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Whole-Genome Analysis of Transporters in the Plant Pathogen *Xylella fastidiosa*

Joao Meidanis,[1] Marilia D. V. Braga,[1] and Sergio Verjovski-Almeida[2]*

Instituto de Computação, Universidade de Campinas, Campinas, São Paulo 13083-970,[1] and Instituto de Química, Universidade de São Paulo, São Paulo, São Paulo 05508-900,[2] Brazil

INTRODUCTION

Xylella fastidiosa is a plant-pathogenic bacterium that causes variegated chlorosis in citrus trees and other diseases in a wide range of plant hosts (15, 34). The citrus pathogen has an insect as its vector, which takes the parasite from tree to tree while feeding on the xylem. Among the plant-pathogenic bacteria, *X. fastidiosa* is the first whose genome has been completely sequenced (44). Annotation of this genome was done by several groups, each focusing on a particular functional category. The categories were based on the classification proposed by Riley (38) for *Escherichia coli*. In this effort, we were responsible for the transport category, and it soon became clear that in the scope of the whole genome paper (44), there was no room to describe all the interesting facts we were finding. For this reason we decided to produce a detailed separate inventory of all transport proteins and the conclusions associated with these findings.

Apart from being the first plant-pathogenic bacterium to be sequenced, *X. fastidiosa* was also one of the least known organisms targeted for sequencing. At the beginning of the sequencing project (1998), only six *Xylella* sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/). The sequencing project made *X. fastidiosa* jump from almost unknown to almost completely known. However, very few, if any, of the functional predictions made for *Xylella* proteins were verified in the laboratory. This is, as far as we know, the status of all the proteins described here: all predictions were made based solely on computational evidence. In this paper we made an effort to suggest experiments that would contribute to verifying some of the most important claims made here.

In addition, many of the important processes of *X. fastidiosa* related to the diseases it causes probably rely on proteins—hemolysins, adhesins, xanthum gum-producing enzymes, virulence factors, detoxification enzymes—involved in one way or another with transport, which provides additional interest for the study of the important class of transport proteins.

SEQUENCE ANALYSIS

The list of all *X. fastidiosa* predicted proteins was obtained from the sequencing project web site (http://www.lbi.ic.unicamp

* Corresponding author. Mailing address: Instituto de Química, Universidade de São Paulo, Av. Prof. Lineu Prestes 748, São Paulo, SP 05508-900, Brazil. Phone: 55-11-3091-2173. Fax: 55-11-3091-2186. E-mail: verjo@iq.usp.br.

.br/xf). The number of transmembrane segments (TMSs) of each *Xylella* protein was estimated using PSORT (25). For comparison, PSORT (25) was used for the prediction of the number of TMS for each of the 18 completely sequenced bacteria that had been analyzed previously by Paulsen et al. (31) as well as for *Saccharomyces cerevisiae*, which has been analyzed by Paulsen et al. (30). For identification of transport families, each *Xylella* sequence was compared to a database consisting of all examples appearing in M. Saier's transport protein Classification web site (http://www-biology.ucsd.edu/≈msaier/transport). This site contains, for each category of transport proteins, a detailed description, including the mode of transport, protein topology, substrate specificity, and species specificity, followed by extensive literature and, more important for our purposes, examples of transport proteins with SWISS-PROT and GenBank reference codes. The site is constantly being updated, so we had to freeze a local copy for our work. We downloaded all relevant pages, corrected some ill-formed reference numbers, fetched all example sequences from the public databases, manually updated the few changes that occurred, and froze our local transport protein database on 9 April 2001.

The comparison consisted of a first phase, in which we used Blast2 (2) and queried all *Xylella* sequences against the transport protein database using default parameters, except that the Blastp search was done without a low-complexity filter, followed by a second phase, in which PRSS (32) was used to evaluate the significance of the sequence similarity score and to check for the absence of sequence composition bias. It is known that α-helical transmembrane domains are rich in L, V, I, and F amino acids and that there is a high repetition rate of these four amino acids in the 18- to 20-amino-acid transmembrane stretches. As a result, a considerable number of putative transmembrane domains are usually filtered out of a Blastp search done with a low-complexity filter. Filtering the transmembrane domains may cause the search to miss important transport family similarities. Thus, search of *Xylella* proteins against the transport protein examples was done using Blastp without a low-complexity filter, and matches having an e value lower than 0.01 were selected in the first phase of the search.

In a second phase we used the homology assessment program PRSS from the FASTA package (32) to confirm that the hits pointed out by Blast2 without a filter were not caused by sequence composition bias. In PRSS we used 200 random shuffles with a local window of 10 amino acid residues and admitted as "good matches" hits that got a score of 0.00001 or lower. Blast E values and PRSS scores are shown in Tables 2 to 7 and are shown in the extended format of Table 2, which is available at http://onsona.lbi.dcc.unicamp.br/≈meidanis/. By selecting the 283 *Xylella* proteins that had a PRSS score of 0.00001 or lower and applying a few manual-scrutiny criteria described below, the list was reduced to 209 transport proteins. It can be seen in Tables 2 to 7 that the highest Blast E value (weakest hit) is 3e-04, which was found for two proteins, XF0437 (1.A.23.1.2) and XF1400 (9.A.19.1.1). It should be noted that nine proteins (XF0103, XF0268, XF0563, XF0777, XF1404, XF1496, XF1978, XF2287, and XF2301) which passed the analysis described above and are included in Tables 2 to 7 do have E values higher than 3e-04 when Blast search against the transport protein database is run with a low-complexity filter. We have placed a remark for each of them in the Comments column in Tables 2 to 7, showing the Blast E value with a filter.

With the good matches, we constructed a preliminary list of transport-related *Xylella* proteins, and each *Xylella* protein automatically acquired one or more TC numbers, inherited from the hits in the examples database. The preliminary list was scrutinized by us, and as a result the final number of transporters was reduced from 283 to 209, as described above. The criteria used included the following. (i) Some proteins were eliminated mainly because they had a much better hit with some protein of another, nontransport function, or because the alignment with the transport example did not cover at least 50% of the sequence described in the transport classification site. In some instances, matches with less than 50% coverage of the example were not eliminated because either a specific conserved domain that is described in the literature as a signature of the corresponding example was also present in the *Xylella* protein or the putative gene was clustered with neighboring *Xylella* genes of the same family that had good matches with TC examples. (ii) When a *Xylella* protein was similar to examples from more than one TC category, a decision had to be made as to which one was most probable. This decision took into account E values from Blast hits and PRSS runs, coincident number of predicted TMSs, prior annotation available from the *Xylella* genome project, proximity to other related proteins in the genome (in the case of ABC transporters, for instance), and scientific literature, including the very well documented transport classification site.

QUANTITATIVE COMPARISON WITH OTHER MICROORGANISMS

Table 1 contains a summary of the number of *X. fastidiosa* proteins predicted by the PSORT algorithm (25) to have a specific number of TMSs; they were separated among six classes with different TMS ranges. For comparison, we used PSORT (25) to predict the number of TMSs for another 18 completely sequenced bacterial genomes that have been analyzed previously by Paulsen et al. (31) and for *S. cerevisiae*, which was analyzed by Paulsen et al. (30). It can be seen that *X. fastidiosa* has 957 proteins with at least one putative TMS. A considerable number of transmembrane proteins will have the potential to be transporters. Among them, 170 proteins (6% of all *Xylella* proteins) have four or more TMSs (Table 1). Typically, in the organisms examined to date, 40 to 60% of the proteins with four or more TMSs are associated with transport (31).

It is interesting that *X. fastidiosa* is the prokaryote with the smallest percentage (6%) of proteins with four or more TMSs (Table 1), which is in agreement with the fact that only 75 major transporters (2.7% of all proteins) were identified in *X. fastidiosa* (see below). Therefore, *X. fastidiosa* is the eubacterium with the smallest proportion of major transporters (2.7%) among the fully sequenced bacteria that were analyzed (Table 1). Only two archaea, *Methanococcus jannaschii* and *Methanobacterium thermoautotrophicum*, have a lower percentage of transporters (2.4%).

TABLE 1. Transmembrane proteins in *X. fastidiosa*, 18 other prokaryotes, and *S. cerevisiae*[a]

Species	No. of ORFs	TM[b]		% of ORFs with *N* TMSs							Major transporters		
		No.	%	0	1	2–3	4–6	7–9	10+	≥ 4	No.	% of total ORFs	No./Mb of DNA
Mycoplasmas													
Mycoplasma genitalium	480	185	38.5	61.5	21.5	7.1	5.0	3.1	1.9	10.0	20	4.3	35
Mycoplasma pneumoniae	677	221	32.6	67.4	18.2	5.5	5.5	1.9	1.6	9.0	22	3.3	27
Gram-positive bacteria													
Bacillus subtilis	4,100	1,349	32.9	67.1	13.4	6.3	6.0	4.0	3.3	13.3	265	6.6	63
Mycobacterium tuberculosis	3,918	1,644	42.0	58.0	22.9	10.1	4.7	1.9	2.4	9.0	113	2.9	26
Gram-negative bacteria													
Escherichia coli	4,289	1,445	33.7	66.3	15.2	5.5	5.0	4.2	3.7	12.9	304	7.1	66
Haemophilus influenzae	1,709	527	30.8	69.2	15.4	3.8	5.4	3.3	2.9	11.6	95	5.7	52
Helicobacter pylori	1,566	504	32.2	67.8	18.3	4.9	4.8	1.9	2.4	9.1	56	3.6	34
Rickettsia prowazekii	834	365	43.8	56.2	22.3	8.5	5.0	4.1	3.8	12.9	38	4.6	34
Chlamydia trachomatis	894	362	40.5	59.5	20.8	9.8	4.9	3.2	1.7	9.8	30	3.4	29
Xylella fastidiosa	2,830	957	33.8	66.1	21.7	6.1	3.3	1.3	1.4	6.0	75	2.7	28
Spirochetes													
Borrelia burgdorferi	850	357	42.0	58.0	23.5	7.4	6.2	3.2	1.6	11.0	36	4.2	25
Treponema pallidum	1,031	408	39.6	60.4	22.3	7.9	5.3	3.1	1.0	9.4	35	3.4	31
Cyanobacterium													
Synechocystis sp. strain PCC6803	3,169	1,214	38.3	61.7	20.3	8.1	5.3	2.7	2.0	10.0	102	3.2	29
Deep-branching bacteria													
Aquifex aeolicus	1,522	468	30.7	69.3	16.3	4.3	5.6	2.6	2.0	10.2	44	2.9	28
Thermotoga maritima	1,846	645	34.9	65.1	18.6	5.4	6.7	2.8	1.4	10.9	86	4.7	46
Archaea													
Methanococcus jannaschii	1,715	526	30.7	69.3	17.3	5.7	4.5	1.8	1.3	7.6	42	2.4	25
Methanobacterium thermoautotrophicum	1,869	569	30.4	69.6	15.9	5.5	5.4	2.5	1.3	9.2	45	2.4	26
Archeoglobus fulgidus	2,407	772	32.1	69.9	17.0	5.5	4.6	3.2	1.7	9.5	71	2.9	33
Pyrococcus horikoshii	2,064	794	38.5	61.5	20.0	8.6	5.7	2.4	1.8	9.9	66	3.2	38
Eukaryote													
Saccharomyces cerevisiae	6,315	1,738	27.5	72.5	15.4	5.3	2.2	2.6	2.1	6.9	258	4.2	22

[a] Data for *X. fastidiosa*, 18 other fully sequenced prokaryotes (previously analyzed by Paulsen et al. [31]), and *S. cerevisiae* (previously analyzed by Paulsen et al. [30]) were calculated with PSORT (25) as described in the text.

[b] TM, transmembrane proteins (proteins with ≥1 TMS), given as number and as a percentage of all ORFs.

Major Transporters

In order to permit a direct comparison of the number of *Xylella* transporters with the 18 previously analyzed fully sequenced bacteria (31), we have opted to use the same classification strategy used by Paulsen et al. (31). In that work, the transport families that were used for computing the number of transport systems only included α-helical channel proteins and did not include the β-porins and pore-forming toxins (31). All electrochemical potential-driven transporters (secondary transporters) were included, but only some of the primary active transporters driven by phosphate bond hydrolysis were included, namely the ABC transporters and F- and P-type ATPases; no type II, III, or IV transport systems or oxidoreduction-driven active transporters or phosphotransferase (PTS) systems were counted (see Table 3 in reference 31). In the same manner, the so-called unclassified transporters were counted (TC 9.A); however, the energizers (TC 2.C), auxiliary transporters (TC 8) (39), and putative uncharacterized transporters (TC 9.B) were not counted (31). We have opted here to call major transporters all those transporters computed in Paulsen et al. (31), and for *X. fastidiosa* to compute separately both the major transporters (Table 1) and all the transport families (Tables 2 to 7 [shown below]) that could be identified by comparison to transport family examples available in Saier's phylogeny-based transport protein classification web site (http://www-biology.ucsd.edu/≈msaier/transport).

Based on the major transport protein families analyzed by Paulsen et al. (31), we have identified 106 *Xylella* proteins which comprise 74 major transporters belonging to 57 families as follows: 5 channels (5 proteins), 39 secondary transporters (44 proteins), 24 primary transporters (51 proteins), and 6 unclassified transporters (6 proteins). The number of major transporters in *X. fastidiosa* is similar to those found for *Archaeoglobus fulgidus* (71 transporters), an archaea, and *Thermotoga maritima* (86 transporters), a deep-branching bacterium (Table 1). The number of major transporters per megabase of DNA is 28 in *X. fastidiosa*. As pointed out by Paulsen et al. (31), this is a fairly constant value for the other

18 prokaryotes analyzed (average value of 36 transporters per Mb); the conspicuous exceptions are *Escherichia coli*, *Bacillus subtilis*, and *Haemophilus influenzae*, which have 66, 63, and 52 transporters per Mb, respectively.

The 39 secondary transporters of *X. fastidiosa* mentioned above correspond to 53% of the major transporter types found in this bacterium. Primary transporters represent 32%, while channels and unclassified transporters account for the remaining 15%. Predominance of secondary transporters over primary ones is in accordance with the fact that *X. fastidiosa* is an aerobic prokaryote in which the respiratory chain is complete. Respiration probably provides most of the energy for generation of a proton motive force that is subsequently used by secondary transporters to effect solute translocation across the membranes. This correlates with previous observations showing that prokaryotic organisms that depend primarily on substrate-level phosphorylation show the highest percentage of primary transporters, while the more aerobic prokaryotes show the reverse tendency (31).

Additional Transporters

In addition to the major transporters described above, we were able to further identify 103 *Xylella* proteins with similarity to other transporters present in Saier's classification (39). They comprise 64 transporters belonging to 34 families: 25 porins and pore-forming toxins (25 proteins), 10 energizers (10 proteins), 8 active transporters (47 proteins), 10 auxiliary transporters (10 proteins), and 11 putative uncharacterized transporter proteins. Thus, a total of 92 different transport families comprising 139 transporters were identified. In all, 209 proteins were classified as constitutive members of transporter families.

It should be noted that there were an additional 45 *Xylella* proteins with four or more putative TMSs which are classified as hypothetical or conserved hypothetical (marked H in Table 7) because they do not match the transport protein database and in addition they either do not match any other GenBank protein or match proteins annotated as hypothetical. Another 10 *Xylella* proteins are classified as undefined because they have four or more TMSs and again do not match the transport protein database; however, they are similar to proteins of other organisms that are given a defined name (see Annotation column for proteins in TC family marked U in Table 7) but have undefined or poorly characterized function (marked U in Table 7). Some 14 of those 55 proteins mentioned above have between 8 and 11 TMSs, and these include many putative transporters, such as an uncharacterized predicted permease (XF0250) with 10 TMSs and unknown function, which has 139 other similar members, all of unknown function, that appear in 16 different bacteria. There is a good chance that of the 55 *Xylella* proteins with unknown function and four or more TMSs, some 22 to 33 additional hypothetical or undefined proteins (40 to 60%) are *Xylella* transporters. It should be interesting to concentrate on experiments that would characterize some of those possible transporters.

TRANSPORTERS PRESENT IN *X. FASTIDIOSA*

Tables 2 through 7 summarize our findings about all transport proteins identified in *X. fastidiosa*. The tables are divided according to the transport family categories described by Saier (39) and provide information about the transport protein example that matches each *Xylella* protein, such as the Transport Category (TC), the putative substrate transported, the name of the example gene, and the E score and PRSS value of the match. In addition, the original *Xylella* annotation (44) is shown along with the cluster of orthologous groups (COG) annotation (46) from http://www.ncbi.nlm.nih.gov/COG/. An additional Table 2 (extended) is available at http://onsona.lbi-.dcc.unicamp.br/≈meidanis/; it has more extensive information that we collected about each match between the *Xylella* protein and the transport example protein.

Alpha-Type Channels

X. fastidiosa possesses only four channel systems (Table 2). One of them is an interesting member of the voltage-gated ion channel superfamily (1.A.1). The *Xylella* protein is similar to other putative voltage-gated channels widely found in eukaryotes and so far only found in *Deinococcus radiodurans* and *Pseudomonas aeruginosa* bacteria. It has significant similarity to the Shal2 voltage-gated potassium channel from *Drosophila melanogaster* (1.A.1.2.3, P17971), a member of the voltage-regulated ion transport family (pfam 00520) (http://pfam.wustl .edu/). In the *Drosophila* protein, transmembrane segment S4 is probably the voltage sensor and is characterized by a series of positively charged amino acids at every third position (48). An identical motif is found in the corresponding transmembrane segment of the *Xylella* protein, as well as in the channel proteins of the other two bacteria. It will be interesting to characterize the functional role in *X. fastidiosa* of such a voltage-regulated ion channel.

Protein XF2267 belongs to the major intrinsic protein family (1.A.8) and is a glycerol facilitator (1.A.8.1.1). There is a protein that serves as a large conductance mechanosensitive channel (1.A.22; MscL), which transports ions nonspecifically, with some selectivity for cations over anions. Large-conductance mechanosensitive channels have been shown to release proteins such as thioredoxin and to protect bacteria from cell lysis during an osmotic downshift (27). In addition, two proteins make up small-conductance mechanosensitive ion channels (1.A.23; MscS) which open in response to pressure changes during osmotic downshift just below those that cause cell disruption and death (22).

It is interesting that no one member of the 15 distinct families of holins are present in *X. fastidiosa*. Holins are channel proteins that organize as homooligomeric complexes that form transmembrane pores which provide the passive transport of murein hydrolases across the cytoplasmic membrane to the cell wall, where these enzymes hydrolyze the cell wall polymer as a step leading to cell lysis (50). In *X. fastidiosa*, no murein hydrolases were found, and phage-related cell wall degradation and lysis are mediated through phage-related lysozymes (XF1564 and XF1669), which apparently have other mechanisms of secretion that are independent of holins.

TABLE 2. Channels, β-porins, and pore-forming toxins[a]

Family and transporter[b]	TC no.	ORF	No. of TMSs	Putative substrate	Occurrence	Comments	E value	PRSS *P* value	*Xylella* annotation	COG
Channels										
VIC	1.A.1.2.3	XF1426	6	Cation	BAE	Voltage-sensitive K^+ channel	1e-21	4.9873e-23	Ion transporter	COG1226 (79 proteins), Kef-type K^+ transport systems, predicted NAD-binding component
MIP	1.A.8.1.1	XF2267	5	Small molecule	BAE	Glycerol facilitator	5e-46	5.8223e-29	Glycerol uptake facilitator protein	COG0580 (36 proteins), glycerol uptake facilitator and related permeases (major intrinsic protein family)
MscL	1.A.22.1.1	XF0039	2	Protein or ion	B	Large mechanosensitive ion channel	8e-35	8.7563e-28	Large-conductance mechanosensitive channel	COG1970 (19 proteins), large-conductance mechanosensitive channel
MscS	1.A.23.1.2	XF0437	5	Ions	BAE	Major MscS mechanosensitive ion channel	3e-04	2.7709e-06	Conserved hypothetical protein	COG0668 (80 proteins), small-conductance mechanosensitive channel
MscS	1.A.23.1.2	XF1258	4	Ions	BAE	Major MscS mechanosensitive ion channel	2e-47	3.2616e-30	Small-conductance mechanosensitive ion channel	COG0668 (80 proteins), small-conductance mechanosensitive channel
β-Porins										
POP	1.B.5.1.2	XF0321	0	Pyrophosphate	B−	Pyrophosphate-selective porin	7e-17	3.0706e-14	Porin O precursor	No COG
POP	1.B.5.1.2	XF0975	0	Pyrophosphate	B−	Pyrophosphate-selective porin	4e-13	8.3331e-14	Polyphosphate-selective porin O	No COG
OOP	1.B.6.1.2	XF0343	1?		B−	OmpF porin	2e-38	5.6084e-35	Outer membrane protein	COG2885 (60 proteins), outer membrane protein and related peptidoglycan-associated (lipo) proteins
FadL	1.B.9.1.1	XF1053	0	Hydrophobic	B−	Fatty acid outer membrane porin	1e-33	1.0349e-23	Outer membrane protein	COG2067 (19 proteins), long-chain fatty acid transport protein
FUP	1.B.11.3.1	XF0081	1	Fimbrial structure	B−	Fimbrial usher	e-119	4.2124e-47	Outer membrane usher protein precursor	COG3188 (31 proteins), PapC-like porin protein involved in fimbrial biogenesis
AT	1.B.12.5.1	XF0267	1	Virulence factor	B−	Autoexporter of serine protease	7e-51	1.5727e-24	Serine protease	2 domains: 1, XF0267_1, COG1404 (68 proteins), subtilisin-like serine proteases: 2, XF0267_2, no COG
AT	1.B.12.5.1	XF1026	1	Virulence factor	B−	Autoexporter of serine protease	8e-48	1.2144e-23	Serine protease	2 domains: 1, XF1026_1, COG1404 (68 proteins), subtilisin-like serine proteases: 2, XF1026_2, no COG
AT	1.B.12.5.1	XF1851	0	Virulence factor	B−	Autoexporter of serine protease	1e-51	1.3276e-26	Serine protease	2 domains: 1, XF1851_1, no COG: 2, XF1851_2, COG1404 (68 proteins), subtilisin-like serine proteases
OMR	1.B.14.1.1	XF2134	0	Iron siderophore	B−	FepA ferric enterobactin receptor	1e-23	3.3329e-20	Ferric enterobactin receptor	COG1629 (205 proteins), outer membrane receptor proteins, mostly Fe transport
OMR	1.B.14.1.1	XF2137	0	Iron siderophore	B−	FepA ferric enterobactin receptor	8e-19	2.4582e-15	Ferric enterobactin receptor	COG1629 (205 proteins), outer membrane receptor proteins, mostly Fe transport
OMR	1.B.14.1.4	XF0599	0	Iron hydroxamate	B−	FhuA ferrichrome receptor	1e-35	2.5692e-26	TonB-dependent receptor for iron transport	COG1629 (205 proteins), outer membrane receptor proteins, mostly Fe transport
OMR	1.B.14.2.3	XF0384	0	Iron siderophore	B−	HemR heme (hemin) receptor	2e-09	8.9988e-11	Outer membrane hemin receptor	COG1629 (205 proteins), outer membrane receptor proteins, mostly Fe transport

OMR	1.B.14.2.3	XF1496	0	Iron siderophore	B−	HemR heme (hemin) receptor; Blast with filter E value, 6e-04	7e-05	4.4425e-08	TonB-dependent receptor for iron transport	COG1629 (205 proteins), outer membrane receptor proteins, mostly Fe transport
OMR	1.B.14.3.1	XF0339	0	Vitamin B_{12}	B−	BtuB cobalamin receptor	2e-19	2.3654e-10	Conserved hypothetical protein	COG1629 (205 proteins), outer membrane receptor proteins, mostly Fe transport
OMR	1.B.14.3.1	XF0550	0	Vitamin B_{12}	B−	BtuB cobalamin receptor	1e-15	1.3333e-11	Conserved hypothetical protein	COG1629 (205 proteins), outer membrane receptor proteins, mostly Fe transport
OMR	1.B.14.3.1	XF2237	0	Vitamin B_{12}	B−	BtuB cobalamin receptor	4e-16	8.6932e-10	Conserved hypothetical protein	COG1629 (205 proteins), outer membrane receptor proteins, mostly Fe transport
OMF	1.B.17.1.1	XF2586	1	Hemolysin, drugs, etc.	B−	TolC outer membrane exporter	4e-62	2.5737e-31	Outer membrane export factor	COG1538 (64 proteins), outer membrane protein
OMA	1.B.18.3.1	XF2370	0	EPS	B−	Capsular polysaccharide export system	3e-11	1.1235e-11	GumB protein	COG1596 (20 proteins), periplasmic protein involved in polysaccharide export
TEC	1.B.20.1.2	XF2550	0	Toxin	B−	Hemolysin secretion protein	1e-27	1.5087e-22	Outer membrane hemolysin activator protein	COG2831 (19 proteins), hemolysin activation/secretion protein
Secretin	1.B.22.1.1	XF1527	0	Protein	B−	PulD protein secretin	7e-39	4.0619e-25	General secretory pathway protein D precursor	COG1450 (39 proteins), general secretory pathway protein D
Secretin	1.B.22.2.1	XF0373	1	Fimbrial protein	B−	PilQ fimbrial subunit; signal peptide missing	2e-95	3.8855e-52	Fimbrial assembly protein	COG1450 (39 proteins), general secretory pathway protein D
Pore-forming toxins										
RTX toxin	1.C.11.1.4	XF1011	2	Pore in host	B	Bifunctional adenylate cyclase-hemolysin toxin precursor	3e-66	1.7905e-14	Hemolysin-type calcium-binding protein	COG2931 (21 proteins), RTX toxins and related Ca^{2+}-binding proteins
RTX toxin	1.C.11.1.4	XF0668	0	Pore in host	B	Bifunctional adenylate cyclase-hemolysin toxin precursor	5e-56	8.5548e-14	Hemolysin-type calcium-binding protein	COG2931 (21 proteins), RTX toxins and related Ca^{2+}-binding proteins
RTX toxin	1.C.11.1.4	XF2407	0	Pore in host	B	Bifunctional adenylate cyclase-hemolysin toxin precursor	8e-72	3.6634e-11	Bacteriocin	COG2931 (21 proteins), RTX toxins and related Ca^{2+}-binding proteins
RTX toxin	1.C.11.1.4	XF2759	0	Pore in host	B	Bifunctional adenylate cyclase-hemolysin toxin precursor	9e-59	2.6427e-15	Hemolysin-type calcium-binding protein	COG2931 (21 proteins), RTX toxins and related Ca^{2+}-binding proteins

[a] Transport families and Transport Commission (TC) numbers were assigned to each Xylella protein identified by its ORF name as described in the text, following the phylogeny-based classification proposed by Saier (39). The number of TMSs for each Xylella protein was calculated using PSORT (25). Putative substrate refers to the substrate transported by the representative member of the family, as described by Saier (39). Occurrence refers to the occurrence of the family in different organisms as follows: B, bacteria; A, archaea; E, eukaryota; B−, gram-negative bacteria; B+, gram-positive bacteria; O, eukaryotic organelles; C, cyanobacteria; B-p, plasmid of gram-negative bacteria; BA?, bacteria, archaea, or perhaps eukaryota; BA, bacteria and archaea. Comments contains the name of the transport protein example having the best hit against the Xylella protein. E value is the expected value of the Blastp match between the Xylella protein and the example protein found in the present review. PRSS P value refers to the P value of the PRSS search of the corresponding Xylella protein against the example protein. Annotation refers to the original annotation of the Xylella genome (44). The COG classification refers to the Cluster of Orthologous Groups of proteins described by Tatusov et al. (46) and available at http://www.ncbi.nlm.nih.gov/COG/.

[b] VIC, voltage-gated ion channel superfamily; MIP, major intrinsic protein; POP, Pseudomonas outer membrane porin; OOP, OmpA-OprF porin; FUP, fimbrial usher porin; AT, autotransporter; OMR, outer membrane receptor; OMF, outer membrane factor; OMA, outer membrane auxiliary protein; TEC, toxin export channel; RTX, repeat in structural toxin; EPS, exopolysaccharides.

TABLE 3. Electrochemical potential-driven transporter[a]

Family and transporter[b]	TC no.	ORF	No. of TMSs	Putative substrate	Occurrence	Comments	E value	PRSS *P* value	*Xylella* annotation	COG
Major facilitator superfamily										
DHA1	2.A.1.2.4	XF0268	8	Tetracycline:H^+ antiporter	B	TetA antiporter; Blast with filter E value, 0.010	2e-05	1.2286e-07	Conserved hypothetical protein	COG0477 (925 proteins), permeases of the major facilitator superfamily
DHA1	2.A.1.2.18	XF1749	11	Drug:H^+ antiporter 1 (sugar efflux)	BE	Lactose and melibiose (and IPTG) efflux	3e-40	2.0382e-18	Transcriptional regulator	COG2814 (42 proteins), arabinose efflux permease
DHA1	2.A.1.2.18	XF1728	10	Drug:H^+ antiporter 1 (sugar efflux)	BE	Lactose and melibiose (and IPTG) efflux	1e-34	7.5481e-15	Transport protein	COG2814 (42 proteins), arabinose efflux permease
DHA2	2.A.1.3.10	XF1765	14	Drug-H^+ antiporter 2	BE	Also similar to 8.A.1	3e-66	2.0866e-25	Drug: proton antiporter	COG0477 (925 proteins), permeases of the major facilitator superfamily
MHS	2.A.1.6.2	XF0087	8	Metabolite	B	α-Ketoglutarate:H^+ symporter	e-157	4.0568e-83	α-Ketoglutarate permease symporter	COG0477 (925 proteins), permeases of the major facilitator superfamily
FHS	2.A.1.7.2	XF1609	10	Glucose/galactose	B	Glucose/galactose permease	e-109	2.3545e-56	Glucose/galactose transporter	COG0738 (16 proteins), fucose permease
FHS	2.A.1.7.2	XF1462	9	Glucose/galactose	B	Glucose/galactose permease	2e-44	3.732e-25	Glucose/galactose transporter	COG0738 (16 proteins), fucose permease
DHA3	2.A.1.21.3	XF0993	8	Drug	B	Tetracycline resistance determinant TetV	2e-21	1.1218e-11	Drug:proton antiporter	COG0477 (925 proteins), permeases of the major facilitator superfamily
PAT	2.A.1.25.2	XF0165	11	Drug, glycopeptide	BE	AmpG penicillin derivative uptake permease	1e-46	6.2703e-36	β-Lactamase induction signal transducer protein	COG0477 (925 proteins) permeases of the major facilitator superfamily
Other secondary porters										
CAT	2.A.3.3.1	XF2208	11	Cationic a.a.	BAE	High-affinity basic a.a. transporter CAT1 of *Musmusculus*	6e-64	7.6968e-36	Cationic amino acid transporter	COG0531 (170 proteins), amino acid transporters
CAT	2.A.3.3.1	XF2207	9	Cationic a.a.	BAE	High-affinity basic a.a. transporter CAT1 of *Mus musculus*	2e-74	1.1345e-43	Cationic amino acid transporter	COG0531 (170 proteins), amino acid transporters
CAT	2.A.3.3.3	XF0408	12	Cationic a.a.	BAE	Similar to amino acid transporter AAT1 of *Arabidopsis thaliana*	2e-58	3.6814e-27	Amino acid transporter	COG0531 (170 proteins), amino acid transporters
CDF	2.A.4.1.1	XF0866	6	Heavy metal	BAE	Cd^{2+}, Zn^{2+}, Co^{2+} efflux permease	1e-84	3.4023e-58	Cobalt-zinc-cadmium resistance protein	COG1230 (28 proteins), Co/Zn/Cd efflux system component
RND (HME)	2.A.6.1.2	XF0243	12	Heavy metal	BAE	Heavy-metal efflux pump (Co^{2+}, Zn^{2+}, Cd^{2+})	3e-73	1.25e-45	Acriflavin resistance protein	COG0841 (73 proteins), cation/multidrug efflux pump
RND (HME)	2.A.6.1.2	XF2083	11	Heavy metal	BAE	Heavy-metal efflux pump (Co^{2+}, Zn^{2+}, Cd^{2+})	3e-71	7.6213e-42	Cation efflux system protein	COG0841 (73 proteins), cation/multidrug efflux pump
RND (HAE1)	2.A.6.2.1	XF2094	12	Multidrug	BAE	Multidrug (acriflavin) resistance pump	0.0	4.0016e-167	Multidrug-efflux transporter	COG0841 (73 proteins), cation/multidrug efflux pump

RND (HAE1)	2.A.6.2.6	XF2385	12	Multidrug	BAE	Multiple drug; N-(3-oxododecanoyl)-L-homoserine lactone autoinducer efflux pump	e-115	1.1486e-70	Acriflavin resistance protein D	COG0841 (73 proteins) cation/multidrug efflux pump
RND (HAE1)	2.A.6.2.7	XF2386	12	Multidrug, aminoglycosides	BAE	Multidrug efflux pump AcrD; exports aminoglycosides	e-108	9.7384e-69	Acriflavin resistance protein	COG0841 (73 proteins), cation/multidrug efflux pump
RND (SecDF)	2.A.6.4.1	XF0225	7	Protein	B	SecD protein	e-136	3.8605e-83	Protein export membrane protein	COG0342 (35 proteins), preprotein translocase subunit SecD
RND (SecDF)	2.A.6.4.1	XF0226	6	Protein	B	SecF protein	2e-53	1.2289e-41	Protein export membrane protein	COG0341 (32 proteins), preprotein translocase subunit SecF
RND (HAE2)	2.A.6.5.1	XF0777	12	Drug, antibiotic	BAE	Antibiotic actinorhodin transport-associated protein ActI13; Blast with filter E value, 0.32	3e-13	2.6152e-08	Membrane protein	No COG
CitMHS	2.A.11.1.2	XF0320	9	Citrate	BE	Citrate:H^+ symporter	e-157	3.1147e-74	Mg^{2+}/citrate complex transporter	COG2851 (10 proteins), H^+/citrate symporter
AAA	2.A.12.1.2	XF1738	10	ATP:ADP antiporter	BE	Similar to Npt1 of Chlamydia trachomatis	1e-16	3.5714e-13	Hypothetical protein	COG3202 (15 proteins), ATP/ADP translocase
POT	2.A.17.1.1	XF1891	10	Oligopeptide: H^+ symporter	BAE	Di- or tripeptide:H^+ symporter	7e-55	2.0679e-25	Di-tripeptide ABC transporter membrane protein	COG3104 (18 proteins), dipeptide/tripeptide permease
SSS	2.A.21.7.1	XF2251	13	Phenylacetate	BAE	Phenylacetate permease	e-178	9.1145e-62	Solute:Na^+ symporter	COG0591 (79 proteins), Na^+/proline, Na^+/panthothenate symporters and related permeases
DAACS	2.A.23.1.2	XF1937	9	a.a., dicarb.	BE	Glutamate, aspartate:Na^+, H^+ symporter	6e-59	3.4059e-32	Proton glutamate symport protein	COG1301 (39 proteins), Na^+/H^+-dicarboxylate symporters
DAACS	2.A.23.1.2	XF0656	7	a.a., dicarb.	BE	Glutamate, aspartate:Na^+, H^+ symporter	1e-56	2.4966e-28	Glutamate symport protein	COG1301 (39 proteins), Na^+/H^+-dicarboxylate symporters
DAACS	2.A.23.1.3	XF0976	8	a.a., dicarb.	BE	C4-dicarboxylate transporter	e-164	1.1088e-67	C4-dicarboxylate transport protein	COG1301 (39 proteins), Na^+/H^+-dicarboxylate symporters
CPA1	2.A.36.3.1	XF2019	12	Na^+?	BE	Putative antiporter (function unknown)	e-114	6.1394e-66	Na^+:H^+ antiporter	COG0025 (39 proteins), NhaP-type Na^+/H^+ and K^+/H^+ antiporters
CPA2	2.A.37.1.1	XF2140	12	K^+ mostly	BAE	Glutathione-regulated K^+ efflux protein C	2e-56	1.7293e-30	Cation:proton antiporter	2 domains: 1 XF2140_1, COG0475 (60 proteins), Kef-type K^+ transport systems, membrane components; 2, XF2140_2, COG1226 (79 proteins), Kef-type K^+ transport systems, predicted NAD-binding component
CPA2	2.A.37.2.1	XF1398	11	Na^+/H^+	BAE	Na^+:H^+ antiporter	9e-11	1.6718e-08	Na^+/H^+ exchange protein	COG0475 (60 proteins), Kef-type K^+ transport systems, membrane components

Continued on following page

TABLE 3—*Continued*

DASS	2.A.47.4.1	XF0785	11	Divalent anion	BAE	Sulfur deprivation response	3e-73	6.3827e-45	Sulfur deprivation response regulator	COG0471 (50 proteins), di- and tricarboxylate transporters
Amt	2.A.49.1.1	XF1844	11	Ammonium	BAE	Ammonium transporter	e-138	1.8823e-49	Ammonium transporter	COG0004 (38 proteins), ammonia permeases
Nramp	2.A.55.1.2	XF1015	9	Metal	BAE	Putative low-affinity Mn^{2+} uptake	2e-73	1.0721e-48	Manganese transport protein	COG1914 (25 proteins), Mn^{2+} and Fe^{2+} transporters of the NRAMP family
Tat	2.A.64.1.1	XF0562	6	Protein	BA?	TatC channel	8e-61	1.0933e-37	Sec-independent protein translocase	COG0805 (31 proteins), Sec-independent protein secretion pathway component TatC
Tat	2.A.64.1.1	XF0177	0	Protein	BA?	TatC protein; exports proteins with leading sequence TRRXFLK	9e-24	6.1298e-22	Conserved hypothetical protein	COG0084 (58 proteins), Mg-dependent DNase
Tat	2.A.64.1.1	XF0563	0	Protein	BA?	TatB protein; Blast with filter E value, 0.024	7e-11	2.0218e-12	Hypothetical protein	COG1826 (51 proteins), Sec-independent protein secretion pathway components
Tat	2.A.64.1.1	XF0564	0	Protein	BA?	TatA protein	2e-09	1.0113e-09	Conserved hypothetical protein	COG1826 (51 proteins), Sec-independent protein secretion pathway components
Tat	2.A.64.1.1	XF1913	0	Protein	BA?	TatD protein	1e-64	2.857e-54	Type V secretory pathway protein	COG0084 (58 proteins), Mg-dependent DNase
MATE	2.A.66.1.1	XF2686	10	Antimicrobials	BE	Multidrug efflux system	1e-71	1.1291e-37	Multidrug efflux protein	COG0534 (83 proteins), Na^{+}-driven multidrug efflux pump
OPT	2.A.67.2.1	XF2261	15	Oligopeptide: H^{+} symporter	BE	Similar to ORF HI0561/560 of Haemophilus influenzae	e-117	6.1557e-43	Oligopeptide transporter	COG1297 (14 proteins), uncharacterized membrane protein
KUP	2.A.72.1.1	XF1903	11	K^{+}	BE	K^{+} uptake permease	e-169	2.7402e-91	Potassium uptake protein	COG3158 (13 proteins), K^{+} transporter
ICT	2.A.73.1.1	XF0103	7	HCO_3^{-}	C	Putative HCO_3^{-}:Na^{+} symporter; Blast with filter E value, = 6e-04	1e-08	1.7299e-06	Membrane protein	COG3307 (22 proteins), lipid A core-O-antigen ligase and related enzymes
RhtB	2.A.76.1.1	XF2730	5	Amino acid	B	Homoserine/homoserine actone/ β-hydroxynorvaline efflux permease	5e-21	5.0767e-16	Amino acid transporter	COG1280 (67 proteins), putative threonine efflux protein
Energizers										
TonB	2.C.1.1.1	XF0010	2	Siderophore, B colicin	B	ExbB protein	9e-17	1.0079e-13	Biopolymer transport ExbB protein	COG0811 (45 proteins), biopolymer transport proteins
TonB	2.C.1.1.1	XF1079	2	Siderophore, B colicin	B	ExbB protein	4e-12	6.5391e-12	Conserved hypothetical protein	COG0811 (45 proteins), Biopolymer transport proteins
TonB	2.C.1.1.1	XF0009	1	Siderophore, B colicin	B	TonB protein	5e-16	4.7175e-07	TonB protein	COG0810 (32 proteins), periplasmic protein TonB, links inner and outer membranes
TonB	2.C.1.1.1	XF0011	1	Siderophore, B colicin	B	ExbD1 protein	7e-17	4.87e-19	Biopolymer transport ExbD1 protein	COG0848 (45 proteins), biopolymer transport protein

TonB	2.C.1.1.1	XF1080	1	Siderophore, B colicin	B	ExbD protein	1e-10	4.9777e-13	Conserved hypothetical protein	COG0848 (45 proteins), biopolymer transport protein
TonB	2.C.1.1.1	XF2287	1	Siderophore, B colicin	B	TonB protein: Blast with filter E value, 0.001	9e-10	7.0449e-06	TonB protein	COG0810 (32 proteins), periplasmic protein TonB, links inner and outer membranes
TonB	2.C.1.1.1	XF2327	1	Siderophore, B colicin	B	TonB protein	5e-13	1.8723e-07	TonB protein	COG0810 (32 proteins), periplasmic protein TonB, links inner and outer membranes
TonB	2.C.1.1.1	XF0012	0	Siderophore, B colicin	B	ExbD2 protein	4e-15	1.4428e-16	Biopolymer transport ExbD2 protein	COG0848 (45 proteins), biopolymer transport protein
TonB	2.C.1.2.1	XF1900	3	Colicin A, DNA		TolQ protein	8e-56	1.9298e-42	TolQ protein	COG0811 (45 proteins), biopolymer transport proteins
TonB	2.C.1.2.1	XF1899	0	Colicin A, DNA	B	TolR protein	2e-19	2.517e-15	TolR protein	COG0848 (45 proteins), biopolymer transport protein

[a] See Table 2, footnote a.

β-Barrel Porins

X. fastidiosa possesses 21 outer membrane proteins (Table 2) that act in conjunction with cytoplasmic membrane transporters to effect the transport of substances from the cytoplasm to the cell exterior.

One of them is an outer membrane factor (1.B.17), which can operate in conjunction with both ATP-binding cassette (ABC) and resistance-nodulation-cell division (RND) transport systems linked to the outer membrane factor protein by a membrane fusion protein (8.A.1). The several proteins involved in this apparatus result in a structure that spans the cytoplasmic membrane, the periplasmic space, and the outer membrane and transports substances directly from the interior of the cell to the outside in one energy-coupled step. This system is used in *X. fastidiosa*, as in most other bacteria in which it is found, for export of multiple drugs, heavy metals, and bacteriocins (28). *X. fastidiosa* has only one outer membrane factor protein, but three membrane fusion proteins that function with RND porters (TC 8.A.1.2), two membrane fusion proteins that function with ABC porters (TC 8.A.1.3), and several RND and ABC systems that export drugs.

X. fastidiosa possesses an outer membrane auxiliary protein (1.B.18), annotated as GumB, which works with two other proteins for the export of exopolysaccharides. One of these extra proteins (GumC) spans the cytoplasmic membrane twice and has an ATP-binding domain, as in several other gram-negative bacteria (29). This is a member of the family of cytoplasmic membrane-periplasmic auxiliary-1 proteins with a cytoplasmic (C) domain (TC 8.A.3) and is located in the genome adjacent to the gene encoding the outer membrane auxiliary protein. The third protein (GumJ) is a member of the polysaccharide transporter family (9.A.1), the gene for which is also close to the other two in the genome. In general, polysaccharide transporter family proteins have 12 TMSs, but the *Xylella* homolog has only nine. A thorough description of the gum-producing machinery of *X. fastidiosa* is under preparation (F. R. Da Silva, personal communication).

Two members of the secretin family (TC 1.B.22) export proteins and fimbrial structures. A member of the fimbrial usher porin family (TC 1.B.11) also exports fimbriae. Two proteins of the *Pseudomonas* outer membrane porin family (TC 1.B.5) export phosphate or pyrophosphate.

Nine outer membrane receptors (1.B.14), which use energy produced by the TonB systems (TC 2.C.1), import a variety of substances into the periplasm. These substances find their way into the cytoplasm mainly via ABC transporters. Based on similarities with homologs from other species, we predict that iron siderophores and vitamin B_{12} are among the substrates imported by this mechanism. Two of the open reading frames (ORFs) classified as vitamin B_{12} receptors (XF0550 and XF2237) have shown much higher similarity to *Schwanella* predicted proteins of unknown function.

There is one protein of the OmpA-OprF porin family (TC 1.B.6.1.2), a large family that includes the functionally well characterized OmpA porin of *E. coli* as well as the OprF porin protein F of *Pseudomonas aeruginosa*. These proteins form eight transmembrane, antiparallel, amphipathic β-strands. They form β-barrels with short turns at the periplasmic barrel ends and long, flexible loops at the external ends (42). OmpA

of *E. coli* is required for bacterial conjugation, maintaining outer membrane stability, and determining cell shape and ability to grow in low-osmolarity medium.

A protein of the FadL family (TC 1.B.9) is present. The *E. coli* FadL protein is responsible for long-chain fatty acid transport across the outer membrane. Residues involved in fatty acid binding and transport have been distinguished and identified (21).

Other transporters in this category include three autotransporters of a serine protease (TC 1.B.12). These autotransporters are virulence factors which cross the cytoplasmic membrane via the Sec (general secretory) pathway (TC 3.A.5), and following cleavage of their N-terminal targeting sequence, they enter the periplasm of the gram-negative bacterial cell envelope. The C-terminal 250 to 300 amino acyl residues fold and insert into the outer membrane to give rise to a putative β-barrel structure with 14 transmembrane β-strands. This structure forms a pore through which the N-terminal virulence factor is transported to the extracellular medium.

Another β-barrel transporter involved in virulence is a toxin export channel (TC 1.B.20), which is involved in export across the outer membranes of gram-negative bacteria of various toxin proteins. For example, the ShlB toxin export channel of *Serratia marcescens* exports hemolysin through the outer membrane after it is secreted by the type II general secretory pathway (TC 3.A.5) system across the cytoplasmic membrane (20). These proteins are thought to form β-barrel channels in the outer membrane through which *Xylella* hemolysin III toxin (XF0175) may be exported.

Certain domains of outer membrane proteins are exposed at the outer surface and therefore may be good candidates for vaccines in gram-negative bacteria. For example, porin F, which is one of the major proteins of the outer membrane of *P. aeruginosa*, has been identified as a candidate for a vaccine because it antigenically cross-reacts in all serotype strains of *P. aeruginosa* of the International Antigenic Typing Scheme (10). Some of the outer membrane channel-forming proteins of *X. fastidiosa* identified above may be considered targets for studies of a possible vaccine.

Pore-Forming Toxins

X. fastidiosa has four RTX toxins (TC 1.C.11), which are bacterial pore-forming exotoxins (Table 2). They are secreted from the bacteria, and after processing, they insert into the membranes of animal cells. There they cause cell rupture by mechanisms that are not well understood.

Electrochemical Potential-Driven Transporters

Xylella has 49 secondary transporters, which are listed in Table 3, that couple proton electrochemical potential of the cytoplasmic membrane to active transport events. Respiration probably provides most of the energy for generation of this proton motive force. A total of 39 major secondary transporters were identified (Table 3), including symporters, antiporters, uniporters, and the resistance-nodulation-cell division (RND) family, along with 10 other energizers (Table 3) of the TonB family.

Symporters, antiporters, and uniporters. (i) Major facilitator superfamily. The major facilitator superfamily is a large and diverse superfamily that includes several hundred sequenced members. They catalyze uniport, solute:cation (H^+ or Na^+) symport, and/or solute:H^+ or solute:solute antiport. Just eight proteins in *X. fastidiosa* belong to the major facilitator superfamily (Table 3). In contrast, 64 proteins of this kind are found in *E. coli*. The *Xylella* members export drugs and import metabolites and fucose.

One interesting member of the major facilitator superfamily is involved in the uptake of oligopeptides (TC 2.A.1.25), including cell wall degradation products (peptides and glycopeptides, including *N*-acetylglucosaminyl-β-1,4-anhydro-*N*-acetylmuramyl-tripeptide) as well as penicillin derivatives. Note also the presence in *X. fastidiosa* of two additional porters involved in the uptake of oligopeptides, TC 2.A.17 and 2.A.67, which are oligopeptide:H^+ symporters. In this respect, it is interesting that no ABC-type active transporter of oligopeptides (TC 3.A.1.5.1) was found in *X. fastidiosa*. The ABC-type oligopeptide transporters also take up amino glycoside antibiotics, such as kanamycin and streptomycin, as well as cell wall-derived peptides such as murein tripeptides. The *oppABCDF* genes of *Salmonella enterica* serovar Typhimurium are well-characterized members of this family, which is represented in all 25 fully sequenced bacteria except *X. fastidiosa*, *Neisseria meningitidis*, and *Rickettsia prowazekii*.

(ii) Other secondary porters. The ATP/ADP translocase antiporter (XF1738) (TC 2.A.12) is the most important member in this class. It has been found only in *Chlamydia* and *Rickettsia* spp., two obligate intracellular bacterial parasites of eukaryotic cells. A homolog has been sequenced in *Arabidopsis thaliana* and is supposed to be localized to the intracellular plastid membrane, where it functions as an ATP importer (18). The *X. fastidiosa* ATP/ADP translocase is similar to the Npt1 translocase of *Chlamydia trachomatis* (E value, 1e-16; 22% identity, 38% similarity, and 90% coverage of the *Chlamydia* example). XF1738 is classified by the cluster of orthologous groups (46) analysis as belonging to COG3202, ATP/ADP translocase. The *Xylella* translocase has 10 TMSs, identical to the number of TMSs predicted by PSORT (25) for the *Chlamydia* ATP/ADP translocase.

In *C. trachomatis* the transporter is an exchange translocase specific for ATP and ADP (47). It functions to take up ATP from the eukaryotic cell cytoplasm into the bacterium in exchange for ADP, thus providing a source of energy for the bacteria (47). It is quite unexpected that a nonintracellular bacterium such as *X. fastidiosa* should have an ATP/ADP translocator, and it is tempting to speculate that *X. fastidiosa* may utilize the so far uncharacterized plant xylem ATP as an additional source of energy. In fact, preliminary results in our laboratory show that *X. fastidiosa* takes up ATP from the culture medium (T. Koide, S. L. Gomes, and S. Verjovski-Almeida, unpublished data) with kinetics very similar to that shown for the *Chlamydia* translocase (47).

Additional porters in *X. fastidiosa* include 37 proteins comprising 23 systems for export or import across the inner membrane of citrates, oligopeptides, amino acids, proteins, drugs, metals, antimicrobial agents, and ions. The most prevalent is the RND family (TC 2.A.6), with six different systems and a total of eight proteins. These porters are ubiquitously present in bacteria, archaea, and eukaryotes. Members of the RND

TABLE 4. Primary active transporters: ABC transporters[a]

Family and transporter	TC no.	ORF	No. of TMSs	Putative substrate	Occurrence	Comments[b]	E value	PRSS *P* value	*Xylella* annotation	COG
Uptake systems										
CUT1	3.A.1.1.3	XF1067	0	Sugar	B	Glycerol-phosphate porter; cytoplasm (ATP binding)	4e-94	5.2223e-64	Sugar ABC transporter ATP-binding protein	COG1130 (175 proteins), ABC-type sugar/spermidine/putrescine/iron/thiamine transport systems, ATPase component
CUT1	3.A.1.1.4	XF2446	5	Sugar	B	Lactose porter; membrane	1e-42	2.5711e-23	ABC transporter sugar permease	COG0395 (111 proteins), sugar permeases
CUT1	3.A.1.1.4	XF2447	4	Sugar	B	Lactose porter; membrane	6e-52	5.0915e-32	ABC transporter sugar permease	COG1175 (110 proteins), ABC-type sugar transport systems, permease components
CUT1	3.A.1.1.7	XF2448	0	Sugar	B	Maltose/trehalose porter; receptor	5e-25	5.5258e-22	ABC transporter sugar binding protein	COG1653 (83 proteins), sugar-binding periplasmic proteins/domains
PAAT	3.A.1.3.1	XF0875	0	Polar a.a.		Histidine/arginine/lysine/ornithine porter; cytoplasm (ATP binding); lacks R	5e-45	1.7196e-35	ABC transporter ATP binding protein	COG1135 (24 proteins), uncharacterized ABC-type transport system ATPase component
PAAT	3.A.1.3.2	XF2695	0	Polar a.a.		Glutamine porter; ATP binding ABC domain	1e-36	1.3515e-28	ABC transporter ATP binding protein	COG2884 (18 proteins), predicted ATPase involved in cell division
PAAT	3.A.1.3.4	XF0874	4	Polar a.a.		Glutamate/aspartate porter; membrane; lacks R	4e-07	2.7183e-08	ABC transporter permease protein	COG2011 (22 proteins), permease component of an uncharacterized ABC transporter
PAAT	3.A.1.3.9	XF0420	3	Glutamate?		Membrane			Toluene tolerance protein	COG0767 (32 proteins), ABC-type toluene export system, permease component
PAAT	3.A.1.3.9	XF0418	0	Glutamate?		Receptor			Toluene tolerance protein	COG2854 (12 proteins), uncharacterized periplasmic protein
PAAT	3.A.1.3.9	XF0419	0	Glutamate?		Receptor			Toluene tolerance protein	COG1463 (53 proteins), permease component of an ABC transporter
PAAT	3.A.1.3.9	XF0421	0	Glutamate		Similar to glutamate porter GluA of *Corynebacterium*; cytoplasm (ATP binding)	2e-29	2.9871e-27	Toluene tolerance protein	COG1127 (24 proteins), uncharacterized ABC-type transport system, ATPase component
HAAT	3.A.1.4.1	XF1409	0	Leu/Ile/Val		Leu/Ile/Val porter (hydrophobic amino acid uptake); cytoplasm (ATP binding), lacks M, R	2e-31	5.8503e-26	ABC transporter ATP-binding protein	COG1137 (24 proteins), ABC-type (unclassified) transport systemATPase component
PepT	3.A.1.5.3	XF1475	0	Nickel		Nickel porter; cytoplasm (ATP binding), lacks M, R	1e-14	2.0481e-15	ABC transporter ATP-binding protein	COG0396 (28 proteins), iron-regulated ABC transporter ATPase subunit SufC
SulT	3.A.1.6.1	XF1345	6	Sulfate	B	Sulfate/thiosulfate porter membrane	8e-75	8.6038e-42	ABC transporter sulfate permease	COG0555 (55 proteins), ABC-type sulfate/molybdate transport systems, permease components
SulT	3.A.1.6.1	XF1346	5	Sulfate	B	Sulfate/thiosulfate porter membrane	2e-71	5.8465e-39	ABC transporter sulfate permease	COG0555 (55 proteins), ABC-type sulfate/molybdate transport systems, permease components
SulT	3.A.1.6.1	XF1344	0	Sulfate	B	Sulfate/thiosulfate porter receptor	e-119	7.8369e-94	ABC transporter sulfate-binding protein	COG1613 (17 proteins), ABC-type sulfate transport system, periplasmic component

Continued on following page

TABLE 4—*Continued*

SulT	3.A.1.6.1	XF1347	0	Sulfate	B	Sulfate/thiosulfate porter; cytoplasm (ATP binding)	8e-80	2.6395e-56	sulfate ABC transporter ATP-binding protein	COG1118 (29 proteins), ABC-type sulfate/molybdate transport systems, ATPase component
PhoT	3.A.1.7.1	XF2142	5	Phosphate	B	Phosphate porter; membrane	5e-90	1.3785e-40	ABC transporter phosphate permease	COG0573 (34 proteins), ABC-type phosphate transport system, permease component
PhoT	3.A.1.7.1	XF2143	5	Phosphate	B	Phosphate porter; membrane	9e-86	3.1053e-48	ABC transporter phosphate permease	COG0581 (34 proteins), ABC-type phosphate transport system, permease component
PhoT	3.A.1.7.1	XF2144	1	Phosphate	B	Phosphate porter; cytoplasm (ATP binding)	1e-97	4.0756e-75	Phosphate ABC transporter ATP-binding protein	COG1117 (38 proteins) ABC-type phosphate transport system, ATPase component
PhoT	3.A.1.7.1	XF2141	0	Phosphate	B	Phosphate porter; receptor	4e-95	2.4942e-50	ABC transporter phosphate-binding protein	COG0226 (46 proteins) ABC-type phosphate transport system, periplasmic component
VB12T	3.A.1.13.1	XF1604	0	Vitamin B_{12}	B	Vitamin B_{12} porter; receptor; lacks C, M	2e-35	1.0273e-33	ABC transporter vitamin B_{12} uptake permease	COG0386 (25 proteins) Glutathione peroxidase
NitT	3.A.1.16.3	XF0411	8	Bicarbonate	B	Bicarbonate porter; membrane; lacks R	2e-15	7.395e-11	ABC transporter nitrate permease	COG0600 (50 proteins) ABC-type nitrate/sulfonate/taurine/bicarbonate transport systems, permease components
NitT	3.A.1.16.3	XF0412	0	Bicarbonate	B	Bicarbonate porter; cytoplasm (ATP-binding); lacks R	2e-44	4.6747e-33	Nitrate ABC transporter ATP-binding protein	[2 domains] (1) XF0412_1: COG1116 (49 proteins) ABC-type nitrate/sulfonate/taurine/bicarbonate transport systems, ATPase components; (2) XF0412_2: NO COG
Export systems										
LOSE	3.A.1.102.1	XF1602	1	Lipooligosaccharide	B	Lipooligosaccharide exporter (nodulation protein); cytoplasm (ATP binding), lacks M	1e-18	1.509e-17	ABC transporter ATP-binding protein	COG1131 (228 proteins) ABC-type multidrug transport system, ATPase component
LOSE	3.A.1.102.1	XF1302	0	Lipooligosaccharide	B	Lipooligosaccharide exporter (nodulation protein); cytoplasm (ATP binding), lacks M	2e-28	4.5271e-23	ABC transporter ATP-binding protein	COG1127 (24 proteins) Uncharacterized ABC-type transport system, ATPase component
LPSE	3.A.1.103.1	XF2567	5	Lipopolysaccharide	B	Lipopolysaccharide exporter; membrane	1e-13	1.844e-09	ABC transporter permease protein	COG1682 (27 proteins) Membrane permeases involved in cell wall biosynthesis
LPSE	3.A.1.103.1	XF2568	0	Lipopolysaccharide	B	Lipopolysaccharide exporter; cytoplasm (ATP binding)	4e-45	4.8821e-39	ABC transporter ATP-binding protein	COG1134 (25 proteins) ABC-type polysaccharide/polyol phosphate transport system, ATPase component
DrugE1	3.A.1.105.1	XF1222	5	Drug	B	Daunorubicin; doxorubicin (drug resistance) exporter; membrane	5e-06	2.3754e-07	Conserved hypothetical protein	COG0842 (129 proteins) ABC-type multidrug transport system, permease component
DrugE1	3.A.1.105.1	XF1223	0	Drug	B	Daunorubicin; doxorubicin (drug resistance) exporter; cytoplasm (ATP binding)	2e-36	1.189e-27	ABC transporter ATP-binding protein	COG1131 (228 proteins), ABC-type multidrug transport system, ATPase component
LipidE	3.A.1.106.1	XF1081	6	Lipid	B	Putative lipid A exporter (flippase); cytoplasm (ATP binding), plus M together	e-131	5.5729e-93	ABC transporter ATP-binding protein	COG1132 (204 proteins), ABC-type multidrug/protein/lipid transport system ATPase component

LipidE	3.A.1.106.1	XF2582	3	Lipid	B	Putative lipid A exporter (flippase); cytoplasm (ATP binding), plus M together	2e-84	1.3328e-59	ABC transporter ATP-binding protein	COG1132 (204 proteins), ABC-type multidrug/protein/lipid transport system, ATPase component
HemeE	3.A.1.107.1	XF2456	7	Heme	B	Putative heme exporter; membrane	2e-41	1.0691e-19	Heme ABC transporter membrane protein	COG2386 (11 proteins), ABC-type transport system involved in cytochrome c biogenesis, permease component
HemeE	3.A.1.107.1	XF2457	6	Heme	B	Putative heme exporter; membrane	2e-49	3.8318e-30	Heme ABC transporter membrane protein	COG0755 (36 proteins), ABC-type transport system involved in cytochrome c biogenesis, permease component
HemeE	3.A.1.107.1	XF2455	0	Heme	B	Putative heme exporter; cytoplasm (ATP binding)	2e-20	1.718e-21	Heme ABC transporter ATP-binding protein	COG1131 (228 proteins), ABC-type multidrug transport system, ATPase component
Prot1E	3.A.1.109.1	XF2397	5	Protein	B	α-Hemolysin exporter; cytoplasm (ATP binding), plus M together	0.0	2.5016e-148	Toxin secretion ABC transporter ATP-binding protein	COG2274 (19 proteins), ABC-type bacteriocin/lantibiotic exporters, contain an N-terminal double glycine peptidase domain
Prot2E	3.A.1.110.2	XF1220	5	Protein	B	Colicin V exporter; cytoplasm (ATP binding), plus M together	e-152	1.0121e-102	Colicin V secretion ABC transporter ATP-binding protein	COG2274 (19 proteins), ABC-type bacteriocin/lantibiotic exporters, contain an N-terminal double glycine peptidase domain
DevE	3.A.1.114.1	XF1077	0	Glycolipid	B	Probable glycolipid exporter (envelope formation); cytoplasm (ATP binding) lacks M	2e-38	1.2501e-31	ABC transporter ATP-binding protein	COG1136 (121 proteins), ABC-type transport systems, involved in lipoprotein release, ATPase components
NatE	3.A.1.115.1	XF2328	6	Sodium	B	ABC transporter sodium permease; membrane	9e-32	1.2142e-23	ABC transporter sodium permease	COG1668 (20 proteins), ABC-type Na^+ efflux pump, permease component
NatE	3.A.1.115.1	XF2329	1	Sodium	B	Sodium ABC transporter ATP-binding protein; cytoplasm (ATP binding)	1e-59	1.2537e-46	Sodium ABC transporter ATP-binding protein	COG1131 (228 proteins), ABC-type multidrug transport system, ATPase component
DrugRA1	3.A.1.120.1	XF2617	1	Drugs	B	Macrolide ATPase; cytoplasm (ATP-binding), lacks M	3e-59	2.2893e-39	ABC transporter ATP-binding protein	COG0488 (93 proteins), ATPase components of ABC transporters with duplicated ATPase domains
DrugRA1	3.A.1.120.1	XF2133	0	Drugs	B	Macrolide ATPase; cytoplasm (ATP binding)	9e-51	8.1429e-35	ABC transporter ATP-binding protein	COG0488 (93 proteins), ATPase components of ABC transporters with duplicated ATPase domains
DrugRA1	3.A.1.120.2	XF0944	1	Drugs	B	Tylosin ATPase; cytoplasm (ATP binding)	2e-58	4.0421e-41	ABC transporter ATP-binding protein	COG0488 (93 proteins), ATPase components of ABC transporters with duplicated ATPase domains

[a] See Table 2, footnote a.
[b] C, cytoplasmic subunit; M, membrane component; R, receptor component.

superfamily all probably catalyze substrate efflux via an H^+ antiport mechanism. The substrates are either heavy metals (e.g., Co^{2+}, Zn^{2+}, Cd^{2+}, and Ni^{2+}), multiple drugs, or proteins.

Two interesting systems deserve special mention. One of them is the type V secretion pathway or twin-arginine transporter (TC 2.A.64), which gets its name because it transports proteins characterized by a leading sequence (S/T)RRXFLK with two arginines. In *E. coli* this system has five components, TatABCDE, with 1, 1, 6, 0, and 1 TMSs, respectively, and sizes of 98, 171, 258, 264, and 67 amino acids, respectively, forming a gene cluster. In *X. fastidiosa* we found five homologs, but they are not in one-to-one correspondence with the *E. coli* proteins, and only three of them are clustered together. *Xylella* ORFs XF0564, XF0563, and XF0562, with 0, 0, and 6 TMSs, respectively, and sizes of 71, 140, and 246 amino acids, respectively, are similar to TatA, TatB, and TatC, respectively. Two homologs of TatD exist, XF0177 and XF1913, both with 0 TMSs and sizes of 260 and 268 amino acids, respectively. However, the similarity of XF1913 to TatD is much higher, and XF0177 shows higher similarity to an unrelated protein (YiiV of *E. coli*) than to TatD. Component TatE was not found, perhaps due to its small size. Also, TatA and TatE may have similar roles, as they can partially substitute for each other in the *E. coli* type V secretion pathway system. These findings support the existence of a twin-arginine transporter system in *X. fastidiosa*. In addition, *X. fastidiosa* has one protein (XF0842) with the leading signal motif TRRXFLK. In fact, XF0842 has the leading sequence 3TRRTFLR9, with a conservative replacement of R for K in the seventh position of the motif. XF0842 is similar (E score, 3e-79) to a putative secreted protein from *Streptomyces coelicolor* (CAB61925).

The other interesting note concerns the inorganic carbon transporter (TC 2.A.73). *X. fastidiosa* has an ORF with similarity to *ictB*, an Na^+:HCO_3^- symporter protein so far found only in the cyanobacterium *Synechocystis* (3) and believed to effect transport of inorganic carbon in the form of HCO_3^- to be processed in photosynthesis in *Synechocystis*. This is surprising and suggests that *X. fastidiosa* may count on HCO_3^- from the xylem of citrus plants.

Energizers

Among its predicted genes, *X. fastidiosa* has three clusters and two isolated predicted genes whose translations were classified in the TonB family (TC 2.C.1). The cytoplasmic membrane protein TonB couples the proton electrochemical potential of the cytoplasmic membrane to active transport events at the outer membrane of gram-negative bacteria via outer membrane receptors (members of the outer membrane receptor family, TC 1.B.14). All the *Xylella* proteins hit the *E. coli* example systems TonB-ExbB-ExbD and TolA-TolQ-TolR. TonB and TolA are believed to serve the same function, and ExbB and TolQ are homologous, as are ExbD and TolR.

One of the *Xylella* clusters (XF0009, XF0010, XF0011, and XF0012) contains four sequences with high similarity to the TonB-ExbB-ExbD1-ExbD2 system of *E. coli*. A second cluster (XF1899 and XF1900) contains only two sequences, similar to TolQ and TolR, but lacks a TolA homolog. However, the two isolated *X. fastidiosa* predicted genes (XF2287 and XF2327) are both similar to TonB, and we propose that one of them could function as TolA for this cluster. Finally, the third cluster (XF1079 and XF1080) also contains two sequences, one of them similar to both ExbB and TolQ, and the other similar to both ExbD and TolR.

Primary Active Transporters

A total of 32 primary active transport systems which couple either the hydrolysis of the phosphate bond of ATP or the oxidoreduction of NADH:ubiquinone to the active transport of ions and other small molecules were identified in *Xylella*. *Xylella* primary active transporters are identified in Tables 4 and 5 below.

Transport driven by phosphate bond hydrolysis. (i) ABC superfamily. The ATP-binding cassette (ABC) transporter superfamily are present in all forms of life and use the energy released by hydrolysis of ATP to promote active transport of substances across the cytoplasmic membrane. Some systems effect uptake while others effect export, but no known system has both functions. A typical ABC system for uptake of solutes is composed of three units appearing together as a gene cluster: a receptor, a membrane component, and a cytoplasmic, ATP-binding component. The receptors are usually not required in the ABC export systems. The best-conserved component is the ATP-binding one; even simple Blast searches made with a typical member can easily uncover all the others. This motif is quite conserved, and usually no false-positives result.

In *X. fastidiosa*, the ATP-binding cassette superfamily is the best represented superfamily, and we found 23 systems comprising a total of 43 proteins (Table 4). Twelve of them are predicted based solely on the presence of the ATP-binding component, and therefore the specified subfamily is tentative. In all such cases we have indicated in the tables that the membrane component is lacking, and when appropriate, we also indicate that the receptor is lacking for the putative uptake systems. Finding the specific substrates for those transporters will be an interesting subject for future study.

(a) Uptake ABC systems. Nine systems were classified as belonging to uptake families (TC 3.A.1.1 to 3.A.1.99), including members for the import of sugar (CUT1), glutamate and other polar amino acids (PAAT), hydrophobic amino acids (HAAT), sulfate (SulT), phosphate (PhoT), vitamin B_{12} (VB12T), nickel (PepT), and bicarbonate (NitT). The CUT1 system is well identified, although its cytoplasmic component gene is located far from the other components, which cluster together in the chromosome. The VB12T system is not well identified, with both the cytoplasmic and membrane components missing and just the receptor present. One of the PAAT systems (XF0874 and XF0875) lacks the receptor, as does the NitT system. The other putative PAAT system has four clustered genes; however, only the cytoplasmic ATP-binding component (XF0421) has homology to the glutamate porter gene *gluA* of *Corynebacterium glutamicum*, a typical example of an ABC four-member uptake porter. This ORF also has good similarity to the *ttg2A* gene of *Pseudomonas putida*, a member of the ABC family that has been implicated in toluene resistance (19) and is not listed among Saier's examples.

The remaining three ORFs in this *Xylella* cluster (XF0420,

XF0419, and XF0418) have similarity to genes *ttg2BCD*, which are part of the *P. putida* operon involved in toluene resistance (19). It is expected that toluene resistance would be related to extrusion of toluene; however, the operon in *P. putida* (19) has been shown to be a four-member system typical of uptake ABC family systems. It is possible that knockout of the *ttg2* genes (19) has made the otherwise toluene-resistant *P. putida* strain nonviable by affecting the uptake of energy fuel and not necessarily by interfering directly with the toluene resistance mechanism. In fact, the authors who described the *ttg2* gene of *P. putida* pointed out that the *ttg2* mutant is very sensitive to short-term treatment with toluene, suggesting the importance of this transporter in toluene resistance; however, as they state in their article, at present it is not clear whether this gene encodes a protein acting as a toluene pump (19). The authors suggested alternatively that the gene might encode a transporter protein functioning in outer membrane synthesis, which is an important barrier to penetration by growth inhibitors (19). It should be noted that *P. putida* has a *ttg3* gene that codes for a toluene efflux pump, which plays an important role in *P. putida* toluene resistance (19), whereas *X. fastidiosa* does not have any gene similar to *ttg3*.

Further functional characterization of this putative PAAT *Xylella* system would clarify its possible glutamate substrate specificity as well as the direction of transport. The HAAT and PepT systems have only the cytoplasmic unit, both lacking the membrane and receptor components; specificity has been assigned based on the GenBank (http://www.ncbi.nlm.nih.gov/) best match of the cytoplasmic component, and further experimental characterization of a membrane unit for these systems would be of utmost relevance. Both SulT and PhoT are well identified, each having four clustered units, including two membrane components.

(b) Export ABC systems. The remaining 14 systems are for export. Thus, we have export of lipopolysaccharides (LPSE) and lipooligosaccharides (LOSE), drugs (DrugE1 and DrugRA1), lipids (LipidE), heme (HemeE), proteins (Prot1E and Prot2E), and sodium (NatE). The best characterized are the LPSE, DrugE1, HemeE, and NatE systems.

The ABC export systems involved in drug transport (ProtE1, ProtE2, DrugE1, and three members of DrugRA1) are probably responsible for conferring resistance to antibiotics and for excretion of antibiotics (macrolides) and toxins.

It is noteworthy that *X. fastidiosa* has a sodium export system (NatE) that is similar to the NatAB system of *Bacillus subtilis*, in which the extrusion of sodium has been very well characterized (6). Sodium extrusion via an ABC transport system is found in all eight fully sequenced archaea except *Thermoplasma acidophilum*. Among the 24 fully sequenced eubacteria, only two gram-positive ones, *B. subtilis* and *D. radiodurans*, have such sodium extrusion ABC transporter, along with *Thermotoga maritima*, a hyperthermophilic bacterium that is closely related to the low-G+C gram-positive bacteria (16). In *T. maritima* it has been proposed that there has been extensive lateral gene transfer with archaea (26) as part of the mechanism of evolution towards thermophilicity. It will be interesting to study the physiology of sodium ion transport in *X. fastidiosa* to understand the role of such a sodium transport system in adapting *X. fastidiosa* to the plant xylem.

Four of the export systems (LPSE, HemeE, DrugE1, and NatE) exhibit their components in gene clusters and have a membrane as well as a cytoplasmic component. The other 10 have only the cytoplasmic unit, and no gene in the vicinity is related to the ABC superfamily. Again, experimental confirmation of the specific substrates that are exported by those transporters will be an interesting subject for future study.

(ii) Other phosphate bond-driven transporters. Seven systems comprising 30 proteins make up the rest of the phosphate bond hydrolysis-driven transporters (Table 5). *X. fastidiosa* has an F-ATPase (TC 3.A.2.1.1) with eight subunits, very similar to *E. coli* and other bacterial systems. This F-ATPase or ATP synthase system is complete in *X. fastidiosa*, indicating that ATP synthesis is driven by a chemiosmotic proton gradient.

It is interesting that no P-type cation transport ATPase (TC 3.A.3) was found in *X. fastidiosa*. The P-type ATPase family of ion transport proteins is a very well characterized system, and the transport mechanism involves phosphorylation (by the gamma-phosphate of ATP) of the aspartyl residue in the conserved motif DKTGT(L/I)T during the catalytic and transport cycle (17). All fully sequenced archaea, bacteria, and eukaryotes have P-type ATPases which transport protons, sodium, potassium, and other ions at the expense of ATP. There are four exceptions: *Pyrococcus horikoshii* is the only archaea among the eight fully sequenced that does not have a P-type ATPase. Among the 24 fully sequenced eubacteria, only *R. prowazekii*, *Borrelia burgdorferi*, and *X. fastidiosa* do not have a P-type cation transport ATPase.

It is interesting that the three eubacteria have in common the fact that they are not free-living organisms (*X. fastidiosa* lives in the plant xylem, *B. burgdorferi* is a blood parasite, and *R. prowazekii* is an intracellular parasite) that are transmitted by insect vectors. It is tempting to speculate that transmission via an insect vector has obviated the need for this ATP-driven cation transport. In turn, it is possible that this adaptation has caused *X. fastidiosa* to have a distinct way of regulating its cation concentration. Characterization of ion transport regulation in *X. fastidiosa* should be an interesting subject for study, since this might be a special target for controlling and interfering with its growth.

A type II or general secretory pathway (TC 3.A.5) is complete. Notice that in our tables, proteins SecD and SecF (XF0225 and XF0226, respectively) are present as examples both in this family and in their own specific family as secondary transporters (TC 2.A.6.4, SecDF), and YajC (XF0224) is also cross-listed here and in the SecDF-associated single transmembrane protein family (TC 9.B.18).

It should be emphasized that no type III (virulence-related) secretory pathway system (TC 3.A.6) was found in *X. fastidiosa*. The type III system is responsible for injection of virulence-related bacterial proteins directly into the cytoplasm of host cells (13, 49). In bacterial plant pathogens, type III secretory systems are responsible for injecting the so-called avirulence (*avr*) genes into host plant cells (1), which in turn elicits plant responses that will ultimately result in a limited host range, often confined to members of a single plant species or genus. In accordance with the absence of a type III secretory system, no genes with similarity to the known avirulence genes were found in the *Xylella* genome. It is apparent that these genes are not required for *Xylella*-host interactions, and it is possible that the insect-mediated mechanism of transmission and vascular

TABLE 5. Primary active transporters other than ABC transporters[a]

Family and transporter[b]	TC no.	ORF	No. of TMSs	Putative substrate	Occurrence	Comments	E value	PRSS *P* value	*Xylella* annotation	COG
Phosphate bond-driven active transporters—F-type ATPases										
F-ATPase	3.A.2.1.1	XF1149	5	H^+	BO	ATP synthase, A chain	2e-68	4.2235e-37	ATP synthase, A chain	COG0356 (29 proteins), F_0F_1-type ATP synthase a subunit
F-ATPase	3.A.2.1.1	XF1148	1	H^+	BO	ATP synthase, C chain	6e-17	3.018e-12	ATP synthase, C chain	COG0636 (53 proteins), F_0F_1-type ATP synthase c subunit/ archaeal/vacuolar-type H^+-ATPase subunit K
F-ATPase	3.A.2.1.1	XF1142	0	H^+	BO	ATP synthase, epsilon chain	2e-27	1.2088e-22	ATP synthase, epsilon chain	COG0355 (30 proteins), F_0F_1-type ATP synthase epsilon subunit (mitochondrial delta subunit)
F-ATPase	3.A.2.1.1	XF1143	0	H^+	BO	ATP synthase, beta chain	0.0	3.4964e-128	ATP synthase, beta chain	COG0055 (31 proteins), F_0F_1-type ATP synthase beta subunit
F-ATPase	3.A.2.1.1	XF1144	0	H^+	BO	ATP synthase, gamma chain	2e-95	3.282e-72	ATP synthase, gamma chain	COG0224 (32 proteins), F_0F_1-type ATP synthase gamma subunit
F-ATPase	3.A.2.1.1	XF1145	0	H^+	BO	ATP synthase, alpha chain	0.0	1.2431e-115	ATP synthase, alpha chain	COG0056 (31 proteins), F_0F_1-type ATP synthase alpha subunit
F-ATPase	3.A.2.1.1	XF1146	0	H^+	BO	ATP synthase, delta chain	3e-21	2.6852e-18	ATP synthase, delta chain	COG0712 (31 proteins), F_0F_1-type ATP synthase delta subunit (mitochondrial oligomycin sensitivity protein)
F-ATPase	3.A.2.1.1	XF1147	0	H^+	BO	ATP synthase, B chain	4e-33	1.5018e-19	ATP synthase, B chain	COG0711 (38 proteins), F_0F_1-type ATP synthase b subunit
Additional phosphate bond-driven active transporters										
IISP	3.A.5.1.1	XF1172	10	Protein	B	SecY subunit	e-141	8.9569e-70	Preprotein translocase SecY subunit	COG0201 (46 proteins), preprotein translocase subunit SecY
IISP	3.A.5.1.1	XF0225	7	Protein	B	SecD protein	e-136	3.8605e-83	Protein export membrane protein	COG0342 (35 proteins), preprotein translocase subunit SecD
IISP	3.A.5.1.1	XF0226	6	Protein	B	SecF protein	2e-53	1.2289e-41	Protein export membrane protein	COG0341 (32 proteins), preprotein translocase subunit SecF
IISP	3.A.5.1.1	XF2639	2	Protein	B	SecE protein	6e-13	1.4354e-11	Preprotein translocase subunit	COG0690 (33 proteins), preprotein translocase subunit SecE
IISP	3.A.5.1.1	XF0073	1	Protein	B	Ffh (signal recognition particle protein homologue)	e-154	7.5182e-76	Signal recognition particle protein	COG0541 (44 proteins), signal recognition particle GTPase
IISP	3.A.5.1.1	XF0224	1	Protein	B	YajC subunit	6e-17	4.8984e-21	Preprotein translocase YajC subunit	COG1862 (27 proteins), preprotein translocase subunit YajC
IISP	3.A.5.1.1	XF0304	1	Protein	B	SecG protein	1e-14	1.9945e-14	Protein export membrane protein	COG1314 (24 proteins), preprotein translocase subunit
IISP	3.A.5.1.1	XF1910	1	Protein	B	FtsY (signal recognition particle receptor homologue)	1e-83	1.5495e-61	Cell division protein	COG0552 (46 proteins), signal recognition particle GTPase
IISP	3.A.5.1.1	XF0806	0	Protein	B	SecA subunit	0.0	2.2789e-172	Preprotein translocase SecA subunit	COG0653 (35 proteins), preprotein translocase subunit SecA (ATPase, RNA helicase)

IVSP	3.A.7.1.1	XFa0011	7	DNA-protein complex	B-p	VirB6	4e-06	1.5005e-07	Conjugal transfer protein>	COG3704 (8 proteins), predicted membrane protein involved in conjugal plasmid transfer
IVSP	3.A.7.1.1	XFa0006	2	DNA-protein complex	B-p	VirB3	2e-07	1.0811e-08	Conjugal transfer protein	COG3702 (3 proteins), uncharacterized membrane protein
IVSP	3.A.7.1.1	XF2049	1	DNA-protein complex	B-p	VirB10	5e-16	1.2192e-11	Conjugal transfer protein	COG2948 (15 proteins), VirB10 component of type IV secretion system a0040 a0014
IVSP	3.A.7.1.1	XFa0012	1	DNA-protein complex	B-p	VirB8	3e-17	4.9039e-18	Conjugal transfer protein	No COG
IVSP	3.A.7.1.1	XFa0014	1	DNA-protein complex	B-p	VirB10	6e-51	5.1851e-28	Conjugal transfer protein	COG2948 (15 proteins), VirB10 component of type IV secretion system a0040
IVSP	3.A.7.1.1	XFa0040	1	DNA-protein complex	B-p	VirB10	2e-15	1.2046e-11	Conjugal transfer protein	COG2948 (15 proteins), VirB10 component of type IV secretion system a0014
IVSP	3.A.7.1.1	XFa0007	0	DNA-protein complex	B-p	VirB4—ATPase	e-135	2.8766e-100	Conjugal transfer protein	COG3451 (15 proteins), predicted ATPase
IVSP	3.A.7.1.1	XFa0013	0	DNA-protein complex	B-p	VirB9	3e-30	5.3519e-27	Conjugal transfer protein	COG3504 (13 proteins), component of conjugal plasmid transfer system a0042
IVSP	3.A.7.1.1	XFa0015	0	DNA-protein complex	B-p	VirB11—ATPase	1e-45	5.2895e-38	Conjugal transfer protein	COG0630 (48 proteins), predicted ATPases involved in pilus and flagellum biosynthesis, VirB11 family
DNA-T	3.A.11.1.1	XF1078	10	ssDNA	B	DNA translocase	1e-45	1.0811e-42	DNA uptake protein	2 domains: 1, XF1078_1, COG0658 (30 proteins), predicted multitransmembrane, metal-binding protein; 2, XF1078_2, COG2333 (17 proteins), predicted hydrolases of metallo-β-lactamase fold
DNA-T	3.A.11.1.1	XF0252	0	ssDNA	B	DNA translocase	1e-06	1.2545e-11	ATP-dependent RNA helicase	COG0513 (165 proteins), superfamily II DNA and RNA helicases
DNA-T	3.A.11.1.1	XF0593	0	ssDNA	B	DNA translocase	3e-11	5.4989e-12	Conserved hypothetical protein	COG1555 (24 proteins), DNA uptake protein and related DNA-binding proteins
FPE	3.A.12.2.1	XF1518	3	Protein	B	Pilin (PilA) secretion/fimbrial assembly system; membrane component	9e-60	8.6426e-52	General secretory pathway protein F	COG1459 (31 proteins), general secretory pathway protein F
FPE	3.A.12.2.1	XF2538	3	Protein	B	Pilin (PilA) secretion/fimbrial assembly system; membrane component	e-131	2.6194e-83	Fimbrial assembly protein	COG1459 (31 proteins), general secretory pathway protein F
FPE	3.A.12.2.1	XF1517	0	Protein	B	Pilin (PilA) secretion/fimbrial assembly system; cytoplasmic ATPase component	e-100	2.8292e-70	General secretory pathway protein E	COG2804 (33 proteins), predicted ATPases involved in pilus biogenesis, PilB homologs
FPE	3.A.12.2.1	XF2544	0	Protein	B	Pilin (PilA) secretion/fimbrial assembly system; cytoplasmic ATPase component	0.0	7.1114e-11	Pilus biogenesis protein	COG2804 (33 proteins), predicted ATPases involved in pilus biogenesis, PilB homologs

Continued on following page

TABLE 5—*Continued*

Oxidoreduction-driven activetransporters										
NDH	3.D.1.1.1	XF0318	13	H^+	BO	NuoN subunit	3e-64	3.4453e-26	NADH-ubiquinone oxidoreductase, NQO14 subunit	COG1007 (23 proteins) NADH: ubiquinone oxidoreductase subunit 2 (chain N)
NDH	3.D.1.1.1	XF0316	12	H^+	BO	NuoL subunit	1e-27	2.1006e-15	NADH-ubiquinone oxidoreductase, NQO12 subunit	COG1009 (38 proteins), NADH: ubiquinone oxidoreductase subunit 5 (chain L)/Multisubunit Na^+/H^+ antiporter, MnhA subunit
NDH	3.D.1.1.1	XF0317	10	H^+	BO	NuoM subunit	1e-93	3.061e-38	NADH-ubiquinone oxidoreductase, NQO13 subunit	COG1008 (24 proteins), NADH: ubiquinone oxidoreductase subunit 4 (chain M)
NDH	3.D.1.1.1	XF0312	6	H^+	BO	NuoH subunit	1e-64	4.6176e-31	NADH-ubiquinone oxidoreductase, NQO8 subunit	COG1005 (24 proteins), NADH: ubiquinone oxidoreductase subunit 1 (chain H)
NDH	3.D.1.1.1	XF0314	4	H^+	BO	NuoJ subunit	3e-16	2.647e-13	NADH-ubiquinone oxidoreductase, NQO10 subunit	COG0839 (26 proteins), NADH: ubiquinone oxidoreductase subunit 6 (chain J)
NDH	3.D.1.1.1	XF0305	3	H^+	BO	NuoA subunit	3e-13	1.7523e-13	NADH-ubiquinone oxidoreductase, NQ07 subunit	COG0838 (22 proteins), NADH: ubiquinone oxidoreductase subunit 3 (chain A)
NDH	3.D.1.1.1	XF0313	2	H^+	BO	NuoI subunit	3e-21	1.2041e-23	NADH-ubiquinone oxidoreductase, NQO9 subunit	COG1143 (36 proteins), formate hydrogenlyase subunit 6/NADH:ubiquinone oxidoreductase 23-kDa subunit (chain I)
NDH	3.D.1.1.1	XF0311	1	H^+	BO	NuoG subunit	3e-51	6.7891e-40	NADH-ubiquinone oxidoreductase, NQO3 subunit	COG1034 (21 proteins), NADH dehydrogenase/NADH:ubiquinone oxidoreductase 75-kDa subunit (chain G)
NDH	3.D.1.1.1	XF0306	0	H^+	BO	NuoB subunit	4e-41	1.1315e-38	NADH-ubiquinone oxidoreductase, NQO6 subunit	COG0377 (26 proteins), NADH: ubiquinone oxidoreductase 20-kDa subunit and related Fe-S oxidoreductases
NDH	3.D.1.1.1	XF0307	0	H^+	BO	NuoC subunit	3e-17	1.5812e-18	NADH-ubiquinone oxidoreductase, NQO5 subunit	COG0852 (27 proteins), NADH: ubiquinone oxidoreductase 27-kDa subunit
NDH	3.D.1.1.1	XF0308	0	H^+	BO	NuoD subunit	1e-81	2.4758e-66	NADH-ubiquinone oxidoreductase, NQ04 subunit	COG0649 (27 proteins), NADH: ubiquinone oxidoreductase 49-kDa subunit 7
NDH	3.D.1.1.1	XF0309	0	H^+	BO	NuoE subunit	5e-20	7.2428e-24	NADH-ubiquinone oxidoreductase, NQO2 subunit	COG1905 (21 proteins), NADH: ubiquinone oxidoreductase 24-kDa subunit
NDH	3.D.1.1.1	XF0310	0	H^+	BO	NuoF subunit	2e-92	1.8278e-69	NADH-ubiquinone oxidoreductase, NQO1 subunit	COG1894 (21 proteins), NADH: ubiquinone oxidoreductase NADH binding (51 kDa) subunit
NDH	3.D.1.1.1	XF0315	1	H^+	BO	NuoK subunit	4e-34	4.5288e-27	NADH-ubiquinone oxidoreductase, NQO11 subunit	COG0713 (22 proteins), NADH: ubiquinone oxidoreductase subunit 11 or 4L (chain K)
QCR	3.D.3.1.1	XF0909	8	H^+	BO	Ubiquinol cytochrome c oxidoreductase, cytochrome b subunit, also hit 3.E.2-PRC	5e-90	2.3737e-43	Ubiquinol cytochrome c oxidoreductase, cytochrome b subunit	COG1290 (30 proteins), cytochrome b subunit of the bc complex
QCR	3.D.3.1.1	XF0908	1	H^+	BO	Ubiquinol cytochrome c oxidoreductase, iron-sulfur subunit; also hit 3.E.2-PRC	1e-24	1.6097e-25	Ubiquinol cytochrome c oxidoreductase, iron-sulfur subunit	COG0723 (35 proteins), Rieske Fe-S protein

QCR	3.D.3.2.1	XF0910	1	H^+	BO	Ubiquinol cytochrome c oxidoreductase, cytochrome c_1 subunit	2e-11	1.4333e-14	Ubiquinol cytochrome c oxidoreductase, cytochrome c_1 subunit	COG2857 (18 proteins), cytochrome c_1
COX	3.D.4.5.1	XF1389	14	H^+	BO	Cytochrome o ubiquinol oxidase, subunit I	0.0	6.6568e-104	Cytochrome o ubiquinol oxidase, subunit I	COG0843 (30 proteins), heme/copper-type cytochrome/quinol oxidases, subunit 1
COX	3.D.4.5.1	XF1388	5	H^+	BO	Cytochrome o ubiquinol oxidase, subunit III	2e-77	1.1358e-44	Cytochrome o ubiquinol oxidase, subunit III	COG1845 (27 proteins), heme/copper-type cytochrome/quinol oxidase, subunit 3
COX	3.D.4.5.1	XF1390	2	H^+	BO	Cytochrome o ubiquinol oxidase, subunit II	1e-91	1.5195e-64	Cytochrome o ubiquinol oxidase, subunit II	COG1622 (33 proteins), heme/copper-type cytochrome/quinol oxidases, subunit 2

[a] See Table 2, footnote a.
[b] IISP and IVSP, type II and type IV general secretory pathway, respectively; DNA-T, DNA transformation; FPE, fimbrillin/protein exporter; NDH, NADH dehydrogenase I; QCR, quinol:cytochrome c reductase; COX, cytochrome oxidase; ssDNA, single-stranded DNA.

restriction of the bacterium obviates the necessity of host cell infection.

A type IV (conjugal DNA-protein transfer or VirB) secretion pathway (TC 3.A.7) is found on the large plasmid with one duplicated gene (*virB10*, XF2049) in the main chromosome. The type IV secretion pathway system is a multisubunit protein complex that spans the two membranes of the gram-negative bacterial cell envelope and exports DNA-protein complexes out of the cell and into the cytoplasm of a recipient cell, which can be a bacterial, yeast, or plant cell. The VirB system of agrobacterial species is the typical type IV system and is specifically designed to transfer T-DNA (transferred DNA) into plant cells (7). *X. fastidiosa* has all six protein homologs of the VirB system (VirB4 and VirB11 are ATPases, VirB6 and VirB10 form an integral membrane transport pore, and VirB7 and VirB9 form an outer membrane complex) which comprise the minimum structural and catalytic elements of the dual-membrane channel complex. In fact, VirB7 was not detected by Glimmer (a gene prediction software tool) and was not present in the original annotation of the genome (44), being recently identified by a detailed search against members of the VirB family by Marques et al. (23).

Although members of the type IV secretion family share many characteristics, not all systems contain the same sets of genes. Thus, additional members of the VirB family such as VirB3 and VirB8 are found in *X. fastidiosa*, while VirB2 and VirB5 are not readily identifiable by similarity. A thorough description of this *Xylella* conjugative system has been reported by Marques et al. (23). Given the conservation between the genes involved in the conjugation process and in the processes of pathogen-host DNA transfer mediated by the VirB system, it is possible that a type IV pathway has a role in *X. fastidiosa* pathogenesis (23).

X. fastidiosa has a bacterial competence-related DNA transformation transporter (TC 3.A.11), a system that is found in many gram-negative as well as gram-positive bacteria and confers natural competence, i.e., the bacteria are able to take up DNA under normal physiological conditions. DNA binds to the cell surface via a type IV pilus; the DNA is usually cleaved, generating double-strand breaks, and one of the two DNA strands is taken up while the other strand is degraded on the external surface of the cell (9). The best-characterized uptake system is that in *B. subtilis*, in which a DNA-binding receptor protein, ComEA, on the external surface extracts the DNA molecule from the type IV pilus and feeds it into the transmembrane channel protein, ComEC, that spans the membrane 10 times (9). Both ComEA and ComEC are required for DNA transport into the cytosol, and similar proteins were found in *X. fastidiosa* (XF0593 and XF1078, respectively). In *B. subtilis*, ComFA is an ATP-driven DNA translocase with homology to *E. coli* DNA/RNA helicases. The ComEA-ComEC-ComFA complex probably drives single-stranded DNA through the ComEC channel in a process energized by ComFA-catalyzed ATP hydrolysis (9). Again, an ATP-dependent RNA helicase protein similar to ComFA was found in *X. fastidiosa* (XF0252).

The fimbrilin/protein exporter family (TC 3.A.12) is present in two copies in the *Xylella* genome. Each of the exporters in the fimbrilin/protein exporter family consists of two members, an integral membrane protein having three putative TMSs and an ATPase localized on the cytoplasmic side of the membrane

(11). Both copies are complete in the *Xylella* genome, being similar to the pilin secretion/fimbrial assembly system PilBC of *P. aeruginosa*. These are part of the general secretory pathway (TC 3.A.5) of gram-negative bacteria.

Oxidoreduction-driven transporters. *X. fastidiosa* has a complete proton ion-translocating NADH dehydrogenase I system (Table 5), which is a multisubunit enzyme that couples electron transfer from NADH to ubiquinone with proton translocation from the negative inner to the positive outer side of the bacterial membrane (12). Fourteen units make up the complete NADH dehydrogenase I complex (TC 3.D.1), similar to the one found in *E. coli* (also called NADH:ubiquinone oxidoreductase), which extrudes H^+ (12). Three other proteins compose a complete quinol:cytochrome *c* reductase system (TC 3.D.3), for which we were unable to pinpoint the subfamily. Two of the components (cytochrome *b* and the Rieske Fe2S2 protein) are similar to the TC 3.D.3.1 *Paracoccus denitrificans* examples, while the third one (cytochrome *c*1) is similar to a TC 3.D.2.1 *Bos taurus* example. The quinol:cytochrome *c* reductase system transfers electrons from a quinol to cytochrome *c* and links this electron transfer to proton extrusion (4). The three subunits of a proton-translocating cytochrome oxidase system (TC 3.D.4) are also present. This multisubunit enzyme reduces O_2 to water and concomitantly pumps four protons across the membrane (24).

The three oxidoreduction-driven transport systems described above provide *X. fastidiosa* with a complete respiratory chain that generates an electrochemical proton motive force able to drive ATP synthesis through the F-ATPase or ATP synthase (TC 3.A.2.1.1) complex.

Phosphotransferase System

The phosphotransferase system (PTS) has been described in several bacteria and is used for import of carbohydrates into the cell (36, 37). Sugar molecules are phosphorylated upon transport to keep them inside the cell. The system involves two auxiliary enzymes, which interact with phosphoenolpyruvate, from which they acquire both the energy for transport and the phosphate group that will be transferred to the imported substrate. These auxiliary enzymes have to interact with the permease component, located in the membrane.

The availability of such a system for sugar transport in *X. fastidiosa* remains a mystery. On one hand, both auxiliary enzymes the PTS enzyme I (8.A.7) and the PTS HPr protein (8.A.8) are present in a cluster (XF1402 and XF1403) (Table 6). On the other hand, no permeases have been found. The best candidate in the entire genome is neighboring ORF XF1404, which is weakly similar (e-value, 7e-05; 22% identity, 44% similarity, and 37% coverage) to a mannose permease IIAB component (P08186) of one of the *E. coli* PTSs, the mannose (glucose, glucosamine, and fructose) porter (TC 4.A.6.1.1). However, the other subunits were not found, especially PTS IIC, which is presumed to be the sugar-transporting channel component, and it is doubtful whether just one subunit could make a functioning permease.

It might be that a new subfamily of permeases, not previously described, exists in *X. fastidiosa*. It is noteworthy that *Treponema pallidum, Chlamydia trachomatis*, and *Neisseria meningitidis* retain only a few of the PTS genes and lack a typical PTS permease channel component; the system could play a solely regulatory role in sugar and nitrogen metabolic pathways (41). In fact, the PTS has been shown to participate in multiple physiological control processes that regulate sugar metabolism in bacteria (37, 40, 43, 45). In *E. coli* and many other gram-negative bacteria, regulation is mediated by HPr and enzyme IIA^{Glc} (40) and by the PTS pathway of the Ntr type comprised of enzyme I(Ntr) (35) and enzyme IIANtr (5, 33). *X. fastidiosa* possesses neither $EIIA^{Glc}$ nor EIIANtr; in fact, among the 22 fully sequenced bacteria having an HPr-related protein, *X. fastidiosa* and *Ureaplasma urealyticum* are the only ones that do not possess EIIANtr. Instead, we found that *X. fastidiosa* has a mannose-specific enzyme IIA component (TC 4.A.6.1.1), and it should be of interest to study the eventual role of this PTS component in sugar metabolism control in *X. fastidiosa*.

Yet another neighboring protein, XF1406, is an HPr kinase/phosphatase, known to phosphorylate and dephosphorylate Ser-45 of the phosphoryl carrier HPr protein, which again suggests that a functional, not yet completely identified sugar phosphotransferase system might be present in *X. fastidiosa*.

Auxiliary Proteins

The majority of the auxiliary proteins listed in Table 6 were already mentioned earlier in connection with the system that they support. The only exception is a β-subunit of voltage-gated ion channels (TC 8.A.5), which conforms to the existence of a protein (TC 1.A.1) in *X. fastidiosa*, a potassium channel. Potassium channels in this family are formed by four identical α-subunits, and in some cases also four oxidoreductase β-subunits that co assemble with the tetramer and remain tightly adherent to it. The *X. fastidiosa* voltage-gated ion channel protein is similar to KscA, a *Streptomyces lividans* K^+ channel. The three-dimensional structure of KscA has been solved, and the protein appears as a four-unit tetramer only, without β-subunits (8). Rat K^+ channel structure has recently been resolved, and an α_4-β_4 complex was determined (14), but the biological function of the oxidoreductase β-subunits of K^+ channels remains unsolved. It is not clear at this point what would be the mechanism of interaction and the function of the *X. fastidiosa* channel β-subunit and its voltage-gated ion channel protein.

Unknown and Uncharacterized Transporters

Eighteen transporters are similar to examples from the TC 9.A (unknown transporters) and 9.B (uncharacterized transporters) families (Table 7). We would like to mention two particular cases here. One is the pair XF1400-XF1401, both of which partially match an example in the MgtE family (TC 9.A.19). The matches occur in different parts of the example, and together the two ORFs, which are in two different frames in the genome, almost entirely cover the MgtE sequence. It may be the case either that in *X. fastidiosa* the function of this transporter is performed by two proteins or that the *mgtE* gene is inactive in *X. fastidiosa* because of a frameshift truncation.

The other case is the MarC homolog. In both *E. coli* and *S. enterica* serovar Typhimurium, this gene is encoded in a tran-

TABLE 6. PTS and auxiliary transport proteins[a]

Family and transporter[b]	TC no.	ORF	No. of TMSs	Putative substrate	Occurrence	Comments	E value	PRSS *P* value	*Xylella* annotation	COG
PTS										
Man	4.A.6.1.1	XF1404	0	Mannose		PTS family mannose porter, IIA component; Blast with filter E value, 4e-04	7e-05	3.8004e-08	Hypothetical protein	COG2893 (17 proteins), PTS, mannose/fructose-specific component IIA
Auxiliary proteins										
MFP	8.A.1.2.1	XF0244	2	Heavy metals	B	Function with RND porters	4e-09	5.3194e-09	Membrane fusion protein	COG0845 (140 proteins), membrane fusion protein
MFP	8.A.1.2.1	XF2093	1	Heavy metals	B	Function with RND porters	7e-13	3.843e-11	Precursor of drug resistance protein	COG0845 (140 proteins), membrane fusion protein
MFP	8.A.1.2.1	XF2384	0	Heavy metals	B	Function with RND porters	1e-13	3.1813e-11	Membrane fusion protein precursor	COG0845 (140 proteins), membrane fusion protein
MFP	8.A.1.3.1	XF1216	1	Protein, peptides	B	Function with ABC porters	3e-15	2.3085e-10	Colicin V secretion protein	COG0845 (140 proteins), membrane fusion protein
MFP	8.A.1.3.1	XF2398	1	Proteins, peptides	B	Function with ABC porters	2e-98	1.4915e-61	Hemolysin secretion protein D	COG0845 (140 proteins), membrane fusion protein
MPA1−C or MPA1+C	8.A.3.2.1	XF2301	0	Capsular polysaccharide	B+	CapB component of a PST-type capsular polysaccharide export system; Blast with filter E value, 0.008	3e-05	9.0861e-11	Polysaccharide export protein	COG0489 (55 proteins), ATPases involved in chromosome partitioning
MPA1−C or MPA1+C	8.A.3.3.1	XF2369	2	Protein	B−	Tyrosine protein kinase	4e-11	1.9834e-09	GumC protein	COG3206 (17 proteins), uncharacterized protein involved in exopolysaccharide biosynthesis
VICb	8.A.5.1.1	XF0367	1	K^+	E	Voltage-gated K^+ channel beta subunit	3e-71	1.2276e-62	Voltage-gated potassium channel beta subunit	COGO667 (94 proteins), predicted oxidoreductases (related to aryl-alcohol dehydrogenases)
Enzyme I	8.A.7.1.1	XF1402	1	Sugar	B	Enzyme I of PTS	3e-89	7.6091e-64	PTS enzyme I	COG1080 (31 proteins), phosphoenolpyruvate-protein kinase (PTS enzyme I component in bacteria)
HPr	8.A.8.1.1	XF1403	0	Sugar	B	HPr enzyme of PTS	3e-11	1.1827e-11	PTS HPr enzyme	COG1925 (45 proteins), PTS HPr-related proteins

[a] See Table 2, footnote *a*.
[b] MFP, membrane fusion protein; MPA1, membrane-periplasmic auxiliary-1 protein; VIC, voltage-gated ion channel.

TABLE 7. *Xylella* transporters of unknown classification, uncharacterized transporters, and hypothetical and undefined transporters[a]

Family and transporter	TC no.	ORF	No. of TMSs	Putative substrate	Occurrence	Comments	E value	PRSS *P* value	*Xylella* annotation	COG
Unknown transporters										
PST	9.A.1.3.1	XF2362	9	EPS	B	Probable O-antigen flippase	6e-27	6.7493e-17	GumJ protein	COG2244 (55 proteins), membrane protein involved in export of O-antigen and teichoic acid
Oxa1	9.A.5.3.1	XF2780	4	Protein	BO	60-kDa inner membrane protein	5e-92	7.9937e-73	60-kDa inner membrane protein	COG0706 (40 proteins), preprotein translocase subunit YidC
FeoB	9.A.8.1.1	XF0933	9	Fe^{2+}	BA	Ferrous iron uptake system	3e-86	1.4851e-62	Ferrous iron transport protein B	COG0370 (21 proteins), ferrous ion uptake system protein FeoB (predicted GTPase)
SDT	9.A.16.1	XF1450	5	DNA	B	Septum DNA translocation pore protein	e-127	1.0734e-79	Cell division protein	COG1674 (49 proteins), DNA segregation ATPase FtsK/SpoIIIE and related proteins
MIT	9.A.17.1.1	XF0900	1	Divalent metal	BAE	Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, and Fe^{2+} transport	1e-10	2.1713e-1	Magnesium and cobalt transport protein	COG0598 (47 proteins), Mg^{2+} and Co^{2} transporters
MgtE	9.A.19.1.1	XF1400	1	Mg^{2+}	B−	Frameshift truncates ORF XF1401, which contains 5′ end of Mg^{2+} transporter; XF1400 contains its 3′ end at different frame	3e-04	5.9021e-07	Hypothetical protein	COG2239 (27 proteins), Mg/Co/Ni transporter MgtE (contains CBS domain)
MgtE	9.A.19.1.1	XF1401	1	Mg^{2+}	B−	Frameshift truncates this ORF; XF1400 contains 3′ end of Mg^{2+} transporter	1e-16	1.9612e-14	Mg^{2+} transporter	COG2239 (27 proteins), Mg/Co/Ni transporter MgtE (contains CBS domain)
Uncharacterized transporter families										
MPE	9.B.3.1.1	XF0796	8	Murein precursor	B	Similar to cell division protein FtsW of *E. coli*	6e-82	1.1124e-43	Cell division protein	COG0772 (58 proteins), bacterial cell division membrane protein
MPE	9.B.3.1.2	XF1313	9	Murein precursor	B	Similar to rod shape-determining protein RodA of *E. coli*	1e-94	7.6299e-49	Rod shape-determining protein	COG0772 (58 proteins), bacterial cell division membrane protein
PET	9.B.4.2.1	XF1978	11	?		Putative protein; Blast with filter E value, 0.045	2e-05	3.5186e-08	Conserved hypothetical protein	COG1289 (30 proteins), uncharacterized membrane protein
MarC	9.B.10.1.1	XF2666	6	Antibiotic	BA	Multiple antibiotic resistance locus (*mar*) of *E. coli*	2e-15	3.9824e-11	Conserved hypothetical protein	COG2095 (41 proteins) integral membrane proteins of the MarC family
HEP	9.B.14.1.1	XF2460	11	Heme?		Cytochrome *c* biogenesis protein	e-165	9.1498e-73	*c*-type cytochrome biogenesis membrane protein	COG1138 (17 proteins), cytochrome *c* biogenesis factor
FAT	9.B.17.1.4	XF0287	2	Fatty acid?		Long-chain fatty acyl CoA synthase (ligase)	e-177	1.3668e-126	Regulator of pathogenicity factors	COG0318 (165 proteins), Acyl-CoA synthetases (AMP-forming)/AMP-acid ligases II
SSTP	9.B.18.1.1	XF0224	1	Protein	B	YajC subunit	6e-17	4.8984e-21	Preprotein translocase YajC subunit	COG1862 (27 proteins), preprotein translocase subunit YajC
PerM	9.B.22.1.1	XF0589	8	?	BA	Similar to putative permease PerM of *E. coli*	7e-27	9.307e-15	Permease	COG0628 (71 proteins), predicted permease
PerM	9.B.22.1.2	XF0281	8	?	BA	Similar to putative permease YhhT of *E. coli*	6e-22	1.6155e-12	Transport protein	COG0628 (71 proteins), predicted permease
YebN	9.B.29.1.1	XF2257	5	?	BA	Similar to YebN protein of *E. coli*	4e-43	2.0897e-33	Conserved hypothetical protein	COG1971 (11 proteins), predicted membrane protein
Hly III	9.B.30.1.1	XF0175	6	?	BE	Similar to HlyIII of *Bacillus cereus*	1e-55	1.1243e-28	Hemolysin III protein	COG1272 (23 proteins), predicted membrane proteins, hemolysin III homologs

Hypothetical proteins with ≥4 TMSs					
Hypothetical	H	XF0929	11	Conserved hypothetical protein	COG1807 (17 proteins), uncharacterized protein affecting LPS biosynthesis
Hypothetical	H	XF0250	10	Conserved hypothetical protein	COG0697 (215 proteins), permeases of the drug/metabolite transporter superfamily
Hypothetical	H	XF1359	10	Conserved hypothetical protein	COG1612 (17 proteins), uncharacterized protein required for cytochrome oxidase assembly
Hypothetical	H	XF1641	10	Conserved hypothetical protein	2 domains: 1, XF1641_1, COG0392 (33 proteins), predicted integral membrane protein; 2, XF1641_2, COG2898 (9 proteins), uncharacterized BCR
Hypothetical	H	XF1814	10	Hypothetical protein	No COG
Hypothetical	H	XF1995	10	Conserved hypothetical protein	COG3307 (22 proteins), lipid A core–O-antigen ligase and related enzymes
Hypothetical	H	XF0277	9	Conserved hypothetical protein	2 domains: 1, XF0277_1, COG0586 (57 proteins), uncharacterized membrane-associated protein; 2, XF0277_2, COG0671 (74 proteins), membrane-associated phospholipid phosphatase
Hypothetical	H	XF0986	8	Hypothetical protein	2 domains: 1, XF0986_1, no COG; 2, XF0986_2, COG2199 (234 proteins), diguanylate cyclase/ phosphodiesterase domain 1
Hypothetical	H	XF1514	8	Conserved hypothetical protein	COG0679 (30 proteins), predicted permeases
Hypothetical	H	XF0561	7	Hypothetical protein	No COG
Hypothetical	H	XF0614	7	Hypothetical protein	No COG
Hypothetical	H	XF1054	7	Conserved hypothetical protein	COG0705 (44 proteins), uncharacterized membrane protein (homolog of Drosophila rhomboid)
Hypothetical	H	XF0072	6	Conserved hypothetical protein	COG0755 (36 proteins), ABC-type transport system involved in cytochrome c biogenesis, permease component
Hypothetical	H	XF0139	6	Conserved hypothetical protein	COG0795 (46 proteins), predicted permeases
Hypothetical	H	XF0142	6	Conserved hypothetical protein	COG1835 (49 proteins), predicted acyltransferases
Hypothetical	H	XF0171	6	Conserved hypothetical protein	COG0755 (36 proteins), ABC-type transport system involved in cytochrome c biogenesis, permease component
Hypothetical	H	XF1304	6	Conserved hypothetical protein	COG3163 (5 proteins), uncharacterized ACR
Hypothetical	H	XF2760	6	Conserved hypothetical protein	No COG
Hypothetical	H	XF0016	5	Hypothetical protein	No COG
Hypothetical	H	XF0180	5	Hypothetical protein	No COG
Hypothetical	H	XF0374	5	Hypothetical protein	No COG
Hypothetical	H	XF0856	5	Conserved hypothetical protein	COG1238 (19 proteins), uncharacterized membrane protein

Continued on following page

TABLE 7—*Continued*

Hypothetical	H	XF1076	5	Conserved hypothetical protein	COG0577 (104 proteins), ABC-type transport systems, involved in lipoprotein release, permease components
Hypothetical	H	XF1099	5	Hypothetical protein	No COG
Hypothetical	H	XF1303	5	Conserved hypothetical protein	COG0767 (32 proteins), ABC-type toluene export system, permease component
Hypothetical	H	XF1587	5	Hypothetical protein	No COG
Hypothetical	H	XF1601	5	Hypothetical protein	No COG
Hypothetical	H	XF2175	5	Hypothetical protein	No COG
Hypothetical	H	XF2186	5	Conserved hypothetical protein	COG0705 (44 proteins), uncharacterized membrane protein (homolog of Drosophila rhomboid)
Hypothetical	H	XF2573	5	Conserved hypothetical protein	COG0697 (215 proteins), permeases of the drug/metabolite transporter superfamily
Hypothetical	H	XF2574	5	Conserved hypothetical protein	COG0671 (74 proteins), membrane-associated phospholipid phosphatase
Hypothetical	H	XF2762	5	Hypothetical protein	No COG
Hypothetical	H	XF0140	4	Conserved hypothetical protein	COG0795 (46 proteins), predicted permeases
Hypothetical	H	XF0383	4	Hypothetical protein	No COG
Hypothetical	H	XF0666	4	Hypothetical protein	No COG
Hypothetical	H	XF0757	4	Conserved hypothetical protein	2 domains: 1, XF0757_1, COG0062 (29 proteins), uncharacterized ACR; 2, XF0757_2, COG0063 (32 proteins), predicted sugar kinase
Hypothetical	H	XF0766	4	Conserved hypothetical protein	COG2391 (29 proteins), predicted transporter components
Hypothetical	H	XF0772	4	Hypothetical protein	No COG
Hypothetical	H	XF0947	4	Hypothetical protein	No COG
Hypothetical	H	XF1025	4	Hypothetical protein	No COG
Hypothetical	H	XF1245	4	Hypothetical protein	No COG
Hypothetical	H	XF1454	4	Conserved hypothetical protein	COG0239 (32 proteins), integral membrane protein possibly involved in chromosome condensation
Hypothetical	H	XF1692	4	Hypothetical protein	No COG
Hypothetical	H	XF2001	4	Hypothetical protein	No COG
Hypothetical	H	XF2414	4	Hypothetical protein	No COG
Proteins with undefined functions and ≥4 TMSs					
Undefined	U	XF2366	11	GumE protein	No COG
Undefined	U	XF2420	10	Virulence factor	COG0728 (28 proteins), uncharacterized membrane protein, putative virulence factor
Undefined	U	XF0406	8	Export protein	COG0861 (42 proteins), membrane protein TerC, possibly involved in tellurium resistance
Undefined	U	XF1192	8	Integral membrane protein	COG2899 (5 proteins), uncharacterized BCR
Undefined	U	XF0651	7	Transport protein	COG2311 (14 proteins), uncharacterized membrane protein

Undefined	U	XF0764	5	Membrane protein	COG0730 (93 proteins), predicted permeases
Undefined	U	XF1979	5	Transmembrane protein	No COG
Undefined	U	XF0375	4	Inner membrane protein	COG2194 (33 proteins), predicted membrane-associated, metal-dependent hydrolase
Undefined	U	XF1311	4	Rod shape-determining protein	COG2891 (11 proteins), rod shape-determining protein
Undefined	U	XF2694	4	Cell division protein	COG2177 (17 proteins), cell division protein

[a] Unknown and uncharacterized transporters are those Xylella proteins found to match the corresponding transport families, as described by Saier (39). Hypothetical proteins (H) refer to Xylella proteins that have four or more TMSs, did not match the transport families database, and, in addition, either did not match any other GenBank protein sequence or had a match with GenBank proteins that were classified as hypothetical and were not functionally characterized. Undefined Xylella proteins (U) have four or more TMSs and again do not match the transport families database; however they are similar to proteins of other organisms that are given a defined name but have undefined or poorly characterized function. See Table 2, footnote a.

scriptional unit at one side of the *marO* operator. On the other side, a divergently positioned transcriptional unit encoding the *marRAB* operon is found. None of the other *mar* genes were found in *X. fastidiosa*.

In addition to the 18 proteins described above, which are classified in Saier's transport protein database, we have identified another 55 proteins that do not match any example in the database and have four or more TMSs (Table 7). They were classified either as hypothetical transport proteins (when they did not match any other GenBank protein or matched proteins classified as hypothetical) or as undefined (when they matched GenBank proteins with defined names but not well-defined function) (Table 7). Many of these *Xylella* proteins are classified in the COG database (46), and upon inspection of the assignments, it can be seen that a good number of them are reported as predicted permeases or as uncharacterized membrane-associated proteins (Table 7). We suggest that these proteins are good candidates for exploratory experimentation on the ability of solutes to move across the *Xylella* membrane.

CONCLUSIONS

The detailed analysis of transport systems described here uncovered important characteristics of *X. fastidiosa*, including hints that can lead to control of the disease caused by this bacterium on citrus trees. Our present work confirmed for the most part the observations made in the sequencing paper (44), but also pointed out a few places where an alternative interpretation of the data is possible, as indicated below. The comparison with other bacterial genomes, some of them available only recently, suggested correlations between the absence of certain transport systems and the life cycle of the parasite.

The sequencing paper (44) mentions a total of 140 proteins involved in transport (4.8% of all ORFs); the present analysis has classified 69 new transporters and suggests that in *X. fastidiosa* there are at least 209 transport proteins, making up 7.4% of all ORFs. In addition, more transporters could eventually be found by experimentation focused on the 55 *Xylella* proteins with four or more TMSs that do not match any known transport protein example. Considering the major transporters computed by Paulsen et al. (31), *X. fastidiosa* has only 2.7% of all ORFs identifiable as major transporters, which makes it the eubacterium with the lowest percentage of ORFs involved in transport, closer to two archaea, *M. jannaschii* (2.4%) and *M. thermoautotrophicum* (2.4%) (31). Also, *X. fastidiosa* exhibits a predominance of secondary transporters over primary ones, following an apparently general tendency of aerobic prokaryotes.

Regarding ways of controlling the disease, one possibility is a vaccine. Protein XF0343 (TC 1.B.6) has long, flexible loops exposed on the exterior of the cell and is a good antigen candidate. Other possibilities should arise from the answer to the question of which compounds of the xylem sap are imported by *X. fastidiosa*. Previous analysis (44) suggested that glycerol, certain amino acids, and possibly cellulose should be among the compounds imported. The present study adds ATP and inorganic carbon to the list of possible imported compounds. It is interesting that the bacterium apparently depends strictly on carbohydrates, both as an energy source and as anabolic precursors.

The presence of an ATP/ADP translocator is especially intriguing, since the only other organisms in which it has been found are obligate intracellular parasites. Could it be that *X. fastidiosa* infects plant cells as well as the xylem?

The lack of a P-type ATPase is also surprising. Only two other completely sequenced eubacteria lack this cation transport system, and they have in common with *X. fastidiosa* the fact that all are parasites transmitted by insects. It would be interesting to construct a genetically modified *X. fastidiosa* with, say, a potassium uptake P-type ATPase from *E. coli* and observe the effects on growth speed and interaction with the insect host.

We confirmed by renewed searches the puzzling fact that *X. fastidiosa* has all the apparatus of a PTS except permease components IIB and IIC. If the system is functional for sugar transport, these proteins cannot be missing. The most plausible explanation seems to be that a new family of permeases, not yet characterized as such, is to be found among the hypothetical proteins. Alternatively, PTS might play a solely regulatory role in sugar metabolism.

Finally, our analysis suggests an alternative functional assignment for the *ttg2* cluster of genes, found both in *X. fastidiosa* and in *P. putida*, in which it was proposed to be either a toluene exporter or a transporter indirectly involved in toluene resistance (19). The presence of a receptor in the *X. fastidiosa* ABC system implies that it is an import rather than an export system. Based on the similarity of its cytoplasmic component, we tentatively placed it in the PAAT family as a glutamate importer. The fact that its knockout impaired *P. putida*'s intrinsic resistance to toluene may be due to impaired synthesis of cell wall components (19) or to the lack of the extra energy supply that would be available from glutamate.

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