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REVIEW

Efflux Systems in *Serratia marcescens*

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Abstract—A widespread bacterium *Serratia marcescens* (family *Enterobacteriaceae*) is an opportunistic pathogen and exhibits multiple drug resistance. Active removal of antibiotics and other antimicrobials from the cells by efflux systems is one of the mechanisms responsible for microbial resistance to these compounds. Among enterobacteria, efflux systems of *Escherichia coli* and *Salmonella enterica* ser. Typhimurium have been studied most extensively. Few efflux systems that belong to different families have been reported for *S. marcescens*. In this review, we analyzed available literature about *S. marcescens* efflux systems and carried out the comparative analysis of the genes encoding the RND type systems in different *Serratia* species and in other enterobacteria. Bioinformatical analysis of the *S. marcescens* genome allowed us to identify the previously unknown efflux systems based on their homology with the relevant *E. coli* genes. Identification of additional efflux systems in *S. marcescens* genome will promote our understanding of the physiology of these bacteria, will detect new molecular mechanisms of resistance, and will reveal their resistance potential.

Keywords: Serratia marcescens, efflux pumps, antibiotic resistance, bioinformatical analysis, orthologous genes

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Gram-negative bacteria of the genus *Serratia*, in particular, *S. marcescens*, are ubiquitously present in the environment, including soil, water, plants, insects, and animals; they are opportunistic pathogens capable of causing diseases in humans, animals, and insects [1, 2]. These bacteria are associated with infections of the respiratory and the urinary tract, as well as with wound infections; they may cause septicemia, meningitis, or endocarditis [3–5]. In immune compromised patients, *S. marcescens* infections may constitute a serious threat [6–8]. Treatment of *S. marcescens* infections is often associated with considerable difficulties, because these bacteria possess multiple resistances to a wide spectrum of antibiotics. In particular, they are resistant to penicillin G, macrolides, clindamycin, glycopeptides, and rifampin; many strains are also resistant to ampicillin, amoxycillin, ampicillin/sulbactam, a wide range of cephalosporins, cephamycins, nitrofurantoin, and other compounds [2].

Discovery of novel antibiotics and synthesis of chemotherapeutic compounds transformed human medicine, and now many previously incurable infectious diseases can be treated. However, wide-scale use of antibiotics is also accompanied by ubiquitous spread of resistant bacteria, in particular, bacteria with multiple drug resistance (MDR). Bacterial cells employ several strategies that make them drug-resistant, such as degradation or modification of drug molecules, modification of drug targets, induction of alternative metabolic pathways, and pump-mediated efflux of drugs from the cell [9, 10]. Among the mechanisms of drug resistance, an important role belongs to efflux systems located in the bacterial membrane. To date, a number of MDR efflux systems have been described in many species of gram-positive and gram-negative bacteria [11–13]. However, their physiological functions are much wider than contribution to antibiotic resistance. Efflux systems play a key role in the survival of bacteria within ecosystems: they are required for excretion of toxic metabolites, for the support of cell homeostasis, for intercellular signal transduction, etc. [14–18]. Moreover, efflux systems contribute significantly to bacterial virulence by transporting adhesins, toxins, and proteins involved in infection and colonization of human, animal, or plant cells [12, 19–21]. It was shown that inactivation of the AcrAB–TolC efflux system in *Salmonella enterica* ser. Typhimurium resulted in attenuation of virulence in mice [22] and made the bacteria unable to colonize chicken organs [23]. *Pseudomonas aeruginosa* cells with inactivated MexAB–OprM efflux pump of the RND type did not kill even leukocyte-deficient mice, whereas the original *P. aeruginosa* strain caused lethal infection [19]. It has also been demonstrated that efflux systems are

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Abbreviations used: MDR, multiple drug resistance; MFS, major facilitator superfamily; MATE, multidrug and toxic compound extrusion family; SMR, small multidrug resistance family; RND, resistance–nodulation–cell division superfamily; ORF, open reading frame; OMP, outer membrane protein; DAPI, 4',6-diamino-2-phenylindole.

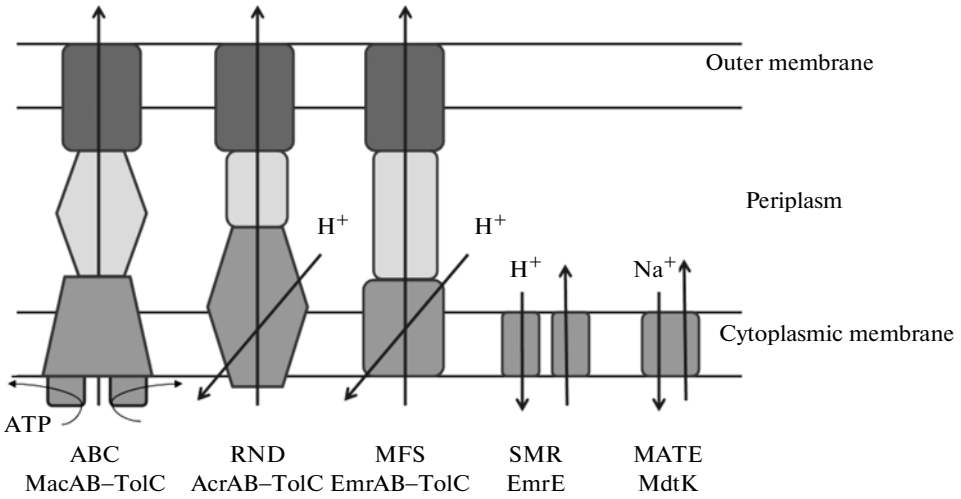

Fig. 1. Prokaryotic efflux pumps (*Escherichia coli*). ABC: ATP-binding cassette transporters (MacAB–TolC); RND: resistance–nodulation–division (AcrAB–TolC); MFS: major facilitator superfamily (EmrAB–TolC); SMR: small multidrug resistance (EmrE); MATE: multidrug and toxic compound extrusion (MdtK).

involved in biofilm formation. In particular, it was shown that expression of certain efflux systems is induced within biofilms [24, 25]. On the other hand, *E. coli* cells within biofilms are more resistant to antibiotics, which may be mediated by various mechanisms, including activation of efflux systems [26]. Efflux system inhibitors suppress the formation of biofilms [27] and are considered as promising antimicrobial compounds [28].

Thus, investigation of efflux systems is of both basic and applied significance. It will help to elucidate the molecular mechanisms governing the behavior of bacteria in populations and their interactions within ecosystems and to overcome multidrug resistance of pathogenic and opportunistic microorganisms. Identification and characterization of efflux systems of pathogenic bacteria, investigation of their physiological functions will provide new targets for the development of efficient drug therapy.

In this review, we discuss the available data on efflux systems of *S. marcescens*, compare the organization of the genes encoding the known RND-type systems, and use database search to identify the homologues of known enterobacterial efflux systems genes in the *S. marcescens* genome.

CLASSIFICATION OF EFFLUX SYSTEMS

MDR efflux pumps are currently classified in two major groups: ATP-binding cassette (ABC) transporters and secondary multidrug resistance transporters [29]. Their principal difference lies in the source of energy utilized for transportation: ABC systems utilize the energy of ATP hydrolysis, whereas secondary transporters employ the proton motive force. ABC transporters form a single family, while secondary transporters are divided into four families depending on their secondary structure and amino acid sequence homology: the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion family (MATE), the small multidrug resistance family (SMR), and the resistance–nodulation–division family (RND) [30–33]. The structures of these types of efflux systems are shown on Fig. 1.

ABC-type transporters are evolutionary conserved from bacteria to humans; they export wide varieties of substrates using ATP hydrolysis. These systems comprise of a transmembrane domain and a nucleotide-binding domain, which may belong to the same or to different protein molecules [34]. Permeases that form a pore in the cytoplasmic membrane usually comprise six transmembrane domains and often form dimers. In gram-negative bacteria, an example of ABC transporters is the MacAB system of *E. coli* [35].

RND-type systems, which function as proton/antibiotic antiporters, have the highest clinical relevance [36]. They are commonly present in gram-negative bacteria and catalyze active expulsion of a wide range of antibacterial compounds, including a number of antibiotics and chemical agents. A typical bacterial RND system is AcrAB–TolC of *E. coli* [37, 38]. This pump includes three components: the inner membrane protein AcrB containing 12 transmembrane domains and two large periplasmic loops, the so-called membrane fusion protein (AcrA), and the TolC protein, which forms a channel in the outer membrane [39–41].

The MFS family includes a large number of proteins and is the most diverse family of secondary transporters. MFS proteins contain 12 or 14 transmembrane domains. Examples of MFS systems are EmrAB and MdfA pumps of *S. enterica* ser. Typhimurium [22].

MATE proteins are topologically similar to MFS proteins, but they are classified in separate families

Table 1. Efflux systems of *Serratia marcescens*

Efflux pump type	System	Reference
RND	SdeAB	[49, 50]
	SdeCDE	[53]
	SdeXY	[58]
MFS	SmfY	[69]
ABC	SmdAB	[73]
SMR	SsmE	[43]

because of low amino acid sequence homology between these two groups. These proteins comprising 12 transmembrane domains serve for sodium-gradient dependent export of toxic compounds, such as fluoroquinolones, aminoglycosides, and cationic toxins. The NorE system of *E. coli* and the MdtK system of *S. enterica* ser. Typhimurium belong to this family [22, 42].

Members of the SMR family are small proteins of 107 to 110 amino acid residues. They contain four transmembrane domains and form tetramers within the cytoplasmic membrane. The number of SMR transporters associated with antibiotic resistance is relatively low. The SsmE system of *S. marcescens* is an example of SMR pumps [43].

Expression of efflux systems is controlled by two-component signaling systems (BaeS–BaeR, CpxA–CpxR, EvgS–EvgA), as well as by specific repressors (AcrR, AcrS, MarA, EmrR, and others) [9]. For instance, expression of the AcrAB–TolC pump is controlled on several levels: locally it is regulated by the AcrR repressor [44], and on a higher level, by stress conditions and by such regulators as MarA, SoxS, and Rob [9, 45, 46].

Among *Enterobacteriaceae*, MDR efflux systems have been extensively studied in *E. coli* and in *S. enterica* ser. Typhimurium. The genes encoding MDR efflux systems of *E. coli* have been cloned, their products classified, and the respective pumps studied [47].

EFFLUX SYSTEMS DESCRIBED IN *S. marcescens*

In *S. marcescens*, several efflux systems representing four families have been characterized (Table 1).

RND efflux systems of *S. marcescens*. Active drug expulsion by *S. marcescens* cells was first described in 2000 [48]. In 2002, Kumar and Worobec reported fluoroquinolone export by SdeAB, an efflux pump of the RND type [49]. Later they identified HasF, a homologue of TolC from *E. coli* representing the outer membrane component of the SdeAB pump, and also cloned the genes and characterized the protein products of the *sdeAB* operon [50]. The functioning of *sdeAB* products was studied using the antibiotic-sensitive *E. coli* strain AG102MB with an *acrB* deletion to determine the role of this pump in bacterial resistance. Introduction of an *sdeAB*-containing plasmid into *E. coli* AG102MB cells increased their resistance to all antimicrobial compounds tested. It was shown that the SdeAB pump can efflux fluoroquinolones, as well as chloramphenicol, SDS (a detergent), ethidium bromide, and *n*-hexane. The only exception was novobiocin [50]. Within *sdeAB, sdeA* encodes a periplasmic adapter protein, while *sdeB* encodes an RND pump transporter. HasF, a protein homologous to TolC of *E. coli*, was identified as the outer membrane component. No other *tolC* homologues were found in the *S. marcescens* genome [51]. The bacterial DNA sequence upstream of *sdeAB* was previously found to contain *sdeR*, a gene transcribed in the opposite direction from *sdeAB*. The amino acid sequence of SdeR has 40% homology to MarA, an *E. coli* protein activating the transcription of the AcrAB–TolC efflux system [45, 52]. *SdeAB*, *hasF* and *sdeR* deletion mutants of *S. marcescens* were obtained to clarify the roles of the respective proteins. Experiments with the mutant strains showed that HasF was the only outer membrane component of the SdeAB pump. It was proposed that SdeR served as a *sdeAB* activator and increased general multidrug resistance of *S. marcescens* [53]. However, it was later found that SdeR participates in the regulation of another efflux system and does not affect SdeAB expression [54]. It was also found that the SdeAB efflux system made bacteria resistant to biocides, in particular, to quaternary ammonium compounds commonly used in hospitals as disinfectants [55]. To evaluate the contribution of the SdeAB efflux pump to fluoroquinolone resistance, *sdeB* expression was assessed in 45 clinical *Serratia* isolates [56]. A real-time PCR assay showed that *sdeB* expression was increased in 20 isolates (44%). Eight of these 20 isolates (40%) were fully resistant to one of the fluoroquinolones tested, whereas the other 12 isolates (60%) were sensitive to all fluoroquinolones. These results suggest that emergence of the multidrug resistant *S. marcescens* phenotype that makes antibiotic therapy of bacterial infections inefficient may result from upregulation of the pump expression. Experiments on transposon-induced mutagenesis identified a gene located upstream from the starting point of *sdeAB, sdeS*, which encodes a protein of 159 amino acids with a high degree of homology to BadM-type transcriptional regulators. It was found that SdeS acts as a *sdeAB* repressor by binding to the operator site in the *sdeAB* promoter. It was shown that *S. marcescens* cells naturally resistant to biocides have a damaged *sdeS* and derepressed *sdeAB* [54]. It was postulated that in natural environments SdeS might be inactivated by various antimicrobial agents. A similar mechanism was suggested for the AcrAB pump regulation by indole, its natural inducer, in *S. enterica* ser. Typhimurium [33]. Indole activates transcription of the regulatory protein RamA, whose overexpression enhances AcrAB expression.

The SdeCDE efflux system has a different substrate specificity [50]. *S. marcescens* strains with knocked-out *sdeCDE* genes did not differ from wild-type bacteria in their sensitivity to any compounds, except for novobiocin. Moreover, novobiocin was the only antibiotic that was accumulated in the mutant cells. These results led to the conclusion that SdeCDE is an RND efflux system with restricted substrate specificity, which makes it different from the SdeAB system [53].

The third RND efflux system, SdeXY, was discovered by Chan et al. [57], who cloned *sdeXY* genes from the chromosomal DNA of *S. marcescens* strain NUSM8906 and characterized the SdeXY proteins and the corresponding efflux pump [58]. A search for protein homologues using the GenBank database showed that SdeY was a member of the RND family of multidrug efflux proteins, and SdeX belonged to membrane-bound proteins. SdeXY was one of the first RND efflux pumps described in a *S. marcescens* strain with multiple drug resistance [58]. Analysis of *sdeXY* expression by real-time PCR and gene inactivation experiments showed that *sdeXY* expression correlated with changes in minimal inhibiting concentrations of tigecycline. Inactivation of the genes encoding SdeY or the outer membrane component HasF decreased the minimal inhibiting concentrations of tigecycline, tetracycline, ciprofloxacin, and cefpirome [59].

Thus, three RND efflux systems have been described in *S. marcescens*: SdeAB, SdeCDE, and SdeXY. With the growing body of data on efflux systems, it is becoming evident that in many cases bacterial resistance to antibiotics used in clinical practice may be a by-product of the physiological function of efflux systems. It is known that RND pumps associated with multidrug resistance are required for export of the virulence factors or for resistance to host-derived antimicrobial agents [60]. The genes encoding RND efflux systems found in *S. marcescens* have orthologs in the genomes of various enterobacteria (Table 2). In particular, nucleotide sequences of orthologous genes encoding efflux systems in *S. proteamaculans* 568 and *S. plymutica* AS9 show a high level of homology (81–88%). The amino acid sequences of the respective proteins have 81–97% homology. *S. marcescens* genes have a considerable homology to the genes of *E. coli* K-12 and *S. enterica* ser. Typhimurium LT2, lying in the range of 66 to 78%. The only exception is the SdeAB system: no *sdeA* homologues were found in the genomes of *E. coli* K-12 and *S. enterica* ser. Typhimurium LT2. However, the level of homology (identity/similarity) between the amino acid sequences of SdeA from *S. marcescens* and AcrA and AcrE from *E. coli* K-12 was 34%/54% and 34%/53%, respectively. The genome of *S. enterica* ser. Typhimurium LT2 also contains a homologous (40%/62%) sequence encoded by the STM0352 locus. Either of the *E. coli* K-12 and *S. enterica* ser. Typhimurium LT2 genomes contains four sequences homologous to *sdeB*. However, these homologous fragments are rather short. In particular, in the *E. coli* K-12 genome, *sdeB* (with an open reading frame (ORF) of 3144 bp) has 69% homology to *acrB* (248/361 bp), 71% homology to *mdtF* (177/250 bp), 66% homology to *acrD* (208/313 bp), and 69% homology to *mdtC* (142/207 bp); in the genome of *S. enterica* ser. Typhimurium LT-2, the respective homology loci are STM0351 (66%, 1004/1510 bp), STM0350 (68%, 577/850 bp), *acrD* (70%, 260/374 bp), and *acrB* (69%, 319/464 bp).

Apart from the species listed in Table 2, many other enterobacteria were found to contain orthologs of the genes encoding RND efflux systems of *S. marcescens*. For example, orthologs of the *sdeAB* genes were found in the genomes of *Klebsiella pneumonia* (82%), *Enterobacter cloaceae* (81%), *Pantoeae* sp. At 9b (80%), and others. Interestingly, other bacterial genomes were found to contain several loci with a high level of homology to *sdeX* and *sdeY* sequences of *S. marcescens*. For instance, the genome of *E. coli* K-12 contains three sequence homologues of *sdeX*: *acrA* (74%), *acrE* (74%), and *mdtE* (65%), as well as three homologues of *sdeY*: *acrB* (77%), *acrF* (72%), and *mdtF* (70%). These data suggest that enterobacterial genomes encode several Acr-type efflux systems. For instance, *E. coli* cells were found to contain AcrEF and AcrD pumps, in addition to the AcrAB system [61, 62]. AcrE and AcrF have 80 and 88% homology to AcrA and AcrB, respectively [63]. The MdtABC system of *E. coli* is an ortholog of the SdeCDE system of *S. marcescens* described above; homology levels between individual genes lie in the range of 70–77%. *SdeCDE* homology to the corresponding genes of *S. proteamaculans* 568 and *S. plymutica* AS9 reaches 82–88% on the nucleotide sequence level and 91–95% identity on the amino acid sequence level.

Structural organization of the RND efflux systems. RND-type efflux systems are composed of three components: a transporter protein of the inner membrane (AcrB in *E. coli*, SdeX in *S. marcescens*), a periplasmic adapter protein (AcrA of *E. coli*, SdeY of *S. marcescens*), and a protein forming the outer membrane channel (TolC of *E. coli*, HasF of *S. marcescens*). Comparison of the genes encoding components of RND efflux systems in different bacteria showed a high level of homology among their nucleotide and amino acid sequences (>70% and >80% identity, respectively) within and among species: *E. coli* (*acrB*/AcrB), *P. aeruginosa* (*mexB*/MexB), *Campylobacter jeujuni* (*cmeB*/CmeB), *Neisseria gonorrhoeae* (*mtrD*/MtrD) [63]. For example, AcrA and AcrB of *S. enterica* ser. Typhimurium have 94 and 97% identity to the corresponding *E. coli* proteins [64].

The organization of the genes encoding three-component efflux systems is also similar in different bacterial species. The common feature is that these genes are organized into operons: the gene of the regulatory protein is located next to the gene of the periplasmic protein, which, in turn, is located next to the

Table 2. Homology of the genes encoding the RND-type efflux systems in *Serratia marcescens* and their orthologs in the genomes of other enterobacteria

S. marcescens Db 11		*E. coli* K-12, homology %	*S. enterica* ser. Typhimurium LT2, homology %	*S. proteamaculans* 568, homology %	*S. plymutica* AS9, homology %
gene	genomic position				
sdeX	–strain 396876–398063	74% *acrA* 71% *acrE* 65% *mdtE*	74% *acrA* 69% *acrE*	88% Spro_1127 69% Spro_3699	87% SerAS9_1055 66% SerAS9_2458
sdeY	–strain 393701–396858	77% *acrB* 72% *acrF* 70% *mdtF*	77% *acrB* 74% *acrF* 69% *acrD*	87% Spro_1126 76% Spro_3700 70% Spro_3492	87% SerAS9_1054 71% SerAS9_3681 66% SerAS9_2457
acrR	–strain 398202–398863	66% *acrR*	71% *acrR*	80% Spro_1128	82% SerAS9_1056
hasF	+strain 3729841–3731238	73% *tolC*	72% *tolC*	82% Spro_4268 (*tolC*)	83% SerAS9_4383 (*tolC*)
sdeA	–strain 1259860–1261047	None	65% STM0352*	81% Spro_1930	83% SerAS9_1878
sdeB	–strain 1256692–1259835	69% *acrB* 71% *mdtF* 66% *acrD* 69% *mdtC*	66% STM0351 68% STM0350 70% *acrD* 69% *acrB*	86% Spro_1929	87% SerAS9_1877
sdeR	–strain 1261299–1261703	71%* *marA*	73%* *rob* (STM4586)	85% Spro_1931	87% SerAS9_1879
sdeC	+strain 3107438–3108790	73% *mdtA*	72% *mdtA*	82% Spro_3552 (*mdtA*)	84% *mdtA*
sdeD	+strain 3110014–3111909	76% *mdtB*	75% *mdtB*	86% Spro_3553 (*mdtB*)	88% *mdtB*
sdeE	+strain 3111918–3114986	78% *mdtC*	76% *mdtC*	86% Spro_3554 (*mdtC*)	88% *mdtC*

Nucleotide sequence homology was determined using the software available at http://blast.ncbi.nlm.nih.gov and http://asap.ahabs.wisc.edu; genomic positions of *S. marcescens* genes were determined using the software available at http://www.sanger.ac.uk.

* In the genomes of *E. coli* K-12 and *S. enterica* ser. Typhimurium LT2, the genes homologous to *S. marcescens sdeR* have overlapping regions of 66/93 and 55/75 bp, respectively. In the genome of *S. enterica* ser. Typhimurium LT2, a short sequence (115/178, 65%) homologous to the *sdeA* was found at the locus STM0352.

gene of the transporter protein. The gene encoding the outer membrane protein (OMP) may also be located nearby. The OMP- and transporter-encoding genes are often co-transcribed. In some systems or species, the OMP-encoding gene is not co-localized with other genes of the same system, e.g., *acrAB* and *tolC* in *E. coli*, or *mexXY* and *oprM* in *P. aeruginosa* [63, 66].

We compared the organization of efflux system genes in the genomes of *S. marcescens* and other enterobacteria. The diagram on Fig. 2 shows the positions of the genes encoding efflux systems of *S. marcescens* (SdeAB, SdeXY, and SdeCDE) in comparison to other enterobacteria. Our data indicate that the SdeAB and SdeXY efflux systems of *S. marcescens* are very similar to the corresponding efflux systems of other *Serratia* species, as well as to the AcrAB systems of *E. coli* and *S. enterica* ser. Typhimurium. The genes encoding proteins of the pump are organized into an operon preceded by the gene of the regulatory protein (*acrR, sdeR, spro_1128*), which is transcribed from the opposite DNA strand (Fig. 2).

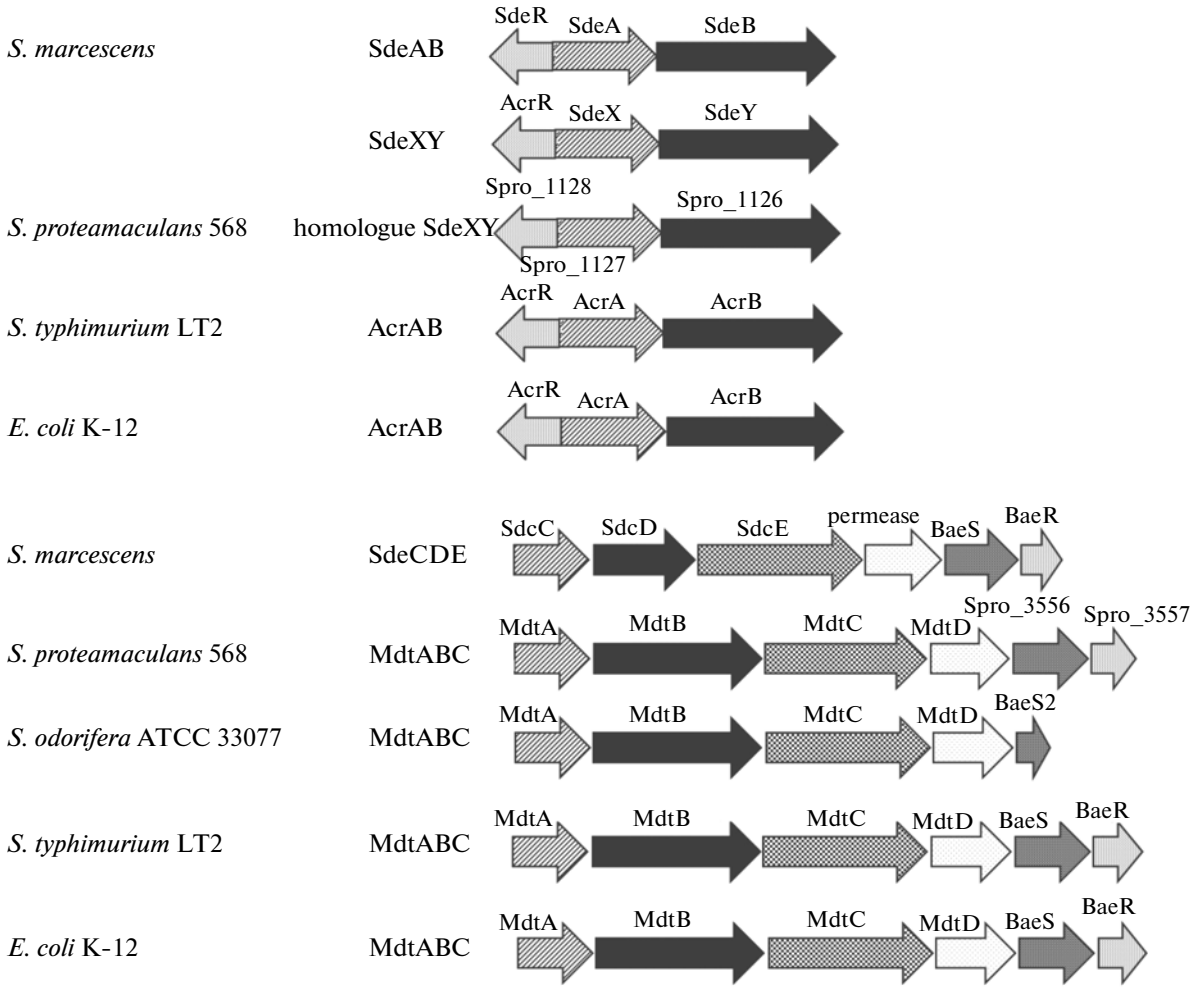

Fig. 2. Genomic organization of RND-type efflux systems of *S. marcescens* and other enterobacteria. SdeA, SdeX, Spro_1127, SdeC, MdtA, membrane fusion proteins. SdeD, MdtB, AcrB, Spro_1126, SdeB, SdeE, MdtC, inner membrane proteins (transporters); BaeS, Spro_3556, sensor proteins of two-component systems; BaeR, Spro_3557, regulatory proteins of two-component systems; SdeR, AcrR, Spro_1128, regulatory proteins (repressors).

The SdeCDE system of *S. marcescens* is organized similarly to MdtABC of *S. proteamaculans* 568 and *S. odorifera, S. enterica* ser. Typhimurium LT2 and *E. coli* K-12. The three genes of the efflux system, *mdtA, mdtB*, and *mdtC* are combined into an operon. The neighboring gene *mdtD* was predicted to encode the transporter protein. However, mutations in this gene did not result in the MDR$^-$ phenotype. The gene order within the operon is identical across bacterial species.

In all of these species, the *baeSR* operon—which is transcribed in the same direction and encodes proteins of the two-component signaling system—is located next to *MdtABC* (Fig. 2). It is known that BaeS–BaeR regulates expression of MdtABC in *S. enterica* ser. Typhimurium and *E. coli*. It was also shown to regulate the expression of another system, AcrD, in *S. enterica* ser. Typhimurium and *E. coli* [67, 68]. Therefore, we may propose that BaeSR also participates in the regulation of the corresponding efflux systems in *S. marcescens* and *S. proteamaculans*. However, in *S. odorifera, baeR* is absent and *baeS* is truncated, which indicates that the MdtAB system in this organism might be regulated in a different manner.

Thus, both the genes encoding the proteins of RND efflux systems and their chromosomal organization are conserved. The fact that bacteria with multiple resistances can be isolated from natural environments, along with the high level of conservation of the corresponding genes, suggests that the genes encoding multiple resistance pumps were not acquired by bacterial pathogens in response to antibacterial therapy. In contrast, they are ancient elements of the genome, extremely important for physiology and ecological adaptation of all living organisms, including bacteria [14].

MFS efflux systems of *S. marcescens*. *S. marcescens* cells also contain efflux systems that belong to

Table 3. Homology of genes encoding efflux systems of *Serratia marcescens* to their orthologs in other enterobacterial genomes

S. marcescens Db 11			*E. coli* K-12, homology %	*Salmonella enterica* ser. Typhimurium LT2, homology %	*S. proteamaculans* 568, homology %	*S. plymutica* AS9, homology %
type, system	gene	genomic position				
ABC, Smd AB	*smdA*	+strain 380867–382642	74% *mdlA*	73% *mdlA*	86% Spro_1107	86% SerAS9_1020
	smdB	+strain 382635–384413	75% *mdlB*	74% *mdlB*	86% Spro_1108	87% SerAS9_1021
MFS, SmfY	*smfY*	+strain 2075177–2077287	None	70% *smvA**	80% Spro_2686	80% SerAS9_2723
SMR, SsmE	*ssmE*	–strain 1966336–1966668	None	None	84% Spro_2603	85% SerAS9_2598

Nucleotide sequence homology was determined using the software available at http://blast.ncbi.nlm.nih.gov and http://asap.ahabs.wisc.edu; genomic positions of *S. marcescens* genes were determined using the software available at http://www.sanger.ac.uk.

* A short homologous sequence (232/331 bp) within a 2112 bp-long gene.

different types. In 2007, a clinical isolate of *S. marcescens* strain NUSM8903 was used to clone the *smfY* gene from chromosomal DNA and to characterize its product, SmfY, a protein belonging to MFS transporters [69]. It was shown that introduction of *smfY*-carrying plasmid to hypersensitive mutant *E. coli* strain KAM32 resulted in significant increase of the minimal inhibiting concentrations of various compounds, including DAPI (4',6-diamino-2-phenylindole), norfloxacin, benzalkonium chloride, acriflavine, and ethidium bromide. SmfY was shown to mediate energy-dependent expulsion of ethidium bromide and acriflavine. We performed a BLAST search and showed that both *S. marcescens* ATCC 13880 and *S. marcescens* Db11 genomes contains only one ORF corresponding to *smfY* with 97% (2054/2112 bp) and 94% homology, respectively. Other *Serratia* genomes also harbor *smfY* orthologs with 80% homology (Table 3). Sequence alignment showed that *smfY* (2112 bp) has 70% homology to *smvA* of *S. enterica* ser. Typhimurium (for the alignment length of 232/331 bp), whereas the homology between the respective proteins was 41%/63% (identity/similarity). At the same time, no similar nucleotide sequences were found in *E. coli* K-12, although there was an amino acid sequence homologous to SmfY at the 29%/48% (identity/similarity) level.

ABC-type efflux systems of *S. marcescens*. Prokaryotic ABC systems have been characterized in detail [70–72]. SmdAB is an ABC efflux system first described in *S. marcescens* NUSM8906 [73]. Individual introduction of *smdA* or *smdB* into hypersensitive *E. coli* strains did not increase the minimal inhibiting concentration of DAPI. Apparently, both SmdA and SmdB are required for efficient efflux of DAPI. These results suggest that both proteins, SmdA and SmdB, may form a heterodimeric efflux pump of the ABC-transporter family in *S. marcescens*. A homology search showed that SmdAB systems were also present in *S. proteamaculans* 568 and *S. plymutica* AS9 (86–87% homology) (Table 3), as well as in *E. coli* K-12 and *S. enterica* ser. Typhimurium (73–75%), and other enterobacteria (*smdA: Cronobacter sakazakii* (78%), *Klebsiella oxytoca* (78%), *Citrobacter rodentium* (78%), *Enterobacter cloaceae* (77%), *Erwinia billingiae* (76%); *smdB: Citrobacter rodentium* (77%), *Klebsiella pneumonia* (78%), *Shigella sonnei* (75%), *Pantoea ananatis* (75%), and others). The fact that homologues of SmdAB-like ABC pumps are commonly present in gram-negative bacteria indicates their physiological importance; its comprehension will require further analysis of the functioning of SmdAB systems and their homologs.

SMR efflux pumps of *S. marcescens*. The recently cloned *ssmE* gene of *S. marcescens* encodes the SsmE protein responsible for energy-dependent expulsion of ethidium bromide [43]. A BLAST search showed that its amino acid sequence was highly similar to those of the SMR efflux pumps of other bacteria: its homology to proteins from different *Serratia* species reached 89%/96% (identity/similarity), *SsmE* homology to *S. plymutica* AS9 and *S. proteamaculans* 568 genes was 84–85% (Table 3). Interestingly, highly homologous sequences were also found in the genomes of different yersiniae: *Yersinia enterocolitica* (74%), *Y. pseudotuberculosis* (72%), and *Y. pestis* (71%), as well as in *Proteus mirabilis* (70%). The genomes of *E. coli* K-12 and *S. enterica* ser. Typhimurium contain the sequences encoding peptides with 56%/76% and 56%/75% homology (identity/similarity), respectively. Previously it was shown that SsmE is a homologue of the EmrE pump of *E. coli* [43].

Table 4. Efflux systems of *Serratia marcescens* identified based on the sequences of orthologous genes of *Escherichia coli* K-12

E. coli K-12			*S. marcescens* Db11	
type	efflux system	genes	homology % (alignment length, bp)	genomic position
ABC	MacAB	*macA*	72% 723/994	+1010164–1011145
		macB	69% 1371/1961	+1011176–1013122
RND	AcrD	*acrD*	76% 2398/3126	+3051095–3054199
			68% 2151/3142	+393754–396858 (*sdeY*)
			64% 2044/3142	+1838813–1841916
MFS	Fsr	*fsr*	74% 878/1163	+1134164–1133068
	Bcr	*bcr*	71% 844/1183	+2740408–2741538
	Ycel (MdtH)	*mdtH*	72% 857/1190	+2186585–2187769
	YidY	*mdtL, yidY*	55% 532/964	–2441213–2442118
	YebQ	*yebQ*	69% 941/1362	–1449134–1450479
	MdfA	*mdfA*	71% 860/1211	–4491834–4493081
	SetA	*setA*	60% 659/1095	+2721774–2722840
	ErmAB	*ermA*	70% 843/1189	+3302697–3303869
		ermB	77% 1198/1539	+3303893–3305425
	MntH	*mntH*	73% 910/1243	–2975648–2976880
MATE	YdhE (MdtK)	*mdtK*	72% 1007/1389	–1531582–1532958
SMR	MdtIJ	*mdtI*	70% 236/333	–2150408–2150737
		mdtJ	69% 261/373	–2150724–2151093
	SugE	*sugE*	59% 159/269	+3079658–3079911
	ErmE	*ermE*	None	

Nucleotide sequence homology and genomic positions were determined using the software available at http://www.sanger.ac.uk.

in silico IDENTIFICATION OF EFFLUX SYSTEMS IN *S. marcescens*

Genomic analysis showed that the genes encoding efflux pumps of drug resistance are widely present in most bacterial genomes [74, 75]. In the *E. coli* genome, 37 ORFs encoding the proteins of multidrug efflux systems have been identified [47]. These genes were cloned, and their expression was studied in antibiotic-sensitive mutant cells. Among these 37 ORFs, 20 were shown to encode the products contributing to *E. coli* resistance. This study showed that genomic analysis is an important approach for identification of new MDR efflux systems [47, 74].

To date, efflux systems have been most extensively characterized in *E. coli* . For this reason, our search for efflux system genes in the genome of *S. marcescens* was based on the known sequences of *E. coli* genes. Using different databases and software packages (e.g., BLAST, NCBI [http://blast.ncbi.nlm.nih.gov], ASAP [https://asap.ahabs.wisc.edu], the Sanger Institute database [http://www.sanger.ac.uk/resources/downloads/bacteria/serratia-marcescens.html]) to analyze the *S. marcescens* genome, we identified the hypothetical efflux systems homologous to those of *E. coli* (Table 4).

To date, SmdAB is the only ABC-type efflux system that has been described in *S. marcescens*: [73]. In the genome of *S. marcescens* Db11, we identified sequences with 72 and 69% homology to *macA* and *macB* of *E. coli*, respectively; therefore, an ABC system similar to MacAB is present in *Serratia*. It was shown that inactivation of the MacAB efflux pump in *S. enterica* ser. Typhimurium resulted in attenuation of the strain's virulence [22].

It is known that *E. coli* cells harbor two RND pumps: AcrAB–TolC and AcrAD–TolC. These systems differ in their inner membrane proteins (AcrB and AcrD), which determine the specificity of both systems [37, 61]. The genome of *S. marcescens* was found to contain three sequences with 76% (2398/3126 bp), 68% (2151/3142 bp), and 64% (2044/3142 bp) homology to *acrD*. Among them, the

sequence with 68% homology corresponds to *sdeY*, while the other two encode hypothetical proteins. These data suggest that *S. marcescens* cells also harbor an AcrAD-like efflux pump.

To date, only one MFS efflux system has been described in *S. marcescens*: SmfY, whereas *E. coli* cells possess several pumps of this family: Fsr, Bcr, YceI (MdtH), YebQ, MdfA, MntH, and ErmAB (Table 4) [76]. A search through the *S. marcescens* genome detected the genes orthologous to these systems with 55 to 73% homology. The *S. marcescens* genome also contains an ortholog (72% homology) of *ydhE*, which encodes a MATE efflux system.

E. coli cells possess three SMR efflux systems: EmrE, MdtIJ, and SugE. Comparative analysis showed that EmrE was homologous to the SsmE system isolated and characterized from *S. marcescens* strain NUSM8903 [43]. So far, no homologues of *E. coli* systems SugE and MdtIJ have been described in *S. marcescens*. However, our search in the genome of *S. marcescens* Db11 identified sequences with 59–70% homology to the genes of these pumps (Table 4).

Thus, in silico analysis of the *S. marcescens* genome identified orthologous genes of potential efflux systems; the corresponding proteins have not been characterized yet, but may play an important role in bacterial resistance to various antibiotics and antimicrobial compounds. Cloning, study of gene expression, and biochemical analysis of the corresponding protein products are required to improve our understanding of the functions of these efflux systems.

Post-genomic studies showed that bacteria possess a large number of genes encoding for multidrug efflux pumps[9]. It was established that under normal growth conditions, most pump components are expressed at a low level. Their expression can be upregulated in response to antibiotic treatment in clinical practice or may be induced by other stress factors. An increase in the efflux pump expression can result from mutations in the genes encoding regulatory proteins, or induced by the environment in the course of infection. It is possible that some bacterial efflux systems are induced within the host organism, as the pumps serve not only for drug resistance but also for the virulence of bacteria [9]. Under these considerations, it is important to identify all potential efflux systems, as well as to determine the pathways of their regulation and physiological substrates of individual efflux systems, in order to comprehend their role in bacterial resistance and their contribution to virulence.

Moreover, since efflux systems affect bacterial virulence and resistance, they are attractive targets for development of new generation drugs. Development of efficient and specific efflux pump inhibitors would provide new treatment options aimed at suppressing bacterial multidrug resistance and virulence.

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