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XENOBIOTIC EFFLUX IN BACTERIA AND FUNGI: A GENOMICS UPDATE

Ravi D. Barabote,

Department of Plant Sciences, University of California, Davis, California

Jose Thekkiniath,

Department of Biological Sciences, Texas Tech University, Lubbock, Texas

Richard E. Strauss,

Department of Biological Sciences, Texas Tech University, Lubbock, Texas

Govindsamy Vedyappan,

Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, Texas

Joe A. Fralick, and

Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, Texas

Michael J. San Francisco

Department of Biological Sciences, Texas Tech University, and Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, Texas

I. Introduction

“...not enough to kill the streptococci but enough to educate them to resist penicillin” (Alexander Fleming, Nobel Prize lecture, Dec. 11, 1945). These prophetic words underscore the arms race in which we find ourselves today. Large populations and mutable genomes give microbes a profound capacity to respond to changing environmental conditions. The misuse of antibiotics in human health and agriculture has contributed to continuing microbial drug resistance. Thus, 65 years later, in 2010, we continue to battle microorganisms and strive to design novel and useful antimicrobial agents (1).

II. Bacterial Efflux Pumps

A. TYPES AND SUBSTRATES

The efflux of antibiotics was discovered in 1980 by Steward Levy and co-workers, who were studying the mechanism of tetracycline resistance in *Escherichia coli* (2). Since that time it has been demonstrated that a single efflux pump can provide resistance to multiple antibiotics (MDR efflux pumps). It has also been found that MDR-like transporters are highly abundant and ubiquitous in nature and represent, on average, more than 10% of the total number of transporters per organism (3).

Although MDR efflux pumps play an important role in the inherent resistance of bacteria to antibiotics, these pumps appear to be evolutionarily ancient transporters that have a wide variety of physiological functions, beyond antibiotic resistance, which contributes to adaptation to a wide variety of environments (4–6).

Phylogenetically, bacterial antibiotic efflux pumps belong to one of five families (Figure 1): (1) SMR or small multidrug resistance subfamily of the DNT (drug/metabolite transporters) superfamily (3, 7); (2) MATE (multi-antimicrobial toxic compound extrusion) subfamily of the MOP (multidrug/oligosaccharidyl-lipid/polysaccharide flippases) superfamily (8, 9); (3) MFS (major facilitator superfamily) (10); (4) RND (resistance–nodulation–division) superfamily (11); and (5) ABC (ATP-binding cassette) superfamily (12). MDR transporters can also be classified into two main groups based on the mode of energy coupling for transport/efflux: (1) primary active transporters that belong to the ABC superfamily and utilize ATP hydrolysis to expel the drug from the cell, and (2) secondary transporters that utilize the proton-motive force or ion gradient for drug expulsion. SMR, MATE, MFS, and RND pumps are secondary transporters or antiporters. Other classifications have been proposed (13, 14), and several reviews have been dedicated to the classification and descriptions of MDR transporter families (15–22).

1. ABC Pumps—ABC (ATP-binding cassette) transporters are found in all living organisms and are classified as primary active transporters that belong to the ABC superfamily and utilize the free energy of ATP hydrolysis to expel the drug from the cell. Historically, the first MDR transporters characterized were members of the ABC superfamily of eukaryotic origin such as P-glycoprotein (23–25). Since that time, ABC pumps are also found in pathogenic fungi and parasitic protozoa, where they impart resistance to antimicrobial drugs (26). The first bacterial ABC MDR pump (LmrA, *Lactococcus lactis*) was reported in 1996 (27). ABC pumps have been found to be widespread among bacteria (14) and appear to play an important role in drug resistance in some pathogenic bacteria, such as *Enterococcus faecalis* and *Streptococcus pneumoniae* (28) (Table 1). Many of these MDR efflux pumps are homologs of the heterodimeric LmrCD pump of *L. lactis*.

ABC transporters have structural characteristics that set them apart from other efflux pumps. They usually have two similar halves, each containing two parts: a transmembrane domain (TMD) that is usually arranged into six transmembrane-spanning α -helices, and a nucleotide-binding domain (NBD), also known as the ATP-binding cassette domain, containing the Walker A, Walker B, and ABC signature motifs (29). The NBDs are responsible for the binding and hydrolysis of ATP and hence generation of the energy of the translocation of the substrate, while the TMDs form the translocation pathway for the transported substrates to cross the cytoplasmic membrane. In most cases a single protein contains a TMD–NBD–TMD–NBD structure (24). However, in bacterial transporters, a TMD fused to a NBD forms a half-transporter, which homo- or heterodimerizes with another half-transporter to form a functional full-size transporter (30): for example, the homodimeric LmrA or heterodimeric LmrCD ABC pump of *L. lactis*. There are exceptions, however; DrrA and DrrB found in *Streptomyces peucetius* contain a single NBD or TMD domain, respectively, and are each thought to function as a tetramer (31).

The generally accepted mechanism by which ABC transporters function is often explained in terms of a two-cylinder engine mechanism (30, 32). In this model, drugs enter a high-affinity site in the TMDs of the ABC transporter from the cytosol side of the cytoplasmic membrane and then, upon the binding/hydrolysis of ATP (at the NBDs to provide the power stroke) and through conformational cycling, the substrate is occluded at the high-affinity site and progresses to a low-affinity release site on the extracellular side of the membrane (Figure 2). As depicted in Figure 1, in some gram-negative bacteria, ABC MDR efflux pumps have periplasmic accessory or membrane fusion proteins (MFPs). These MFPs interact with outer membrane channel or efflux proteins (OEPs) to form a tripartite pump that bridges both membranes of the gram-negative envelope to mediate the extrusion of substrates from the cell, such as the MacAB–TolC ABC MDR pump. MacA is a periplasmic protein of the MFP family, TolC is an outer membrane channel protein, and MacB is a half-type ABC transporter with four putative TMD segments and an N-terminal NBD (34, 35). Table 1 lists examples of ABC MDR pumps found in bacteria and their substrates.

2. SMR Pumps—SMR (small multidrug resistance)-mediated multiple drug resistance is widespread among bacteria (16). These bacterial MDR efflux pumps are among the smallest known pumps and are made up of proteins that are typically 100 to 115 amino acid residues in length. SMR pumps have a four-transmembrane α -helical topology (7) and a highly conserved residue, Glu14 (49, 50), that has been shown to be essential and involved directly in drug and proton binding (51, 52). It has been assumed that these pumps function as oligomeric complexes, perhaps as dimers, and that the Glu14 of both protomers in a dimer form a shared binding pocket (51, 53). The genes encoding SMR pumps are found in a variety of plasmids from clinical isolates of *S. aureus* and other staphylococci (54–56) as well in the chromosomes of many bacteria (57–59) (Table 2). The substrates of SMR pumps typically share similar physical properties but may differ in size and shape and are almost exclusively monovalent hydrophobic cations.

The SMR pump EmrE of *E. coli* is one of the better characterized of the SMR pumps (49, 52, 60). EmrE transports a diverse array of aromatic, positively charged substrates in exchange for protons (61). A model for its translocation cycle has been suggested in which the binding of the drug to its binding site (Glu14) deprotonates the Glu14 residues in the functional antiparallel dimer and causes the transporter to undergo a conformational change in which the binding sites close behind the substrate and open in front of the substrate to expose it to the outer face of the cytoplasmic membrane (Figure 3). The release of the drug is thought to be catalyzed by protons that protonate the two Glu14's, thus coupling drug export to H⁺ import. However, the precise mechanism by which the proton-induced conformational changes bring about translocation of substrate across the membrane remains to be deciphered. Table 2 includes some examples of SMR pumps and their substrates.

3. MATE Pumps—MATE (multidrug and toxic compound extrusion) MDR pumps are a relatively new family of secondary efflux pumps (67). Early members, NorM and its *E. coli* homolog YdhG, were originally classified as MFS pumps because they possessed 12 putative transmembrane domains (68). However, it was later discovered that there was little sequence homology of these pumps with other MDR efflux pumps, and they were

reclassified as members of a new family of MDR pumps (MATE) (8). Since that time over 1000 members of this family, including representatives from all three kingdoms (Eukarya, Archaea, and Eubacteria) have been identified (8, 19) and placed into three large subfamilies: (1) bacterial (2) eukaryotic, and (3) bacterial and archaeobacterial transporters. MATE proteins range in size from 400 to 700 amino acid residues with 12 putative transmembrane α -helices. No apparent consensus sequence is found in all MATE sequences but they do share approximately 40% sequence similarity (67).

Bacterial MATE MDR pumps are energized by either H^+ - or Na^+ - coupled antiporters (68–70). These secondary MDR pumps can remove cationic drugs such as ethidium bromide, tetraphenylphosphonium, acriflavine, norfloxacin, and berberine, and at least one, MepA of *S. aureus*, when overexpressed, can confer resistance to tigecycline, a new glycylcycline antibiotic that is effective against methicillin resistant *S. aureus* cells (MRSA) (71). Because of their recent discovery, MATE MDR efflux pumps are the least well characterized and relatively little is known about their structure–function relationship. Most studies have focused on describing their presence and the antibiotic resistance they provide. However, with the recent crystallization of NorM from *Neisseria gonorrhoeae* (72, 73), this may change. Table 3 provides some examples of MATE MDR efflux pumps and their substrates.

4. MFS Pumps—The MFS (major facilitator superfamily) pumps are proton-dependent secondary transporters. Approximately 25% of all known membrane transport proteins in bacteria belong to this superfamily (20, 85), which contains over 50 distinct families and more than 1500 members (15, 17, 86, 87). Structurally, most MFS transporters are 400 to 600 amino acid residues in length and contain 12 or 14 TMDs (17), although there are at least two exceptions: one family has only six TMD and another 24 (15). There is also good evidence for an internal tandem gene duplication, indicating a common ancestral gene. The best characterized MDR MFS pump protein is EmrD, an efflux pump that exports amphipathic compounds, such as carbonylcyanide-*m*-chlorophenylhydrazone (CCCP), across the *E. coli* cytoplasmic membrane. Its crystal structure has been solved to 3.5-Å resolution (88). It is 394 amino acids in length and has 12 TMDs organized as a pair of six-helix domains that surrounds a hydrophobic pore. Two long loops extend into the inner leaflet of the cytoplasmic membrane that are thought to determine substrate specificity, called a *substrate specificity filter*, and which may facilitate transport (88). It has been postulated that the mechanism by which MFS transporters move substrates across a membrane is via a rocker switch mechanism alternating-access model coupled with an H^+ antiport (Figure 4). This model was originally based on the crystal structures of GlpT (89), LacY (90), and more recently, EmrD (88). Table 4 presents some examples of MFS MDR efflux pumps and their substrates.

Some multidrug-resistant MFS systems have a tripartite structure (as do some ABC and RND pumps) such as the VceABC pump of *Vibrio cholerae* (91, 92) and the EmrAB–TolC pump of *E. coli* (93, 94). These pumps are responsible for the removal of substrates across both membranes of the gram-negative bacterial envelope. These systems are comprised of an MFS transporter containing 14 TMD (EmrB, VceB), a periplasmic MFP (EmrA, VceA), and an OEP (TolC, VceC). Interestingly, unlike the tripartite AcrAB–TolC pump, where AcrB and TolC have been shown to be trimers, electron microscopy studies of reconstructed

EmrAB suggest that they exist as dimers (94). More structural studies are warranted to decipher the interactions and architecture of MDR MFS tripartite efflux pumps.

5. RND Pumps—RND superfamily transporters are found in Eukarya, Archaea, and Eubacteria and have been placed into eight phylogenetically distinct families that correlate with their substrate specificity (123). RND transporters are larger than MFS transporters and range in size from 700 to over 1300 amino acid residues in length (124). Like MFS transporters, RND transporters are predicted to adopt a 12-TMD structure, and the sequences of the first and second halves of the RND transporter are similar, suggesting that they have also arisen through gene duplication (124). However, unlike MFS transporters, RND transporters possess large periplasmic domains (34, 125, 126).

The crystal structure of the RND transporter, AcrB, has been solved (125, 127–129). It is a homotrimer, the monomer of which is 1049 amino acids in length and contains 12 transmembrane α -helices and two expansive periplasmic loops that determine substrate specificity (126, 130). Topologically, the core of AcrB is formed by a bundle of the 12 transmembrane α -helices, two of which (TM4 and TM10) extend approximately 70Å into the periplasm, forming a distal TolC docking domain and a porter/pore domain, the latter being closest to the plane of the outer leaflet of the cytoplasmic membrane. In the center of the trimer, the TolC docking domain produces a funnel-shaped structure with a narrow diameter that leads to a central pore that is located in the porter domain. It is this domain with which AcrB docks to TolC. The central pore leads to a central cavity approximately 35Å in width. Three vestibules located near the cytoplasmic membrane have been implicated as entrances by which substrates may gain access to the central cavity (129).

The initial structural studies were conducted on crystals with threefold symmetry. However, recently, reports describing an asymmetric AcrB trimer have been published (131, 132). The asymmetric structure reveals three different monomer conformations, presumably representing three consecutive states in the transport cycle and suggest a model for drug transport based on conformational cycling of the monomers by the RND pump (131–134). The three different monomer conformations are designated as loose (L), tight (T), and open (O) (131, 132, 135, 136). In this model, conformational changes from loose to tight to open and then back to loose enable the substrate access to a tunnel through which substrates are translocated to the outside via the TolC channel. The mechanism by which this is accomplished is based on occlusion migrating from the entrance toward the central tunnel similar to that of a peristaltic pump. The energy for the conformational cycling is envisioned to be provided by electronmotive force (emf) to this transporter [see the article by Pos (135) for an excellent review of this mechanism].

6. Tripartite Pumps—RND, MFS, and ABC transporters can form tripartite pumps in gram-negative bacteria. The components of these tripartite MDR pumps, as described previously, are periplasmic MFPs, outer membrane OEPs, and the respective transporter protein (RND/MFS/ABC). All three components are essential for their function. The composite structure spans the cytoplasmic membrane, the periplasmic space, and the outer membrane allowing for the removal of the substrate from the cytosol/cytoplasmic membrane to the outside of the cell envelope (Figure 5). This provides a huge advantage for the gram-

negative bacterial cell because once exported, the drug must negotiate the outer membrane barrier to reenter the cell. Thus, as was so insightfully pointed out by Nikaido, these MDR pumps work synergistically with the outer membrane barrier (137, 138). That both the outer membrane barrier and MDR efflux pumps play an important role in the intrinsic resistance to various hydrophobic inhibitors was shown by the additive effect of deep rough (affects outer membrane permeability to hydrophobic agents) and MDR efflux (TolC) mutants (139).

The most extensively studied tripartite pumps are the MexA–MexB–OprM pump of *Pseudomonas aeruginosa* and the AcrA–AcrB–TolC pump of *E. coli*, both of which are considered to play a major role in antibiotic resistance for their respective bacteria. The RND transporters MexB–AcrB determine the substrate specificity of their tripartite pump, which is quite large compared to other MDR pumps and combined include bile salts, organic solvents, dyes, and compounds that are anionic, cationic, zwitterionic, and a broad range of different antibiotics (133) (Table 5).

i. MFPS: Membrane fusion proteins are associated with their cytoplasmic membrane as either a lipoprotein or via a TMD near the N-terminal, with the preponderance of the protein residing in the periplasm (181). Partial crystal structures of membrane fusion proteins MexA (140), AcrA (141), and MacA (142) are available (i.e., missing their extreme N- and C-terminal regions). The structures of AcrA and MexA share a significant degree of sequence and structure similarity. Both are elongated, sickle-shaped molecules composed of three domains: a β -barrel domain, a centrally located lipoyl domain, and a coiled-coil α -helical hairpin at the other end of the molecule. Chemical cross-linkage and mutagenesis studies have shown that the α -helical coiled-coil hairpin of AcrA–MexA docks with the coiled coils of the OEP (TolC–OprM) (143, 144). The β -barrel domain is the probable site of interaction with the transporter protein (AcrB–MexB). The stoichiometry and oligomeric state of the assembled MFPS are unknown [see the article by Zgurskaya et al. (145) for an excellent review on this topic].

ii. OEPs: The architecture of the OEPs whose crystal structures have been solved (*E. coli* TolC, *P. aeruginosa* OprM, and *Vibrio cholerae* VceC) are remarkably similar, even though their amino acid sequence identity or similarity is quite low (146–148). In each case the homotrimers of these proteins make up a long cannon-shaped structure consisting of a 40 Å long β -barrel, which passes through the outer membrane and a 100 Å long α -helical barrel which projects into the periplasm, which is closed at its periplasmic end (146). Based on this structure and the crystal structures of AcrB (129) and AcrA (141) and with the evidence that TolC could be cross-linked independently to either AcrA or AcrB (149–152), models have been proposed which attempt to explain the assembly and function of MDR pumps (143, 152–158). In such models, the periplasmic ends of a trimeric AcrB and a trimeric TolC are envisioned to dock in such a manner as to form a continuous channel that crosses the periplasm and spans the outer membrane. The periplasmic contact between AcrB and TolC has been suggested to involve the TolC entrance coils and the apex (TolC docking domain) of AcrB (129, 154). In these models this connection is bridged and stabilized by the MFP, which is anchored to the cytoplasmic membrane and may play a role in the recruitment of TolC to the AcrB antiporter (159). During assembly of the MDR pump, the periplasmic end

of the OEP must open in order for the pump to function. This transition to the open state has been likened to an “irislike” realignment of the entrance helices (146, 160). This opening of TolC is thought to occur through conformational changes in TolC via its interaction(s) with either AcrB or AcrA or both (146). However, the details by which tripartite pumps are assembled is only beginning to be deciphered and is currently an active area of investigation [see the literature (138, 155) for excellent reviews on this topic].

There are several different mechanisms by which an organism can become resistant to antimicrobial drugs. However, resistance mediated by multidrug-resistance (MDR) efflux pumps appears to be a dominant paradigm among microbial human pathogens. Mobile genetic elements such as plasmids and transposons carrying genes encoding MDR pumps are thought to play an important role in the lateral acquisition of drug resistance by bacteria. The emergence and increasing numbers of drug-resistant pathogenic bacteria pose a great threat to human health. Therefore, it is imperative to study the origin, evolution, and organismal distribution of these xenobiotic transporters, especially in order to develop effective strategies to combat human diseases.

B. PHYLOGENY AND EVOLUTION OF BACTERIAL EFFLUX PUMPS

As noted above, one-tenth of the transporters encoded in a bacterial genome are involved in MDR efflux. The transporter classification system (14) currently recognizes approximately 650 transporter families that include about a dozen large superfamilies (<http://www.tcd.org/>). A majority of these families are associated with solute uptake, and only a few constitute exporters. Furthermore, only a half-dozen exporter families (that include four of the superfamilies) contain members that extrude xenobiotic compounds. Despite the fewer proportions of these xenobiotic-extruding transporters in organisms and their relatively high importance, these classes of exporters remain less well understood than were the uptake systems (13).

The capability to export xenobiotic drugs appears to have evolved across independent lineages of transporters. As described above, thus far, functionally characterized bacterial drug exporters and their sequence homologs identified from genome analyses belong to one of five phylogenetically distinct and ubiquitously found transporter families: the MFS superfamily, the RND superfamily, the DMT superfamily, the MATE family, and the ABC superfamily (13). It should be noted that not all families within each of these superfamilies are involved in drug efflux, and that many carry out solute uptake or export. It is likely that the drug efflux pumps function to export cellular metabolites and other molecules, and perhaps simple modifications in their protein sequence can confer additional capabilities to export either specific or multiple xenobiotics.

The MFS superfamily comprises of approximately 65 families of transporters, of which only six contain characterized drug efflux pumps (Table 6). Similarly, only eight families within the ABC, two families within the RND, and only one family in the DMT superfamily (i.e., the SMR family) function in drug efflux in bacteria (Table 6).

III. Genomics of Bacterial Efflux Pumps

Since the first complete genome sequence of *Haemophilus influenzae* that was completed by the Institute for Genomic Research (TIGR), hundreds of bacterial genomes have now been sequenced. Data from these genome sequences are constantly revealing many new uncharacterized transport proteins. In comparison to the few multidrug efflux transporters that have been characterized functionally, the genome-sequencing efforts have identified numerous putative xenobiotic efflux pumps. Previous analyses of complete genomes indicate that approximately 10% of the transporters encoded in bacterial genomes are involved in multidrug efflux (3).

Comparative genomic analyses offer a comprehensive overview of the distribution of transporters across a wide group of organisms. Such analyses offer distinct advantages that can help answer many biological questions. For example:

1. Are xenobiotic efflux pumps encoded in all bacterial genomes or do they occur only in certain species?
2. Is the distribution of the efflux types uniform or species specific?
3. Did specific efflux transporters arise specifically in response to the use of drugs?

In this section we focus on answering some of these questions.

A. METHODOLOGY

Sequences of all the proteins predicted in the complete genome sequences (which include chromosomes and plasmids) of 854 bacteria were downloaded onto our local computer from the National Center for Biotechnology Information (NCBI) through their ftp site (<ftp.ncbi.nih.gov>). A total of 3,092,197 proteins sequences were obtained. The Basic Local Alignment Search Tool (BLAST) was also downloaded from the NCBI ftp site. All transporter proteins present in the Transporter Classification Database [TCDB; (184)] were also downloaded onto our local machine. A total number of 5238 functionally described transport proteins were obtained from TCDB. Of these, 236 transport proteins belong in the drug efflux transporter families described in Table 6. All 3,092,197 predicted proteins were searched against the 5238 known transport proteins using the BLAST tool on our local machine. The expected value (E-value) cutoff of 10^{-6} was used for the BLAST search. This cutoff has been found from our previous studies to yield true hits and minimized false positives (185, 186). Proteins that showed one of the 236 MDR proteins in TCDB as their topmost BLAST hit were identified and inspected carefully for any false positives. A total of 30,564 putative MDR efflux transport proteins from all the five major MDR efflux transporter classes were identified from the 8454 bacterial genomes. The organismal and taxonomical distributions of these transporter proteins are described below. It must be mentioned that a few distant homologs of the MDR pumps that fall below our search threshold (i.e., false negatives) may have been missed. However, from our previous experiences, we anticipate that the numbers of such false negatives would be extremely low.

B. DISTRIBUTION OF DRUG EFFLUX PUMPS IN BACTERIAL GENOMES

The genome sizes of the bacteria analyzed ranged between 0.25 and 10 Mb, with the exception of a myxobacterium belonging to the deltaproteobacteria subdivision, *Sorangium cellulosum* So ce 56, which has a 13-Mb genome that is 71% G + C-rich and encodes 9381 predicted proteins (187). Our computational analyses identified a total of 64 MDR proteins in its genome. The smallest genome (0.25 Mb) analyzed was that of *Candidatus sulcia muelleri* GWSS, a gut symbiont of the blue-green sharpshooter and several other leafhopper species (188). The genome of this organism is only 22% G + C-rich and is predicted to encode just 227 proteins. We could not identify any xenobiotic efflux proteins in the genome of this organism, representing the only organism lacking any recognizable xenobiotic transporters. At least one MDR efflux transport protein was identified in each of the remaining 854 bacterial genomes.

Of the collection of 30,564 drug transport proteins identified in the 854 genomes, approximately 33% (10,013 proteins) belong to the ABC superfamily, 31% (9349 proteins) are MFS type, 27% (8235 proteins) RND type, 6% (1810 proteins) MATE type, and 4% (1157 proteins) are SMR type. A majority of MFS- and MATE-type pumps are generally composed of a single membrane translocator, while RND-type transporters commonly consist of two membrane-associated proteins. Although several SMR pumps are known to contain a single protein, there are many examples of SMR pumps composed of more than one protein. The drug-transporting ABC pumps may be composed of a single protein with both the membrane-spanning and ATP-hydrolyzing domains fused, or may consist of two separate proteins containing the two individual domains. Therefore, based on the adjusted calculations, we estimate that the relative abundance of the different drug efflux pumps would be MFS (41%) > ABC (29%) > RND (18%) > MATE (8%) > SMR (4%). This shows that the MFS- and ABC-type pumps are abundantly present in bacteria, while the MATE and SMR families have restricted representation. All five types were found to occur in a wide variety of bacteria and are not restricted to pathogenic bacteria, as noted above.

C. CORRELATION WITH GENOME SIZE

In general, the total number of proteins dedicated to xenobiotic efflux in the bacterial genomes correlated ($R^2 = 0.72$, $p < 0.05$) with their genome size (Figure 6). The slope of the best-fit line was 10.36 (Figure 6), indicating that approximately 10 drug transport proteins are encoded per megabase of bacteria genome. This is in good agreement with previous observations that 10% of all transport proteins in prokaryotes are involved in multidrug efflux (3). In general, a megabase of genome in bacteria encodes roughly 1000 proteins, and approximately 10 to 15% of all proteins predicted in bacterial genomes are transport proteins (189). Therefore, one would expect to find about 100 to 150 transport proteins encoded per megabase of genome, of these 10 to 15 proteins would be involved in xenobiotic efflux. In our analyses, the organism encoding the highest number of drug efflux proteins was *Burkholderia multivorans* ATCC 17616, which has a genome size of 7 Mb (190); it encodes 158 xenobiotic efflux proteins. This number is more than double the number of MDR efflux proteins expected for its genome size. *B. multivorans* is associated with infections in cystic fibrosis patients and is an important opportunistic pathogen that colonizes the lungs. It is

associated with a decrease in long-term survival of patients. A minority of patients with *B. multivorans* infection develop cepacia syndrome, which is frequently fatal (191).

Figure 6 also shows the correlation between genome size and the numbers of each of the five types of xenobiotic efflux pumps. The data show a relative abundance of MFS and ABC proteins followed by the RND homologs. The MATE and SMR types occur in substantially lower proportions in the genomes. There appears to be an expansion of MFS-type transporters with genome size, and some of the larger genomes contain abundant MFS transporters. This is likely because most MFS pumps consist of a single protein, and a single gene duplication can give rise to a functional new pump. Such an expansion is less pronounced with ABC- and RND-type pumps, probably because of the multicomponent nature of these systems. Evolution of a new functional pump would involve coordinated duplication of multiple genes, which perhaps occurs less frequently. *B. multivorans* ATCC 17616 encodes the highest number of MFS and RND proteins (66 and 48 proteins, respectively) among all bacteria compared in our study, this is reflective of the large complement of drug transporters in this opportunistic pathogen. However, *Streptomyces griseus* subsp. *griseus* NBRC 13350, which is slightly larger (8.5 Mb), encodes the largest number of ABC transport proteins (58 homologs). It is followed by seven other actinobacteria, two of which contain smaller genomes. *Corynebacterium glutamicum* ATCC 13032 with a genome size of 3.3 Mb, and *Beutenbergia cavernae* DSM 12333 with a genome size of 4.7 Mb, both encode 44 ABC proteins each. This indicates a relative expansion and likely importance of ABC family drug transporters in actinomycetes, soil-dwelling microbes that are known to produce a wide range of antibiotics. Both SMR and MATE transporters do not appear to increase in numbers linearly with genome size. Most organisms appear to encode two to four MATE-type and one or two SMR-type drug efflux transporters. *Bacillus licheniformis* ATCC 14580 encodes an expanded repertoire of 12 SMR family proteins. This organism is a soil-dwelling endospore-forming microbe that is used extensively in the industrial production of important enzymes such as proteases, penicillinases, and amylases as well as smaller compounds such as the antibiotic bacitracin and various organic metabolites. Its 4.2-Mb genome encodes 125 drug efflux transporter proteins (three times the expected number) representing all five families. The organism with the largest number of MATE family proteins is *Eubacterium eligens* ATCC 27750, a firmicute and member of the normal human gut microflora. This organism (with a 2.8-Mb genome) encodes a total of 46 drug efflux proteins that include the 23 ABC, 21 MATE, and 2 RND family proteins but no MFS or SMR pumps. Thus, expansion of the MATE transporters may compensate for the lack of MFS facilitators in this organism.

D. ORGANISMS LACKING ONE OR MORE TRANSPORTER TYPES

Although just one organism was found to lack any recognizable drug efflux pumps as mentioned above, less than half (47%) of the bacteria analyzed contain all five types of drug transport proteins (Table 7). This group of bacteria contains representatives from most of the major taxonomic subdivisions of eubacteria. Fifty-three percent of bacteria (455 genomes) were found to lack one or more of the five transporter types (Table 7). Approximately, 5% of bacteria (39 genomes) carry just one type of drug efflux transporter; many of these are intracellular pathogens and obligate symbionts. The genome sizes of these organisms ranged

from 0.42 to 1.9 Mb; all except one [*Baumannia cicadellinicola* str. Hc (*Homalodisca coagulata*)] lacked the secondary carriers (i.e., MFS, RND, SMR, and MATE porters) and contained 1 to 10 proteins of just the ABC-type transporters. These organisms include 23 mycoplasmas and 13 chlamydiae that are known to have undergone genome reduction. A reduced genome (0.42 Mb) gammaproteobacterium, *Buchnera aphidicola* str. Cc (*Cinara cedri*), also contains just 2 ABC-type drug transport proteins; however, other *B. aphidicola* strains additionally contain MFS-type transporters. A bacteroidetes with 1.9-Mb genome, *Candidatus amoebophilus asiaticus* 5a2, encodes 10 ABC-type drug efflux proteins, but none of the other types. This organism is an obligate endosymbiont specific to its protozoan host, *Acanthamoeba* sp. TUMSJ-321, isolated from lake sediment in Malaysia (192). *B. cicadellinicola* str. Hc, a gammaproteobacterium, has a 0.69-Mb genome (193) and encodes just three MFS-type drug efflux proteins; it lacks any recognizable drug transporters of the other four types. This newly discovered organism is an obligate endosymbiont of the leafhopper insect *Homalodisca coagulata* (Say), also known as the glassy-winged sharpshooter, which feeds on the xylem of plants.

Forty-one (5%) organisms encode only two out of the five efflux types, many of which also are intracellular pathogens and endosymbionts. Of these, 26 contain just MFS- and ABC-type drug efflux transporters, seven encode MFS- and RND-type pumps, four encode ABC- and RND-type pumps, three contain ABC- and MATE-type transporters, and only one organism encodes the RND and MATE family of transporters but not the other types. The organisms containing both and MFS- and ABC-type transporters comprise predominantly of firmicutes (17 species, mostly *Streptococcus pyogenes* strains), gammaproteobacteria (four *B. aphidicola* strains), Alphaproteobacteria (three), and Actinobacteria (two *Tropheryma whippelii* strains). The remaining organisms that do not contain either MFS- or ABC-type efflux pumps are alphaproteobacteria (seven), spirochetes (two), bacteroidetes (two), gammaproteobacteria (two), a firmicute, and an unclassified bacterium; most encode fewer than 10 drug transporter proteins. However, two organisms encoded as many as 19 and 30 efflux proteins each and are discussed briefly below. *Halorhodospira halophila* SL1, a gammaproteobacterium, has a genome of 2.7-Mb genome and encodes eight ABC-type drug transport proteins and 11 RND-type efflux transporter proteins. *H. halophila* SL1, formerly *Ectothiorhodospira halophila*, was isolated from salt lake mud and is one of the most halophilic eubacteria known (194). The other organism, *Eubacterium rectale* ATCC 33656, with a 3.4-Mb genome, encodes an equal number (15 each) of ABC- and MATE-type drug transport proteins. *E. rectale* ATCC 33656, a firmicute, was isolated from human feces. *Eubacterium* spp. are thought to play a beneficial role in maintaining the normal ecology of the large intestine, in part by producing chemicals such as butyric acid which act to inhibit the growth of other bacteria. These organisms are occasionally isolated from wounds and abscesses and may be an opportunistic pathogen. This genus has also been isolated from sewage and soil.

One hundred and seventeen (14%) of the bacteria that contain three types of drug efflux transporters are predominated by bacteria (83 genomes) that contain MFS, RND, and ABC proteins (83 organisms), while 21 bacteria lack the MFS and SMR homologs, 11 lack the RND and SMR pumps, and two organisms lack RND and MATE transport proteins. The organisms that lack the MFS proteins comprise of spirochaetes (eight), firmicutes (four),

gammaproteobacteria (four), bacteroidetes/chlorobi (two), thermotogae (two), and fusobacteria (one). Finally, 30% (258) of bacteria lack just one type of transporter, of which a majority lack SMR-type pumps (170 genomes), followed by MATE-type pumps (82 genomes). The MFS and RND pumps are absent in three genomes each, while there were no organisms that lacked just the ABC-type drug efflux transporters.

E. TAXONOMICAL DISTRIBUTION OF DRUG EFFLUX PUMPS

The bacteria surveyed in our present study can be classified into 19 different taxonomical groups. Gammaproteobacteria, firmicutes, and alphaproteobacteria are well represented, with 221, 189, and 111 bacterial genomes, respectively. While planctomycetes, fusobacteria, and acidobacteria are poorly represented, having just one or two completely sequenced members, another 13 taxonomic subdivisions contain five to 69 bacterial species with complete genomes. The relatively well-represented groups offer an opportunity to assess any taxonomic bias with respect to the occurrence of drug efflux pumps. The average number of drug efflux transport proteins per megabase of genome within each group of bacteria is shown in Figure 7. These data show that both firmicutes and gammaproteobacteria contain the highest density of drug transport proteins per megabase of genome, while the genomes of chlamydiae/verrucomicrobia contain a fourfold lower density of these proteins (Figure 7). Spirochaetes and cyanobacteria encode half as many drug transporters as firmicutes per unit length of the genome. Interestingly, the relative density of each of the five transporter families varies remarkably across the bacterial groups (Figure 7). Firmicutes and thermotogae encode more ABC proteins per megabase of genome, whereas betaproteobacteria, epsilonproteobacteria, spirochaetes, chlamydiae/verrucomicrobia, and aquificae encode only half as many of these transport proteins. Although spirochaetes and chlamydiae/verrucomicrobia encode fewer ABC transporters, the largest number of drug transporters in these bacteria is the ABC type, reflective of the overall lower abundance of drug transporters in these bacteria.

Actinobacteria and betaproteobacteria along, with firmicutes and gammaproteobacteria, encode three to five MFS proteins per megabase of genome, higher than most other groups. The highest number of drug transporters in the deinococcus-thermus group is the MFS type, and these bacteria encode two to three MFS transporters per megabase of genome. Bacteroidetes/chlorobi as well as all proteobacteria encode more RND transporters per unit of genome, while firmicutes have a much lower representation of RND pumps in their genomes. MATE and SMR pumps generally occur in substantially lower proportions in bacterial genomes. Interestingly, the single member of the fusobacteria analyzed encodes as many as four MATE family proteins per megabase of genome in comparison to the zero to two homologs in other groups of bacteria. *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586 has a 2.17-Mb genome and encodes 19 drug efflux transport proteins, of which eight are MATE type, six are ABC proteins, and five are RND-family proteins. It appears to lack MFS- or SMR-type drug transporters completely. *F. nucleatum* belongs to the normal microflora of the human oral and gastrointestinal tracts (195). This bacterium is capable of forming coaggregates with other pathogenic and nonpathogenic bacteria in the mouth. Its MATE-type efflux transporters may play a role in resistance to antimicrobials produced by other inhabitants of the oral microflora.

F. DIFFERENCES AMONG STRAINS

Approximately, half of the bacterial species analyzed (431 genomes) are represented by a single sequenced strain, while 111 different bacterial species have multiple strains (ranging between two and 27 strains) whose genomes have been sequenced completely, accounting for the remaining 423 genomes. Thirty-seven bacterial species with four or more completely sequenced strains are discussed below (Table 8).

Escherichia coli has the most strains sequenced (27 strains); these include both pathogenic and nonpathogenic strains. Six other species contains 10 or more sequenced strains: *Salmonella enterica* (15 strains), *Staphylococcus aureus* (14 strains), *Streptococcus pyogenes* (13 strains), *Prochlorococcus marinus* (12 strains), *Streptococcus pneumoniae* (11 strains), and *Clostridium botulinum* (10 strains). Surprisingly, the strain-level variation in the numbers of the different drug efflux pumps appears to be very low in *S. aureus* and *S. pyogenes*, both pathogenic in humans.

Six sequenced strains of *Acinetobacter baumannii* show large variations in the numbers of drug transporters (Table 8). Most notable are the differences in the numbers of MFS transporters, followed by RND, SMR, and ABC proteins in descending order. *A. baumannii* is an aerobic gram-negative bacillus that is an opportunistic pathogen in humans. Infections by this organism are becoming increasingly problematic, due to the high number of resistance genes found in clinical isolates. Some strains are now resistant to all known antibiotics. Most of these resistance genes appear to have been transferred horizontally from other organisms. Many of them cluster into a single genomic island in strain AYE, as compared to strain SDF.

A similar high level of variation is observed in six strains of a nonpathogenic bacterium, *Rhodopseudomonas palustris*, which is commonly found in soil and water environments (Table 8). However, the most noted differences were observed in the numbers of ABC- and RND-type pumps, and to a lesser extent in the number of MFS drug transporters. Intermediate-level differences are found among strains of other pathogenic species, such as *Bacillus anthracis*, *Bacillus cereus*, *Coxiella burnetii*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Yersinia pestis*, as well as nonpathogenic species such as *Pseudomonas putida*, *Shewanella* sp., and *Synechococcus* sp. (Table 8). In the six pathogens cited above, the differences are mostly in the numbers of MFS and ABC transporters, except in the case of *C. burnetii* and *E. coli*, whose strains mostly differ in the complements of MFS and RND families of proteins. A few additional pathogens with interesting profiles are as follows: *S. enterica* strains differ in the number of SMR proteins besides MFS and RND; *C. botulinum* strains show differences in the number of MATE homologs in addition to ABC and MFS proteins; strains of *Francisella tularensis* and *Burkholderia cenocepacia* vary mostly in the number of MFS-type drug efflux pumps predicted, and *S. pneumoniae* strains mostly show differences in the number of ABC proteins (Table 8). Strains of all other species show relatively fewer differences in the number of drug efflux transporters encoded in their genome (Table 8).

The organization differences between prokaryotic (bacterial) and eukaryotic cells (animals, plants, fungi) in part impose requirements for transport proteins to move molecules into and

out of cells. Gram-positive bacteria are enclosed by a single cytoplasmic membrane, whereas gram-negative bacteria possess two membranes: an inner cytoplasmic membrane and a lipopolysaccharide-containing outer membrane. Movement of molecules into and out of gram-positive bacteria generally requires single-component transporters. Transport of molecules in gram-negative bacteria requires passage through the periplasm enclosed between the two membranes. Thus, many transporters in gram-negative bacteria are multicomponent, as described in this chapter. The cytoplasm of fungi is enclosed by a single plasma membrane, and therefore most transporters are products of a single gene (described below). However, unlike gram-positive bacteria, fungi also possess transport proteins in membranes of intracellular organelles. While the majority of fungal efflux pumps reside in the cytoplasmic membrane, some pumps are located in the membranes of vacuoles (196, 197).

IV. Fungi

The fungi represent a large, diverse group of eukaryotic microorganisms that range in size from macroscopic multicellular mushrooms to microscopic unicellular forms. Fungi inhabit most environments on the planet, but their primary reservoir is the soil. Although most may appear invisible, many are essential for carbon and nutrient recycling in nature, others are important in the food, pharmaceutical, and beverage industries, and many are serious pathogens of plants and animals. Fungi are also known to synthesize a vast array of compounds, many of which are toxins. Fungi may form threadlike tubular walled structures, hyphae that branch and anastomose into complex matlike structures known as mycelia. Hyphae may be segmented into individual cell-like units connected by pores that permit movement of organelles, nuclei, and cytoplasm (198). Some fungi may exist as unicellular forms with cell walls and a single nucleus per cell. These yeast forms occur in many fungal groups. Dimorphic fungi possess both yeast and mycelial stages in their life histories, and each growth form may provide novel functional capabilities to the organism (199–202). Thus plant- and animal-pathogenic fungi may utilize one developmental form during interaction with the host and the other during growth outside the host. Some fungi have no known mycelial stage and form saclike cells which may be multinucleate (yeasts). Some of these fungi have motile flagellated forms as part of their life cycle, and these often play a role in the initial interaction with the host (203). Almost all groups of fungi have members that are important pathogens of plants, animals, and humans. Over the past two decades we have observed a sharp increase in the occurrence of invasive fungal infections of humans, many of which are associated with morbidity and mortality (204). More recently, fungi have been implicated as the causative agents in the white-nose bat syndrome (*Gleomyces* sp.) and in the global extinction crisis of amphibians (*Batrachochytrium dendrobatidis*) (205, 206).

A. ANTIFUNGAL AGENTS AND THEIR USE

Because of their eukaryotic nature, fungi are inherently difficult to treat without causing damage to the host. Furthermore, their relatively slow growth rate (compared to bacteria) adds to the loss in efficacy of certain antifungal agents. These features therefore restrict the array of antifungal drugs that can be used therapeutically (207). The limited number of useful drugs to treat fungal infections is under the additional burden because of rapidly

developing antifungal drug resistance (207, 208). Thus, the development of new drugs that impair uniquely fungal biological processes with limited side effects and ease of delivery is of vital importance. Seven major classes of drugs are currently used to treat fungal infections therapeutically: the triazoles, polyenes, echinocandins, allylamines, nucleoside analogs, morpholines, and griseofulvin-type. Among drugs currently used to treat fungal infections are those that target an enzyme in a unique fungal sterol biosynthesis pathway absent in plants and animals. The azoles (fluconazole, ketokonazole, itraconazole) are inhibitors of fungal P450_{14DM} (lanosterol 14 α -demethylase), which causes the accumulation of C-14 methyl sterols in fungi and impairs normal membrane function (209–214). The polyenes (amphotericin B and nystatin) intercalate into membranes containing the unique fungal sterol ergosterol, causing ion leakage and cell death (215). Echinocandins (e.g., caspofungin and micafungin) inhibit 1,3- β -D-glucan synthase, required for fungal cell wall synthesis. Loss of wall integrity can result in cell lysis and death. The azoles, polyenes, and echinocandins are the only three of the seven antifungal drug classes that can be used to treat systemic infections (216). The allylamines (naftifine and terbinafine) inhibit squalene oxidase, a key step in ergosterol biosynthesis (required for fungal plasma membranes). Morpholine (amorolfine) impairs ergosterol biosynthesis by inhibiting cytochrome P450 enzymes and nucleoside analogs (flucytosine), and griseofulvin-type substances (grifulvinV, fulvicin U/F) interfere with DNA and RNA synthesis and mitotic spindle formation, respectively (215). In nature, fungi encounter a large variety of antifungal substances that are made by a broad spectrum of organisms (217, 218). These compounds include peptides, fatty acids, proteins, alkaloids, quinones, and statins. Survival therefore necessitates employment of effective antitoxin mechanisms. The most common processes used by fungi to become resistant to antifungal agents are destruction of the agent, change in the target enzyme or pathway by mutation, and active efflux to maintain low intracellular concentrations (213, 219).

B. FUNGAL EFFLUX TRANSPORT AND GENOMICS

Data from genome sequences available at the Broad Institute and the J. Craig Venter Institute (Transporter Protein Analysis Database) were used in this chapter (<http://www.broadinstitute.org/>; <http://www.membranetransport.org/>). A significant proportion of fungal genomes are devoted to transport proteins. Transport proteins are important for nutrient uptake, intracellular ion concentration maintenance, secretion of proteins, secretion of secondary metabolites, and efflux of toxins and xenobiotics. In the yeasts, *Saccharomyces* sp., and in filamentous fungi, *Aspergillus* sp., *Neurospora* sp. and *Cryptococcus* sp., the number of transport proteins per megabase of genome is between 13 and 30 (220). By comparison, the closely related fungal group, the oomycetes, have fewer than 5, and the model eukaryotes *Arabidopsis thaliana* have 9.7, *Caenorhabditis elegans* 6.7, *Drosophila melanogaster* 5, *Entamoeba histolytica* 8.5, and *Mus musculus* 0.4 (220). This underscores the importance of transport proteins to the survival of fungi in different environments. For example, in the soil, fungi have to contend with bacterial and fungal antibiotics, plant root exudates, chemicals from pollution, protozoans, and insects. Pathogenic fungi have to survive in potentially toxic host plant or animal environments which necessitate effective efflux processes. Additionally, many fungi produce toxins and secondary metabolites that must be secreted into their hosts or the environment (221). Two major classes of transport

proteins are found in fungi: the ATP-binding cassette superfamily (ABC) and the major facilitator superfamily (MFS) (222).

These two superfamilies of transporters make up between 12 and 22% and 76 and 85%, respectively, of the total number of transporters in many fungi. The ABC transporters belong to a large superfamily of membrane proteins that are found in other eukaryotes and bacteria. The nucleotide-binding domain (ATP-binding cassette) is the most highly conserved region among members of this superfamily. The energy that drives the movement of molecules across the membrane through these transporters is derived from ATP. Members of the superfamily are important in the import of sugars, amino acids, peptides, ions and efflux of proteins, secondary metabolites, and xenobiotics. All fungi examined thus far possess ABC superfamily transporters. Table 9 shows the proportion of fungal and related oomycetes and *Phytophthora infestans* efflux transporters out of the total number of transporters of each superfamily, ABC and MFS, from a distribution of pathogenic and nonpathogenic fungi. Greater than 50% and up to 75% of the ABC transporters in these organisms are devoted to efflux purposes. Although the number of *P. infestans* ABC transporters is large, they actually represent 0.67 per megabase genome and the number of ABC efflux transporters per megabase genome is 0.5. On the other hand, the fungi *Aspergillus fumigatus*, *A. nidulans*, *C. neoformans*, and *S. cerevisiae* encode approximately 1 ABC efflux transporter per megabase of genome. However, *Neurospora crassa* encodes just 0.4 ABC efflux transporters per megabase of genome. These observations suggest that a sizable portion of the transport protein-encoding genome in some fungi is committed to maintenance of an intracellular environment low in potentially harmful metabolites and xenobiotics or for secretion of toxins. Most of these data are based on bioinformatic evidence, and more experimental evidence is required to support these observations. Interestingly, there appears to be no correlation between the percentage of efflux transporters and whether a fungus is a pathogen or a nonpathogen (Table 9). This suggests that efflux pumps play a variety of roles in addition to those required to survive in a toxic host environment. It is conceivable that resistance capabilities may have evolved from proteins required for other cellular and ecological processes. A similar concept had previously been discussed regarding fungi, where efflux pump gene expression is required during mitosis (223) and where important physiological substrates such as steroids may be transported by efflux pumps (224, 225). Similar suggestions have also been made regarding bacteria, where efflux pumps play roles in endurance in their ecological niches, such as attachment, invasion, colonization, and persistence (226).

1. Fungal ABC Transporters—ABC transporters may be organized into different configurations; transmembrane domains (TMDs) followed by nucleotide-binding domains (NBDs), (TMD–NBD)₂, reverse (NBD–TMD)₂ or NBD–TMD. Each nucleotide-binding domain contains characteristic sequences [Gx4GK (ST)] Walker A box and [(RK)X3GX3L(hydrophobic)] Walker B box, separated by 90 to 120 amino acids (227). In fungi, full-size transporters typically comprise 1200 amino acids, between 12 and 20 TMDs, and two NBDs (TMD–NBD)₂ or (NBD–TMD)₂, whereas half-size transporters have between 5 and 10 TMDs and one NBD (NBD–TMD) (228). While the TMDs probably function in substrate translocation across the membrane, the driving energy derived from

ATP is harvested by the NBDs. The ABC superfamily of transporters comprises seven families: ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, and ABCG. Of these, families ABCB, ABCC, and ABCG are implicated in active efflux and are also known as the multidrug resistance (MDR), multidrug resistance–associated protein (MRP), and the pleiotropic drug resistance (PDR) families (229–231). Families ABCE and ABCF that do not have TMDs are not discussed further (232, 233).

Saccharomyces cerevisiae is the best and most extensively studied model fungus. Consequently, we have much biochemical, physiological, and molecular biological evidence to support the bioinformatic information on the organism. The *S. cerevisiae* genome encodes 24 ABC superfamily transporters, and these represent approximately 34% of the ATP-dependent transporters (220). Representatives of the ABCG family (PDR5) are in a (NBD–TMD)₂ configuration and are involved in multidrug resistance. In this fungus, the family is also known as cluster I and includes Pdr5p, Snq2p, and YOI075C proteins (228, 234). *S. cerevisiae* ABCC family representatives (MRP; cluster II.1) Ycg1p, Btp1p, Ybt1p/Bat1p, and Yor1 have a (TMD–NBD)₂ configuration and are full-size transporters. The *S. cerevisiae* ABCB family representative (cluster II.2) Ste6p is a full-size transporter. Cluster II.3 representatives of this family, Atm1p, Mdl1p, and Mdl2p, are half-size transporters.

i. *Saccharomyces* Species: Substrates of efflux proteins Pdr5p, Snq2p, and Yor1 were studied using single, double, and triple mutants with 349 substrates (235). This study demonstrated that these pumps share overlapping, though not identical substrate preferences. Triple mutants showed full sensitivity to itraconazole, miconazole, nystatin, antimycin, and tetradecylammonium bromide. Pdr5p was observed to provide resistance to cycloheximide, benomyl, and phenarpmyl, Snq2p to resazurin and quinoline oxides, and Yor1p to propanil, ferbam, oligomycin, and thiram. In an elegant study using point mutations with Pdr5p, it was demonstrated that substrate specificity is in part determined by protein folding and that pump inhibitor sites are functionally separated from substrate interacting sites (235). Pdr5p has also been shown to mediate resistance to certain mycotoxins and is involved in the transport of glucocorticoids (236, 237). ABCC family proteins of cluster II.1—Ycg1p, Btp1p, and Ybt1p/Bat1p—are capable of transporting bile acids and glutathione conjugates. ABCB (cluster II.2) family protein Ste6p is required for transport of mating pheromone (factor α). Cluster II.3 proteins are all localized to the mitochondrial membranes and play a role in export of mitochondrial peptides. While most of the *S. cerevisiae* ABC efflux transporters are localized to the plasma membrane (e.g., Pdr5p, Snq2, Yor1p, Pdr10p, Pdr15p, Ste6p), others may be localized to vacuolar membranes (e.g., Btp1p, Ybt1p, Ycf1p) (230).

ii. *Aspergillus* Species: The genus *Aspergillus* contains members that have considerable impact on our lives and environment. *Aspergillus fumigatus* is an opportunistic pathogen of animals, while *A. flavus* infects grain crops and is responsible for the production of aflatoxin. *A. nidulans* is generally nonpathogenic and is a soilborne fungus. *A. fumigatus* persists in the environment as airborne spores, and consequently, the human respiratory tract is constantly exposed to the fungus. Infections of immunocompromized individuals by *A. fumigatus* are very high, and the mortality rate is as high as 50%. Recent observations of

increased *A. fumigatus* resistance to triazoles have been attributed partly to enhanced drug efflux pump activity (238, 239). This fungus encodes 45 ABC transporters, of which 75% are involved in efflux. Of these, 12 pumps belong to the ABCG family, 13 to the ABCB family, and 10 to the ABCC family. BLAST pairwise alignment shows that 12 efflux transporters in *A. fumigatus*, two transporters from *A. nidulans*, and 10 transporters from *A. flavus* are related to the prototypical mammalian P-glycoprotein (ABCB family). *A. flavus* has 23 transporters that belong to the ABCG family based on pairwise alignment, while *A. nidulans* has 14 representatives. ABC proteins AfuMdr1 (*A. fumigatus*) and *A. flavus* AflMdr1 are similar to the *Schizosaccharomyces pombe* leptomycin B resistance protein and also to the human Mdr1. *A. fumigatus*, AfuMdr2, is similar to two MDR-like genes of *S. cerevisiae* and confer resistance to the echinocandin B analog, cilofungin (240).

The genome of the nonpathogen *A. nidulans* encodes 45 ABC transporters, of which 75% are involved in efflux. In *A. nidulans*, ABC superfamily efflux transporters bearing a resemblance to the *S. cerevisiae* Pdr5p and Snq2p transporters have been described experimentally (241). The proteins AtrA and AtrB also share homology to the dimorphic fungus *Candida albicans*, Cdr1, and mammalian P-glycoprotein-type transporters (see below). AtrB expression in *S. cerevisiae* was capable of countering the drug hypersensitivity of a *S. cerevisiae* Pdr5p mutant. Interestingly, transcription of these *A. nidulans* transporters was found to be enhanced following exposure of the fungus to azoles or plant defense chemicals. *A. nidulans* AtrA, B, C, and D genes were expressed differentially when the fungus was grown in the presence of the structurally unrelated compounds camptothecin, imazalil, itraconazole, hygromycin, and 4-nitroquinoline oxide (4-NQO) (242). In the presence of 4-NQO, AtrA expression was increased approximately 14-fold, but AtrB was increased more than 4500-fold. AtrC expression was enhanced more than 62-fold in the presence of hygromycin, and AtrD expression was enhanced more than 250-fold. Itraconazole in the growth medium enhanced AtrB and AtrD expression 39- and 23-fold, respectively, while exposure to camptothecin resulted in decreased expression of AtrA and AtrC. AtrA was also repressed in the presence of imazalil and itraconazole. These observations suggest that resistance to certain compounds may be provided preferentially by certain transporters. Alternatively, induction and repression of transport protein-encoding genes may respond differently to varying stimuli.

iii. Candida Species: *Candida albicans* is a dimorphic fungus capable of causing opportunistic systemic infections in immunocompromised individuals and superficial mucosal infections in healthy individuals (243). Many systemic infections often result in mortality. In fact, 50% of nosocomially acquired fungal infections are caused by this fungus (244, 245). Dimorphism in this fungus is characterized by a yeast-like budding form and a hyphal form that can develop into a mycelium. The hyphal–mycelial stage is often recognized with the onset of pathogenesis. In *C. albicans*, out of 17 predicted ABC proteins, 13 proteins are identified as resembling the ABCG family (Pdr5p-like), two proteins bear significant identity to the ABCB family (α -factor export), and two proteins show significant identity to the ABCC family (oligomycin resistance). In *C. albicans*, Cdr1p (ABCG) provides resistance to azoles and in certain clinical isolates has been shown to be important in resistance to fluconazole, ketoconazole, and itraconazole (245, 246). Indeed,

overexpression of the transporter is believed to play a significant role in azole resistance in clinical isolates of the fungus (247). Using photoaffinity labels in competition studies, Cdr1p has been shown to possess separate substrate-binding sites for nystatin and myconazole (248). Site-directed mutagenesis of a conserved cysteine residue in the Walker A motif of Cdr1p suggests that the nucleotide-binding domains respond asymmetrically to substitutions in this amino acid (249–251). Structure and function studies with this protein suggest that specific amino acid residues in the NBD are either indispensable or are important determinants in substrate affinity interactions (251). Furthermore, conformational changes in Cdr1p are unaffected by specific amino acid changes in Cdr1p but are impaired in others (251). In an elegant experiment, Cdr1p was functionally reconstituted into sealed membrane vesicles and shown to carry out drug efflux and also translocate phospholipids (252). Energy-dependent efflux using rhodamine, a fluorescent dye, has been measured in *Candida* species (253).

While the ABC efflux transporters of *C. albicans* provide resistance to azoles, they have not been implicated in resistance to the cell wall inhibiting echinocandins (254). Interestingly, in *C. dubliniensis*, however, the role of Cdr1p in fluconazole resistance has been questioned (255). This suggests that utilization of certain efflux pumps by different species of a fungal genus may vary and may provide clues to alternate mechanisms or pumps employed for drug resistance.

Compared to the animal pathogenic fungi, biochemical and molecular biological experimental data in the plant pathogens are lacking. However, many fungal plant pathogen genomes have been sequenced and provide useful information. Plant pathogen efflux transporters have recently been reviewed (229).

Two broad categories of these pumps have been designated: those involved in secretion of virulence factors and toxins (mycotoxins, e.g., aflatoxin and gliotoxin, and host-specific toxins, e.g., vitorin, botrydyl, and cercosporin) and those involved in the removal of plant-derived antifungal agents. The efflux transporters of the first class are not described further in this chapter. Fungal plant pathogens have been shown to gain resistance to fungicides when exposed to low levels of the antifungal agents. Strobilurin is a *quinine outside inhibiting* (QoI) fungicide and is particularly useful against two different classes of fungi, the ascomycetes and the basidiomycetes, as well a related group, the oomycetes. The target of QoI fungicides is the mitochondrion, and fungi have evolved different mechanisms to provide resistance to these compounds (256).

In *Pyrenophora tritici-repentis*, exposure of the fungus to sublethal concentrations of QoI fungicides showed increased efflux-based resistance to strobilurin and azole fungicides (257). Analysis of the *P. tritici-repentis* genome shows that it encodes 15 ABC transporters that belong to the ABCG subfamily, 47 ABCB subfamily proteins, and 38 ABCC proteins ($E < 10^{-12}$). *Puccinia graminis-tritici*, a pathogen of wheat, barley, and oats (258), encodes approximately 10 ABCG subfamily proteins, 15 ABCB proteins, and 15 ABCC subfamily transporters. The plant pathogen *Magnaporthe grisea* also shows enhanced ABC efflux transporter gene expression following low-level exposure to antifungal agents (259). This ABC efflux transporter has been shown to be an important pathogenicity factor during the

infection of rice (259). Gene expression of ABC1 was enhanced following exposure to toxins and a rice antifungal phytoalexin, sakuranetin. Interestingly, a mutation in the gene did not result in hypersensitivity to antifungal agents tested (inhibitors of protein synthesis, sterol biosynthesis, protein secretion, and a phytoalexin). The mutant was, however, unable to cause disease in rice plant bioassays. These results suggest that in vitro susceptibility testing may not always provide an accurate perspective of the role of a protein or proteins during infection and/or that the MgAbc1 protein may play additional roles during the infection process (e.g., secretion of toxins or efflux of compounds not tested in vitro). The ABC3 transporter in *M. grisea* has been shown through mutational analysis to provide fungal resistance to peroxide and other cytotoxic agents of plant origin during the early stages of infection (260). Analysis of the genome of *M. grisea* indicates that it encodes approximately 15 proteins of the ABCG subfamily, 66 proteins of the ABCB subfamily, and 52 of the ABCC subfamily ($E < 10^{-12}$). The genus *Fusarium* causes disease in over 200 species of plants, many of which are of agricultural importance. Among these are legumes, cereals, and wheat. Three genera—*F. oxysporum*, *F. graminearum*, and *F. verticilloides*—encode 50, 36, and 31 proteins that belong to the ABCG subfamily, 56, 57, and 55 of the ABCB family, and 56, 57, and 55 ABCC proteins, respectively, signifying the importance of these efflux transporters to these pathogens. Recent documentation of increased benzimidazole resistance in *F. graminearum* in China (261) may be due to enhanced activity of these transporters. *Verticillium dahliae* and *V. albo-atrum* pathogens of woody plants (e.g., ash, elm, oak, maple) encode 22 and 27 ABCG proteins, 49 and 48 ABCB transporters, and 34 and 35 ABCC transporters, respectively. *Ustilago maydis*, a basidiomycete pathogen on grain crops, encodes 13 ABCG proteins and 44 ABCB transporters ($E < 10^{-12}$). *Mycosphaella graminicola*, a pathogen of wheat, has been shown experimentally to have resistance to azoles conferred by ABC transporters (262). *Botrytis cinerea*, a pathogen of grapes, ornamentals, fruits, and vegetables encodes approximately 21 ABCG subfamily proteins, 47 ABCB proteins, and 47 ABCC transporters ($E < 10^{-12}$). Reduced susceptibility of this pathogen to the fungicides fludioxonil and fenpiclonil is moderated by the ABC efflux transporter, BcatrB (263). A mutant with a disruption in the BcATR gene showed higher accumulation of fludioxonil and reduced accumulation in strains in which the gene was overexpressed. The use of azoles to control plant pathogenic fungi has been considered from the perspective of enhancing resistance to these compounds in animal pathogenic fungi (233, 264). Although the debate is far from over, it is apparent that use of antimicrobial agents in agriculture may result in them leaching into water systems and soils, thereby exposing other pathogens to sublethal concentrations that may result in increased resistance broadly.

The amphibian pathogen *Batrachochytrium dendrobatidis*, a chytrid fungus, has been implicated in the global decline of frog populations (265). This pathogen infects the keratinized mouth parts of tadpoles and keratinized epithelial cells of adults. Little is known of how the fungus infects its host, survives within the host epithelial cells, or survives in the environment. Analysis of the genome sequence shows the presence of 17 ABCG efflux transporters, 64 ABCB transporters, and 33 ABCB subfamily transporters ($E < 10^{-12}$). No experimental data are available on the role of these efflux transporters in the survival and infective stages of the pathogen.

iv. Phylogenetic Analysis of Fungal ABC Transporters: Because evolutionary convergence of function implies convergence of DNA or protein sequences, a phylogenetic perspective can be used to assess whether pathogenic fungal species are more similar in sequence than is expected from their phylogenetic relationships. Several methods are available for evaluating convergence within a phylogenetic context (266–268). One such method is illustrated here, based on a phylogenetic hypothesis (Figure 8) of the relationships among a set of 27 species of ascomycota fungi based on amino acid sequences of the *Candida* ABC efflux transporter (*Cdr1p*), which has been characterized biochemically. About half of the species (14 of 27, indicated by a “P” following the strain identifier) are pathogenic; the remaining 13 species (indicated by “N”) are nonpathogens. To the extent that the pathogens are convergent in protein sequence, they should tend to cluster together on the tree; if their sequences are not convergent, the tree should, instead, reflect their evolutionary relationships. There is no obvious clustering of pathogenic and nonpathogenic species, independent of taxonomic relationships. The basal branch of the tree (estimated by midpoint rooting) divides the species into the Saccharomycetales and eurotiomycetiales (Ascomycetes). Although some of the relationships among genera are unexpected (e.g., the inclusion of *Coccidioides*, *Penicillium*, and *Neosartorya* within *Aspergillus*), such a lack of resolution is common in single-gene analyses.

Ideally, the tree should be compared against a comparable phylogenetic tree, for the same species, based on multigene or phylogenomic analyses (269–273). Because such a phylogenetic study has yet to be done, the tree of Figure 8 can be compared more crudely with a classification based on the Linnean hierarchy (derived from the Global Biodiversity Information Facility; www.gbif.net/species/browse/taxon/13140889), using the method of Podani et al. (274) to detect hierarchical levels at which the two dendrograms show maximum agreement. The resulting comparison indicated strong similarities at all levels in the trees, with marginally significant disagreement only in the dispersion of *Aspergillus* species. Thus, the phylogenetic tree based on *Cdr1p* sequences is strongly consistent with the accepted Linnean classification, with no evidence supporting either sequence convergence or pathogenic patterns conserved among species (273).

2. Fungal MFS Transporters—The major facilitator superfamily (MFS) proteins in the fungi are relatively poorly understood compared to the ABC transport proteins. A recent review of fungal MFS proteins and their roles in fungal physiology underscores this point (280). Major facilitator superfamily efflux transporters possess either 12 (drug/proton antiporter, DHA1) or 14 (DHA2) TMDs, with a large cytoplasmic loop between domains 6 and 7. Analysis of the *S. cerevisiae* genome sequence indicates the presence of approximately 85 MFS transporters, of which 21% are involved in efflux (Table 9). Only three of these proteins have been characterized functionally. Among other fungi the percentages of MFS–MDR transporters range between 29 and 39% (Table 9). Curiously, in the fungal-related group, the oomycetes (*P. infestans*), less than 2% of the MFS proteins are involved in efflux. This is particularly interesting because the percentage of ABC efflux transporters in *P. infestans* is not notably different from the other fungi.

One of the first MFS proteins involved in drug resistance in fungi was identified in *S. cerevisiae* through mutant selection and complementation experiments (281). The MFS

efflux transporter Dtr1p, known to provide resistance to organic acids and antimalarial drugs, has also been shown to play an essential role in *S. cerevisiae* spore wall maturation by secreting the building block, bisformyl tyrosine, from the cytoplasm (282). This study provides evidence for an additional role of the multidrug transporter in the development of the fungus. In the fission yeast *S. pombe*, the MFS drug efflux transporter Caf5, together with a brefeldin A–resistance protein-encoding gene, Bfr1, was found to be up-regulated in cells where Int6CT was overexpressed (283). Int6CT is a C-terminal fragment of translation initiation factor Int6 that may promote transcriptional activity of Pap1. Pap1 plays a role in oxidative stress resistance and enhancement of HBA2 efflux pump gene expression (284).

In *A. fumigatus*, itraconazole resistance is moderated in part by MFS AfuMDR3 (285). Expression of this gene was enhanced following exposure to the azole. As a dermatophyte, *A. fumigatus* infects keratinized cells and keratin-rich tissue. Sulfite efflux is required to provide a reducing environment for keratin breakdown where cystine is converted to cysteine and *S*-sulfocysteine. Reduced proteins are more accessible to secreted fungal proteases (286). Sulfite secretion is moderated by a MFS tellurite-resistance/dicarboxylate transporter (TDT) family protein. A study on the transportome of *C. albicans* has recently been published (287). Of the 95 putative MFS-encoding genes, 22 and 9 representatives represent the DHA1 and DHA2 subfamilies, respectively. *Candida* strains demonstrate resistance to a variety of antifungal agents. The protein CaMdr1p (BEN^R) provides resistance to benomyl, fluconazole, and methotrexate (288–290). Analysis of a mutation in MDR1 that impairs expression of the gene has demonstrated its importance in the virulence of the fungus (291, 292). Recent studies to elucidate the structural and functional aspects of domains of CaMdr1p utilized both tagging of the protein with green-fluorescent protein (GFP) and alanine-scanning mutagenesis of transmembrane domain 5, believed to contribute to drug/H⁺ transport (293). These studies demonstrated that this transmembrane domain, bearing the conserve motif G(X₆) G(X₃) G(X₃) GP(X₂) G, is essential for drug/H⁺ transport. Resistance to fluconazole, cycloheximide, 4-nitroquinolone, and phenanthroline has also been shown to be provided by the FLU1, TMP1, and TMP2 genes of this family (294, 295). An interesting observation using a variety of clinical isolates of *C. albicans* suggests that overexpression of FLU1 did not always correlate with drug resistance (296).

Among the plant pathogens, a novel MFS transporter from *Botrytis cinerea*, Bcmfs1, has been shown to provide tolerance toward the natural toxins camptothecin and cercosporin as well as fungicides (297). In *Mycosphaerella graminicola*, the MFS drug efflux transporter MgMFS1 (DHA14) provides resistance to fungicides and naturally occurring toxins, particularly strobilurin and cercosporin (298). Interestingly, in bioassays, virulence of the isolate harboring a disruption in the gene was observed to be similar to the control parent strain. In field studies, expression of the gene was found to be elevated under conditions where a sublethal concentration of the fungicide trifloxistrobin was present (299). These two studies demonstrate that some drug efflux transporters play important roles in survival of the fungus when present outside the host.

C. REGULATION OF EFFLUX PUMP GENE EXPRESSION

Recent reviews have discussed the regulation of multidrug gene expression in fungi (207, 229). Gulshan and Moye-Rowley (207) provide an excellent overview of the significant regulatory interactions governing pleiotropic drug resistance in *S. cerevisiae*. Coleman and Mylonakis provide a much-needed overview of drug efflux transporters in plant pathogens and their genomics. Because of its clinical importance and relative ease of genetic manipulation, studies with *C. albicans* are in abundance. Our understanding of the regulation of ABC transporters in *C. albicans* has come from studies with clinical isolates showing increased drug resistance and through mutational analyses. The transcriptional regulatory protein Tac1p, known to moderate expression of CDR1 and CDR2 genes, has been shown to harbor a single-point mutation that confers increased drug resistance through these pumps (300). Tac1p has also been shown to play a role in the oxidative stress response and lipid metabolism in part through interaction with its own promoter (301). Transcription factor Ndp80p also regulates Cdr1p (302). A negative regulator, Rep1p, first identified in *S. cerevisiae*, has been shown to moderate Mdr1p efflux pump gene expression in *C. albicans* (261). Overexpression of this negative regulator heterologously in *S. cerevisiae* increased susceptibility to fluconazole. Furthermore, a mutation in the gene encoding the protein in *C. albicans* enhanced drug resistance. Another regulatory protein, Mrr1p (multidrug resistance regulator), has been shown to moderate *C. albicans* MDR1 gene expression. Clinical isolates of the fungus with increased resistance to fluconazole showed an ability to coordinate up-regulation of both the transcription factor and the ABC efflux pump (303).

Analysis of the gene encoding the regulatory protein showed that two-point mutations contribute to high-level constitutive expression of the gene, which results in increased drug resistance. In *C. albicans*, MDR1 is under complex regulation involving oxidative stress response, drug exposure, and multiple transcriptional regulators, including Cap1, Mrr1p, Upc2p, and Mcm1p (304–306). Other studies with *C. albicans* show that uncoupling oxidative phosphorylation in petite mutants of the fungus resulted in reduced sensitivity to fluconazole and voriconazole (but no change in the resistance to ketoconazole, itraconazole, and amphotericin B), and this phenotype could be attributed to overexpression of MDR1 (291). Recent studies with *C. glabrata* show that CgCdr1-, CgCdr2-, and CgSnq2-encoding genes, which moderate azole resistance, may not be coordinately regulated. Gain-of-function (GOF) mutations in the transcription factor encoding gene CgPDR1 increased azole tolerance in vitro through differential expression of CgCdr1-, CgCdr2-, and CgSnq2-encoding genes. Furthermore, strains carrying the GOF mutations also showed enhanced virulence in an in vivo model compared to wild-type strains (307).

While many studies rely on gene disruptions to assess contributions to a specific phenotype or capability, a recent study with *S. cerevisiae* highlights an important issue related to multiple drug resistance in fungi. Deletion of YOR1- and SNQ2-specific regions resulted in increased efflux in Pdr5p efflux substrates. Additionally, increased transcript production and resistance to Yor1p and Snq1p substrates increased in a PDR5 deletion strain (308).

D. IDENTIFICATION OF NOVEL AND USEFUL EFFLUX PUMP INHIBITORS

The use of chemicals that can work synergistically with useful antifungal agents can reduce concentrations of the drugs currently used, and possibly reduce the likelihood of exposure-based drug resistance. A 1.8 million member D-octapeptide combinatorial peptide library was recently used to screen for inhibitors of an ABC (Pdr5p) hyperexpressing strain of *S. cerevisiae* that carried deletions in five other ABC efflux pumps (309). This study identified a noncompetitive inhibitor of ATPase activity and sensitized the strain to fluconazole and increased the permeability of the plasma membrane to rhodamine. A naturally occurring compound tetrandrine was shown to increase rhodamine 123 accumulation in *C. albicans* (310). Cerulenin is an inhibitor of fatty acid synthesis and substrate for ABC and MFS efflux transport in *C. albicans*. Structural analogs of the compound were screened for their ability to increase sensitivity to brefeldin A, and several were found effective against CaMdr1p-mediated resistance (311). A natural product of turmeric, curcumin, known to block ABC transport activity (ABCB1, ABCC1, and ABCG2) in mammalian cancer cells, was shown to be effective in vitro (312). In these studies, rhodamine 6G (R6G) efflux was measured in *S. cerevisiae* cells expressing *C. albicans* Cdr1p and Cdr2p proteins. Treatment with curcumin resulted in decreased extracellular R6G in both expressing cell lines, suggesting that the compound impaired efflux pump function. More detailed studies with curcumin also revealed that this compound enhances the effectiveness of certain antifungal products (ketoconazole, miconazole, and itraconazole) but not others (fluconazole, voriconazole, anisomycin, and cyclohexamide). These observations suggest that curcumin may be a valuable additive when used with conventional antifungal drugs. Curcumin may also be used in more detailed structure–function studies of efflux pumps to identify amino acid residues essential for drug interaction and transport.

E. BIOFILMS

Microbes are known to form biofilms on surfaces. Biofilms represent a communal aggregate of microorganisms in a matrix with varying amounts of extracellular polysaccharide, protein, and nucleic acid. Microbes in biofilms show loss of motility functions, lowered metabolism, and enhanced drug and antibiotic resistance (313). The medical importance of biofilms cannot be overemphasized. Biofilms form on in-dwelling medical devices and in wounds. Thus, treatment of microbes in biofilms with drugs and antibiotics is a continuous challenge in medicine. *A. fumigatus* biofilms were studied in vitro and on bronchial epithelial cells for their resistance to a variety of drugs. Azoles were found to be more effective than echinocandins against mature (48 h) biofilms (314). Overall, less antifungal drug susceptibility was observed in biofilms than in planktonic cells. Antifungal drug resistance in *Candida* species has been reviewed recently (315, 316). The role of drug efflux pumps, in biofilms, however, remains somewhat enigmatic (317). Efflux pump mutants (Cdr1p, Cdr2p, Mdr1p) of *C. albicans* were allowed to form biofilms and were studied with the parental nonmutant at three stages (6, 12, and 48 h) for their resistance to fluconazole (318). The results showed that efflux pumps play a role in resistance to this antibiotic in the early but not at the later 12- and 48-h stages. In planktonic cells, however, expression of the three pumps was observed at only 12 and 48 h, suggesting that in *C. albicans*, efflux pump gene expression is regulated differentially and in a phase-specific manner. Gene expression of CDR- and MDR-encoding genes was examined in planktonic and biofilm cells of *C.*

albicans and found to be up-regulated in biofilms (319). When single- and double-mutant cells were examined for fluconazole resistance, planktonic cells showed predicted sensitivity, but biofilm cells were resistant. These results suggest a multifactorial-based mechanism of resistance when cells are in a biofilm (319). Research with *C. albicans* on cell density influence on drug resistance suggests that azole tolerance at high cell densities (found in biofilms) cannot be attributed to drug efflux pumps (320). A strain lacking functional drug efflux pump-encoding genes CDR1, CDR2, and MDR1 was susceptible to azoles at low cell densities but was resistant at high cell densities and when present in a biofilm. Cell wall protein production in *C. albicans* has been the subject of a recent review, and linkage among cell wall proteins, biofilm formation, and drug resistance has been postulated (321).

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Figure 1.

Bacterial antibiotic drug transporters: the five classes of MDR efflux pumps. Not all MFS and ABC MDR pumps are tripartite in structure. MFS, major facilitator superfamily; RND, resistance–nodulation–division; MATE, multidrug and toxic compound extrusion; SMR, small multi-drug resistance; ABC, ATP-binding cassette; OM, outer membrane; P, periplasm; CM, cytoplasmic membrane; TETs, tetracyclines; CAT, chloramphenicol; FQs, fluoroquinolones; CDDs, cationic dyes and detergents; AMGs, aminoglycosides; MACs, macrolides; BLAs, β -lactams. [Adapted from (22).]

Figure 2.

Hypothetical mechanism of ABC transporters: the closure and dimerization of cytosolic NBDs, which provides the “power stroke” of the two-cylinder engine mechanism for ABC transporters (30, 32) that pulls the TMDs from an inward-to-outward facing conformation. The ABC transporter is heterodimeric (black and white). CM, cytoplasmic membrane; TMD, transmembrane domain; NBD, nucleotide-binding domain. [Adapted from (33).]

Figure 3.

Hypothetical mechanism of SMR transporters: the opening and closing of the antiparallel SMR dimer. The substrate (stars) binds and deprotonates Glu14 residues in the binding pocket of the SMR dimer, causing the transporter to undergo conformational changes, which, in turn, cause the transporter to close behind the substrate and open on the other side of the cytoplasmic membrane (CM). The release of the substrate is coupled to the protonation of the two Glu14 of the dimer, thus coupling drug export to H^+ import.

Figure 4.

Hypothetical mechanism for substrate transport by EmrD: The drug can enter the hydrophobic internal cavity of EmrD either from the cytoplasm or from the inner leaflet of the cytoplasmic membrane (CM). The drug is then transported through a rocker-switch alternating-access process coupled through a proton antiport. [Adapted from (88).]

Figure 5.

Tripartite RND efflux pump: a tripartite MDR efflux pump consisting of an RND transporter, a periplasmic (PP) membrane fusion protein (MFP), and an outer membrane (OM) channel or efflux protein (OEP). The circular arrow depicts the rotational conformational changes of the substrate-binding sites in the monomers of the transporter, leading to the peristaltic mechanism of transport.

Figure 6.

Correlation between the total number of transporters encoded in a genome of 854 bacteria and their genome sizes in megabases. Linear regression of the correlation between the total number of transporters and the genome size was calculated. The R^2 value obtained was 0.72. The equation of the best-fit line is $y = 10.36x - 2.73$. Note that the y-axis scale in the top left panel and the remaining plots is different.

Figure 7.

Average number of transporters encoded per megabase of genome in the different taxonomical groups of bacteria. The 19 taxonomical groups (and the number of bacterial genomes sequenced in each group), numbered 1 to 19 (bottom to top) on the y-axis, are firmicutes (189), gammaproteobacteria (221), betaproteobacteria (67), actinobacteria (69), bacteroidetes/chlorobi (27), epsilonproteobacteria (25), alphaproteobacteria (111), fusobacteria (1), thermotogae (10), other bacteria (16), deltaproteobacteria (27),

acidobacteria (2), aquificae (5), deinococcus-thermus (5), chloroflexi (10), spirochaetes (18), cyanobacteria (36), planctomycetes (1), and chlamydiae/verrucomicrobia (14), respectively.

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Figure 8.

Phylogenetic relationships among 27 species (35 strains) of ascomycota fungi based on amino acid sequences of the Cdr1p encoding gene. Sequences were obtained from BLAST comparisons with the *Candida* Cdr1p sequence and were aligned using the MEGA v4.0.2 interface to ClustalW (275) with a gap-opening penalty of 15 and a gap extension penalty of 6.6 (276). The mean overall Jukes–Cantor distance was 0.06, indicating consistency with the parsimony algorithm. Shown is the bootstrap consensus tree (277), based on 500 bootstrap replicates of the three most parsimonious trees (length = 6130) with missing and ambiguous

states deleted (278). Of the 1267 positions in the final data set, 871 were parsimony informative. Consistency index = 0.57, retention index = 0.73, and composite index = 0.41. Maximum parsimony trees were estimated using the close-neighbor-interchange algorithm (276) with search level 2, in which initial trees were obtained with the random addition of sequences (10 replicates). Phylogenetic analyses were conducted in MEGA4 (279).

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TABLE 1

Examples of ABC MDR Efflux Pumps and Their Substrates

Efflux System	Substrates ^a	Gene Location	Organism	Refs.
Msr(A)	ML, SG-B	Plasmid	<i>Staphylococcus</i> spp.	(36)
Msr(C)	ML, SG-B	Chromosome	<i>Enterococcus faecium</i>	(37, 38)
Msr(D)	ML, KL	Chromosome	<i>Streptococcus pneumoniae</i>	(39)
Vga(A/B)	SG-A	Plasmid	<i>Staphylococcus aureus</i>	(40)
Lsa	LS, SG	Chromosome	<i>Enterococcus faecalis</i>	(39–41)
EfrAB	CP, NOR, TC	Chromosome	<i>E. faecalis</i>	(42)
Lsa(B)	CD	Plasmid	<i>Staphylococcus sciuri</i>	(43)
MacAB-TolC	ML, CD, OFL	Chromosome	<i>Escherichia coli</i>	(44)
LmrA	BL, TC	Chromosome	<i>Lactococcus lactis</i>	(45)
LmrA	AG, CP, OFL	Chromosome	<i>L. lactis</i>	(45)
VcaM	CP, NOR, TC	Chromosome	<i>Vibrio cholerae</i>	(46)
Rv2686c–Rv2687c– Rv2688c	FQ	Chromosome	<i>Mycobacterium tuberculosis</i>	(47)
MD1, MD2	CP	Chromosome	<i>Mycoplasma hominis</i>	(48)

^aAC, acriflavine; AG, aminoglycoside; AH, aromatic hydrocarbons; AZ, azithromycin; BER, berberine; BL, β -lactams; BS, bile salts; CCCP, carbonyl cyanide m-chlorophenylhydrazone; CH, cholate; CLH, chlorhexidine; CL, cerulenin; CD, clindamycin; CM, chloramphenicol; CP, ciprofloxacin; CPC, cetylpyridinium chloride; CV, crystal violet; DAR, daunorubicin; DOC, deoxycholate; DXR, doxorubicin; DAPI, 6-diamidino-2-phenyl indole dihydrochloride; EB, ethidium bromide; ER, erythromycin; FA, fatty acids; FQ, fluoroquinolones; FU, fusidic acid; FL, florfenicol; GL, glycolylcyclines; GM, gentamicin; KL, ketolides; KM, kanamycin; LC, lipophilic cations; LS, linocyclins; ML, macrolides; MOX, moxifloxacin; MN, minocycline; MV, methyl viologen; NAL, nalidixic acid; NOR, norfloxacin; NV, novobiocin; OFL, ofloxacin; OZ, oxazolidinones; PCP, pentachlorophenol; PI, pentamidine isothionate; PMA, phenylmercuric acetate; PM, puromycin; PY, pyronine; QA, quaternary amine compounds; RD, rhodamine; RF, rifampicin; RM, roxythromycin; SAL, salicylate; SDS, sodium dodecyl sulfate; SG-A, type A streptogramins; SG-B, type B streptogramins; SM, sulfonamides; SP, spiramycin; SPR, sparfloxacin; STM, streptomycin; SO, safranin O; TC, tetracycline; TL, thiolactomycin; TG, tigecycline; TS, triclosan; TP, trimethoprim; TX, Triton X-100.

TABLE 2

Examples of SMR Efflux Pumps and Their Substrates

Efflux System	Substrates ^a	Gene Location	Organisms	Ref.
Mmr	CP, NOR, AC, EB	Chromosome	<i>Mycobacterium smegmatis</i>	(62)
Smr/QacC	EB, CV, MV, QA	Plasmid	<i>Staphylococcus aureus</i>	(55)
EmrE	EB, AC, MV	Chromosome	<i>Escherichia coli</i>	(63)
EbrAB	EB, AC, PY, SO	Chromosome	<i>Bacillus subtilis</i>	(58)
YkkCD	EB, CV, PY, MV, TC, SP, STM, PM, CPC	Chromosome	<i>B. subtilis</i>	(64)
Tbsmr	AC, EB, MV	Chromosome	<i>Mycobacterium tuberculosis</i>	(59)
Pasmr	AC, EB, MV	Chromosome	<i>Pseudomonas aeruginosa</i>	(65)
QacE	EB, QA, SM	Plasmid	Gram-negative bacteria	(66)

^aSee Table 1 footnote for substrate abbreviations.

TABLE 3

Examples of MATE Transporters, Substrates and Gene Locations

Efflux System	Substrates ^a	Gene Location	Organisms	Refs.
MepA	GL, EB	Chromosome	<i>Staphylococcus aureus</i>	(71, 74)
NorE (YdhE)	NOR, AC, CP, KM, STP	Chromosome	<i>Escherichia coli</i>	(68, 75)
NorM	NOR, CP, AC, EB, BER	Chromosome	<i>Neisseria gonorrhoeae</i> , <i>Neisseria Meningitidis</i>	(73)
AbeM	NOR, CP, KM, ER, CM, AC, DAR, DXR, TS, GM	Chromosome	<i>Acinetobacter baumannii</i>	(70)
BexA	NOR, AC, EB, MV	Chromosome	<i>Bacteroides thetaiotaomicron</i>	(76)
HmrM	NOR	Chromosome	<i>Haemophilis influenzae</i>	(77)
PmpM	NOR	Chromosome	<i>Pseudomonas aeruginosa</i>	(78)
NorM	NOR, CP, EB	Chromosome	<i>Vibrio parahaemolyticus</i>	(68)
VmrA	AC, EB, DAPI	Chromosome	<i>V. parahaemolyticus</i>	(79)
CdeA	NOR, AC, EB	Chromosome	<i>Clostridium difficile</i>	(80)
VcmA	NOR, CP, EB, KM, STM, DAR, DXR	Chromosome	<i>Vibrio cholerae</i>	(81)
VcrM	CM, AC, EB	Chromosome	<i>V. cholerae</i>	(82)
VcmB, D, H and VcmN	CP, NOR, KM, EB, OFL	Chromosome	<i>V. cholerae</i>	(83)
MdtK	NOR, DXR, AC	Chromosome	<i>Salmonella typhimurium</i>	(84)

^aSee Table 1 footnote for substrate abbreviations.

TABLE 4

Examples of MFS MDR Transporters, Substrates, and Their Gene Locations

Efflux System	Substrates ^a	Gene Location	Organisms	Refs.
Cml, CmlA, CmlB	CM	Mostly plasmid; some chromosome	<i>Pseudomonas aeruginosa</i> , <i>Enterobacter aerogenes</i> , <i>Klebsiella pneumonia</i> , <i>Salmonella enterica</i> serovar <i>typhimurium</i>	(95)
Cml, Cmlv	CM	Plasmid	<i>Streptomyces</i> spp., <i>Corynebacterium</i> spp.	(95)
Mef(A)	ML	Chromosome	<i>Streptococcus</i> spp., <i>Corynebacterium</i> spp., <i>Acinetobacter</i> spp., <i>Enterococcus</i> spp., <i>Staphylococcus</i> spp., <i>Neisseria</i> spp., etc.	(38, 96)
PmrA	CP, NOR	Chromosome	<i>Streptococcus pneumoniae</i>	(97, 98)
MdfA	CM, ER	Chromosome	<i>Escherichia coli</i>	(99, 100)
EmrAB–TolC	LC, CCCP, NAL, TL	Chromosome	<i>E. coli</i>	(93)
EmrKY–TolC	ML	Chromosome	<i>E. coli</i>	(101)
Flo, FloR	CM	Plasmid	<i>E. coli</i> , <i>K. pneumoniae</i> ,	
pp-Flo	FL	Chromosome	<i>Vibrio cholerae</i> , <i>S. enterica</i> serovar <i>typhimurium</i>	(95)
Mef(A)	ML	Chromosome	<i>Streptococcus</i> spp.	(38, 96)
EmeA	CP, NOR	Chromosome	<i>Enterococcus faecalis</i>	(102, 103)
Lde	CP, NOR	Chromosome	<i>Listeria monocytogenes</i>	(104)
Bmr, Bmr3, and Blt	FQ	Chromosome	<i>Bacillus subtilis</i>	(97, 105)
NorA	NOR, CP	Chromosome	<i>Staphylococcus aureus</i> , <i>Bacteroides fragilis</i>	(97, 106, 107)
NorB	NOR, CP, MOX, SPR	Chromosome	<i>S. aureus</i>	(108)
MdeA	ML, LS, SG-A	Chromosome	<i>Staphylococcus aureus</i> , <i>S. hemolyticus</i> , <i>Bacillus cereus</i> , <i>B. subtilis</i>	(109)
QacA	EB, QA, CLH, PI	Plasmid	<i>S. aureus</i>	(110)
LmrB	LS	Chromosome	<i>B. subtilis</i> , <i>Corynebacterium glutamicum</i>	(111–113)
Cme	ER	Chromosome	<i>Clostridium difficile</i>	(114)
VceAB–VceC	DOC, CCCP, NAL, SAL, CM, ER, PMA, PCP	Chromosome	<i>V. cholerae</i>	(91, 92)
Tet (A, B, C, D, E, G, H, J, & Y, Z)	TC	Plasmid	Gram-negative bacteria	(115–117)
Tet (K, L)	TC	Plasmid/chromosome	Gram-positive bacteria	(115, 116)
Tet38	TC	Chromosome	<i>S. aureus</i>	(108)
Tet(V)	TC	Chromosome	<i>Mycobacterium tuberculosis</i> , <i>Mycobacterium fortuitum</i>	(118–120)
Rv1258/Tap	OFL	Chromosome	<i>M. tuberculosis</i>	(121)
Rv1634 FQ	—	Chromosome	<i>M. tuberculosis</i>	(119)
P55/Rv1410	TC	Chromosome	<i>M. tuberculosis</i> , <i>Mycobacterium bovis</i>	(122)
EfpA, LfrA	FQ, CP, NOR	Chromosome	<i>Mycobacterium smegmatis</i>	(62)

^aSee Table 1 footnote for substrate abbreviations.

TABLE 5

Examples of RND Family of MDR Efflux Pumps and Their Substrates

Efflux System	Substrates ^a	Gene Location	Organisms	Refs.
AcrAB–TolC	ML, ER, EB, LS, DOC, GL, BS, CV, CP, NV, KL, AC, AZ, BL, CH, OZ, FQ, CM	Chromosome	<i>Escherichia coli</i> and other gram-negative bacteria	(100, 138, 155, 159, 161–166)
AcrEF–TolC	FA, FQ, FU, NAL, NV, RF, TC, SDS, TX, OZ	Chromosome	<i>E. coli</i> , <i>Salmonella</i> spp.	(166, 167)
MexAB–OprM	AC, AH, BL, CL, CM, CV, EB, FQ, ER, NV, SM, SDS, TG, RD, TL, TS	Chromosome	<i>Pseudomonas aeruginosa</i>	(65, 99, 168)
MexCD–OprJ	ER, SDS, TL, TP, FQ, RM, CM	Chromosome	<i>P. aeruginosa</i>	(99, 169)
MexEF–OprN	TP, TS, FQ	Chromosome	<i>P. aeruginosa</i>	(99, 169)
MexJK–OprM	AH, CM, ER, FQ, TP, TS	Chromosome	<i>P. aeruginosa</i>	(99, 170)
MexXY–OprN	AG, ER, FQ, TC	Chromosome	<i>P. aeruginosa</i>	(99, 171)
MexHI–OpmD	EB, AC, NOR, RD	Chromosome	<i>P. aeruginosa</i>	(172)
MexVW–OprM	FQ, CM, TC	Chromosome	<i>P. aeruginosa</i>	(173)
SmeABC and SmeDEF	FQ, TC, ER, BL	Chromosome	<i>Stenotrophomonas maltophilia</i>	(100, 174)
CmeABC and CmeDEF	FQ	Chromosome	<i>Campylobacter jejuni</i>	(175)
SdeAB	FQ	Chromosome	<i>Serratia marcescens</i>	(176)
SdeXY	NOR	Chromosome	<i>S. marcescens</i>	(177)
MtrCDEML	FQ	Chromosome	<i>Neisseria gonorrhoeae</i>	(178–180)
CeoAB–OpcM	FQ	Chromosome	<i>Burkholderia cepacia</i> (<i>cenocepacia</i>)	(181)
AcrAB–TolC	BL, SDS, AC, MN	Chromosome	<i>Haemophilis influenzae</i>	(99, 182)
AdeABC	FQ, CP, TC, CM, ER	Chromosome	<i>Acinetobacter baumannii</i>	(99, 183)

^aSee Table 1 footnote for substrate abbreviations.

TABLE 6

Phylogeny of the Bacterial Drug Efflux Pump Families^a

TC Family (TC#)	Examples
(1) Major facilitator superfamily (2.A.1)	
Drug:H ⁺ antiporter-1 (DHA1) family (2.A.1.2)	CmlA of <i>Pseudomonas aeruginosa</i>
Drug:H ⁺ antiporter-2 (DHA2) family (2.A.1.3)	QacA of <i>Staphylococcus aureus</i>
Sugar efflux transporter (SET) family (2.A.1.20)	SetA of <i>Escherichia coli</i>
Drug:H ⁺ antiporter-3 (DHA3) family (2.A.1.21)	Cmr of <i>Corynebacterium glutamicum</i>
Putative aromatic compound/drug exporter (ACDE) family (2.A.1.32)	YitG of <i>Bacillus subtilis</i>
Fosmidomycin resistance (Fsr) family (2.A.1.35)	Fsr of <i>E. coli</i>
(2) Resistance–nodulation–cell division (RND) superfamily (2.A.6)	
(Largely gram-negative bacterial) hydrophobe/amphiphile efflux-1 (HAE1) family (2.A.6.2)	AcrAB of <i>E. coli</i>
(Gram-positive bacterial putative) hydrophobe/amphiphile efflux-2 (HAE2) family (2.A.6.5)	MmpL7 of <i>Mycobacterium tuberculosis</i>
(3) Drug/metabolite transporter (DMT) superfamily (2.A.7)	
4 TMD small multidrug resistance (SMR) family (2.A.7.1)	Smr of <i>S. aureus</i>
(4) Multi-antimicrobial extrusion (MATE) family (2.A.66.1)	NorM of <i>Vibrio parahaemolyticus</i>
(5) ATP-binding cassette (ABC) superfamily (3.A.1)	
Drug exporter-1 (DrugE1) family (3.A.1.105)	DrrAB of <i>Streptomyces peucetius</i>
Lipid exporter (LipidE) family (3.A.1.106)	Sav1866 of <i>S. aureus</i>
Drug exporter-2 (DrugE2) family (3.A.1.117)	LmrA of <i>Lactococcus lactis</i>
Drug/siderophore exporter-3 (DrugE3) family (3.A.1.119)	TetAB of <i>Corynebacterium striatum</i>
(Putative) drug resistance ATPase-1 (Drug RA1) family (3.A.1.120)	SrmB of <i>Streptomyces ambofaciens</i>
(Putative) drug resistance ATPase-2 (Drug RA2) family (3.A.1.121)	MsrA of <i>Staphylococcus epidermidis</i>
Multidrug/hemolysin exporter (MHE) family (3.A.1.130)	CylA/B of <i>Streptococcus agalactia</i>
Drug exporter-4 (DrugE4) family (3.A.1.135)	YdaG/YdbA of <i>L. lactis</i>

^aMore information on each of the drug transporter families is available at the TCDB web site (<http://www.tcdb.org>).

TABLE 7
Enumeration of Organisms Against Different Profiles Showing the Presence of Each of the Five Transporter Family Homologs^a

Number of Drug Transporter Types	Organisms		Transporter Type				
	Number	Percent	ABC	MFS	RND	MATE	SMR
Five	398	47	Yes	Yes	Yes	Yes	Yes
Four	170	20	Yes	Yes	Yes	Yes	—
	82	10	Yes	Yes	Yes	—	Yes
	3	0.4	Yes	—	Yes	Yes	Yes
	3	0.4	Yes	Yes	—	Yes	Yes
Three	83	10	Yes	Yes	Yes	—	—
	21	2	Yes	—	Yes	Yes	—
	11	1	Yes	Yes	—	Yes	—
	2	0.2	Yes	Yes	—	—	Yes
Two	26	3	Yes	Yes	—	—	—
	7	1	—	Yes	Yes	—	—
	4	0.5	Yes	—	Yes	—	—
	3	0.4	Yes	—	—	Yes	—
	1	0.1	—	—	Yes	Yes	—
One	38	4.4	Yes	—	—	—	—
	1	0.1	—	Yes	—	—	—
None	1	0.1	—	—	—	—	—

^aThe table is sorted in the descending order of the number of organisms under each category listed in the first column.

TABLE 8

Strain-Level Variation in the Number of Drug Efflux Proteins Encoded^a

Genus and Species	Number of Strains	MFS	ABC	RND	MATE	SMR	Total MDR Proteins
<i>Acinetobacter baumannii</i>	6	16 (6–21)	10 (8–12)	14 (10–17)	2 (2–3)	4 (2–7)	46 (30–54)
<i>Bacillus anthracis</i>	5	43 (42–45)	22 (20–26)	5 (5–5)	5 (4–6)	6 (6–6)	81 (77–88)
<i>B. cereus</i>	9	46 (42–51)	23 (21–26)	5 (4–5)	4 (4–5)	6 (5–7)	84 (78–91)
<i>Buchnera aphidicola</i>	6	1 (0–1)	3 (2–3)	0 (0–1)	0 (0–1)	0 (0–0)	4 (2–4)
<i>Burkholderia cenocepacia</i>	4	48 (45–50)	13 (12–14)	35 (34–37)	3 (3–3)	3 (2–3)	102 (98–104)
<i>B. mallei</i>	4	26 (24–27)	9 (7–11)	16 (15–17)	2 (2–3)	2 (2–2)	55 (52–60)
<i>B. pseudomallei</i>	4	28 (28–28)	11 (11–11)	22 (22–22)	3 (3–3)	2 (2–2)	66 (66–66)
<i>Campylobacter jejuni</i>	5	5 (4–6)	4 (4–4)	5 (4–5)	2 (1–2)	4 (4–4)	19 (17–21)
<i>Chlamydia trachomatis</i>	5	0 (0–0)	2 (2–2)	0 (0–0)	0 (0–0)	0 (0–0)	2 (2–2)
<i>Chlamydomonas reinhardtii</i>	4	0 (0–0)	2 (2–2)	0 (0–0)	0 (0–0)	0 (0–0)	2 (2–2)
<i>Clostridium botulinum</i>	10	4 (3–8)	19 (15–23)	1 (1–2)	11 (10–14)	0 (0–0)	35 (31–39)
<i>Coxiella burnetii</i>	5	12 (8–14)	6 (5–8)	6 (4–8)	1 (0–1)	0 (0–1)	25 (19–31)
<i>Cyanobacterium</i> sp.	4	3 (2–5)	11 (8–15)	10 (9–11)	1 (0–2)	0 (0–0)	25 (22–29)
<i>Escherichia coli</i>	27	21 (18–25)	15 (14–16)	8 (7–13)	3 (2–4)	4 (3–6)	51 (46–58)
<i>Francisella tularensis</i>	7	11 (9–15)	7 (5–8)	6 (5–6)	0 (0–0)	0 (0–0)	23 (20–29)
<i>Haemophilus influenzae</i>	4	3 (2–3)	7 (6–8)	3 (3–3)	1 (1–1)	0 (0–0)	14 (12–15)
<i>Helicobacter pylori</i>	7	2 (2–2)	2 (2–3)	3 (3–3)	2 (2–2)	0 (0–0)	9 (9–10)
<i>Legionella pneumophila</i>	4	15 (14–15)	16 (14–16)	15 (13–17)	0 (0–0)	1 (1–1)	46 (43–49)
<i>Mycobacterium tuberculosis</i>	5	13 (12–13)	12 (12–13)	15 (14–16)	0 (0–0)	1 (1–1)	41 (39–43)
<i>Neisseria meningitidis</i>	4	3 (3–3)	5 (5–6)	5 (5–5)	1 (1–1)	1 (0–1)	15 (14–16)
<i>Prochlorococcus marinus</i>	12	1 (1–1)	8 (6–9)	3 (2–6)	0 (0–0)	0 (0–0)	12 (10–16)
<i>Pseudomonas aeruginosa</i>	4	23 (20–24)	15 (13–19)	34 (33–35)	3 (3–3)	6 (6–6)	81 (76–86)
<i>P. putida</i>	4	19 (16–22)	16 (13–18)	27 (22–30)	2 (2–2)	3 (2–4)	67 (57–73)
<i>Rhodobacter sphaeroides</i>	4	6 (5–6)	12 (9–14)	8 (8–9)	2 (1–2)	2 (2–3)	30 (28–31)
<i>Rhodospirillum rubrum</i>	6	10 (7–12)	17 (11–27)	29 (23–34)	2 (2–3)	3 (2–3)	60 (50–77)
<i>Salmonella enterica</i>	15	21 (18–22)	15 (14–16)	9 (7–11)	2 (1–3)	4 (2–7)	51 (46–55)
<i>Shewanella baltica</i>	4	12 (11–12)	14 (14–15)	24 (23–25)	7 (6–7)	1 (1–1)	58 (57–60)
<i>Shewanella</i> sp.	4	12 (9–17)	13 (13–14)	31 (28–36)	6 (6–6)	1 (1–1)	63 (60–73)

Genus and Species	Number of Strains	MFS	ABC	RND	MATE	SMR	Total MDR Proteins
<i>Staphylococcus aureus</i>	14	23 (22–24)	4 (4–6)	3 (3–3)	1 (1–1)	0 (0–0)	31 (30–33)
<i>Streptococcus pneumoniae</i>	11	1 (1–2)	14 (12–16)	1 (1–2)	3 (3–3)	0 (0–0)	20 (17–21)
<i>S. pyogenes</i>	13	5 (4–6)	10 (9–12)	0 (0–0)	0 (0–1)	0 (0–0)	16 (14–18)
<i>S. suis</i>	5	1 (1–2)	21 (19–23)	1 (1–1)	4 (3–6)	0 (0–0)	27 (24–32)
<i>Synechococcus</i> sp.	9	2 (1–4)	9 (7–16)	5 (3–8)	0 (0–0)	1 (0–1)	17 (12–25)
<i>Xanthomonas campestris</i>	4	9 (8–9)	9 (8–9)	23 (21–27)	2 (2–2)	2 (1–2)	44 (42–46)
<i>Xylella fastidiosa</i>	4	1 (0–3)	5 (4–5)	8 (7–9)	1 (1–1)	0 (0–0)	14 (13–18)
<i>Yersinia pestis</i>	7	15 (13–17)	11 (7–16)	11 (10–12)	2 (2–2)	4 (3–4)	44 (37–51)
<i>Y. pseudotuberculosis</i>	4	17 (17–18)	13 (13–13)	13 (12–13)	2 (2–2)	4 (4–4)	49 (48–49)

^aTotal number of completely sequenced strains for each organism is provided in column 2. The table lists the average number of transport proteins of each type in each species as well as provides in parentheses the minimum and maximum numbers of these transporters across the strains in a given species.

TABLE 9

Distribution of Efflux Transporters in Some Fungi

Organism	Total ABC	Efflux ABC	% ^a	Total MFS	Efflux MFS	% ^b	P/N ^c	Genome (Mb)
<i>Saccharomyces cerevisiae</i>	24	13	54	85	18	21	N	13
<i>Aspergillus fumigatus</i>	45	35	75	275	96	35	P	33
<i>Cryptococcus neoformans</i>	29	19	65	192	54	28	P	19
<i>Neurospora crassa</i>	31	17	55	141	55	39	N	40
<i>Aspergillus nidulans</i>	45	35	75	356	99	28	N	31
<i>Aspergillus oryzae</i>	72	60	83	507	180	35	P	32
<i>Phytophthora infestans</i>	160	116	72	102	2	<2	P	237

^aSource: Data acquired from Transporter Protein Analysis Database (<http://www.membranetransport.org/>).^bPercentage ABC efflux transporters out of total ABC transporters.^cPercentage MFS efflux transporters out of total MFS transporters.^dP/N, pathogen/nonpathogen.