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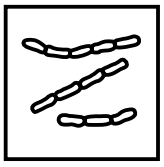

Research Paper

Genomic analyses of transport proteins in *Ralstonia metallidurans*

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Abstract

Ralstonia (Wautersia, Cupriavidus) metallidurans **(Rme) is better able to withstand high concentrations of heavy metals than any other well-studied organism. This fact renders it a potential agent of bioremediation as well as an ideal model organism for understanding metal resistance phenotypes. We have analysed the genome of Rme for genes encoding homologues of established and putative transport proteins; 13% of all genes in Rme encode such homologues. Nearly one-third of the transporters identified (32%) appear to function in inorganic ion transport with three-quarters of these acting on cations. Transporters specific for amino acids outnumber sugar transporters nearly 3 : 1, and this fact plus the large number of uptake systems for organic acids indicates the heterotrophic preferences of these bacteria. Putative drug efflux pumps comprise 10% of the encoded transporters, but numerous efflux pumps for heavy metals, metabolites and macromolecules were also identified. The results presented should facilitate genetic manipulation and mechanistic studies of transport in this remarkable bacterium. Copyright © 2005 John Wiley & Sons, Ltd.**

Keywords: **bioinformatics; transport proteins; comparative genomics**

Introduction

Ralstonia metallidurans (Rme; previously *Alcaligenes eutrophus,* renamed in 2004 *Wautersia metallidurans* and then *Cupriavidus metallidurans*; Goris *et al*., 2001; Vandamme and Coenye, 2004; Vaneechoutte *et al*., 2004), is a Gram-negative facultative chemolithoautotrophic β-proteobacterium. It was first identified in 1976, when it was isolated from industrial sediments, soils and wastes that were polluted with high concentrations of various heavy metals, such as cobalt, zinc, nickel and cadmium (Mergeay *et al*., 1985). The concentrations of these metals that can exist in the habitats of Rme greatly exceed the values that are lethal to almost any other living organisms. Rme is related to the important plant pathogen *Ralstonia solanacearum* (Boucher *et al*., 2001), which is resistant to a wide variety of drugs and toxic compounds. The complete genome sequence of the latter organism is available (Salanoubat *et al*., 2002).

The properties of Rme render it potentially important for purposes of bioremediation, such as for the degradation of aromatic compounds and xenobiotics, even in the presence of heavy metals as additional pollutants. Rme is also able to synthesize polyhydroxyalkalonates (PHAs), which accumulate as carbon and energy sources and might be useful for the development of biodegradable plastics. The extraordinary heavy metal resistance of Rme and its ability to accumulate these metals on its surface make it a candidate for a variety of clean-up purposes (Legatzki *et al*., 2003a; Mergeay *et al*., 2003; Nies, 2003).

Two low copy number plasmids, pMOL30 (238 kb; Mergeay *et al*., 1985) and pMOL28 (180 kb; Taghavi *et al*., 1997), that are stably carried by Rme strain CH34, are primary determinants of the remarkable heavy metal resistance characteristic of Rme (Legatzki *et al*., 2003a,b). Both are self-transferable at low frequencies, potentially offering a new approach for inserting resistance genes into other organisms. Rme lacks the RecBCD

pathway for DNA degradation — a property that allows it to serve as an acceptor for foreign resistance genes. The fact that specific transport systems responsible for the uptake and export of various metabolites and heavy metals (Andres *et al*., 2000; Borremans *et al*., 2001; Goris *et al*., 2001; Juhnke *et al*., 2002; Mergeay *et al*., 2003; Nies, 2003; Roux *et al*., 2001) have been better characterized in Rme than in any other bacterium (Nies, 2003), renders Rme a model organism for basic research on metal resistance and homeostasis.

It has been suggested that the resistance of Rme to heavy metals and toxic compounds results from multiple layers of efflux pumps with overlapping substrate specificities (Juhnke *et al*., 2002; Nies, 2003; Silver, 2003). However, comprehensive genome analyses of the transporters in Rme are still lacking. In this paper we correct this deficiency, reporting bioinformatic studies of all recognizable transporters encoded within the genome of Rme.

Computer methods

The protein sequences of Rme were extracted from the JGI database and downloaded for all of the analyses reported here. The sequencing work done at JGI (**http://genome.jgi-psf.org/draft_microbes/ralme/ralme.home.html**) and the annotation project performed by the CH34 annotation consortium (**http://genome.ornl.gov/microbial/rmet/**) formed the basis of this work and are acknowledged at this point. Since the names of the CH34 genes have changed many times in the past, as has the name of the organism, cross-reference tables are supplied as supplementary material (**http://bionomie.mikrobiologie.uni-halle.de/SupMat/SupplMat.htm**). Computer-aided searches were conducted to retrieve all proteins encoded within the genome that are recognizably homologous to transport system constituents included in the Transporter Classification Database (TCDB; Busch and Saier 2002; Tran *et al*., 2003). Briefly, all proteins encoded within the genome were blasted in an automated manner (using BLASTP) against TCDB. Additional databases used for protein functional analysis were the non-redundant SWISSPROT and TrEMBL protein sequence databases. Several protein pattern databases (conserved domain databases at NCBI and Pfam) were also used. Charge bias analyses of membrane protein topology were performed using the TMHMM (Krogh *et al*., 2001) and WHAT (Zhai and Saier, 2001) programs.

Results and discussion

Topological predictions for membrane transporter homologues

The proteome of Rme was analysed for topological predictions; 59% (4072) of the 6985 proteins identified have no predicted TMSs, while 21% (1434) have only one putative TMS. While most of the former proteins are likely to be cytoplasmic, many of the latter will undoubtedly prove to be periplasmic and outer membrane proteins; 8% (580) have two or three TMSs, 5% (320) have four to six TMSs, and 3% each (196 and 223) have seven to 10 and >10 TMSs, respectively. Relative to most other prokaryotes analysed, Rme has increased proportions of integral membrane proteins of all topological types (Paulsen *et al*., 2000).

All putative transport protein constituents recognized in the proteome of Rme were similarly analysed for topology; 932 putative transporter proteins (13%) were recognized in the proteome of Rme. This percentage is higher than observed for most other organisms with fully sequenced genomes (Paulsen *et al*., 2000). About 24% (227) of these proteins may be cytoplasmic, as they exhibit no putative TMSs. All others are potential integral membrane constituents. Of these, 21% (196) are predicted to have one TMS, 9% (88) have two or three TMSs, 16% (146) have four to six TMSs, 10% (94) have seven to 10 TMSs, and 19% (179) have $\geqslant$11 TMSs. Many of the one-TMS proteins displayed typical leader sequences at their respective amino-termini and may be secreted via the Sec and Tat export systems (see below). They may be receptors for ABC-, TRAP-T- and TTT-type transport systems (see below). Since transporter families include proteins that are almost always concerned exclusively with transport (Saier, 2003), it is probable that nearly all of these proteins function in transmembrane transport.

Classes of transporters found in *R. metallidurans*

According to the transporter classification (TC) system, transporters are classified into five well-defined categories (classes 1–5) and two poorly defined categories (classes 8 and 9). The well-defined categories are; (a) channels; (b) secondary carriers; (c) primary active carriers; (d) group translocators; and (e) transmembrane electron flow carriers (Busch and Saier, 2002; Saier, 2000). The less well-defined proteins include (8) auxiliary transport proteins and (9) transporters or putative transporters of unknown mechanism of action or function (Saier, 2000).

Table 1 summarizes the distribution of the 932 transporter protein constituents from Rme in each of the major TC categories and also provides a breakdown of these proteins found in the various TC subclasses; 123 channel proteins, most of them outer membrane porins, were identified. However, the majority of defined transport proteins found are secondary carriers (304) and constituents of primary active transporters (343).

Only one phosphoenolpyruvate-dependent, sugar transporting phosphotransferase system (PTS) permease, which catalyses group translocation of hexoses, was found. Further, only 10 transmembrane electron flow system constituents were identified. This latter fact may in part reflect the limited representation of transmembrane electron flow carriers in the Transporter Classification Database (TCDB). Thirty-one auxiliary proteins of TC class 8 and 65 putative transporters of TC class 9 were identified (Table 1). The probable functional identities of the individual proteins will be discussed below.

Classes of substrates transported

Table 2 summarizes the numbers of transporter proteins concerned with the transport of various types of substrates; 300 proteins are putative transport protein homologues concerned with the uptake or efflux of inorganic ions, and nearly three-quarters of them are concerned with inorganic cation transport. This observation undoubtedly relates to the remarkable heavy metal resistance of Rme.

Forty-one systems specific for sugars and their derivatives and 110 systems specific for amino acids and their derivatives were identified. These facts suggest that amino acid metabolism may be more important to Rme than sugar metabolism for heterotrophic growth. This substrate preference of Rme has been observed before (Mergeay *et al*., 1985). Rme has 142 transport protein homologues putatively concerned with carboxylate transport, which also agrees with the substrate spectrum of this bacterium (Mergeay *et al*., 1985). This fact, together with the greater number of secondary carriers relative to primary active transporters, points to a strong metabolic dependency on respiration rather than fermentation. Ninety-one

Table 1. Categories of recognized transport proteins found in *Ralstonia metallidurans*

TC class	No. of transporters (%)	TC subclass	No. of transporters (%)
1 Channels	123 (13)	1.A. α-Type channel-forming proteins and peptides	27 (3)
		1.B. Outer membrane porins (ß-structure)	94 (10)
		1.C. Pore-forming toxins (proteins and peptides)	1 (0.1)
		1.E. Holins	1 (0.1)
2 Secondary carriers	304 (33)	2.A. Carrier-type facilitators	299 (32)
		2.C. Ion-gradient-driven energizers	5 (1)
3 Primary transporters	343 (37)	3.A. P-P bond hydrolysis-driven transporters	290 (31)
		3.B. Decarboxylation-driven active transporters	2 (0.2)
		3.D. Oxidoreduction-driven active transporters	51 (5)
4 Group translocators (PTS)	2 (0.2)	4.A. Phosphotransferase systems	2 (0.2)
5 Transmembrane electron carriers	10 (1)	5.A. Transmembrane electron transfer carriers	10 (1)
8 Auxiliary transport proteins	31 (3)	8.A. Auxiliary transport proteins	31 (3)
9 Poorly-defined systems	65 (7)	9.A. Transporters of unknown classification	10 (1)
		9.B. Putative uncharacterized transporters	55 (6)
Unclassified	54 (6)	Unclassified	54 (6)
Total number	932 (100)		932 (100)

Table 2. Breakdown of transport proteins according to predicted substrate types in *Ralstonia metallidurans*

Substrate class	No. of transporters (%)	Substrate subclass	No. of transporters (%)
1 Inorganic compounds	300 (32)	Cations	221 (24)
		Anions	78 (8)
		H_2O	1 (0.1)
2 Organic compounds	400 (43)	Sugars/sugar metabolites	41 (4)
		Amino acids/polyamines	110 (12)
		Mono-, di-, tricarboxylates	
		Fatty acids	142 (15)
		Drugs/toxic compounds	91 (10)
		Nucleotides/nucleosides	4 (0.4)
		Aromatics	13 (1)
3 Macromolecules	102 (11)	Lipoproteins/proteins	75 (8)
		Lipopolysaccharides/polysaccharides	20 (2)
		DNA	5 (0.5)
		Lipids	1 (0.1)
4 Miscellaneous/unknown	130 (14)	Miscellaneous	15 (2)
		Unknown	115 (12)
Total	932 (100)		932 (100)

proteins are predicted to be concerned with transport of drugs and hydrophobic substances, while 130 proteins fall into the miscellaneous/unknown category.

Global analysis of transporters in Rme and their family associations

Table 3 summarizes the results of our detailed analyses of transporters found in Rme. On the left, the family TC number, the name of the family and its standard abbreviation can be found (columns 1–3). Column 4 presents the types of substrates known to be transported by members of the respective family. Column 5 gives the number of family members identified in Rme, while column 6 presents the gene designation used in the draft version (02jul03) of the Rme genome analysed here. A full version of this table that contains all of the various names of the CH34 genes is provided as supplementary material (**http://bionomie.mikrobiologie.uni-halle.de/SupMat/Roz_05/Table 3.htm**). Column 7 gives the protein size in number of amino acyl residues, and column 8 provides an estimate of the number of putative transmembrane spanning regions (TMSs) for each protein. The TC number of the protein in TCDB that shows greatest similarity to the Rme ORF under consideration is presented in column 9. Finally, column 10 presents the level of confidence for the functional assignment (1 = sure, 2 = probable, 3 = uncertain or unknown).

Channels

In category 1A (α-type channels), Rme possesses two members of the VIC family (1.A.1), both probably K^+ channels. Two members of the MIP family of aqua/glycerol porins are also present. Four putative chloride channels (ClC family) were found, as well as one CytB homologue. This last system may function primarily in transmembrane electron flow, but no bacterial member of this family has been characterized (Kimball and Saier, 2002).

MscL (1.A.22), MscS (1.A.23) and MIT (1.A.35) families are all well represented with one, nine and four members, respectively. All four MIT family members are probably divalent cation transporters, while the MscL and MscS proteins are most likely non-specific channels for protection against osmotic stress (Busch and Saier, 2002; Nottebrock *et al*., 2003; Pivetti *et al*., 2003). Rme exhibits two paralogues within the hsp70 family of chaperone proteins, some of which have been shown to be capable of forming transmembrane channels (Arispe and De Maio, 2000). No other channel-type proteins of TC class 1.A could be recognized.

A tremendous number of putative outer membrane porins were identified. For example, just within the general bacterial porin (GBP) family (1.B.1), 29 paralogues were found. Most of these proteins are of 300–400 amino acids in length and probably consist largely of β-structure. A trimeric

Table 3. Putative transport proteins identified in *Ralstonia metallidurans*[a]

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
1.A. α-Type channel-forming proteins and peptides									
1.A.1	Voltage-gated ion channel	VIC	Na^+, K^+, Ca^{2+}, multiple cations		Contig372gene5732	307	5	1.A.1.2.3(1)	3
				2	Contig373gene6187	229	7	1.A.1.13.1(1)	3
1.A.8	Major intrinsic protein	MIP	H_2O, glycerol, urea, polyols, NH_3, CO_2		Contig375gene7720	250	6	1.A.8.13.1(1)	3
				2	Contig375gene8643	234	6	1.A.8.3.1(1)	2
1.A.11	Chloride channel	ClC	Cl^-, anions		Contig365gene3384	376	4	1.A.11.6.1(1)	3
					Contig365gene3245	657	12	1.A.11.6.1(1)	3
					Contig352gene1115	560	8	1.A.11.6.1(1)	3
				4	Contig367gene3837	522	10	1.A.11.6.1(1)	3
1.A.20	$gp91_{phox}$ Phagocyte NADPH oxidase-associated cytochrome b558	CytB	H^+	1	Contig363gene2857	447	6	1.A.20.6.1(1)	2
1.A.22	Large conductance mechanosensitive ion channel	MscL	Proteins, ions (slightly cation selective)	1	Contig367gene3927	144	2	1.A.22.1.3(1)	2
1.A.23	Small conductance mechanosensitive ion channel	MscS	Ions (slight anion selectivity)		Contig350gene938	456	6	1.A.23.1.1(1)	2
					Contig373gene6759	357	4	1.A.23.1.1(1)	3
					Contig375gene8191	275	3	1.A.23.1.1(1)	3
					Contig375gene8051	832	9	1.A.23.1.1(1)	2
					Contig375gene7869	288	4	1.A.23.2.1(1)	2
					Contig372gene5633	284	4	1.A.23.2.1(1)	2
					Contig371gene5100	771	13	1.A.23.3.1(1)	3
					Contig358gene1757	570	5	1.A.23.4.1	3
				9	Contig361gene2514	447	5	1.A.23.4.1(1)	3
1.A.30	H^+- or Na^+-translocating bacterial flagellar motor ExbBD outer membrane transport energizer	Mot/Exb-Mot	H^+, Na^+		Contig371gene5340	299	4	1.A.30.1.1(2)	2
				2	Contig371gene5341	325	1	1.A.30.1.1(2)	2
1.A.33	Cation channel-forming heat shock protein-70	Hsp70	Ions, polypeptides		Contig370gene5054	621	0	1.A.33.1.2(1)	2
				2	Contig372gene5852	648	0	1.A.33.1.2(1)	2
1.A.35	CorA metal ion transporter	MIT	Heavy-metal ions, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+}, Al^{3+}, Mn^{2+}		Contig363gene2888	320	2	1.A.35.1.2(1)	2

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
					Contig374gene7317	383	3	1.A.35.3.1(1)	3
					Contig365gene3224	362	3	1.A.35.3.1(1)	3
				4	Contig367gene3951	393	2	1.A.35.3.1(1)	3
1.B. Outer membrane porins (ß-structure)									
1.B.1	General bacterial porin	GBP	Ions, small (M_r of <1000 Da) molecules		Contig358gene1907	367	1	1.B.1.4.1(1)	2
					Contig340gene366	432	3	1.B.1.4.1(1)	3
					.00Dec2000-Contig485gene1110			1.B.1.4.1(1)	3
					Contig373gene6049	381	1	1.B.1.4.1(1)	3
					Contig373gene6801	393	6	1.B.1.4.1(1)	3
					Contig374gene7003	111	1	1.B.1.4.1(1)	2
					Contig338gene292	382	1	1.B.1.4.1(1)	3
					Contig375gene8510	352	0	1.B.1.4.1(1)	2
					Contig375gene8258	379	1	1.B.1.4.1(1)	2
					Contig375gene8489	355	3	1.B.1.4.1(1)	2
					Contig375gene9145	353	1	1.B.1.4.1(1)	2
					Contig375gene9285	341	0	1.B.1.4.1(1)	3
					Contig354gene1343	377	1	1.B.1.4.1(1)	2
					Contig364gene3106	371	1	1.B.1.4.1(1)	2
					Contig375gene8681	352	1	1.B.1.4.1(1)	2
					Contig373gene6282	386	1	1.B.1.4.1(1)	2
					Contig371gene5423	391	1	1.B.1.4.1(1)	2
					Contig372gene5670	387	2	1.B.1.4.1(1)	2
					Contig372gene5912	358	0	1.B.1.4.1(1)	2
					Contig372gene5687	362	0	1.B.1.4.1(1)	2
					Contig370gene4663	361	1	1.B.1.6.1(1)	3
					Contig373gene6196	382	1	1.B.1.6.1(1)	2
					Contig357gene1691	374	1	1.B.1.6.1(1)	2
					Contig358gene1746	354	1	1.B.1.6.1(1)	2
					Contig361gene2442	371	1	1.B.1.6.1(1)	2
					Contig373gene6139	355	3	1.B.1.6.1(1)	2
					Contig373gene6638	371	1	1.B.1.6.1(1)	3
					Contig371gene5227	363	1	1.B.1.6.1(1)	2
				29	Contig369gene4254	355	3	1.B.1.6.1(1)	2
1.B.6	OmpA-OmpF porin	OOP	Ions, small molecules		Contig370gene4785	217	0	1.B.6.1.1(1)	2
					Contig365gene3355	643	2	1.B.6.1.2(1)	3
				3	Contig373gene6115	217	1	1.B.6.1.3(1)	3
1.B.9	FadL outer membrane protein	FadL	Fatty acid, toluene, *m*-xylene and benzyl alcohol	1	Contig343gene479	464	1	1.B.9.2.1(1)	2

				Contig358gene1879	761	0	1.B.11.3.1(1)	2	
1.B.11	Outer membrane fimbrial usher porin	FUP	Protein folding and subunit assembly						
				Contig365gene3393	854	1	1.B.11.3.1(1)	2	
			3	Contig354gene1279	850	1	1.B.11.3.1(1)	2	
1.B.12	Autotransporter	AT		Contig365gene3360	1741	1	1.B.12.1.3(1)	2	
1.B.14	Outer membrane receptor	OMR	N-terminal protein domains Iron-siderophore complexes, vitamin B_{12}, Cu^{2+}, colicin, DNA of various phages	Contig374gene7240	733	1	1.B.14.1.2(1)	3	
				Contig372gene5928	731	1	1.B.14.1.2(1)	2	
				Contig372gene5930	717	0	1.B.14.1.2(1)	2	
				Contig370gene4894	764	1	1.B.14.1.4(1)	2	
				Contig361gene2288	742	1	1.B.14.1.4(1)	2	
				Contig374gene7149	744	1	1.B.14.1.4(1)	2	
				Contig374gene7151	804	0	1.B.14.1.4(1)	3	
				Contig363gene2909	753	1	1.B.14.1.4(1)	2	
				Contig369gene4344	728	1	1.B.14.1.4(1)	2	
				Contig369gene4384	761	2	1.B.14.1.4(1)	3	
				Contig375gene8531	741	0	1.B.14.1.4(1)	2	
				Contig374gene6949	719	0	1.B.14.1.6(1)	3	
				Contig373gene6356	661	0	1.B.14.1.6(1)	2	
				Contig375gene8072	821	1	1.B.14.1.8(1)	3	
				Contig366gene3585	698	1	1.B.14.3.1(1)	2	
				Contig375gene8595	724	0	1.B.14.4.1(1)	2	
			17	Contig369gene4334	815	1	1.B.14.9.1(1)	2	
1.B.17	Outer membrane factor	OMF	Heavy metal cations, drugs, oligosaccharides, proteins, etc.	Contig366gene3474	493	0	1.B.17.1.1(1)	2	
				Contig369gene4234	448	1	1.B.17.2.1(1)	1	
				Contig368gene3997	418	0	1.B.17.2.1(1)	1	
				Contig357gene1641	460	0	1.B.17.2.2(1)	1	
				Contig371gene5461	445	0	1.B.17.2.2(1)	3	
				Contig374gene7266	431	1	1.B.17.2.2(1)	1	
				Contig375gene8615	418	0	1.B.17.2.2(1)	1	
				Contig374gene7202	520	2	1.B.17.2.3(1)	3	
				Contig373gene6079	433	0	1.B.17.2.3(1)	3	
				Contig375gene9177	496	0	1.B.17.3.1(1)	2	
				Contig375gene8190	485	1	1.B.17.3.1(1)	2	
				Contig353gene1195	455	0	1.B.17.3.2(1)	3	
				Contig360gene2100	519	0	1.B.17.3.2(1)	2	
				Contig354gene1322	418	1	1.B.17.3.2(1)	2	
				Contig364gene3065	504	1	1.B.17.3.2(1)	2	
				Contig358gene1809	486	2	1.B.17.3.3(1)	2	
				Contig375gene7574	497	1	1.B.17.3.3(1)	2	
				Contig359gene2067	488	1	1.B.17.3.3(1)	2	

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
					Contig353gene1181	488	1	1.B.17.3.3(1)	2
					Contig373gene6314	518	1	1.B.17.3.3(1)	2
					Contig373gene6558	511	3	1.B.17.3.3(1)	2
					Contig375gene8564	495	0	1.B.17.3.3(1)	2
					Contig358gene1815	519	0	1.B.17.3.4(1)	2
					Contig373gene6386	589	0	1.B.17.3.4(1)	3
					Contig362gene2648	512	0	1.B.17.3.4(1)	2
					Contig353gene1190	497	1	1.B.17.3.5(1)	2
					Contig375gene8587	476	0	1.B.17.3.5(1)	2
				28	Contig375gene7766	491	2	1.B.17.3.5(1)	2
1.B.18	Outer membrane auxiliary protein	OMA	Exo- or capsular polysaccharide		Contig375gene8672	606	0	1.B.18.1.2(1)	2
				2	Contig372gene5594	362	0	1.B.18.3.1(1)	2
1.B.19	Glucose-selective OprB porin	OprB	Ions, small molecules	1	Contig359gene1948	492	1	1.B.19.1.1(1)	3
1.B.20	Two-partner secretion	TPS	Proteins		Contig373gene6550	588	0	1.B.20.1.1(1)	2
				2	Contig371gene5256	558	1	1.B.20.3.1(1)	3
1.B.22	Outer bacterial membrane secretin	Secretin	Proteins		Contig371gene5305	473	0	1.B.22.1.1(1)	2
					Contig365gene3336	620	1	1.B.22.1.2(1)	3
					Contig375gene7610	783	1	1.B.22.1.2(1)	2
					Contig367gene3787	710	1	1.B.22.2.1(1)	2
					Contig375gene9238	734	1	1.B.22.4.1(1)	2
				6	Contig368gene4122	600	0	1.B.22.7.1(1)	3
1.B.39	Bacterial porin, OmpW	OmpW	Methyl viologen and benzyl viologen		Contig375gene9331	286	1	1.B.39.1.1(1)	3
				2	Contig372gene5565	245	0	1.B.39.1.1(1)	3
1.C. Pore-forming toxins (proteins and peptides)									
1.C.1	Channel-forming colicin	Colicin	Ions, small molecules	1	Contig353gene1187	443	1	1.C.1.3.1(1)	3
1.E. Holins									
1.E.14	LrgA holin	LrgA Holin	Zn^{2+}, Fe^{2+}	1	Contig372gene5735	128	3	1.E.14.1.1(1)	3
2.A. Carrier type facilitators									
2.A.1	Major facilitator superfamily	MFS	Various small molecules						
		- SP (1)	Sugars	1	Contig373gene6468	484	12	2.A.1.1.15(1)	3
		- DHA1 (12 spanner) (2)	Drugs		Contig374gene7546	418	12	2.A.1.2.4(1)	3
					Contig358gene1790	411	12	2.A.1.2.4(1)	3
					Contig370gene4856	418	12	2.A.1.2.7(1)	2
					Contig375gene9203	426	11	2.A.1.2.7(1)	2
					Contig369gene4402	634	12	2.A.1.2.9(1)	3
					Contig375gene8690	408	12	2.A.1.2.8(1)	3
					Contig371gene5419	415	12	2.A.1.2.8(1)	3

		Contig373gene6337	404	12	2.A.1.2.10(1)	3	
		Contig373gene6438	275	6	2.A.1.2.10(1)	3	
		Contig375gene8780	408	12	2.A.1.2.10(1)	3	
		Contig369gene4487	426	11	2.A.1.2.10(1)	3	
		Contig373gene6352	388	12	2.A.1.2.14(1)	2	
		Contig375gene9023	421	12	2.A.1.2.14(1)	2	
		Contig375gene7618	400	12	2.A.1.2.14(1)	3	
		Contig375gene7724	398	12	2.A.1.2.18(1)	3	
16		Contig369gene4338	395	12	2.A.1.2.20(1)	3	
	Drugs	Contig360gene2102	537	14	2.A.1.3.2(1)	2	
- DHA2 (14 spanner) (3)							
		Contig358gene1921	532	14	2.A.1.3.2(1)	2	
		Contig364gene3067	519	14	2.A.1.3.2(1)	2	
		Contig366gene3427	396	12	2.A.1.3.5(1)	3	
		Contig375gene7796	528	14	2.A.1.3.5(1)	2	
		Contig339gene328	480	14	2.A.1.3.11(1)	2	
		Contig335gene226	548	14	2.A.1.3.12(1)	2	
		Contig373gene6800	504	14	2.A.1.3.17(1)	3	
		Contig374gene7112	490	14	2.A.1.3.17(1)	2	
		Contig375gene8085	473	14	2.A.1.3.17(1)	2	
		Contig373gene6605	513	14	2.A.1.3.17(1)	2	
		Contig351gene1053	508	14	2.A.1.3.18(1)	2	
		Contig375gene8189	524	13	2.A.1.3.18(1)	2	
		Contig375gene8551	545	14	2.A.1.3.18(1)	2	
15		Contig371gene5277	525	14	2.A.1.3.18(1)	3	
	Dicarboxylates, tricarboxylates	Contig373gene6395	434	12	2.A.1.6.1(1)	2	
- MHS (6)		Contig361gene2390	429	12	2.A.1.6.1(1)	2	
		Contig374gene7196	476	12	2.A.1.6.1(1)	2	
		Contig375gene8112	436	12	2.A.1.6.1(1)	2	
		Contig373gene6395	434	0	2.A.1.6.1(1)	3	
		Contig354gene1283	262	7	2.A.1.6.1(1)	2	
		Contig372gene5669	443	12	2.A.1.6.1(1)	2	
		Contig375gene7621	461	12	2.A.1.6.1(1)	2	
		Contig372gene5548	437	12	2.A.1.6.3(1)	2	
		Contig352gene1110	565	0	2.A.1.6.4(1)	3	
		Contig358gene1834	255	6	2.A.1.6.5(1)	2	
		Contig358gene1835	300	6	2.A.1.6.5(1)	2	
		Contig352gene1110	565	12	2.A.1.6.5(1)	2	
		Contig373gene6129	459	12	2.A.1.6.6(1)	2	
15		Contig375gene8177	439	12	2.A.1.6.6(1)	2	
	Nitrate, nitrite	Contig375gene7921	437	12	2.A.1.8.4(1)	2	
- NNP (8)		Contig360gene2126	427	12	2.A.1.8.11(1)	2	
		Contig360gene2127	460	12	2.A.1.8.11(1)	2	
3		Contig364gene3127	441	12	2.A.1.11.1(1)	2	
- OFA (11)	Oxalate, formate						

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
					Contig355gene1391	1472	12	2.A.1.11.1(1)	3
				3	Contig357gene1707	475	12	2.A.1.11.1(1)	3
		- SHS (12)	Sialate, lactate, pyruvate	1	Contig359gene2081	397	12	2.A.1.12.1(1)	3
		- ACS (14)	Organic acids		Contig353gene1192	443	12	2.A.1.14.1(1)	3
					Contig375gene9216	453	12	2.A.1.14.1(1)	2
					Contig371gene5115	433	12	2.A.1.14.1(1)	2
					Contig365gene3217	444	12	2.A.1.14.1(1)	2
					Contig365gene3305	418	12	2.A.1.14.2(1)	3
					Contig346gene689	437	12	2.A.1.14.3(1)	2
					Contig361gene2423	441	12	2.A.1.14.3(1)	2
					Contig359gene1940	453	12	2.A.1.14.8(1)	2
				9	Contig375gene8175	432	12	2.A.1.14.8(1)	3
		- AAHS(15)	Aromatic acids		Contig364gene3071	413	12	2.A.1.15.1(1)	3
					Contig375gene8062	459	12	2.A.1.15.1(1)	2
					Contig371gene5360	441	12	2.A.1.15.1(1)	2
					Contig373gene6741	395	12	2.A.1.15.3(1)	3
				5	Contig375gene9515	441	12	2.A.1.15.4(1)	2
		- CP (17)	Cyanate	1	Contig370gene4875	423	12	2.A.1.17.1(1)	3
		- OCT (19)	Organic cations	1	Contig373gene6048	526	12	2.A.1.19.4(1)	2
		- SET (20)	Sugars	1	Contig373gene6742	434	0	2.A.1.20.2(1)	3
		- DHA3 (12 spanner) (21)	Drugs	1	Contig351gene976	493	12	2.A.1.21.3(1)	3
		- VNT (22)	Neurotransmitter	1	Contig375gene7913	514	12	2.A.1.22.1(1)	3
		- BST (23)	Unknown	1	Contig375gene8194	436	12	2.A.1.23.1(1)	3
		- PAT (25)	Peptides, AcCoA	1	Contig374gene6932	466	12	2.A.1.25.2(1)	2
		- UMC-terminal fragment (26)	Unknown	1	Contig368gene4202	413	12	2.A.1.26.1(1)	3
		- PPP (27)	Phenylpropionate	1	Contig369gene4405	365	11	2.A.1.27.1(1)	2
		- ADT (30)	Abietane diterpenoid	1	Contig372gene5630	468	12	2.A.1.30.1(1)	3
		- Nre (31)	Ni^{2+}	1	Contig369gene4238	408	12	2.A.1.31.1(1)	2
		- Fsr (35)	Fosmidomycin	1	Contig375gene7801	409	12	2.A.1.35.1(1)	2
		- AtoE (37)	Short chain fatty		Contig364gene2968	467	14	2.A.1.37.1(1)	2
	total 83			2	Contig375gene9151	484	14	2.A.1.37.1(1)	2
2.A.3	Amino acid-polyamine-organocation	APC	Amino acids, polyamines, choline						
		- AAA (1)	Amino acids		Contig361gene2312	493	12	2.A.3.1.2(1)	2
					Contig375gene8013	510	12	2.A.3.1.2(1)	2
					Contig375gene8010	462	12	2.A.3.1.3(1)	2

				Contig373gene6637	475	12	2.A.3.1.9(1)	2	
			5	Contig373gene6757	474	12	2.A.3.1.9(1)	2	
		- CAT (3)	1	Contig374gene7465	469	14	2.A.3.3.1(1)	2	
	total 6	CDF		Contig375gene8618	316	5	2.A.4.1.1(1)	1	
2.A.4	Cation diffusion facilitator			Contig375gene9479	337	6	2.A.4.1.1(1)	3	
			3	Contig374gene6900	436	6	2.A.4.1.2(1)	2	
2.A.5	Zinc (Zn^{2+})—iron (Fe^{2+}) permease	ZIP	1	Contig356gene1473	291	6	2.A.5.4.1(1)	3	
2.A.6	Resistance-nodulation-cell division	RND							
		- HME (1)		Contig369gene4237	1076	11	2.A.6.1.1(1)	1	
				Contig368gene3999	1076	12	2.A.6.1.1(1)	1	
				Contig331gene151	1043	12	2.A.6.1.2(1)	1	
				Contig371gene5463	840	0	2.A.6.1.X	3	
				Contig371gene5464	184	5	2.A.6.1.X	1	
				Contig373gene6557	1039	12	2.A.6.1.2(1)	1	
				Contig373gene6563	1045	12	2.A.6.1.2(1)	1	
				Contig375gene8617	1063	12	2.A.6.1.2(1)	1	
				Contig361gene2416	1036	12	2.A.6.1.2(1)	1	
				Contig375gene8282	1023	11	2.A.6.1.2(1)	1	
				Contig363gene2863	1009	7	2.A.6.1.2(1)	1	
				Contig375gene8486	691	6	2.A.6.1.2(1)	1	
				Contig375gene8119	365	5	2.A.6.1.2(1)	1	
				Contig373gene6081	1055	14	2.A.6.1.3(1)	2	
				Contig357gene1642	384	0	2.A.6.1.4(4)	3	
				Contig369gene4482	521	1	2.A.6.1.4(4)	2	
			17	Contig369gene4483	1056	14	2.A.6.1.4(1)	2	
		- HAE1 (2)		Contig375gene7765	1050	12	2.A.6.2.2(1)	2	
		Hydrophobe/amphiphile substrates							
				Contig369gene4331	1044	12	2.A.6.2.7(1)	2	
				Contig358gene1808	1063	12	2.A.6.2.9(1)	2	
				Contig375gene7572	1051	12	2.A.6.2.12(1)	2	
				Contig375gene7573	1100	12	2.A.6.2.12(1)	2	
				Contig365gene3373	1066	0	2.A.6.2.12(1)	2	
				Contig365gene3373	1066	12	2.A.6.2.12(1)	2	
				Contig353gene1179	1065	12	2.A.6.2.12(1)	2	
			9	Contig375gene7759	1069	12	2.A.6.2.12(1)	2	
		- SecDF (4)		Contig372gene5750	636	5	2.A.6.4.1(2)	2	
		Sec secretory accessory proteins	2	Contig372gene5751	324	6	2.A.6.4.1(2)	2	
		- HAE2 (5)	1	Contig364gene3205	858	9	2.A.6.5.1(1)	3	
		Hydrophobe/amphiphile substrates							

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
	total 30	- ORF4 (8)	Hydrophobe/amphiphile substrates	1	Contig364gene3146	786	11	2.A.6.8.1(1)	2
2.A.7	Drug/metabolite transporter	DMT	Multiple drugs and dyes (mostly cationic)						
		- SMR (1)	Drugs		Contig338gene297	109	4	2.A.7.1.3(1)	3
				2	Contig374gene7258	123	5	2.A.7.1.3(1)	3
		- BAT (2)	Unknown		Contig356gene1583	362	11	2.A.7.2.1(1)	3
				2	Contig375gene9035	143	5	2.A.7.2.1(1)	2
		- DME (3)	Drugs, metabolites		Contig375gene9463	297	10	2.A.7.3.2(1)	2
					Contig364gene3008	306	10	2.A.7.3.2(1)	3
					Contig374gene7235	312	10	2.A.7.3.2(1)	3
					Contig352gene1130	301	10	2.A.7.3.2(1)	3
					Contig375gene7949	297	10	2.A.7.3.2(1)	3
					Contig363gene2886	347	11	2.A.7.3.2(1)	3
					Contig375gene8660	337	11	2.A.7.3.3(1)	3
					Contig370gene4864	532	10	2.A.7.3.3(1)	3
					Contig361gene2346	300	10	2.A.7.3.4(1)	3
					Contig375gene8332	345	10	2.A.7.3.4(1)	3
					Contig348gene822	319	10	2.A.7.3.6(1)	3
				12	Contig375gene7919	288	10	2.A.7.3.6(1)	2
		- RarD (7)	Chloramphenicol		Contig375gene7722	342	10	2.A.7.7.1(1)	2
	total 18			2	Contig375gene8931	311	10	2.A.7.17.1(1)	2
2.A.9	Cytochrome oxidase bio-genesis	Oxa1	Proteins	1	Contig375gene9312	555	4	2.A.9.3.1(1)	2
2.A.10	2-Keto-3-deoxygluconate transporter	KDGT	2-Keto-3-deoxygluconate	1	Contig356gene1558	327	10	2.A.10.1.1(1)	2
2.A.11	Citrate-Mg^{2+} : H^+ (CitM) Citrate-Ca^{2+} : H^+ (CitH) Symporter	CitMHS	Citrate	1	Contig375gene7824	485	11	2.A.11.1.1(1)	3
2.A.12	ATP : ADP antiporter	AAA	ATP, ADP	1	Contig375gene7893	453	10	2.A.12.3.1(1)	3
2.A.14	Lactate permease	LctP	Lactate	1	Contig372gene5556	566	16	2.A.14.1.2(1)	2
2.A.19	Ca^{2+}:cation antiporter	CaCA	Ca^{2+}	1	Contig375gene7970	360	11	2.A.19.1.1(1)	2
2.A.20	Inorganic phosphate transporter	PiT	Inorganic phosphate	1	Contig344gene557	336	9	2.A.20.2.4(1)	2
2.A.21	Solute : sodium symporter	SSS	Sugars, amino acids, vitamins, nucleosides, inositols, iodide, urea		Contig375gene7737	461	13	2.A.21.4.1(1)	2
					Contig375gene8758	683	14	2.A.21.7.1(1)	2
					Contig372gene5917	553	14	2.A.21.7.1(1)	2
					Contig359gene1964	478	13	2.A.21.8.1(1)	2
				5	Contig374gene7322	967	4	2.A.21.9.1(1)	3
2.A.23	Dicarboxylate/amino acid : cation (Na^+ or H^+) symporter	DAACS	C4-dicarboxylates, acidic and neutral amino acids	5	Contig365gene3380	435	9	2.A.23.1.2(1)	2
					Contig369gene4393	430	8	2.A.23.1.2(1)	2

				Contig375gene7654	467	10	2.A.23.1.3(1)	2	
				Contig369gene4353	452	8	2.A.23.1.3(1)	2	
				Contig374gene7480					
2.A.24	Citrate : cation symporter	CCS	Mono-, di-, and tricarboxylates	Contig373gene6794	448	13	2.A.24.2.5(1)	2	
2.A.36	Monovalent cation : proton antiporter-1	CPA1	Na^+/H^+, Na^+ or K^+/H^+	Contig373gene6805	435	12	2.A.36.6.1(1)	3	
2.A.37	Monovalent cation : proton antiporter-2	CPA2	Na^+/H^+ or K^+/H^+	Contig358gene1749	404	12	2.A.37.1.1(2)	2	
				Contig375gene9498	219	0	2.A.37.1.1(2)	2	
				Contig375gene9499	604	13	2.A.37.1.1(2)	2	
				Contig374gene7542	674	13	2.A.37.1.2(2)	2	
				Contig375gene8748	408	13	2.A.37.1.2(2)	2	
			6	Contig375gene9414	406	12	2.A.37.1.2(2)	3	
2.A.40	Nucleobase : cation symporter-2	NCS2	Nucleobases, urate	Contig370gene4820	482	13	2.A.40.1.1(1)	2	
				Contig372gene5621	453	13	2.A.40.1.1(1)	3	
			3	Contig371gene5375	445	14	2.A.40.3.1(1)	2	
2.A.45	Arsenite–antimonite	ArsB	Arsenite, antimonite	Contig375gene8078	419	12	2.A.45.1.1(1)	3	
2.A.46	Benzoate : H^+ symporter	BenE	Benzoate	Contig338gene295	395	12	2.A.46.1.1(1)	2	
2.A.47	Divalent anion : Na^+ symporter	DASS	Dicarboxylates, phosphate, sulphate	Contig369gene4367	181	5	2.A.47.3.1(1)	2	
				Contig369gene4366	532	7	2.A.47.3.1(1)	2	
				Contig365gene3403	507	15	2.A.47.3.3(1)	2	
			4	Contig373gene6264	530	15	2.A.47.3.3(1)	3	
2.A.49	Ammonium transporter	Amt	Ammonium	Contig375gene7868	400	0	2.A.49.X	2	
			2	Contig374gene7490	510	13	2.A.49.1.1(1)	2	
2.A.51	Chromate ion transporter	CHR	Chromate, sulphate (uptake or efflux)	Contig355gene1410	193	5	2.A.51.1.1(1)	3	
				Contig371gene5134	390	12	2.A.51.1.1(1)	1	
				Contig368gene4196	401	12	2.A.51.1.(0)	1	
			4	Contig375gene7933	408	11	2.A.51.1.2(1)	3	
2.A.52	Ni^{2+}-Co^{2+} transporter	NiCoT	Ni^{2+}, Co^{2+}	Contig334gene209	278	7	2.A.52.1.2(1)	3	
2.A.53	Sulphate permease	SulP	Sulphate	Contig325gene78	603	11	2.A.53.1.4(1)	2	
				Contig375gene8514	492	13	2.A.53.3.1(1)	2	
				Contig365gene3367	599	11	2.A.53.4.1(1)	2	
				Contig375gene8575	578	11	2.A.53.4.1(1)	2	
			5	Contig371gene5371	586	13	2.A.53.4.1(1)	2	
2.A.56	Tripartite ATP-independent periplasmic transporter	TRAP-T	C4-dicarboxylates, acidic amino acids, sugars?	Contig369gene4416	434	13	2.A.56.1.1(3)	3	
				Contig361gene2330	327	1	2.A.56.1.1(3)	3	
				Contig361gene2332	436	11	2.A.56.1.1(0)	2	
				Contig369gene4417	343	1	2.A.56.1.1(3)	2	
				Contig366gene3497	180	4	2.A.56.1.2(0)	2	
			6	Contig366gene3498	574	13	2.A.56.1.2(0)	2	

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
2.A.58	Phosphate : Na^+ symporter	PNaS	Inorganic phosphate		Contig353gene1224	632	9	2.A.58.2.1(1)	2
				2	Contig374gene7545	558	9	2.A.58.2.1(1)	2
2.A.59	Arsenical resistance-3	ACR3	Arsenite	1	Contig359gene2078	354	10	2.A.59.1.1(1)	3
2.A.64	Twin arginine targeting	Tat	Redox proteins		Contig367gene3817	77	1	2.A.64.1.1(4)	3
					Contig367gene3818	168	1	2.A.64.1.1(4)	3
					Contig367gene3819	260	5	2.A.64.1.1(4)	2
				4	Contig367gene3820	401	1	2.A.64.1.1(4)	2
2.A.66	Multidrug/oligosaccharidyl-lipid/polysaccharide	MOP	Drugs, lipid-linked oligosaccharide precursors						
		- MATE (1)	Drugs		Contig362gene2523	449	12	2.A.66.1.1(1)	2
					Contig367gene3916	455	12	2.A.66.1.1(1)	3
				3	Contig375gene8978	492	12	2.A.66.1.3(1)	3
		- PST (2)	Polysaccharides	1	Contig366gene3637	419	12	2.A.66.2.4(1)	2
		- MVF (4)	Unknown	1	Contig372gene5715	534	14	2.A.66.4.1(1)	2
2.A.67	Oligopeptide transporter	OPT	Peptides		Contig372gene5640	668	17	2.A.67.3.1(1)	3
				2	Contig363gene2777	676	18	2.A.67.4.1(1)	2
2.A.69	Auxin efflux carrier	AEC	Auxin (efflux)		Contig356gene1506	293	10	2.A.69.1.1(1)	3
				2	Contig375gene9534	351	10	2.A.69.2.1(1)	3
2.A.72	K^+ uptake permease	KUP	K^+ (uptake)	1	Contig349gene850	656	11	2.A.72.1.1(1)	2
2.A.75	L-Lysine exporter	LysE	Basic amino acids	1	Contig371gene5136	216	6	2.A.75.1.1(1)	2
2.A.76	Resistance to homoserine/threonine	RhtB	Neutral amino acids and their derivatives		Contig355gene1462	205	5	2.A.76.1.1(1)	3
					Contig362gene2710	223	6	2.A.76.1.1(1)	3
					Contig355gene1382	209	6	2.A.76.1.1(1)	3
					Contig363gene2791	212	6	2.A.76.1.1(1)	3
					Contig371gene5511	208	6	2.A.76.1.1(1)	3
					Contig374gene7486	214	6	2.A.76.1.1(1)	2
					Contig375gene7623	212	6	2.A.76.1.1(1)	3
					Contig372gene5890	212	6	2.A.76.1.1(1)	3
					Contig373gene6271	205	5	2.A.76.1.2(1)	3
					Contig373gene6137	203	6	2.A.76.1.2(1)	3
				11	Contig372gene5541	204	6	2.A.76.1.2(1)	3
2.A.78	Branched chain amino acid exporter	LIV-E	Carboxylates, amino acids, amines (efflux)	1	Contig352gene1134	265	4	2.A.78.1.1(2)	3
2.A.80	Tricarboxylate transporter	TTT	Tricarboxylate		Contig373gene6710	326	1	2.A.80.1.1(3)	3
					Contig364gene3003	322	1	2.A.80.1.1(3)	3
					Contig364gene2995	320	1	2.A.80.1.1(3)	3
					Contig358gene1831	337	0	2.A.80.1.1(3)	3
					Contig370gene4730	554	1	2.A.80.1.1(3)	3

Contig345gene580	327	4	2.A.80.1.1(3)	3	
Contig360gene2191	326	1	2.A.80.1.1(3)	3	
Contig373gene6749	322	3	2.A.80.1.1(3)	3	
Contig373gene6096	328	3	2.A.80.1.1(3)	3	
Contig373gene6578	330	0	2.A.80.1.1(3)	3	
Contig371gene5514	328	4	2.A.80.1.1(3)	3	
Contig371gene5517	325	2	2.A.80.1.1(3)	3	
Contig373gene6354	322	0	2.A.80.1.1(3)	3	
Contig370gene4900	323	4	2.A.80.1.1(3)	3	
Contig357gene1683	334	4	2.A.80.1.1(3)	3	
Contig375gene9115	328	2	2.A.80.1.1(3)	3	
Contig358gene1825	327	1	2.A.80.1.1(3)	3	
Contig373gene6531	321	0	2.A.80.1.1(3)	3	
Contig357gene1608	332	1	2.A.80.1.1(3)	3	
Contig372gene5872	334	0	2.A.80.1.1(3)	3	
Contig335gene234	311	0	2.A.80.1.1(3)	2	
Contig361gene2500	327	1	2.A.80.1.1(3)	3	
Contig341gene384	336	1	2.A.80.1.1(3)	3	
Contig353gene1176	332	4	2.A.80.1.1(3)	3	
Contig374gene7144	366	0	2.A.80.1.1(3)	3	
Contig374gene7146	327	0	2.A.80.1.1(3)	3	
Contig373gene6763	348	0	2.A.80.1.1(3)	3	
Contig345gene622	323	1	2.A.80.1.1(3)	3	
Contig345gene628	363	1	2.A.80.1.1(3)	3	
Contig357gene1594	329	2	2.A.80.1.1(3)	3	
Contig366gene3580	336	3	2.A.80.1.1(3)	3	
Contig354gene1306	353	2	2.A.80.1.1(3)	3	
Contig371gene5098	332	2	2.A.80.1.1(3)	3	
Contig371gene5502	341	0	2.A.80.1.1(3)	3	
Contig370gene4704	500	0	2.A.80.1.1(3)	2	
Contig370gene4705	325	0	2.A.80.1.1(3)	3	
Contig375gene8996	328	1	2.A.80.1.1(3)	3	
Contig358gene1912	333	0	2.A.80.1.1(3)	3	
Contig375gene7952	334	0	2.A.80.1.1(3)	3	
Contig370gene4597	333	0	2.A.80.1.1(3)	3	
Contig375gene8980	500	12	2.A.80.1.1(3)	2	
Contig375gene8884	330	2	2.A.80.1.1(3)	3	
Contig356gene1500	337	2	2.A.80.1.1(3)	3	
Contig373gene6518	331	0	2.A.80.1.1(3)	3	
Contig354gene1258	345	2	2.A.80.1.1(3)	3	
Contig375gene8567	348	0	2.A.80.1.1(3)	3	
Contig375gene8579	353	0	2.A.80.1.1(3)	3	
Contig371gene5324	346	4	2.A.80.1.1(3)	3	

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
					Contig367gene3835	323	1	2.A.80.1.1(3)	3
					Contig364gene3052	320	0	2.A.80.1.1(3)	3
					Contig364gene3101	382	2	2.A.80.1.1(3)	3
					Contig373gene6242	333	0	2.A.80.1.1(3)	3
					Contig373gene6267	322	1	2.A.80.1.1(3)	3
					Contig373gene6280	330	1	2.A.80.1.1(3)	3
					Contig373gene6586	331	4	2.A.80.1.1(3)	3
					Contig375gene7775	323	1	2.A.80.1.1(3)	3
					Contig371gene5154	322	1	2.A.80.1.1(3)	3
					Contig371gene5178	326	2	2.A.80.1.1(3)	3
					Contig371gene5421	318	3	2.A.80.1.1(3)	3
					Contig371gene5426	326	1	2.A.80.1.1(3)	3
					Contig371gene5429	320	1	2.A.80.1.1(3)	3
					Contig371gene5443	325	2	2.A.80.1.1(3)	3
					Contig373gene6160	341	1	2.A.80.1.1(3)	3
					Contig373gene6671	329	1	2.A.80.1.1(3)	3
					Contig373gene6675	320	0	2.A.80.1.1(3)	3
					Contig372gene6001	328	1	2.A.80.1.1(3)	3
					Contig375gene9477	330	3	2.A.80.1.1(3)	3
					Contig375gene9392	385	1	2.A.80.1.1(3)	3
					Contig375gene7729	322	1	2.A.80.1.1(3)	2
					Contig375gene7731	504	13	2.A.80.1.1(3)	2
					Contig375gene8159	333	1	2.A.80.1.1(3)	2
					Contig375gene8161	551	12	2.A.80.1.1(3)	2
					Contig375gene8171	329	0	2.A.80.1.1(3)	3
				74	Contig375gene8944	513	11	2.A.80.1.1(3)	2
2.A.81	Aspartate : alanine exchanger	AAE	Aspartate, alanine		Contig344gene526	561	11	2.A.81.1.1(1)	2
				2	Contig375gene7947	567	11	2.A.81.1.1(1)	2
2.C. Ion-gradient-driven energizers									
2.C.1	TonB-ExbB-ExbD/TolA-TolQ-TolR family of auxiliary proteins for energization of outer membrane receptor (OMR)-mediated active transport	TonB	H^+?, drives solute uptake across outer bacterial membranes		Contig375gene8338	243	3	2.C.1.1.1(3)	3
					Contig366gene3670	227	3	2.C.1.2.1(6)	2
					Contig366gene3671	145	1	2.C.1.2.1(6)	3
				5	Contig366gene3673	446	1	2.C.1.2.1(6)	3

3.A P-P bond hydrolysis-driven transporters								
3.A.1 ATP-binding cassette	ABC	All sorts of inorganic and organic molecules of small, intermediate, and large sizes, from simple ions to macromolecules						
			Contig375gene8339	137	1	2.C.1.1.1(3)	3	
	- CUT1 (1)	Sugars, metabolites						
			Contig360gene2245	293	6	3.A.1.1.3(4)	2	
			Contig360gene2246	282	6	3.A.1.1.3(4)	2	
			Contig360gene2247	367	1	3.A.1.1.3(4)	2	
			Contig362gene2677	395	1	3.A.1.1.X	2	
			Contig362gene2678	366	0	3.A.1.1.X	2	
			Contig362gene2679	294	6	3.A.1.1.X	2	
			Contig362gene2680	276	6	3.A.1.1.X	2	
			Contig362gene2682	580	1	3.A.1.1.X	3	
			Contig375gene7943	352	1	3.A.1.1.12(4)	2	
			Contig365gene3399	279	0	3.A.1.1.16(4)	2	
			Contig375gene9297	464	0	3.A.1.1.X	3	
			Contig375gene9298	371	1	3.A.1.1.X	2	
			Contig375gene9299	310	6	3.A.1.1.X	2	
		15	Contig375gene9300	295	6	3.A.1.1.X	2	
	- CUT2 (2)	Sugars, metabolites	Contig349gene903	298	9	3.A.1.2.1(4)	3	
			Contig370gene4724	537	0	3.A.1.2.X	2	
			Contig370gene4725	364	10	3.A.1.2.X	3	
		4	Contig370gene4726	306	9	3.A.1.X	3	
	- PAAT (3)	Polar amino acids	Contig346gene661	302	1	3.A.1.3.4(4)	2	
			Contig369gene4394	303	0	3.A.1.3.4(4)	3	
			Contig359gene1941	302	1	3.A.1.3.4(4)	2	
			Contig346gene653	282	1	3.A.1.3.4(4)	2	
			Contig346gene654	231	5	3.A.1.3.4(4)	2	
			Contig346gene655	447	6	3.A.1.3.4(4)	2	
			Contig346gene656	249	0	3.A.1.3.4(4)	2	
			Contig374gene7255	304	0	3.A.1.3.4(4)	2	
			Contig338gene294	310	1	3.A.1.3.4(4)	2	
			Contig359gene1987	299	0	3.A.1.3.4(4)	2	
			Contig359gene1988	242	5	3.A.1.3.4(4)	2	
			Contig359gene1989	227	5	3.A.1.3.4(4)	2	
			Contig359gene1990	244	0	3.A.1.3.4(4)	2	
		14	Contig371gene5478	274	0	3.A.1.3.10(3)	2	
	- HAAT (4)	Hydrophobic amino acids	Contig350gene948	384	9	3.A.1.4.1(6)	2	
			Contig350gene949	258	0	3.A.1.4.1(6)	2	
			Contig350gene950	238	0	3.A.1.4.1(6)	2	
			Contig374gene7171	361	0	3.A.1.4.X	3	
			Contig374gene7172	285	7	3.A.1.4.X	2	

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
					Contig374gene7174	255	0	3.A.1.4.1(6)	2
					Contig361gene2483	437	11	3.A.1.4.1(6)	3
					Contig361gene2484	259	0	3.A.1.4.1(6)	2
					Contig361gene2485	237	0	3.A.1.4.1(6)	2
					Contig355gene1439	479	1	3.A.1.4.1(6)	2
					Contig355gene1440	308	9	3.A.1.4.1(6)	2
					Contig370gene4963	425	1	3.A.1.4.1(6)	3
					Contig355gene1441	424	11	3.A.1.4.1(6)	2
					Contig355gene1442	255	0	3.A.1.4.1(6)	2
					Contig355gene1443	233	0	3.A.1.4.1(6)	2
					Contig340gene370	398	3	3.A.1.4.1(6)	3
					Contig375gene8086	287	7	3.A.1.4.X	2
					Contig375gene8087	342	10	3.A.1.4.X	2
					Contig375gene8088	254	1	3.A.1.4.1(6)	2
					Contig375gene8089	235	2	3.A.1.4.1(6)	2
					Contig375gene8090	390	1	3.A.1.4.X	3
					Contig349gene849	238	0	3.A.1.4.1(6)	2
					Contig372gene5996	379	2	3.A.1.4.1(6)	3
					Contig374gene7472	313	0	3.A.1.4.1(6)	2
					Contig374gene7473	304	8	3.A.1.4.1(6)	2
					Contig374gene7474	358	10	3.A.1.4.X	3
					Contig374gene7476	271	0	3.A.1.4.1(6)	2
					Contig375gene9380	257	0	3.A.1.4.X	2
					Contig375gene9381	241	0	3.A.1.4.1(6)	2
					Contig375gene9382	402	1	3.A.1.4.X	3
					Contig375gene9387	382	1	3.A.1.4.X	3
					Contig375gene9388	350	9	3.A.1.4.1(6)	3
					Contig375gene9389	617	10	3.A.1.4.1(6)	2
					Contig375gene9390	247	0	3.A.1.4.X	2
					Contig361gene2481	416	1	3.A.1.4.X	3
					Contig361gene2482	323	8	3.A.1.4.2(5)	3
					Contig375gene9184	288	7	3.A.1.4.2(5)	3
					Contig366gene3541	389	1	3.A.1.4.2(5)	3
					Contig374gene6823	263	0	3.A.1.4.2(5)	2
					Contig350gene946	401	1	3.A.1.4.3(4)	2
					Contig375gene9383	294	8	3.A.1.4.3(4)	2
					Contig375gene9384	344	9	3.A.1.4.3(4)	3
					Contig375gene9412	383	1	3.A.1.4.3(4)	2
					Contig358gene1906	412	1	3.A.1.4.4(5)	3
				45	Contig370gene5000	230	0	3.A.1.4.4(5)	3
		- PepT (5)	Peptide, opine, nickel		Contig373gene6123	348	0	3.A.1.5.X	2

		Contig373gene6124	337	0	3.A.1.5.X	2	
		Contig373gene6120	527	0	3.A.1.5.X	3	
		Contig373gene6122	299	5	3.A.1.5.X	2	
		Contig336gene262	306	0	3.A.1.5.2(5)	2	
		Contig336gene263	300	6	3.A.1.5.X	2	
		Contig336gene265	275	0	3.A.1.5.2(5)	2	
		Contig362gene2744	661	1	3.A.1.5.2(5)	3	
		Contig362gene2745	349	6	3.A.1.5.2(5)	2	
		Contig362gene2746	376	6	3.A.1.5.2(5)	2	
		Contig362gene2747	549	0	3.A.1.5.2(5)	2	
		Contig370gene4823	344	0	3.A.1.5.2(5)	3	
		Contig374gene7247	318	6	3.A.1.5.X	2	
		Contig374gene7248	308	6	3.A.1.5.X	2	
		Contig374gene7249	575	1	3.A.1.5.X	2	
		Contig374gene7250	325	0	3.A.1.5.X	2	
		Contig374gene7251	367	0	3.A.1.5.X	2	
		Contig355gene1375	545	1	3.A.1.5.4(5)	2	
		Contig357gene1662	259	1	3.A.1.5.3(5)	2	
		Contig340gene353	535	1	3.A.1.5.X	2	
		Contig340gene354	325	6	3.A.1.5.X	2	
		Contig340gene355	309	5	3.A.1.5.X	2	
		Contig340gene356	332	0	3.A.1.5.X	2	
		Contig340gene357	354	0	3.A.1.5.X	2	
		Contig358gene1735	289	1	3.A.1.5.X	3	
		Contig358gene1736	347	6	3.A.1.5.X	2	
		Contig358gene1737	279	6	3.A.1.5.X	2	
		Contig358gene1738	547	0	3.A.1.5.X	2	
		Contig361gene2374	586	0	3.A.1.5.X	2	
		Contig361gene2375	316	6	3.A.1.5.X	2	
		Contig361gene2376	295	5	3.A.1.5.X	2	
		Contig361gene2377	359	0	3.A.1.5.X	2	
		Contig361gene2378	337	0	3.A.1.5.X	2	
33	- SulT (6)	Contig351gene1029	335	1	3.A.1.6.1(5)	2	Sulphate, tungstate
		Contig351gene1036	335	6	3.A.1.6.1(5)	2	
		Contig351gene1037	305	6	3.A.1.6.1(5)	2	
		Contig351gene1038	367	0	3.A.1.6.1(5)	2	
		Contig374gene6978	279	0	3.A.1.6.1(5)	2	
6	- PhoT (7)	Contig367gene3795	232	0	3.A.1.6.3(4)	2	Phosphate
		Contig362gene2718	343	1	3.A.1.7.1(4)	2	
		Contig362gene2719	321	6	3.A.1.7.1(4)	2	
		Contig362gene2720	300	6	3.A.1.7.1(4)	2	
		Contig362gene2721	262	0	3.A.1.7.1(4)	2	
		Contig372gene5607	333	1	3.A.1.7.1(4)	2	
6		Contig375gene8133	355	3	3.A.1.7.1(4)	2	

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
		- MolT (8)	Molybdate		Contig372gene5726	232	5	3.A.1.8.1(3)	3
					Contig375gene7940	272	6	3.A.1.8.1(3)	2
					Contig365gene3396	258	1	3.A.1.8.1(3)	2
				4	Contig365gene3398	238	5	3.A.1.8.1(3)	2
		- PhnT (9)	Phosphonate		Contig349gene904	264	0	3.A.1.9.1(3)	2
					Contig370gene4744	326	0	3.A.1.9.1(3)	2
					Contig370gene4745	349	6	3.A.1.9.1(3)	2
					Contig372gene5787	279	0	3.A.1.9.1(3)	2
					Contig372gene5788	292	1	3.A.1.9.1(3)	3
				6	Contig372gene5789	266	5	3.A.1.9.1(3)	2
		- POPT(11)	Polyamine, opine, phosphonate		Contig364gene3011	364	0	3.A.1.11.1(4)	2
					Contig364gene3012	338	6	3.A.1.11.1(4)	2
					Contig364gene3013	260	6	3.A.1.11.1(4)	2
					Contig364gene3014	362	1	3.A.1.11.X	2
					Contig371gene5479	259	6	3.A.1.11.1(4)	3
					Contig375gene7939	340	1	3.A.1.11.X	3
					Contig375gene7942	291	6	3.A.1.11.X	3
				8	Contig372gene5727	229	0	3.A.1.11.2(4)	2
		- QAT (12)	Quaternary amine		Contig370gene4824	217	5	3.A.1.12.6(3)	3
					Contig357gene1649	516	6	3.A.1.12.3(4)	2
					Contig370gene4872	316	1	3.A.1.12.4(4)	2
					Contig370gene4873	216	5	3.A.1.12.4(4)	2
				5	Contig370gene4874	398	0	3.A.1.12.4(4)	2
		- VB12T(13)	Vitamin B_{12}	1	Contig366gene3515	300	0	3.A.1.13.1(3)	2
		- FeCT (14)	Iron chelate		Contig366gene3586	335	9	3.A.1.14.X	2
					Contig366gene3587	269	0	3.A.1.14.X	2
					Contig373gene6359	283	1	3.A.1.14.5(3)	2
					Contig373gene6360	333	9	3.A.1.14.5(3)	2
				5	Contig373gene6361	261	0	3.A.1.14.5(3)	2
		- MZT (15)	Manganese, zinc, iron chelate	1	Contig375gene9102	264	0	3.A.1.15.1(3)	3
		- NitT (16)	Nitrate, nitrite, cyanate		Contig374gene6840	434	3	3.A.1.16.X	2
					Contig374gene6841	303	6	3.A.1.16.1(4)	2
					Contig374gene6842	267	1	3.A.1.16.1(4)	2
					Contig360gene2192	347	2	3.A.1.16.2(3)	3
					Contig360gene2193	270	0	3.A.1.16.2(3)	2
					Contig362gene2587	341	1	3.A.1.16.2(3)	3
					Contig362gene2588	347	8	3.A.1.16.2(3)	2
					Contig362gene2589	262	0	3.A.1.16.X	2
					Contig350gene955	317	3	3.A.1.16.X	3

			Contig350gene956	291	6	3.A.1.16.2(3)	2
			Contig350gene957	259	0	3.A.1.16.2(3)	2
			Contig364gene3098	256	1	3.A.1.16.2(3)	2
			Contig375gene8294	304	0	3.A.1.16.2(3)	2
		14	Contig367gene3893	581	12	3.A.1.16.3(4)	3
	- TauT (17)	Taurine	Contig360gene2194	263	5	3.A.1.17.1(3)	2
			Contig351gene1030	345	1	3.A.1.17.2(3)	2
			Contig351gene1032	268	6	3.A.1.17.2(3)	2
			Contig351gene1033	291	0	3.A.1.17.2(3)	2
			Contig350gene955	317	3	3.A.1.17.1(3)	3
			Contig359gene1981	352	1	3.A.1.17.1(3)	3
			Contig359gene1982	281	0	3.A.1.17.1(3)	2
			Contig359gene1983	259	6	3.A.1.17.X	2
			Contig367gene3894	448	0	3.A.1.17.1(3)	2
			Contig364gene3097	348	1	3.A.1.17.X	3
			Contig375gene8293	341	0	3.A.1.17.X	3
			Contig364gene3099	298	7	3.A.1.17.X	3
		13	Contig375gene8295	388	7	3.A.1.17.X	3
uptake—total 181	- BIT (20)	Fe^{3+}	Contig375gene7762	371	1	3.A.1.20.1(6)	3
	- CPSE (101)	Capsular polysaccharides	Contig375gene8669	262	6	3.A.1.101.1(2)	2
		2	Contig375gene8670	221	0	3.A.1.101.1(2)	2
	- LOSE (102)	Lipo-oligosaccharide	Contig358gene1866	919	6	3.A.1.102.1(2)	2
			Contig355gene1354	303	1	3.A.1.102.1(2)	2
			Contig355gene1355	285	6	3.A.1.102.1(2)	2
			Contig359gene2065	328	1	3.A.1.102.1(2)	2
			Contig367gene3801	316	0	3.A.1.102.1(2)	2
			Contig362gene2639	83	0	3.A.1.102.1(2)	3
		7	Contig362gene2647	372	7	3.A.1.102.1(2)	3
	- DrugEI (105)	Drugs	Contig359gene2066	384	6	3.A.1.105.2(2)	3
		2	Contig375gene9090	316	1	3.A.1.105.2(2)	2
	- HemeE (107)	Heme	Contig375gene8794	207	0	3.A.1.107.1(3)	2
			Contig375gene8795	228	6	3.A.1.107.1(3)	2
			Contig375gene8796	245	6	3.A.1.107.1(3)	2
			Contig357gene1721	211	0	3.A.1.107.1(3)	2
			Contig357gene1722	222	6	3.A.1.107.1(3)	3
		6	Contig357gene1723	259	6	3.A.1.107.1(3)	2
	- Prot1E (109)	Proteins	Contig370gene4796	767	6	3.A.1.109.2(1)	2
	- Prot2E (110)	Proteins	Contig372gene5991	232	1	3.A.1.110.2(1)	3
	- Drug RA1 (120)	Drugs	Contig342gene443	540	0	3.A.1.120.1(1)	2
			Contig355gene1406	659	0	3.A.1.120.1(1)	2
			Contig373gene6751	536	0	3.A.1.120.2(1)	2

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
					Contig353gene1211	554	0	3.A.1.120.3(1)	3
				5	Contig367gene3831	670	0	3.A.1.120.4(1)	2
		- Drug RA2 (121)	Drugs	1	Contig336gene249	347	0	3.A.1.121.2(1)	2
		- MacB (122)	Macrolide	1	Contig353gene1239	234	0	3.A.1.122.1(1)	2
		- LPT (125)	Lipoproteins		Contig361gene2469	208	0	3.A.1.125.1(3)	2
					Contig370gene5060	416	5	3.A.1.125.1(3)	2
				3	Contig370gene5061	249	0	3.A.1.125.1(3)	2
	export -total 30	- HMT (210)	Heavy metals		Contig355gene1389	610	6	3.A.1.210.2(1)	2
	total 211			2	Contig359gene1980	630	7	3.A.1.210.3(1)	2
3.A.2	H^+- or Na^+-translocating F-type, V-type and A-type ATPase	F-ATPase	H^+, Na^+		Contig375gene9399	289	6	3.A.2.1.1(8)	2
					Contig375gene9400	88	0	3.A.2.1.1(8)	3
					Contig375gene9401	156	1	3.A.2.1.1(8)	3
					Contig375gene9402	180	0	3.A.2.1.1(8)	2
					Contig375gene9403	513	0	3.A.2.1.1(8)	2
					Contig375gene9404	291	0	3.A.2.1.1(8)	2
					Contig375gene9405	467	1	3.A.2.1.1(8)	2
				8	Contig375gene9406	138	0	3.A.2.1.1(8)	2
3.A.3	P-type ATPase	P-ATPase	Na^+, H^+, K^+, Ca^{2+}, Mg^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Ag^+, phospholipids (flipping)		Contig373gene6510	920	10	3.A.3.2.4(1)	2
					Contig375gene9376	813	8	3.A.3.5.1(1)	2
					Contig369gene4263	805	8	3.A.3.5.5(1)	2
					Contig375gene7707	66	0	3.A.3.5.5(1)	3
					Contig375gene8429	752	0	3.A.3.5.7(1)	3
					Contig373gene6415	829	6	3.A.3.6.1(1)	2
					Contig374gene7074	794	6	3.A.3.6.4(1)	2
					Contig375gene8357	984	8	3.A.3.6.4(1)	2
					Contig373gene6441	799	8	3.A.3.6.4(1)	2
					Contig374gene7319	610	12	3.A.3.7.1(3)	2
					Contig374gene7320	743	7	3.A.3.7.1(3)	2
				12	Contig374gene7321	203	1	3.A.3.7.1(3)	2
3.A.5	General secretory pathway	IISP	Proteins		Contig363gene2920	463	0	3.A.5.1.1(11)	2
					Contig363gene2773	930	1	3.A.5.1.1(11)	2
					Contig374gene6838	948	0	3.A.5.1.1(11)	2
					Contig367gene3758	447	10	3.A.5.1.1(11)	2
				5	Contig372gene5749	108	1	3.A.5.1.1(11)	3
3.A.6	Type III (virulence-related) secretory pathway	IIISP	Proteins		Contig373gene6292	156	0	3.A.6.1.2(10)	3

				Contig373gene6293	186	2	3.A.6.1.2(10)	3
				Contig373gene6294	264	4	3.A.6.1.2(10)	2
				Contig373gene6295	89	2	3.A.6.1.2(10)	3
				Contig373gene6296	253	6	3.A.6.1.2(10)	2
				Contig373gene6256	563	2	3.A.6.1.2(10)	2
				Contig373gene6258	278	0	3.A.6.1.2(10)	2
				Contig373gene6259	486	0	3.A.6.1.2(10)	2
				Contig37 1 gene5351	380	4	3.A.6.1.2(10)	2
			10	Contig37 1 gene5352	695	8	3.A.6.1.2(10)	2
3.A.7	Type IV (conjugal DNA-protein transfer or VirB) secretory pathway	IVSP	Proteins, protein–DNA complexes	Contig35 1 gene1012	423	0	3.A.7.4.1(10)	2
				Contig342gene418	669	2	3.A.7.X	2
				Contig342gene420	358	0	3.A.7.4.1(10)	2
				Contig35 1 gene1006	819	0	3.A.7.4.1(10)	2
				Contig35 1 gene1007	245	1	3.A.7.4.1(10)	3
				Contig35 1 gene1009	459	7	3.A.7.X	3
				Contig35 1 gene1010	234	1	3.A.7.4.1(10)	2
				Contig35 1 gene1011	330	0	3.A.7.4.1(10)	2
				Contig342gene423	809	0	3.A.7.4.1(10)	2
				Contig342gene424	241	1	3.A.7.4.1(10)	3
				Contig342gene427	234	0	3.A.7.4.1(10)	3
				Contig342gene428	333	0	3.A.7.4.1(10)	2
				Contig342gene429	422	1	3.A.7.4.1(10)	2
				Contig368gene4065	818	2	3.A.7.4.1(10)	2
				Contig368gene4066	252	1	3.A.7.4.1(10)	3
				Contig368gene4116	303	0	3.A.7.4.1(10)	3
				Contig368gene4117	414	2	3.A.7.4.1(10)	2
				Contig37 1 gene5307	438	0	3.A.7.5.1(10)	2
				Contig365gene3342	456	0	3.A.7.5.1(10)	2
			20	Contig368gene4062	349	0	3.A.7.5.1(10)	2
3.A.11	Bacterial competence-related DNA transformation transporter	DNA-T	Single-stranded DNA	Contig370gene5063	851	9	3.A.11.1.1(3)	2
3.A.12	Septal DNA translocator	S-DNA-T	DNA, DNA–protein complexes	Contig357gene1647	1123	3	3.A.12.1.2(1)	2
			2	Contig346gene649	775	4	3.A.12.1.2(1)	2
3.A.13	Filamentous phage exporter	FPhE	DNA	Contig374gene7161	358	0	3.A.13.1.1(1)	2
3.A.15	Outer membrane protein secreting main terminal branch	MTB	Pilin/fimbrilin	Contig363gene2929	573	1	3.A.15.2.1(10)	3
				Contig363gene2930	421	4	3.A.15.2.1(10)	2
				Contig363gene2931	289	7	3.A.15.2.1(10)	2
				Contig346gene667	154	1	3.A.15.2.1(10)	3
				Contig372gene5842	347	0	3.A.15.2.1(10)	2
				Contig372gene5843	381	0	3.A.15.2.1(10)	2
				Contig375gene9231	202	1	3.A.15.X	2

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
					Contig375gene9232	442	3	3.A.15.X	2
					Contig375gene9233	568	0	3.A.15.X	2
					Contig352gene1091	182	1	3.A.15.2.1(10)	3
					Contig352gene1092	234	1	3.A.15.2.1(10)	3
					Contig374gene7449	147	2	3.A.15.2.1(10)	3
					Contig375gene8624	167	2	3.A.15.2.1(10)	3
					Contig368gene4125	635	0	3.A.15.2.1(10)	3
					Contig375gene7605	284	1	3.A.15.1.1(14)	3
					Contig375gene7606	327	1	3.A.15.1.1(14)	3
					Contig375gene7611	513	0	3.A.15.1.1(14)	2
				18	Contig375gene7612	405	4	3.A.15.1.1(14)	2
3.B. Decarboxylation-driven active transporters									
3.B.1	Na^+-transporting carboxylic acid decarboxylase	NaT-DC	Na^+		Contig358gene1826	539	4	3.B.1.1.2(5)	2
				2	Contig365gene3364	535	3	3.B.1.1.2(5)	2
3.D. Oxidoreduction-driven active transporters									
3.D.1	Proton-translocating NADH dehydrogenase	NDH	H^+ or Na^+ (efflux)		Contig356gene1471	467	3	3.D.1.1.1(14)	2
					Contig370gene4970	119	3	3.D.1.2.1(14)	2
					Contig370gene4971	160	1	3.D.1.2.1(14)	2
					Contig370gene4972	199	0	3.D.1.2.1(14)	2
					Contig370gene4973	417	0	3.D.1.2.1(14)	2
					Contig370gene4974	168	1	3.D.1.2.1(14)	3
					Contig370gene4975	431	1	3.D.1.2.1(14)	2
					Contig370gene4976	828	1	3.D.1.2.1(14)	2
					Contig370gene4977	354	8	3.D.1.2.1(14)	2
					Contig370gene4979	163	0	3.D.1.2.1(14)	2
					Contig370gene4980	225	5	3.D.1.2.1(14)	3
					Contig370gene4981	101	3	3.D.1.2.1(14)	2
					Contig370gene4982	692	17	3.D.1.2.1(14)	2
					Contig370gene4984	491	14	3.D.1.2.1(14)	2
					Contig365gene3228	518	2	3.D.1.X	2
					Contig365gene3229	957	0	3.D.1.X	2
					Contig369gene4352	414	2	3.D.1.1.1(14)	2
					Contig364gene3085	402	1	3.D.1.1.1(14)	3
				19	Contig370gene4983	488	14	3.D.1.3.1(14)	2
3.D.2	Proton-translocating transhydrogenase	PTH	H^+ (efflux)		Contig334gene207	101	3	3.D.2.2.1(3)	3
					Contig334gene208	457	10	3.D.2.2.1(3)	2
					Contig326gene94	257	1	3.D.2.2.1(3)	2
					Contig372gene5764	401	0	3.D.2.2.1(3)	2

					Contig372gene5765	152	3	3.D.2.2.1(3)	2
				6	Contig372gene5766	490	10	3.D.2.2.1(3)	2
3.D.3	Proton-translocating quinol : cytochrome *c* reductase	QCR	H^+ (efflux)		Contig367gene3822	205	1	3.D.3.1.1(3)	2
					Contig367gene3823	467	13	3.D.3.1.1(3)	2
				3	Contig367gene3824	247	2	3.D.3.X	3
3.D.4	Proton-translocating cytochrome oxidase	COX	H^+ (efflux)		Contig375gene8425	518	13	3.D.4.2.1(1)	3
					Contig360gene2255	482	12	3.D.4.3.1(1)	3
					Contig364gene3091	529	13	3.D.4.3.1(1)	3
					Contig370gene4992	322	3	3.D.4.5.1(5)	2
					Contig370gene4993	657	14	3.D.4.5.1(5)	2
					Contig370gene4994	214	5	3.D.4.5.1(5)	2
					Contig370gene4995	116	3	3.D.4.5.1(5)	3
					Contig374gene7516	308	9	3.D.4.5.1(5)	2
					Contig374gene7508	536	12	3.D.4.6.1(2)	2
					Contig374gene7512	286	7	3.D.4.X	2
					Contig375gene8971	391	3	3.D.4.X	3
					Contig375gene8972	585	12	3.D.4.X	2
					Contig375gene8973	222	5	3.D.4.X	3
					Contig375gene8974	234	5	3.D.4.X	3
					Contig372gene5641	319	3	3.D.4.5.1(5)	2
					Contig372gene5642	658	15	3.D.4.5.1(5)	2
					Contig372gene5643	226	5	3.D.4.5.1(5)	2
					Contig372gene5644	121	3	3.D.4.5.1(5)	3
					Contig375gene8913	349	4	3.D.4.5.1(5)	2
					Contig375gene8914	667	15	3.D.4.5.1(5)	2
					Contig375gene8915	218	5	3.D.4.5.1(5)	2
					Contig375gene8916	142	0	3.D.4.5.1(5)	3
				23	Contig374gene7507	422	3	3.D.4.7.1(3)	3
4. Phosphotransferase systems									
4.A.6	PTS mannose–fructose–sorbose	Man	Glucose, mannose, fructose, sorbose, etc.		Contig362gene2525	316	3	4.A.6.1.1(3)	3
				2	Contig374gene7493	151	0	4.A.6.1.2(4)	3
5.A. Transmembrane electron transfer carriers									
5.A.1	Disulphide bond oxido-reductase D	DsbD	$2\ e^-$		Contig375gene8804	278	4	5.A.1.1.1(1)	3
				2	Contig367gene3767	624	9	5.A.1.1.1(1)	2
5.A.2	Disulphide bond oxido-reductase B	DsbB	$2\ e^-$	1	Contig336gene256	255	4	5.A.2.1.1(1)	3
5.A.3	Prokaryotic molybdopterin-containing oxidoreductase	PMO	Proton translocation		Contig340gene359	701	0	5.A.3.2.1(3)	3
					Contig366gene3609	1025	1	5.A.3.2.1(3)	2

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
					Contig366gene3610	226	0	5.A.3.2.1(3)	3
					Contig366gene3612	418	6	5.A.3.2.1(3)	2
					Contig360gene2128	1252	0	5.A.3.1.1(3)	2
					Contig360gene2129	517	0	5.A.3.1.1(3)	2
				7	Contig360gene2131	227	5	5.A.3.1.1(3)	2
8.A. Auxiliary transport proteins									
8.A.1	Membrane fusion protein	MFP	Proteins, peptides, lipopolysaccharides, drugs, dyes, signalling molecules, heavy metal ions, etc.		Contig358gene1816	378	1	8.A.1.1.1(1)	2
					Contig374gene7200	349	1	8.A.1.1.1(1)	3
					Contig360gene2101	413	2	8.A.1.1.1(1)	2
					Contig354gene1324	322	1	8.A.1.1.1(1)	3
					Contig375gene9176	328	1	8.A.1.1.1(1)	3
					Contig375gene8188	381	1	8.A.1.1.1(1)	2
					Contig375gene8550	380	2	8.A.1.1.1(1)	2
					Contig375gene8586	392	3	8.A.1.1.1(1)	3
					Contig364gene3066	423	2	8.A.1.1.1(1)	2
					Contig371gene5462	405	0	8.A.1.2.1(1)	3
					Contig373gene6080	505	1	8.A.1.2.1(1)	2
					Contig373gene6556	385	1	8.A.1.2.1(1)	3
					Contig373gene6562	404	1	8.A.1.2.1(1)	3
					Contig375gene8616	520	0	8.A.1.2.1(1)	1
					Contig361gene2415	523	0	8.A.1.2.1(1)	3
					Contig363gene2862	407	1	8.A.1.2.1(1)	3
					Contig369gene4235	93	1	8.A.1.2.1(1)	3
					Contig369gene4236	292	0	8.A.1.2.1(1)	1
					Contig368gene3998	395	1	8.A.1.2.1(1)	1
					Contig329gene132	387	1	8.A.1.6.1(1)	2
					Contig353gene1238	387	0	8.A.1.6.1(1)	3
					Contig358gene1807	412	0	8.A.1.6.1(1)	3
					Contig353gene1180	398	2	8.A.1.6.1(1)	2
					Contig375gene7758	407	3	8.A.1.6.1(1)	2
				25	Contig375gene7764	415	0	8.A.1.6.1(1)	2
8.A.3	Cytoplasmic membrane-periplasmic auxiliary-1 (MPA1) protein with cytoplasmic (C) domain	MPA1	Complex polysaccharides		Contig366gene3603	362	0	8.A.3.2.2(2)	3
					Contig372gene5596	748	1	8.A.3.3.1(1)	2
				3	Contig372gene5968	777	2	8.A.3.3.2(1)	2

8.A.4	Cytoplasmic membrane-periplasmic auxiliary-2	MPA2	Complex polysaccharides	1	Contig375gene8671	368	2	8.A.4.1.1(1)	2
8.A.7	Phosphotransferase system enzyme I	EI	Sugars	1	Contig374gene7495	585	0	8.A.7.1.1(1)	2
8.A.8	Phosphotransferase system HPr	HPr	Sugars	1	Contig374gene7494	89	1	8.A.8.1.1(1)	3
9.A. Transporters of unknown classification									
9.A.2	MerTP mercuric ion (Hg^{2+}) permease	MerTP	Hg^{2+} (uptake)		Contig375gene8504	88	0	9.A.2.1.1(1)	3
					Contig375gene8370	95	0	9.A.2.1.1(1)	3
				3	Contig369gene4509	91	0	9.A.2.1.1(1)	2
9.A.8	Ferrous iron uptake	FeoB	Fe^{2+} (uptake)	1	Contig372gene5560	620	11	9.A.8.1.1(1)	2
9.A.10	Oxidase-dependent Fe^{2+} transporter	OFeT	Fe^{2+} (uptake)		Contig375gene9242	504	1	9.A.10.1.1(2)	3
					Contig369gene4470	614	1	9.A.10.1.1(2)	3
				3	Contig375gene8124	605	1	9.A.10.1.1(2)	3
9.A.17	Lead	PbrT	Lead resistance		Contig373gene6437	642	7	9.A.17.1.1(1)	2
				2	Contig369gene4270	254	1	9.A.17.1.1(1)	2
9.A.21	ComC DNA uptake competence	ComC	DNA, proteins	1	Contig375gene8629	1102	1	9.A.21.1.1(1)	3
9.B. Putative uncharacterized transporters									
9.B.3	Putative bacterial murein precursor exporter	MPE	Lipid-linked murein precursors such as NAG-NAM-pentapeptide pyrophosphoryl undecaprenol (lipid II)		Contig363gene2762	413	9	9.B.3.1.1(1)	2
				2	Contig374gene7332	380	9	9.B.3.1.2(1)	2
9.B.4	Putative efflux transporter	PET	Unknown		Contig367gene3857	790	12	9.B.4.1.1(1)	2
					Contig353gene1242	664	11	9.B.4.1.2(1)	3
					Contig354gene1321	728	12	9.B.4.1.2(1)	3
				4	Contig375gene9174	659	11	9.B.4.1.2(1)	2
9.B.10	6 TMS putative MarC transporter	MarC	Multiple antibiotic resistance	1	Contig367gene3809	207	6	9.B.10.1.1(1)	3
9.B.14	Putative heme exporter protein	HEP	Heme		Contig375gene8799	653	15	9.B.14.1.1(1)	2
				2	Contig357gene1726	680	15	9.B.14.1.1(1)	2
9.B.17	Putative fatty acid transporter	FAT	Fatty acyl CoA ligases (fatty acyl CoA synthases), camitine CoA ligases, and putative transporters		Contig349gene843	549	4	9.B.17.1.4(1)	2
					Contig353gene1184	617	2	9.B.17.1.4(1)	2
					Contig340gene362	560	1	9.B.17.1.4(1)	2
					Contig362gene2701	629	2	9.B.17.1.4(1)	2
					Contig362gene2706	553	0	9.B.17.1.4(1)	2
					Contig373gene6071	631	0	9.B.17.1.4(1)	3
					Contig358gene1823	548	1	9.B.17.1.4(1)	2

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
					Contig355gene1404	516	1	9.B.17.1.4(1)	2
					Contig374gene7145	564	0	9.B.17.1.4(1)	2
					Contig345gene613	555	2	9.B.17.1.4(1)	2
					Contig366gene3582	517	0	9.B.17.1.4(1)	2
					Contig375gene8255	630	0	9.B.17.1.4(1)	2
					Contig366gene3706	566	0	9.B.17.1.4(1)	2
					Contig373gene6519	510	0	9.B.17.1.4(1)	2
					Contig373gene6406	515	0	9.B.17.1.4(1)	2
					Contig375gene8238	509	0	9.B.17.1.4(1)	2
					Contig367gene3834	500	0	9.B.17.1.4(1)	2
					Contig371gene5171	545	0	9.B.17.1.4(1)	2
					Contig371gene5425	523	0	9.B.17.1.4(1)	2
					Contig371gene5430	510	3	9.B.17.1.4(1)	2
					Contig371gene5447	501	0	9.B.17.1.4(1)	2
					Contig365gene3363	570	0	9.B.17.1.4(1)	2
					Contig373gene6687	517	0	9.B.17.1.4(1)	2
					Contig372gene5610	518	0	9.B.17.1.4(1)	2
					Contig375gene8757	660	0	9.B.17.1.6(1)	2
					Contig370gene4532	626	1	9.B.17.1.6(1)	2
					Contig373gene6475	545	1	9.B.17.1.6(1)	2
					Contig371gene5414	567	2	9.B.17.1.6(1)	3
					Contig373gene6157	527	1	9.B.17.1.6(1)	2
				30	Contig373gene6682	525	1	9.B.17.1.6(1)	2
9.B.20	Putative Mg^{2+} transporter-C	MgtC	Mg^{2+}		Contig375gene8497	152	4	9.B.20.2.1(1)	3
				2	Contig385gene222	240	4		
9.B.22	Putative permease	PerM	Unknown	1	Contig357gene1729	361	7	9.B.22.1.3(1)	3
9.B.24	Testis-enhanced gene transfer	TEGT	Glucose (and fructose?) uptake or metabolism, cell death	1	Contig360gene2157	235	7	9.B.24.2.1(1)	2
9.B.26	PC-terminal fragment 7	PC-terminal fragment 7	Unknown	1	Contig370gene4655	241	5	9.B.26.1.1(1)	3
9.B.30	Hly III	Hly III	Unknown	1	Contig375gene8924	205	7	9.B.30.1.1(1)	2
9.B.32	Putative vectorial glycosyl polymerization	VGP	Polysaccharides		Contig353gene1189	658	7	9.B.32.1.3(1)	3
				2	Contig369gene4253	367	2	9.B.32.1.3(1)	3
9.B.37	HlyC/CorC	HCC	Ions?		Contig353gene1170	530	7	9.B.37.1.2(1)	3
					Contig346gene665	438	5	9.B.37.2.1(1)	2
				3	Contig375gene7878	437	3	9.B.37.2.1(1)	2
9.B.40	DotA/TraY	DotA/TraY	Unknown	1	Contig366gene3548	751	7	9.B.40.1.2(1)	2
9.B.42	ExeAB	ExeAB	Secretin	1	Contig375gene8813	277	0	9.B.42.1.1(2)	3
9.B.43	YedZ	YedZ	Unknown	1	Contig367gene3778	224	6	9.B.43.1.1(1)	2

9.B.45	YnfA	YnfA	Unknown	1	Contig375gene8050	105	4	9.B.45.1.1(1)	2
9.B.53	Unknown IT-6	UIT6	Unknown	1	Contig347gene696	476	12	9.B.53.1.1(0)	3
Unclassified									
	Unclassified	Unclassified	Unknown						
					Contig358gene1867	397	6	N/A(0)	3
					Contig353gene1240	384	4	N/A(0)	3
					Contig370gene4727	380	0	N/A(0)	3
					Contig370gene4852	195	0	N/A(0)	3
					Contig355gene1383	409	10	N/A(0)	3
					Contig366gene3534	372	6	N/A(0)	3
					Contig366gene3535	387	6	N/A(0)	3
					Contig375gene7560	363	9	N/A(0)	3
					Contig358gene1814	388	8	N/A(0)	3
					Contig375gene8797	63	1	N/A(0)	3
					Contig365gene3385	367	7	N/A(0)	3
					Contig342gene421	127	0	N/A(0)	3
					Contig342gene426	461	9	N/A(0)	3
					Contig342gene428	333	0	N/A(0)	3
					Contig373gene6385	3750	1	N/A(0)	3
					Contig367gene3796	258	6	N/A(0)	3
					Contig367gene3797	179	1	N/A(0)	3
					Contig375gene9280	83	0	N/A(0)	3
					Contig372gene5736	241	6	N/A(0)	3
					Contig362gene2646	389	6	N/A(0)	3
					Contig371gene5477	273	1	N/A(0)	3
					Contig371gene5133	335	1	N/A(0)	3
					Contig375gene9091	273	6	N/A(0)	3
					Contig375gene8503	115	3	N/A(0)	3
					Contig374gene6814	955	0	N/A(0)	3
					Contig374gene6977	376	6	N/A(0)	3
					Contig374gene7342	316	10	N/A(0)	3
					Contig365gene3237	268	1	N/A(0)	3
					Contig356gene1538	232	7	N/A(0)	3
					Contig352gene1090	231	7	N/A(0)	3
					Contig352gene1095	336	0	N/A(0)	3
					Contig375gene8369	116	3	N/A(0)	3
					Contig375gene8372	562	0		3
					Contig364gene3037	253	7	N/A(0)	3
					Contig372gene5975	467	12	N/A(0)	3
					Contig367gene3796	258	6	N/A(0)	3
					Contig375gene7603	174	1	N/A(0)	3
					Contig375gene7604	134	1	N/A(0)	3

Table 3. Continued

Family (1)	Family (2)	Abbreviation (3)	Typical substrates (4)	Total # (5)	Gene (02jul03) (6)	Length (#aas) (7)	# TMSs (8)	Nearest homologue in TCDB (9)	Evidence (10)
					Contig375gene7607	509	0	N/A(0)	3
					Contig375gene7608	188	1	N/A(0)	3
					Contig375gene7609	268	1	N/A(0)	3
					Contig369gene4471	427	0	N/A(0)	3
					Contig369gene4472	132	1	N/A(0)	3
					Contig369gene4473	305	8	N/A(0)	3
					Contig369gene4474	158	0	N/A(0)	3
					Contig369gene4481	435	0	N/A(0)	3
					Contig375gene9429	402	12	N/A(0)	3
					Contig375gene8485	366	0	N/A(0)	3
					Contig375gene8120	419	1	N/A(0)	3
					Contig375gene8125	360	1	N/A(0)	3
					Contig375gene8126	128	0	N/A(0)	3
					Contig369gene4508	116	3	N/A(0)	3
					Contig368gene4000	351	10	N/A(0)	3
					Contig368gene4195	324	0	N/A(0)	3
				55	Contig368gene4197	197	0	N/A(0)	3
				932					

[a] A full version of the table containing all the various names of the CH34 genes is provided as on-line supplementary material at: **http://bionomie.mikrobiologie.uni-halle.de/SupMat/Roz_05/Table 3.htm**

structure is established for several members of this family. Three members of the OmpA-OmpF porin (OOP) family and a single FadL homologue, presumably concerned with transport of fatty acids across the outer membrane, were identified.

The next two families listed in Table 3, the FUP and AT families, with three members and one member, respectively, are concerned with export of proteins across the outer membrane. The three FUP ushers probably export fimbrial subunits for the assembly of 3 structurally and functionally distinct fimbriae. AT family members export their own N-terminal domains, which in this case may be a large cell surface protein. However, no surface layer could be observed for Rme (D. Neumann and D. H. Nies, unpublished data).

Seventeen OMR family members were identified. Fifteen of these are probably concerned with uptake of iron siderophore complexes (subfamilies 1 and 9). One is probably the Rme vitamin B_{12} porin (subfamily 3). The single member of subfamily 4 may be concerned with copper acquisition.

Outer membrane factors (OMFs; TC #1.B.17) generally mediate efflux of heavy metals, drugs and macromolecules across the outer membrane in conjunction with an active efflux pump in the inner membrane. Twenty-eight homologues were identified. Of these, one is in subfamily 1 (a general OMF able to interact with multiple efflux pumps), eight are in subfamily 2 (concerned with heavy metal ion efflux), and 19 are in subfamily 3 (concerned with export of macromolecules, drugs and metals). Two members of this last subfamily resemble oligosaccharide exporters; four most resemble protein exporters; seven may be involved in export of drugs and other hydrophobic substances; and three may function in copper ion efflux.

Two members of the OMA family (1.B.18) are presumed to function in exopolysaccharide export, one member of the OprB family (1.B.19) probably allows facilitation of small molecules across the outer membrane, and the two members of the TPS family (1.B.20) most likely export proteins. Most of the six secretins (1.B.22) also probably function in protein export. Finally, the two OmpW family members (1.B.39) may export drugs and other hydrophobic molecules.

A channel-forming colicin-like protein (1.C.1), resembling colicin A of *Citrobacter freundii*, was found. A single holin (1.E.14), presumably involved in autolysin export for the purpose of promoting cell death, is also present.

Secondary carriers

By far the largest number secondary carriers encoded within the Rme genome are members of the major facilitator superfamily (MFS). Rme has 83 recognizable MFS carriers. As shown in Table 3 and summarized in Table 4, 32 of these MFS permeases are putative drug/amphiphile/hydrophobe transporters of MFS families DHA1 (16 members), DHA2 (15 members) and DHA3 (1 member) (Busch and Saier, 2002). Some of these are likely to serve as lipid exporters, but others undoubtedly play primary roles in defence, in toxic substance export or in metabolite export.

Just one sugar transporter (SP family), one organophosphate porter (OPA family), 15 metabolite transporters (MHS family), three nitrate/nitrite transporters (NNP family), and three oxalate : formate antiporters (OFA) of the MFS allow uptake of essential nutrients. Additionally, one SHS porter, nine ACS porters, five AAHS porters, and one CP porter all probably function to bring organoanions into the cell. The OCT porter may transport organocations. Other MFS paralogues represented, with usually a single protein member in any one family, undoubtedly transport a wide range of other substances (Table 3).

Six amino acid/polyamine/organocation (APC) superfamily members were identified. Two of the subfamilies in the APC superfamily are represented. These porters are predicted to transport a range of zwitterionic and basic amino acids.

The CDF family and the ZIP family of heavy metal divalent cation transporters are represented with three and one members, respectively. All three CDF proteins have been characterized in detail (Anton *et al*., 2004; Munkelt *et al*., 2004). They belong to different clusters of the CDF protein family (Nies, 2003) and transport Cd^{2+}, Co^{2+}, Zn^{2+}, Fe^{2+} and Ni^{2+}. A single member of the NiCoT family (TC #2.A.53), probably a Ni^{2+} transporter, was also identified. A related protein is involved in nickel uptake for synthesis of the hydrogenases in the related bacterium *Ralstonia eutropha* (Degen and Eitinger, 2002; Eberz *et al*., 1989; Eitinger and Friedrich, 1991, 1994; Eitinger *et al*., 1997; Wolfram *et al*., 1991, 1995).

Table 4. Family associations including subfamilies within the MFS, APC, RND, DMT, MOP and ABC superfamilies of transporter constituents

Family	Abbreviation	Typical substrates	No. of members (%)
1.A.1	VIC	Na^+, K^+, Ca^{2+}, multiple cations	2 (0.2)
1.A.8	MIP	H_2O, glycerol, urea, polyols, NH_3, CO_2	2 (0.2)
1.A.11	ClC	Cl^-, anions	4 (0.4)
1.A.20	CytB	H^+	1 (0.1)
1.A.22	MscL	Proteins, ions (slightly cation-selective)	1 (0.1)
1.A.23	MscS	Ions (slight anion selectivity)	9 (1)
1.A.30	Mot/Exb-Mot	H^+, Na^+	2 (0.2)
1.A.33	Hsp70	Ions, polypeptides	2 (0.2)
1.A.35	MIT	Heavy-metal ions, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+}, Al^{3+}, Mn^{2+}	4 (0.4)
1.B.1	GBP	Ions, small (M_r < 1000 Da) molecules	29 (3.1)
1.B.6	OOP	Ions, small molecules	3 (0.3)
1.B.9	FadL	Fatty acid, toluene, *m*-xylene and benzyl alcohol	1 (0.1)
1.B.11	FUP	Protein folding and subunit assembly	3 (0.3)
1.B.12	AT	N-terminal protein domains	1 (0.1)
1.B.14	OMR	Iron–siderophore complexes, vitamin B_{12}, Cu^{2+}, colicin, DNA of various phages	17 (1.8)
1.B.17	OMF	Heavy metal cations, drugs, oligosaccharides, proteins, etc.	28 (3)
1.B.18	OMA	Exo- or capsular polysaccharide	2 (0.2)
1.B.19	OprB	Ions, small molecules	1 (0.1)
1.B.20	TPS	Proteins	2 (0.2)
1.B.22	Secretin	Proteins	6 (0.6)
1.B.39	OmpW	Methyl viologen and benzyl viologen	2 (0.2)
1.C.1	Colicin	Ions, small molecules	1 (0.1)
1.E.14	LrgA Holin	Zn^{2+}, Fe^{2+}	1 (0.1)
2.A.1	MFS	Various small molecules	Total 83 (8.9)
	-SP (1)	Sugars	1 (0.1)
	-DHA1 (12 spanner) (2) drugs	Drugs	16 (1.7)
	-DHA2 (14 spanner) (3) drugs	Drugs	15 (1.6)
	-OPA (4)	Sugars, glycerol	1 (0.1)
	-MHS (6)	Dicarboxylates, tricarboxylates	15 (1.6)
	-NNP (8)	Nitrate, nitrite	3 (0.3)
	-OFA (11)	Oxalate, formate	3 (0.3)
	-SHS (12)	Sialate, lactate, pyruvate	1 (0.1)
	-ACS (14)	Organic acids	9 (1)
	-AAHS (15)	Aromatic acids	5 (0.5)
	-CP (17)	Cyanate	1 (0.1)
	-OCT (19)	Organic cations	1 (0.1)
	-SET (20)	Sugars	1 (0.1)
	-DHA3 (12 spanner) (21) drugs	Drugs	1 (0.1)
	-VNT (22)	Neurotransmitter	1 (0.1)
	-BST (23)	Unknown	1 (0.1)
	-PAT (25)	Peptides, AcCoA	1 (0.1)
	-UMC-terminal fragment (26)	Unknown	1 (0.1)
	-PPP (27)	Phenylpropionate	1 (0.1)
	-ADT (30)	Abietane diterpenoid	1 (0.1)
	-Nre (31)	Ni^{2+}	1 (0.1)
	-Fsr (35)	Fosmidomycin	1 (0.1)
	-AtoE (37)	Short chain fatty	2 (0.2)
2.A.3	APC	Amino acids, polyamines, choline	Total 6 (0.6)
	-AAA (1)	Amino acids	5 (0.5)
	-CAT (3)	Cationic amino acids	1 (0.1)
2.A.4	CDF	Cd^{2+}, Co^{2+}, Zn^{2+}	3 (0.3)
2.A.5	ZIP	Zn^{2+}, Fe^{2+}	1 (0.1)

Table 4. Continued

Family	Abbreviation	Typical substrates	No. of members (%)
2.A.6	RND	Heavy metal ions, multiple drugs, oligosaccharides, organic solvents, fatty acids, phospholipids, cholesterol	Total 30 (3.2)
	-HME (1)	Heavy metals	17 (1.8)
	-HAE1 (2)	Hydrophobe/amphiphiles	9 (1)
	-SecDF(4)	Sec secretory accessory proteins	2 (0.2)
	-HAE2 (5)	Hydrophobe/amphiphiles	1 (0.1)
	-ORF4 (8)	Hydrophobe/amphiphiles	1 (0.1)
2.A.7	DMT	Multiple drugs and dyes (mostly cationic)	Total 18 (1.9)
	-SMR (1)	Drugs	2 (0.2)
	-BAT (2)	Unknown	2 (0.2)
	-DME (3)	Drugs, metabolites	12 (1.3)
	-RarD (7)	Chloramphenicol	2 (0.2)
2.A.9	Oxa1	Proteins	1 (0.1)
2.A.10	KDGT	2-Keto-3-deoxygluconate	1 (0.1)
2.A.11	CitMHS	Citrate	1 (0.1)
2.A.12	AAA	ATP, ADP	1 (0.1)
2.A.14	LctP	Lactate	1 (0.1)
2.A.19	CaCA	Ca^{2+}	1 (0.1)
2.A.20	PiT	Inorganic phosphate	1 (0.1)
2.A.21	SSS	Sugars, amino acids, vitamins, nucleosides, inositols, iodide, urea	5 (0.5)
2.A.23	DAACS	C_4-dicarboxylates, acidic and neutral amino acids	5 (0.5)
2.A.24	CCS	Mono-, di-, and tricarboxylates	1 (0.1)
2.A.36	CPA1	Na^+/H^+, Na^+ or K^+/H^+	1 (0.1)
2.A.37	CPA2	Na^+/H^+ or K^+/H^+	6 (0.6)
2.A.40	NCS2	Nucleobases, urate	3 (0.3)
2.A.45	ArsB	Arsenite, antimonite	1 (0.1)
2.A.46	BenE	Benzoate	1 (0.1)
2.A.47	DASS	Dicarboxylates, phosphate, sulphate	4 (0.4)
2.A.49	Amt	Ammonium	2 (0.2)
2.A.51	CHR	Chromate, sulphate (uptake or efflux)	4 (0.4)
2.A.52	NiCoT	Ni^{2+}, Co^{2+}	1 (0.1)
2.A.53	SulP	Sulphate	5 (0.5)
2.A.56	TRAP–T	C_4-dicarboxylates, acidic amino acids, sugars?	6 (0.6)
2.A.58	PNaS	Inorganic phosphate	2 (0.2)
2.A.59	ACR3	Arsenite	1 (0.1)
2.A.64	Tat	Redox proteins	4 (0.4)
2.A.66	MOP	Drugs, lipid-linked oligosaccharide precursors	Total 5 (0.5)
	-MATE (1)	Drugs	3 (0.3)
	-PST (2)	Polysaccharides	1 (0.1)
	-MVF (4)	Unknown	1 (0.1)
2.A.67	OPT	Peptides	2 (0.2)
2.A.69	AEC	Auxin (efflux)	2 (0.2)
2.A.72	KUP	K^+ (uptake)	1 (0.1)
2.A.75	LysE	Basic amino acids	1 (0.1)
2.A.76	RhtB	Neutral amino acids and their derivatives	11 (1.2)
2.A.78	LIV–E	Carboxylates, amino acids, amines (efflux)	1 (0.1)
2.A.80	TTT	Tricarboxylate	74 (8)
2.A.81	AAE	Aspartate, alanine	2 (0.2)
2.C.1	TonB	H^+?, drives solute uptake across outer bacterial membranes	5 (0.5)
3.A.1	ABC	All sorts of inorganic and organic molecules of small, intermediate, and large sizes, from simple ions to macromolecules	Total 213 (23)
	-CUT1(1)	Sugars, metabolites	15 (1.6)

Table 4. Continued

Family	Abbreviation	Typical substrates	No. of members (%)
	-CUT2 (2)	Sugars, metabolites	4 (0.4)
	-PAAT (3)	Polar amino acids	14 (1.5)
	-HAAT (4)	Hydrophobic amino acids	45 (4.8)
	-PepT (5)	Peptide, opine, nickel	33 (3.5)
	-SulT (6)	Sulphate, tungstate	6 (0.6)
	-PhoT (7)	Phosphate	6 (0.6)
	-MolT (8)	Molybdate	4 (0.4)
	-PhnT (9)	Phosphonate	6 (0.6)
	-POPT(11)	Polyamine, opine, phosphonate	8 (0.9)
	-QAT (12)	Quaternary amine	5 (0.5)
	-VB12T(13)	Vitamin B_{12}	1 (0.1)
	-FeCT (14)	Iron chelate	5 (0.5)
	-MZT (15)	Manganese, zinc, iron chelate	1 (0.1)
	-NitT (16)	Nitrate, nitrite, cyanate	14 (1.5)
	-TauT (17)	Taurine	13 (1.4)
	-BIT (20)	Fe^{3+}	1 (0.1)
	-CPSE (101)	Capsular polysaccharides	2 (0.2)
	-LOSE (102)	Lipo-oligosaccharide	7 (0.8)
	-DrugE1(105)	Drugs	2 (0.2)
	-HemeE(107)	Heme	6 (0.6)
	-Prot1E (109)	Proteins	1 (0.1)
	-Prot2E (110)	Proteins	1 (0.1)
	-Drug RA1 (120)	Drugs	5 (0.5)
	-Drug RA2 (121)	Drugs	1 (0.1)
	-MacB (122)	Macrolide	2 (0.2)
	-LPT (125)	Lipoproteins	3 (0.3)
	-HMT (210)	Heavy metals	2 (0.2)
3.A.2	F-ATPase	H^+, Na^+	8 (0.9)
3.A.3	P-ATPase	Na^+, H^+, K^+, Ca^{2+}, Mg^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Ag^+, phospholipids (flipping)	12 (1.3)
3.A.5	IISP	Proteins	5 (0.5)
3.A.6	IIISP	Proteins	10 (1.1)
3.A.7	IVSP	Proteins, protein–DNA complexes	20 (2.2)
3.A.11	DNA-T	Single-stranded DNA	1 (0.1)
3.A.12	S-DNA-T	DNA, DNA–protein complexes	2 (0.2)
3.A.13	FPhE	Viruses	1 (0.1)
3.A.15	MTB	Pilin/fimbrilin	18 (1.9)
3.B.1	NaT-DC	Na^+	2 (0.2)
3.D.1	NDH	H^+ or Na^+ (efflux)	19 (2)
3.D.2	PTH	H^+ (efflux)	6 (0.6)
3.D.3	QCR	H^+ (efflux)	3 (0.3)
3.D.4	COX	H^+ (efflux)	23 (2.5)
4.A.6	Man	Glucose, mannose, fructose, sorbose, etc.	2 (0.2)
5.A.1	DsbD	2 e^-	2 (0.2)
5.A.2	DsbB	2 e^-	1 (0.1)
5.A.3	PMO	Proton translocation	7 (0.8)
8.A.1	MFP	Proteins, peptides, lipopolysaccharides, drugs, dyes, signalling molecules, heavy metal ions, etc.	25 (2.7)
8.A.3	MPA1	Complex polysaccharides	3 (0.3)
8.A.4	MPA2	Complex polysaccharides	1 (0.1)
8.A.7	EI	Sugars	1 (0.1)
8.A.8	HPr	Sugars	1 (0.1)
9.A.2	MerTP	Hg^{2+} (uptake)	3 (0.3)
9.A.8	FeoB	Fe^{2+} (uptake)	1 (0.1)
9.A.10	OFeT	Fe^{2+} (uptake)	3 (0.3)
9.A.17	PbrT	Lead resistance	2 (0.2)
9.A.21	ComC	DNA, proteins	1 (0.1)

Table 4. Continued

Family	Abbreviation	Typical substrates	No. of members (%)
9.B.3	MPE	Lipid-linked murein precursors, such as NAG–NAM–pentapeptide pyrophosphoryl undecaprenol (lipid II)	2 (0.2)
9.B.4	PET	Unknown	4 (0.4)
9.B.10	MarC	Multiple antibiotic resistance	1 (0.1)
9.B.14	HEP	Heme	2 (0.2)
9.B.17	FAT	Fatty acyl CoA ligases (fatty acyl CoA synthases), carnitine CoA ligases, and putative transporters	30 (3.2)
9.B.20	MgtC	Mg^{2+}	2 (0.2)
9.B.22	PerM	Unknown	1 (0.1)
9.B.24	TEGT	Glucose (and fructose?) uptake or metabolism, cell death	1 (0.1)
9.B.26	PC-terminal fragment (7)	Unknown	1 (0.1)
9.B.30	Hly III	Unknown	1 (0.1)
9.B.32	VGP	Polysaccharides	2 (0.2)
9.B.37	HCC	Ions?	3 (0.3)
9.B.40	DotA/TraY	Unknown	1 (0.1)
9.B.42	ExeAB	Secretin	1 (0.1)
9.B.43	YedZ	Unknown	1 (0.1)
9.B.45	YnfA	Unknown	1 (0.1)
9.B.53	UIT6	Unknown	1 (0.1)
	Unclassified	Unknown	1 (0.1)
Total			932 (100)

The RND superfamily of export pumps is well represented, with 30 members. Of these, over half (17) in subfamily 1 are predicted to function in heavy metal efflux. Another nine (in subfamily 2) probably export drugs and other hydrophobic and amphipathic substances. The RND proteins of Rme have been compared to those from other bacteria recently (Nies, 2003). The two SecDF system components (subfamily 4), facilitate protein secretion via the general secretory pathway (Sec; 3.A.5). Lipid (subfamily 5) and pigment (subfamily 8) exporters may also be present.

Another well-represented superfamily encoded within the genome of Rme is the drug/metabolite transporter (DMT) superfamily, with 18 members within four of the families of this superfamily. Most of these transporters (families 1, 2 and 3) probably function in drug and metabolite efflux, but one (family 7) may be a sugar uptake permease.

A single putative 2-keto-3-deoxygluconate uptake permease was identified. Additionally, one member of the CitMHS (citrate uptake) family and one member of the LctP lactate uptake family were found. One system may export Ca^{2+} (CaCA family) while another may import phosphate (PiT family). A surprise was the identification of a member of the ATP : ADP antiporter (AAA) family, because such transporters were previously predominantly identified in intracellular pathogenic organisms and rarely in other bacteria (until now in *Ralstonia eutropha* strain JMP134, *Pseudomonas fluorescens, Pirella, Rhodopirellula baltica* and *Magnetospirillum magnetotacticum*). However, what it could be doing in a free-living organism remains to be determined.

Five members of the SSS family most resemble characterized permeases for organoanions and cations as well as a putative nitrogen sensor. All of the five members of the DAACS family are predicted to transport dicarboxylates. These may include the two dicarboxylate amino acids, aspartate and glutamate. A putative CCS family member is also predicted to take up dicarboxylates. The four DASS family members probably serve similar functions but may also take up tricarboxylate compounds.

Both the CPA1 and CPA2 monovalent cation antiporter families are represented, with one and six members, respectively. CPA1 family members are predicted to be $Na^+ : H^+$ antiporters, while CPA2 family members may be K^+ efflux systems. Three NCS2 nucleobase/nucleoside uptake systems and

two Amt ammonia/ammonium transporters were identified.

Two putative arsenite exporters (one of the ArsB-type and one of the Acr3-type) were found. Four potential chromate resistance (CHR) pumps and five putative sulphate uptake permeases (SulP) may be involved in chromate and sulphate metabolism, respectively. The CHR and SulP porters may be functionally related, since chromate is a sulphate analogue.

Six constituents of the tripartite TRAP-T family (2.A.56) may comprise three distinct systems for dicarboxylate uptake. However, studies indicate that members of this family may transport substrates of diverse structure, rendering substrate identification difficult. Only two TRAP-T receptors but at least three large and one small integral membrane constituents of these systems were identified. Because of rapid sequence divergence of the small integral membrane constituents, some of these proteins may have been missed. This situation can be contrasted with the superficially similar tripartite TTT family (2.A.80), where 74 potential constituents were found. Interestingly, about five proved to resemble the large and 11 the small integral membrane constituents of these systems, while 58 proved to be homologous to TTT family receptors. The occurrence of multiple probable receptors for TTT family systems in some bacteria has been noted before (Antoine *et al*., 2003).

Several additional families of transporters are probably involved in nutrient uptake (BenE, OPT and AAE) and metabolite efflux (AEC, LysE, RhtB and LIV-E). All of these are concerned with transport of peptides, amino acids and their derivatives. The largest of these families is the RhtB family, with 11 members. Additionally, constituents of a TonB–ExbBD system, which probably functions primarily to energize transport across the outer membrane by a proton electrophoretic mechanism, were identified.

A complete twin arginine targeting (TatABC) system, as well as a single Oxa1 homologue, is encoded within the genome of Rme. These two independently acting systems function in the secretion of a subset of extracellular proteins and in the insertion of integral membrane proteins, including redox enzymes, respectively (Yen *et al*., 2002). Genome analyses of the leader sequences of potential secretory proteins should reveal which are substrates of the Tat system and which are exported via the Sec system.

Primary active transporters

The vast majority of protein constituents of primary active transporters encoded within the Rme genome are members of the ABC superfamily; 213 proteins in Rme belong to this superfamily, 181 putative uptake system proteins and 32 putative efflux system proteins. Most ABC systems consist minimally of two membrane protein (M) and two ATP hydrolysing cytoplasmic protein (C) subunits which may be fused in various combinations. Consequently, the basic unit of an ABC transporter may be encoded by a single gene or up to four distinct genes. Additionally, extracytoplasmic receptors are associated with all uptake systems, and there may be several of these per system. Therefore, it is not possible to estimate accurately the number of intact ABC transporters present. The problem is exacerbated by the fact that the constituents of ABC systems are often encoded within multiple, non-adjacent operons.

Table 4 summarizes the family associations of the various ABC transporter constituents. The ratio of sugar uptake system constituents (CUT1 + CUT2) to amino acid plus peptide uptake systems (PAAT + HAAT + PepT) is 15 : 52 or about 1 : 4. This fact, together with the corresponding analyses of secondary carriers discussed above, reveals the much greater dependency of Rme on amino acid metabolism than carbohydrate metabolism (see also Table 2). Values for numbers of sugar and amino acid transporter constituents can be compared with the total number of organic and inorganic anion and cation uptake transporter constituents (about 20 of each). ABC-type efflux systems are concerned with the export of drugs (10), complex carbohydrates (5), heme (6), proteins (5) and heavy metals (7) (Tables 3 and 4).

Rme has a single multicomponent F-type ATPase for the interconversion of chemical and chemiosmotic energy. It also possesses a dozen paralogous cation transporting P-type ATPases. Three of them have been characterized in detail (Borremans *et al*., 2001; Legatzki *et al*., 2003a) and all of them have been compared to P-type ATPases from other bacteria (Nies, 2003). Recently, the ongoing annotation work (**http://genome.ornl.gov/microbial/rmet/**)

identified another P-type ATPase (ZP_00 273 867) that was not included here.

A complete multicomponent general protein secretory (Sec) system (TC #3.A.5) was found in Rme, and this system undoubtedly serves as the primary protein export system for transport of proteins from the cytoplasm to the periplasm (Cao and Saier, 2003). However, Rme also has types II (MTB), III and IV macromolecular export systems. The first of these functions exclusively to export proteins across the outer membrane, but the latter two transport their substrates across both membranes. Type IV systems may also function in conjugation, and, in plant pathogens, in DNA export to the host cell. Additional potential DNA translocation proteins of the DNA-T, S-DNA-T and FphE families were also identified (Table 3). However, assignment of their specific functional roles must await experimental studies.

The Na^+ transporting carboxylate decarboxylases (TC #3.B.1) are multicomponent systems where the β-subunit catalyses Na^+ export in response to cytoplasm substrate decarboxylation catalysed by the α-subunit. These systems minimally require the presence of α-, β- and γ-subunits (Dimroth *et al*., 2001). One such system may be present in Rme.

Proton pumping electron carriers

Rme has a single member of each of the three proton- or sodium-translocating electron transfer complexes of the NADH dehydrogenase (NDH), quinol : cytochrome *c* reductase (QCR) and cytochrome oxidase (COX) families. It also has at least two multicomponent transhydrogenases (PTH family). Rme therefore has a complete electron transfer chain for oxidizing NADH, using molecular oxygen as electron acceptor. All four electron carrier complexes cited above have the potential to generate an ion motive force as a primary source of energy. These coupled systems probably function together under aerobic conditions. Other transmembrane electron flow systems that can influence cellular energetics (class 5A and 5B) were also identified.

Group translocators

The complete phosphoenolpyuvate–sugar phosphotransferase system (PTS; TC #4.A) is present in Rme. It includes, however, just one mannose (Man)-type PTS permease (Zhang *et al*., 2003). Only one Enzymes I and one HPr were identified. It is clear that Rme possesses a minimal PTS, in agreement with the earlier conclusion, based on secondary and primary active transporter analyses, that Rme is not strongly dependent on sugar metabolism as a source of energy.

Poorly-defined transporters

Among the poorly characterized permeases of TC class 9.A, Rme has systems that probably transport heavy metal ions: mercury, iron, lead and magnesium. Several putative permeases of TC class 9.B were also identified (Table 3), but their functions are not known.

Perspectives and conclusions

We have analysed transporters in the heavy metal-resistant organism, *R. metallidurans* (Rme). This organism possesses several α-type channel proteins. Some are concerned specifically with monovalent or divalent inorganic cation or anion transport, but several non-specific stress response channels also appear to be present. Rme also has a huge repertoire of outer membrane β-barrel porins involved in transport of small molecules as well as macromolecules across the outer membrane. Many (e.g. OMRs) are probably specific for uptake, while others (e.g. OMFs) mediate efflux.

Regarding secondary carriers for sugars, Rme seems to have a very limited repertoire of such systems relative to most other sequenced Gram-negative bacteria, such as *E. coli* and other enteric bacteria. Thus, Rme has only one MFS carbohydrate transporter in the sugar porter family. It has no putative glycoside transporters of the GPH family (TC #2.A.2). It does have a putative 2-keto-3-deoxygluconate transporter of the KDGT family, and it has a few ABC uptake transporters specific for monosaccharides and small oligosaccharides of the CUT1 and CUT2 subfamilies, as well as a complete phosphotransferase system. Rme may only transport hexoses via the one PTS permease identified.

The capacity of Rme to transport carboxylic acids and their derivatives as sources of carbon appears to be fairly extensive. Thus, several families of secondary mono- and dicarboxylate carriers

(MFS, DAACS, DASS and TRAP-T) were identified. It also possesses members of the tricarboxylate transporting CitMHS, CCS and TTT families (Winnen *et al*., 2003). ABC-type carboxylate transporters were also found. Thus, the results point to a strong respiratory-type metabolism, with greater dependency on exogenous organic acids than carbohydrates.

Our genome analyses revealed several transporters that are probably specific for amino acids, peptides and their derivatives. Thus, for the uptake of amino acids, three families of secondary carriers were represented [MFS (MHS), APC and SSS], while members of two ABC families with this specificity (PAAT and HAAT) were found. For the uptake of peptides, two potential families of secondary carriers (OPT, MPE) and one ABC family (PepT) were represented. Finally, for amino acid efflux, members of five potential families were identified (DMT, AEC, LysE, RhtB and LIV-E). It seems clear that the transport and metabolism of amino acids and their derivatives is of considerable importance to the lifestyle of Rme.

Our analyses also revealed a large number of potential drug/hydrophobe/amphiphile export systems. Many of these belong to the DHA1, -2 and -3 families of the MFS. While a few of these efflux pumps may be involved in sugar export (Table 3; Saier, 2000), it is possible that some export amino acids and their derivatives, particularly those of a hydrophobic nature. It should be noted, however, that this has not yet been established for any member of the three MFS DHA families.

Other families, including transporters that probably export hydrophobic substances, include the HAE1 family in the RND superfamily, and the DME family of the DMT superfamily. At least some of these are probably concerned with drug export. Members of the MATE family within the MOP superfamily and several putative drug exporters of the ABC superfamily may serve similar functions. All of these families are represented in Rme. The diversity of substrates exported by these systems has yet to be studied.

As noted in Table 2 and further exemplified in Tables 3 and 4, over 220 transporters in Rme are probably concerned with inorganic ion transport. The following families are represented (see Table 3): (1) for monovalent cations: VIC, CytB, MscL, MscS, CPA1, CPA2, Amt, KUP, F-ATPase, P-ATPase and four proton-translocating electron carriers (NDH, PTH, QCR and COX); (2) for di- or trivalent cations: MIT, NNP(MFS), CDF, ZIP, RND, CaCA, NiCoT, FeCT(ABC), MZT(ABC), P-ATPase and MgtC; and (3) for anions: MFS, Pit, ArsB, DASS, CHR, SulP, PNaS, ACR3, SulT(ABC), PhoT(ABC), MolT(ABC) and NitT(ABC).

Inspection of Table 3 reveals possible transporters for a variety of additional interesting metabolites, such as organic anions (benzoate, phenylacetate, cyanate, phosphonates, sulphonates). Transporters specific for osmolytes, both purine and pyrimidine bases and nucleosides, quaternary ammonium compounds and possibly nucleotides (ADP/ATP in the AAA family), were also identified.

An extensive repertoire of macromolecular exporters was found. Protein secretion and membrane protein insertion systems include the Sec, Tat, Oxa1 and types I–IV systems. Complex carbohydrates can probably be exported via MOP, ABC and VGP family transporters. Possible lipid exporters of the RND superfamily have been identified, and several MFS and ABC systems may similarly catalyse lipid 'flip-flop', which is equivalent to export from the inner leaflet of the cytoplasm membrane bilayer to the outer leaflet. Some of these transporters may also export lipids from the inner membrane to the outer membrane.

Finally, several of the identified transporters could not be assigned even a tentative function. It should also be kept in mind that transporters that belong to functionally uncharacterized families may not be included in the TC system and therefore may not be identified using the computer approaches used here. Although our studies have revealed a disproportionate number of transporters concerned with inorganic ion transport, particularly with heavy metal resistance, and while these studies clearly point to the dominant types of metabolic activity upon which Rme depends for energy, it is clear that we are only at the beginning of an understanding of the scope of molecular transport processes in *Ralstonia metallidurans*.

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