (2.A.72) of K^+ uptake systems is found in 7 of the 11 bacteria, and the Amt family (2.A.49) of NH_3 channel proteins [88] is found in nine. This last family was previously thought to transport NH_4^+ by a carrier-type mechanism, but high-resolution X-ray structures of the *E. coli* AmtB protein clearly suggest that at least this member of the family deprotonates NH_4^+ at the external surface of the pore and transports NH_3 via a simple hydrophobic channel-type mechanism [88,89].

10.3. Anions

Seventeen families of secondary carriers represented in Table 5 include members that probably transport anionic compounds. Most of these are specific for organic anions that can be used as sources of carbon (gluconate, ketodeoxygluconate, tri-, di- and monovalent acids such as citrate, succinate and lactate, respectively, and bile salts), but several can transport inorganic anions as well (formate, nitrate, arsenite, sulfate, phosphate). Perusal of Table 5 reveals that the gluconate porter (GntP) family (2.A.8) is represented in seven organisms. Three additional well-represented families (CCS, DASS and DAACS) primarily transport organic anions while three other well-represented families (PiT, FNT and SulP) transport inorganic anions. Other anion transporting families are represented to a lesser degree.

Interestingly, the poorly characterized auxin efflux carrier (AEC) family (2.A.69) is found in 10 of the 11 organisms examined with between 1 and 5 members per organism. The few characterized prokaryotic members of this family catalyze uptake of dicarboxylates such as malate and malonate [90,91]. Many of these anion-transporting families are members of the IT superfamily [92].

10.4. Amino acids and peptides

In addition to the APC superfamily, several other families represented in Table 5 include members that catalyze amino acid uptake [93]. The NSS family (2.A.22) is represented only in Bli. Glycine and alanine permeases of the AGCS family of semipolar amino acid uptake porters (2.A.25) are represented in 2 of the eleven organisms.

Three families of amino acid efflux porters are represented in Table 5. The first of these is the LysE basic amino acid exporters (2.A.75), present only in Bli. The LysE family is a member of the LysE superfamily, all members of which catalyze substrate efflux [94]. The second amino acid efflux family represented is the RhtB semipolar amino acid exporters (2.A.76), present in Sth, Lme and Bli. This family is also a member of the LysE superfamily [94]. Finally, members of the LIV-E hydrophobic amino acid exporter family (2.A.78) are present in Ppe, Lbr, Lcr, Sth, Lme, Blo and Bli. The LIV-E family is unrelated to the LysE superfamily, and all characterized members of this family export hydrophobic amino acids [95,96].

Peptide uptake permeases represented include those of the POT family (2.A.17) found in 7 organisms, the OPT family (2.A.67) found in 2 organisms, and the AbgT family (2.A.68) found in 1 organism. The Tat family of pmf-driven protein secretion permeases (2.A.64) was found in Sth and Bli. The Oxa1 family (2.A.9), involved in the insertion of integral membrane proteins into the plasma membrane bilayer, is represented in all organisms examined (Table 5).

10.5. Nucleobases and nucleosides

Three families found in Table 5 are concerned primarily with the uptake of nucleobases and nucleosides. These are the NCS1 family (2.A.39) (4 organisms represented), the NCS2 family (2.A.40), present in all 11 organisms, and the CNT family (2.A.41), present in 4 of the 11 bacteria. Multiple such systems are present in all eleven bacteria.

11. Primary active transporters

Primary active transporters couple a primary source of energy to transport, and they are consequently often multicomponent and always multidomain systems. They couple transport to processes such as ATP hydrolysis, organic acid decarboxylation and electron transfer. A quick look at Table 5 reveals that each of the eleven organisms has multiple (66–175) ABC system proteins, a single F-type ATPase, always with 8 dissimilar subunits as expected, several (4–13) P-type ATPases, and a number of ATP-driven macromolecular transport systems. One organism has an organic acid decarboxylation-driven Na^+ exporter, but with the exception of Bli, none has a monovalent cation (H^+ or Na^+)-translocating electron carrier. The individual families of primary active transporters and the members identified will be discussed below. In the following sections we will discuss homologues of known primary active transporters.

11.1. The ABC superfamily (3.A.1)

ABC transporters segregate phylogenetically into uptake and efflux systems [97,98]. The former but not the latter act in conjunction with extracytoplasmic solute binding receptors (R) that feed the solute into the transmembrane channel (M) [99] which is energized by the cytoplasmic ATP hydrolyzing subunit/domain (C) (see TCDB). The receptors can be lipid anchored or attached to non-cleavable, N-terminal, leader sequences. In some cases they are even fused to the transmembrane transporter component. Sometimes several receptors can feed different, but structurally related solutes into a single

ABC channel [100]. Transport is energized by the hydrolysis of ATP, catalyzed by a membrane-associated ATP-binding cassette (ABC) protein or protein domain [101].

Both the integral membrane *c*hannel *f*orming *p*rotein (CFP; M) and the ABC protein (C) are usually present as dimers, each consisting either of a single gene product, yielding a homodimer, or two gene products, yielding a heterodimer [102]. Sometimes, but seldom, these three protein types are fused to form multidomain proteins. ABC uptake permease systems therefore normally consist of 3–5 distinct proteins although the number can be smaller (due to gene fusions) or larger (due to the presence of multiple receptors) (see TCDB). The average number of proteins per ABC uptake system is roughly 4.

By contrast, ABC efflux systems consist only of the ABC (C) and CFPs (M), and these two domains are often fused. Consequently, there are either one or two proteins per ABC exporter; thus, about 1.5 proteins are present on an average per ABC exporter. The quantitation presented in Table 5 is always in terms of numbers of proteins; consequently, these numbers should be divided by about 4 and 1.5 for families of uptake and efflux systems, respectively, in order to estimate the numbers of systems represented.

As summarized in Table 5, ABC transporters occur in substantial numbers in all Gram-positive bacteria studied. The numbers of ABC proteins encoded within the various genomes vary from 66 (for Ppe) to 175 (for Bli). The average number of ABC system proteins per organism is 127.

Two families within the ABC superfamily are concerned exclusively with carbohydrate uptake, the carbohydrate uptake transporter-1 and -2 (CUT1 and CUT2) families. While CUT1 systems are largely concerned with oligosaccharide uptake, CUT2 systems usually exhibit specificity for monosaccharides, particularly aldopentoses and hexoses. CUT1 homologues are found in all organisms except Ppe, Sth and Lme. Blo has 40, probably comprising about 10 systems. It is interesting to note that Bli, with a larger genome, has only 5. Other bacteria probably have 1–4 such transporters. Lcr and Ooe probably have four such systems. CUT2 family homologues are found in all organisms studied, but the numbers of CUT2 family proteins never reach the large numbers sometimes observed for CUT1 family proteins. Note that the PepT family (3.A.1.5) includes members that like CUT1 family members can transport oligosaccharides (see below).

The two principal amino acid uptake families in the ABC superfamily (PAAT and HAAT) are specific for polar and hydrophobic amino acids, respectively. Representation in the former family (11–32 proteins) far exceeds that in the latter family (0–11 proteins). The PepT family, which includes members that transport peptides and oligosaccharides, is well represented (6–40 proteins per organism). The methionine uptake transporter (MUT) family (TC #3.A.1.24) is represented in all 11 organisms.

The next few uptake families listed in Table 5 are largely concerned with inorganic ion transport. Sulfate-transporting SulT family homologues are found in 5 of the 11 bacteria, but all 11 organisms have at least one phosphate transporter of the PhoT family, and five also have a phosphonate transporter of the PhnT family.

The POPT family includes a diverse group of transporters exhibiting specificity for polyamines, opines and phosphonates. One or two systems are present in all bacteria except Ppe and Blo. Similarly, at least one (and sometimes 2 or 3) QAT family systems for the uptake of quaternary amine osmolytes and amino acid derivatives are present in all but three organisms. TauT family systems, specific for taurine, are found in a few bacteria, and these systems may, in addition to allowing utilization of taurine, provide defense against osmotic stress.

The next several families are concerned with heavy metal uptake. They include the FeCT and BIT iron (complex) transporters, the MZT, Mg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+} transporters, the CoT cobalt uptake systems and the NiCoT nickel/cobalt uptake transporters. Surprisingly, the MZT systems are present in all eleven bacteria although the FeCT family is present in only 5 of them, and the BIT and CoT families are represented in only three. A single NitT nitrate/nitrite transporter is found in Lbr. The NiCoT family is represented in all eleven organisms. Homologues of the putative yersiniabactin Fe^{3+} uptake transporter (YbtPQ) family were found in Lbr, Lca and Ooe. Members of all other ABC uptake families are absent from all eleven organisms.

ABC efflux systems fall into two principal subdivisions, those found primarily in prokaryotes (the 3.A.1.100 series) and those found primarily in eukaryotes (the 3.A.1.200 series). Although the capsular polysaccharide exporter (CPSE) family is lacking in the 11 organisms examined, the Lipooligosaccharide Exporter (LOSE) family, previously characterized only in Gram-negative bacteria, is represented in 7 of the 11 organisms. Members of the Lipopolysaccharide Exporter (LPSE) and Teichoic Acid Exporter (TAE) families are also represented. Although Gram-positive bacteria do not make LPS, they do incorporate teichoic acids into their cell walls [103].

Four of the six prokaryotic ABC drug efflux families (Drug E2, Drug RA1, Drug RA2 and MacB) are all nearly universally present in the eleven bacteria, and the two remaining drug export families (Drug E1 and Drug E3) are also present in select organisms. It is interesting that many of these organisms seem to have multiple MacB macrolide exporters. Three bacteria may also have eukaryotic-type MDR pumps, and nine may have a member of the β-exotoxin 1 Exporter (βETE) family.

Four of the five ABC peptide exporter families are represented in Table 5 (the Pep1, 2, 4 and 5E families). While Pep3E pumps are lacking in all organisms, and a Pep1E family member is present in each of four organisms, the other three families, Pep2E, Pep4E and Pep5E, are well represented. Multiple such peptide exporters are found in many of the bacteria. Homologues of the protein exporter (Prot1E) family are found in five of the eleven organisms, while a single homologue of the Prot2E family is present in Bli.

A few other ABC exporter families are represented in Table 5. These include the poorly characterized DevE family of putative glycolipid exporters, the LPT family of lipoprotein translocases, and the lipid exporter (LipidE) family. Two of

these families (DevE and LPT) occur in 8 of the 11 organisms examined. Most surprisingly, the LipidE family is represented in all of the 11 organisms except Ppe with as many as 11 such proteins per organism.

11.2. P-type ATPases (3.A.3)

The eleven organisms studied have between 4 and 13 P-type ATPases per organism. These can be classified according to the substrates most likely transported, based on sequence similarity to functionally characterized systems (data not shown).

One organism (Lca) has a protein most closely resembling eukaryotic Na^+, K^+ ATPases of P-ATPase subfamily 1, a novel finding for a prokaryote. In eukaryotes, these enzymes catalyze uptake of $3K^+$ coupled to efflux of $2Na^+$ in an electrogenic process [104]. All organisms except Bli have multiple Ca^{2+}/Mn^{2+} ATPases of subfamily 2. The numbers of these systems per organism vary between 1 and 5.

In subfamily 3, which includes the characterized Mn^{2+}/Cd^{2+} ATPase of *Lactobacillus plantarum* [105] as well as H^+-translocating ATPases of fungi and protozoans, we find single member representation in two organisms, Lbr and Sth. Lca, Lde, Lga and Lcr each have two such members. These four bacteria also have one member of subfamily 4, similar to the Mg^{2+}/Ni^{2+} ATPase of *Salmonella typhimurium*.

All organisms have at least one, and as many as 3 Cu^+, Ag^+-ATPases of subfamily 5. In *Enterococcus hirae*, two such systems are characterized (TC #3.A.3.5.1 and 3.A.3.5.2) [106]. One catalyzes copper uptake, the other copper efflux, presumably allowing proper maintenance of copper homeostatic conditions in the cell [106].

Finally, all but two organisms (Sth and Blo) have 1–3 broad specificity heavy metal (Zn^{2+}, Co^{2+}, Cd^{2+}, Hg^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+} and Ag^{2+}) efflux pumps of subfamily 6. Surprisingly, no K^+ ATPases of subfamily 7, no phospholipid flippases of subfamily 8, and no monovalent alkali cation (Na^+, K^+) pumps of subfamily 9 were detected.

11.3. Macromolecular secretion systems

Constituents of three protein export systems as well as two DNA translocases were identified. The general secretory pathway (Sec) complex is ubiquitous, being found in every organism for which it has been sought [107]. However, it can consist of as many as 8 proteins and one small (4.5 S) RNA, as is true for *E. coli*, or as few as 6 proteins plus a small RNA, as is true for *Mycoplasma genitalium*. The SecA, SecY, SecE and SecG proteins as well as the Ffh and FtsY proteins (also required for membrane protein insertion) are always present. When proteins are inserted into the membrane, YidC homologues of the Oxa1 family (2.A.9) often participate [108]. The SecDF complex (homologous to RND transporters), which serves an auxiliary function, is present only in *B. linens*. Surprisingly, *B. linens* also has two SecA proteins. Finally, only Ppe has two SecYs, one for general secretion and one for specific secretion of large cell surface adhesions [109–111]. The latter system uses different auxiliary proteins, the Asp1–4 proteins. While Ppe has Asp2–4, Lme has Asp1–3 but lacks a second SecY. What these proteins are doing in these two bacteria has yet to be determined (Table 5).

Gram-negative bacteria have multicomponent conjugal protein/DNA export systems. The Gram-positive bacterium, *Staphylococcus aureus*, also has such a system, the Trs system, which consists of 15 dissimilar proteins [112]. Ten of the eleven bacteria examined here have homologues of this system (3.A.7), but the numbers of such proteins vary between 2 (for Lbr, Lga and Bli) and 8 (for Blo). It is therefore not clear that these bacteria have complete systems.

Constituents of the Bacterial Competence-related DNA Transformation Transporter (DNA-T) family (3.A.11) are found in all of the bacteria, but the numbers of such homologues vary between 2 and 6. All but Lme, Blo and Bli have all three of the proteins required for competence [113]. Similarly, Septal DNA Translocators of the S-DNA-T family (3.A.12) are found in one or two copies in all eleven organisms. These proteins may be required for translocation of chromosomal or plasmid DNA across the septal membrane [114,115].

The Fimbrilin/Protein Exporter (FPE) family is represented in all nine low G+C Gram-positive bacteria under study, but it is not found in the two high G+C Gram-positive bacteria. These systems consist of two proteins, an integral membrane channel protein, and an energizing ATPase. Eight of the low G+C Gram-positive bacteria have both such constituents while Lca has just the ATPase homologue.

11.4. Na^+-translocating organocarboxylate decarboxylases

Nine organisms, seven low G+C and the two high G+C Gram-positive bacteria, have multiple (2–6) subunits of putative Na^+-translocating decarboxylases (3.B.1) [116,117]. Of these, only one (Lca) has the decarboxylase subunit (α), the integral membrane transporter subunit (β) and the small γ-subunit. Consequently, only Lca can be expected to export Na^+ in response to the cytoplasmic decarboxylation of substrate carboxylates.

12. The Phosphotransferase System (PTS)

PTS permeases consist of 3 or 4 domains, the IIA, IIB, IIC (present in all families), and IID (present only in the Mannose (Man) family). These domains can be fused together or detached as separate polypeptide chains [22,118]. Variations in representation of PTS proteins in the eleven organisms were extensive. The two PTS energy-coupling proteins, Enzyme I and HPr, are present in all organisms except in the high G+C Gram-positive bacterium, Bli, which also lacks a PTS permease. The other high G+C Gram-positive bacterium, Blo, has both Enzyme I and HPr and seems to have a single PTS permease, possibly specific for β-glucosides. Lbr has far fewer PTS permeases than the other low G+C Gram-positive bacteria with only two systems, a lactose-type (Lac) system and a Man system.

The representation of PTS permeases in the remaining low G+C lactic acid bacteria varies tremendously. Lca has the most

with 63 PTS permease proteins, and all seven known families of these permeases are encoded within the Lca genome. The first such family, the Glucose/Glucoside (Glc) family (4.A.1) is represented in all of the low G+C lactic acid bacteria except Lbr, with up to 12 proteins belonging to this family per organism. Surprisingly, Sth has 8 of its 15 PTS proteins within this family while Lca, with 4 times as many PTS proteins, has only 12 in the Glc family.

Within the Glc family, putative glucoside transporting systems outnumber hexose-transporting systems 4 to 1, emphasizing the dependency of these bacteria on plant glycosides. The Fructose/Mannitol (Fru) family (4.A.2) is found in seven organisms with 1–7 proteins per organism. The Lactose/Diacetylchitobiose/Lichenan Oligosaccharide (Lac) family (4.A.3) is well represented with approximately four times as many proteins as for the Fru family. This is in agreement with the observation that many of these bacteria are dependent on plant glycosides for growth. It contrasts with the preponderance of Fru systems in γ-proteobacteria [22]. The Glucitol (Gut) family (4.A.4) is represented in only two organisms (Ppe and Lca) while proteins of the GAT family (4.A.5) are present in four (Lbr, Lca, Lga and Ooe). However, Lbr has only the IIC component, implying that the protein does not function by a PTS-type mechanism. It may be a secondary carrier as discussed previously for other organisms [119,120]. The L-ascorbate (Asc) family (4.A.7) is represented in four lactic acid bacteria (Lca, Lcr, Lme and Ooe). All of the low G+C Gram-positive bacteria studied have at least one mannose/fructose/sorbose (Man)-type system (4.A.6), and at least three of them have 3–6 such systems.

13. Transmembrane electron transport systems

When grown in the presence of heme, at least some lactic acid bacteria gain additional metabolic energy in the form of a proton motive force. The mechanisms are not known, and these observations could be a result of indirect effects rather than the direct coupling of electron flow to proton efflux. In fact, none of the currently recognized bacterial H^+- or Na^+-translocating electron flow systems was identified (families of oxidoreduction-driven cation transporters; TC section 3.D).

Transmembrane electron flow systems of TC classes 5.A and 5.B do not transport cations across the membrane, but electrons do flow from one side of it to the other. These systems therefore contribute to or subtract from the membrane potential. Recognized transmembrane electron flow systems are present in only one organism, the large genome high G+C Gram-positive bacterium, Bli. Bli has 4 members of the Disulfide Bond Oxidoreductase (DsbD) family (5.A.1) and three protein members of the Prokaryotic Molybdopterin-containing Oxidoreductase (PMO) family (5.A.3). However, of these three proteins, two are α-subunits and one is a β-subunit. Therefore, only one complete PMO family member may be present. Recognizable pmf-generating or -dissipating electron flow carriers are essentially absent in all other bacteria examined.

14. Auxiliary transport proteins

Auxiliary transport proteins are relegated to TC class 8. Gram-positive bacteria have "membrane fusion proteins" (MFPs) of family 8.A.1. These proteins were named following their discovery in Gram-negative bacteria [121], but they have since been described in Gram-positive bacteria where they can be essential for the activity of particular ABC-type export transporters [122].

Only two of the bacteria studied here have such a protein (Sth and Lme). These probably function in conjunction with peptide efflux pumps of the ABC superfamily [122]. In fact, both Sth and Lme have genes encoding ABC transporters of the Pep2E family (TC #3.A.1.112) adjacent to the genes coding for their MFPs. Two Gram-positive bacterial MFPs have been shown to be essential for ABC transport function [123–125].

Seven organisms have MPA1 family members (8.A.3) that regulate complex capsular polysaccharide export via PST-type transporters of the MOP superfamily (TC #2.A.66.2) [83]. The K^+ Transport/Nucleotide Binding Regulatory Subunit (KTN) family (8.A.5), which controls the activities of Trk family K^+ porters, is found in 9 of the 11 organisms, and all of the low G+C lactic acid bacteria have homologues of the eukaryotic rBAT (1–5 copies per organism). rBAT homologues stimulate amino acid transport via members of the APC superfamily, but they are homologous to various glycosidases [126–128]. There is no evidence that these homologues regulate amino acid transport in bacteria.

15. Poorly characterized transporters

Several poorly characterized transporter families of TC class 9 are sparsely represented in Table 5. These include MerTP Hg^{2+} transporters (9.A.2) (1 organism), the PnuC family (9.A.4) of nicotinamide mononucleotide (NMN) transporters (3 organisms) and the FeoB (9.A.8) and OFeT (9.A.10) families of iron transporters (4 and 2 organisms, respectively). A putative Lead Uptake Porter (PbrT family; 9.A.17) is found in each of two organisms, Sth and Blo. MgtE Mg^{2+} transporters (9.A.19) are found in single copy in Ppe, Lme and Bli.

Several families of unknown function (TC class 9.B) are worthy of brief mention. The putative murein precursor exporter (9.B.3), responsible for export of lipid-linked oligosaccharide precursors of peptidoglycan, is found in all eleven organisms. These transporters may be required for the assembly of the bacterial cell wall. The MgtC family of putative Mg^{2+} transporters (9.B.20) is represented in single copy in 7

Table 6
Numbers of putative transporters predicted to exhibit polar characteristics: channel-type bidirectional transport, uptake, or export

Direction	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli
Bidirectional	7	7	5	3	5	4	4	7	3	5	6
Uptake	47	48	51	48	46	45	48	54	48	49	49
Export	32	33	31	33	31	35	32	29	30	29	30
Unknown[a]	15	13	13	16	17	15	17	10	19	17	16

[a] The polarities of these transporters could not be predicted.

Table 7
Percent distribution of transport proteins according to substrate type

Category	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli
Amino acids and derivatives	19	22	22	33	22	17	32	25	16	19	23
Carbon sources	23	15	23	9	20	18	10	15	23	24	10
Drugs and toxic compounds	10	13	10	8	6	12	7	11	12	11	12
Inorganic molecules	23	28	23	20	23	25	24	30	20	20	30
Macromolecules	11	10	10	15	11	12	13	9	10	10	7
Unknown	14	13	13	15	17	15	15	10	18	17	18

organisms. The AI-2E family (TC #2.A.86; formerly the PerM family), one member of which exports the boron-containing autoinducer AI-2 signalling molecule [129–131] is found in all eleven organisms (1–5 copies per organism). As AI-2 is believed to be required for interspecies communication, it is possible that this type of signalling is important for the lifestyles of LABs and other Gram-positive bacteria.

The Hly III family (9.B.30) of putative hemolysins is present in 10 of the 11 organisms. It will be interesting to know the function of these homologues. Members of the Putative Vectorial Glycosyl Polymerization (VGP) family (9.B.32) are found in 9 of the 11 organisms, and as many as 6 paralogues may be present in a single organism. It can be presumed that these proteins function in the assembly of exopolysaccharides and capsular polysaccharides and possibly teichoic acids as well [132].

16. Conclusions

The fully sequenced genomes of eleven Gram-positive bacteria were searched for homologues in TCDB in order to analyze the transport capabilities of these bacteria. These eleven bacteria devote at least 13 to 18% of their genes to transport. Of the total known transport proteins encoded in these genomes, on average, about 48% constitute uptake systems, 32% constitute efflux systems, and 5% of the proteins encode systems that transport substrates bidirectionally (Table 6). Fifteen percent are of unknown polarity and mechanism of action. Most of the bidirectional transporters are channels that either have low specificity for their substrates or are nonspecific. The putative uptake systems in these lactic acid bacteria transport primarily amino acids, sugars, cations, anions and peptides. The amino acid transporters are the

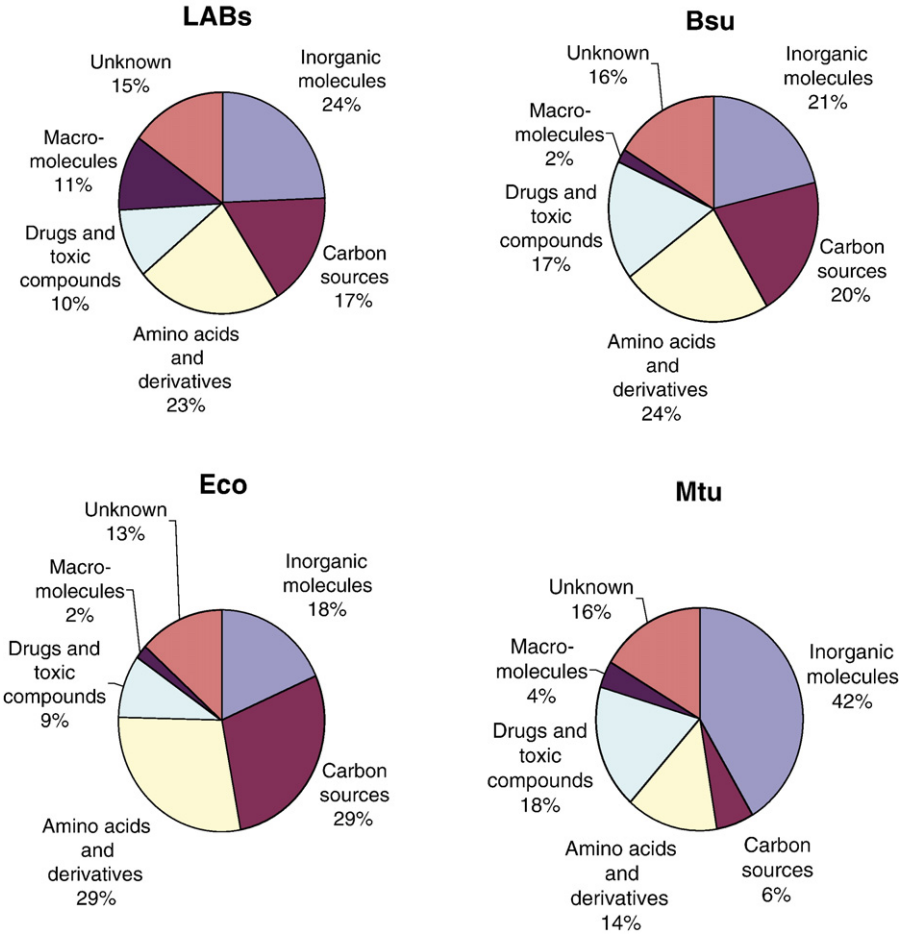

Fig. 4. Comparison of the average percent distribution of transport proteins of various specificities in the nine LABs with that in other bacteria, *Bacillus subtilis* (Bsu), *Mycobacterium tuberculosis* (Mtu) and *Escherichia coli* (Eco).

most abundant while peptide transporters are the least abundant of these uptake systems.

Lactic acid bacteria have many drug efflux systems, but they also have substantial numbers of peptide and macromolecular exporters. Comparison of the relative proportions of transport proteins in lactic acid bacteria with those in *E. coli*, *Bacillus subtilis* and *Mycobacterium tuberculosis* revealed interesting features of the lactic acid bacteria (Table 7 and Fig. 4). Twenty-four percent of the transport proteins in lactic acid bacteria seem to be involved in transport of inorganic molecules, and 23% of the transport proteins may transport amino acids and their derivatives. *E. coli*, *B. subtilis* and *M. tuberculosis* devote about 18%, 21% and 42% to transport of inorganic molecules and 29%, 24% and 14% to transport of amino acids and their derivatives. About 17% of the transport proteins in lactic acid bacteria may be involved in the uptake of carbon sources while in *E. coli*, *B. subtilis* and *M. tuberculosis* these systems constitute 29%, 20% and 6% of the total transport systems, respectively. In all of these respects the LABs most closely resemble *B. subtilis*. Transport proteins that may be involved in efflux of drugs and toxic compounds constitute 10% of the total transport proteins in the LABs unlike *B. subtilis* (17%) and *M. tuberculosis* (18%) but similar to *E. coli* which has 9% of its transport proteins concerned with efflux of these molecules. Eleven percent of the transport proteins in lactic acid bacteria may be involved in transport of macromolecules unlike *E. coli*, *B. subtilis* and *M. tuberculosis* which have only 2%, 2% and 4% of their transport proteins devoted to this function, respectively.

Of the 11 bacteria examined, *B. linens* has the largest genome, and many transporter families are represented that are found in none of the others (see Table 5). Families of secondary carriers that were only found in *B. linens* are the UMF2, YnfM, EntS and NNP families in the MFS, KDGT, CaCA, NSS, APC (ACT), CPA3, CHR, LysE and RND (Hae1 and SecDF) families. Three ABC-type families, Prot2E, DrugE3 and CT (ABCC), were also found only in *B. linens*. Moreover, only in this organism were electron carriers of the DsbD, PMO, QCR and COX families identified. Finally, certain oversized proteins with 19–25 TMS were found only in *B. linens*, and these proteins belong to the FeCT (19 TMSs), UIT10 (19 TMSs) and the CPA3 (24 and 25 TMSs) families.

Analysis of the MFS transporters in these bacteria revealed that the greatest proportion of these systems transport drugs (Table 8). Forty to 83% of MFS transporters in these bacteria probably exhibit specificity for drugs and other amphipathic compounds. MFS transporters that translocate anions are the second most abundant of the MFS carriers followed by those that transport sugars. However, Bli has more MFS transporters that transport anions than drugs or other substrates. Surprisingly, Sth has no sugar transporters of the MFS-type at all. These systems are almost ubiquitous among free-living bacteria.

Table 8
Percent distribution of MFS transporters according to substrate type

Substrate [a]	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli
Drugs	64	60	67	57	48	83	60	57	63	52	40
Anions	18	14	18	21	29	4	20	27	12	19	44
Sugars	9	14	9	14	10	4	0	10	17	22	7
Others	9	11	6	7	14	8	20	7	7	7	9

[a] All drug pumps catalyze efflux while anion and sugar transporters catalyze uptake and/or substrate exchange.

Table 9
Percent distribution of ABC efflux and uptake systems according to substrate type

Category	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo
(A) Efflux										
Drugs	38	30	22	18	12	29	18	23	22	12
Lipids	0	5	16	21	20	2	16	3	8	12
Peptides	8	8	10	16	27	7	11	6	3	2
Complex carbohydrates	4	3	0	0	2	5	3	0	0	2
Proteins	0	0	5	0	2	2	0	3	3	2
Inorganic cations	0	0	2	0	0	2	0	0	3	2
(B) Uptake										
Amino acids	19	14	16	18	15	10	24	31	19	16
Sugars	4	8	5	5	7	17	8	6	17	33
Inorganic cations	8	11	9	5	2	10	8	14	8	7
Peptides	4	5	5	5	2	5	5	3	6	7
Organic cations	8	8	5	5	5	5	5	6	6	0
Inorganic anions	4	8	3	5	5	5	3	3	3	2
Organic anions	4	0	2	0	0	0	0	3	3	2

Analysis of ABC transporters in the eleven bacteria revealed that of these systems, these organisms generally have more uptake systems than efflux systems (Table 9). However, Lca, Lde and Lga have more ABC efflux systems than ABC uptake systems. Drug efflux pumps constitute the largest proportion of the ABC systems, and amino acid uptake systems are the second most abundant. However, the opposite is true for Sth and Lme which have more amino acid uptake systems than drug efflux pumps. Interestingly, in Lde, most ABC systems efflux lipids and hydrophobic compounds while in Lga most ABC systems efflux peptides. In Blo, ABC-type sugar uptake systems dominate. A large number of drug exporters is a common feature of soil bacteria which presumably must cope with such toxic substances frequently.

Bacterial multidrug resistance efflux pumps fall into five superfamilies, MFS (TC #2.A.1), RND (TC #2.A.6), DMT (TC #2.A.7), MOP (TC #2.A.66 and ABC (TC #3.A.1). Several of these superfamilies include multiple families that export drugs

Table 10
Percent of putative multidrug resistance (MDR) efflux pumps according to superfamily

Superfamily [a]	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli
MFS	50	55	58	50	63	54	23	57	72	64	57
ABC	36	29	34	44	31	34	54	29	25	23	29
MOP	4	8	3	0	0	9	23	4	3	14	2
RND	4	5	3	6	6	0	0	7	0	0	10
DMT	7	3	3	0	0	3	0	4	0	0	2

[a] The abbreviations are: MFS, Major Facilitator Superfamily; ABC, ATP-binding Cassette Superfamily; MOP, Multidrug/Oligosaccharidyl-lipid/Polysaccharide Flippase Superfamily; RND, Resistance-Nodulation-Division Superfamily; DMT, Drug/Metabolite Transporter Superfamily.

Table 11
Percent sugar uptake systems falling into the three categories of transporters

Family	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli	Eco	Bsu	Gram-negative Pathogens (Bbu, Tpa, Hin)	Gram-positive Pathogens (Lmo, Spy, Mge)
2 ° carrier	32	70	16	40	16	18	18	52	46	38	58	28	34	8	9
ABC	4	13	8	20	10	32	27	9	21	58	42	23	26	70	16
PTS	64	17	76	40	74	50	55	39	32	4	0	49	40	22	75

and other toxic molecules [80,83,133,134]. We analyzed the distribution of these various types of pumps in the eleven Gram-positive bacteria as well as several other well-characterized bacteria.

Analyses of the putative drug efflux pumps (Table 10) in the eleven bacteria reveals that overall, 55% of these pumps belong to the MFS followed by ABC (33%), MOP (6%), RND (4%) and DMT (2%). Some organisms provided exceptions to this trend. Sth had more ABC family members (54%) than MFS (23%) or MOP (23%), but this was the only organism in which MOP and MFS drug efflux pumps are present in equal numbers. No RND or DMT family members were found in Sth, while no representatives of the MOP or DMT families were found in Lde and Lga. RND and DMT systems were absent in Ooe and Blo. Although the greatest proportions of drug efflux pumps in *E. coli* and *B. subtilis* are MFS-types, the relative proportions of these and other drug efflux pumps in these bacteria differ. *E. coli* has 58% MFS, 16% RND, 14% ABC, 7% DMT and 5% MOP-type systems while *B. subtilis* has 61% MFS, 14% MOP, about 11% each of ABC and DMT and only 3% (one system) of RND-type systems.

Sugar transporters can be secondary carriers, ABC transporters or PTS group translocators. Half of the sugar uptake systems in the low G+C Gram-positive lactic acid bacteria are PTS-type (50%) while 34% are secondary carriers and only 16% are ABC transporters (Table 11). However, in the high G+C Gram-positive bacteria, Bli and Blo, most of the sugar transporters are either secondary carriers (48%) or ABC-type transporters (50%). Bli does not encode any PTS permeases while just one sugar transport system in Blo is a PTS permease.

In three representative Gram-negative bacterial pathogens, *Borrelia burgdorferi* (Bbu), *Treponema pallidum* (Tpa) and *Haemophilus influenzae* (Hin), 70% of all sugar transporters are ABC-type while 22% are PTS and only 8% are secondary carriers (Table 11). However, in three representative Gram-positive pathogens, *Listeria monocytogenes* (Lmo), *Streptococcus pyogenes* (Spy) and *M. genitalium* (Mge), about 75% of all sugar transporters are PTS permeases and 16% are ABC-type while only 9% are secondary carriers. These considerations demonstrate the unique features of the LABs and reveal which organismal types they most resemble.

The low G+C Gram-positive lactic acid bacteria seem to encode slightly more transporters specific for glycosides (51%) as compared with those for free sugars (49%) (Table 12). The two high G+C Gram-positive bacteria, Bli and Blo, show tremendous bias in the utilization of glycosides versus sugars, but in opposite directions. Blo has more than twice as many uptake systems for glycosides as for free sugars while Bli has more than ten times more transporters for free sugars than for glycosides. In comparison, *E. coli* and *B. subtilis* transport more free sugars (65% and 55%, respectively) than glycosides (35% and 45%, respectively). Representative Gram-negative pathogens (Bbu, Tpa and Hin) have five times more uptake systems for free sugars than for glycosides while Gram-positive bacterial pathogens (Lmo, Spy and Mge) have nearly two fold more sugar uptake proteins than glycoside uptake systems. These observations illustrate the unusual sugar utilization capabilities of the Gram-positive bacteria that live primarily in association with plants.

All eleven bacteria studied here have at least one peptide uptake system (Fig. 5), and all but Bli have at least one peptide efflux system. Bli has disproportionately greater numbers of peptide uptake systems (13 systems) while Lga has many more peptide efflux systems (11 systems) than the other bacteria. Lca, Lde, Lga, Sth and Lme have more peptide efflux systems than peptide uptake systems, while Ppe, Ooe and Blo have more uptake systems for peptides than efflux systems. Lbr and Lcr have equal proportions of these two types of systems (Fig. 5).

All low G+C lactic acid bacteria possess mechanosensitive channels for osmotic adaptation, suggesting that these organisms are exposed to extremes of osmotic conditions in their natural habitat. They also have chloride channels that may function in pH homeostasis. For the most part, they lack electron transfer, suggesting a predominantly fermentative mode of energy generation. They have peptide uptake and export systems that function in nutrition, signalling, regulation and biological warfare. They have complements of transporters that distinguish them from other types of bacteria, and the different LABs also exhibit species-specific characteristics. All eleven organisms have competence-related and septal DNA translocation systems although competence has not been demonstrated for any of these bacteria. They all possess one

Table 12
Percent distribution of free sugar versus oligosaccharide/glycoside transport systems

Details	Ppe	Lbr	Lca	Lde	Lga	Lcr	Sth	Lme	Ooe	Blo	Bli	Eco	Bsu	Gram-negative Pathogens (Bbu, Tpa, hin)	Gram-positive Pathogens (Lmo, Spy, Mge)
Free sugars	46	39	59	60	45	36	64	39	50	33	92	65	55	83	63
Oligosaccharides	54	61	41	40	55	64	36	61	50	67	8	35	45	17	37

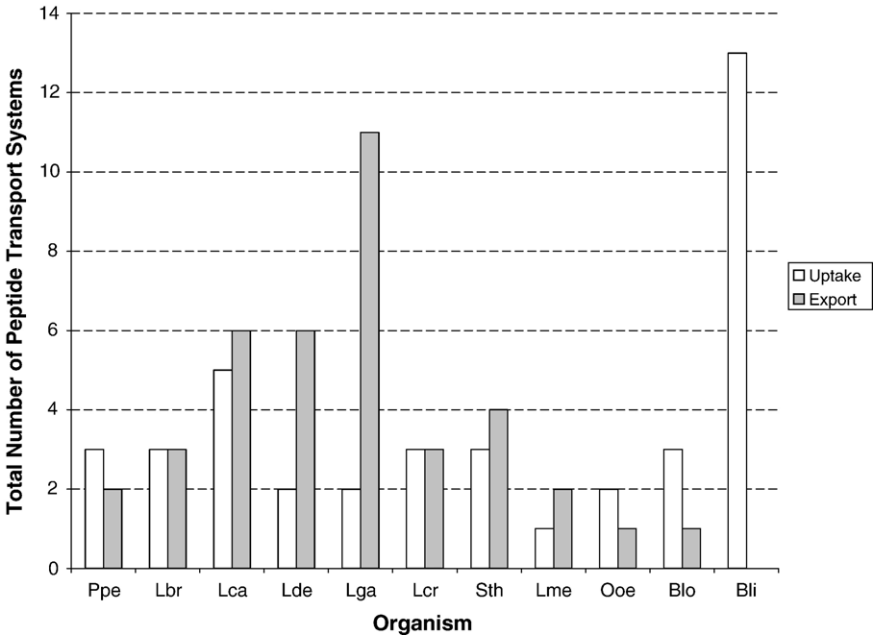

Fig. 5. Distribution of uptake versus efflux transport systems specific for peptides in the eleven Gram-positive bacteria included in this study.

or more putative murine precursor exporter(s), possibly required for extracellular peptidoglycan assembly. Finally, they all possess the Sec/Oxa1 systems for secretion, membrane insertion and assembly of proteins that include all of the transporters discussed in this article.

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