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COMPARATIVE *IN SILICO* ANALYSIS OF TRANSPORTERS CODED WITHIN BIOSYNTHETIC GENES CLUSTERS FOR RAMOPLANIN AND RELATED ANTIBIOTICS

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Glycopeptide antibiotics (GPAs), like teicoplanin and vancomycin, have been the first-line treatment for infections caused by Gram-positive multidrug-resistant pathogens. GPAs appear to be related to ramoplanin-like lipodepsipeptides (LDPs), yet another significant class of lipid II binders. Major compounds among LDPs are ramoplanin (the key representative), enduracidin, and chersinamycin; each with known biosynthetic gene clusters (BGCs). Five additional BGCs for the putative LDPs were recently described. LDP BGCs are poorly investigated; one particular aspect that deserves further investigation is transporters coded within BGCs. These proteins most likely take part in the export of antibiotics out of the cell, as well as in the producer's resistance to its own secondary metabolite. In this work, we performed *in silico* analysis of genes encoding transporters from ramoplanin and other LDP BGCs. We investigated the domain architecture of these transporters, discovered their homologues in BGCs from MIBiG and beyond, generated models of secondary and tertiary structures, and compared the overall LDP BGCs transport genes blueprint. We were able to identify previously uncharacterized gene encoding ABC transporter within ramoplanin BGC – *ramo3*. Ramo1 and Ramo3 in ramoplanin BGC appear to be paralogues coding for a permease subunit of the ABC transporter. In every other LDP BGCs, except for chersinamycin BGC, we found only one corresponding homologue encoding this type of protein. Similarly, we found that Ramo2 and Ramo23 are also homologous proteins, which appear to be ATP-binding subunits of the ABC transporter; Ramo2 and Ramo23 have only one homologue in each other LDP BGCs. Next, we were able to describe Ramo8 as ATP-binding ABC transporter, containing both ATPase and transmembrane parts, similar to those encoded in GPA BGCs. For Ramo8, we modelled 3D structure as well as quaternary structure for homodimer of this protein. Finally, our *in silico* analysis revealed Ramo31 to be a proton membrane antiporter, having distant homologue only in chersinamycin BGC; most likely Ramo31 is not connected to ramoplanin biosynthesis.

Keywords: biosynthetic gene clusters, ramoplanin, membrane transport proteins, secondary metabolites, soil microorganisms

The fast rise of bacteria resistant to existing antibiotics outpaces the present pipeline of new drugs, creating a severe danger to our ability to treat infections successfully. In fact, the antibiotic pipeline is unequipped to deal with the growing bacterial resistance of current antimicrobials. For a long time, glycopeptide antibiotics (GPA), teicoplanin and vancomycin particularly, have represented the frontline treatment of infections caused by Gram-positive multidrug-resistant (MDR) pathogens. The potent action of GPA antibiotics against Gram-positive bacteria depends on their remarkable ability to disrupt cell wall biosynthesis by specifically binding to the D-Ala-D-Ala motif of lipid II [34]. However, teicoplanin and vancomycin-resistant strains of staphylococci and enterococci have continued to emerge in the last decades [30].

Another promising class of antimicrobials are lipodepsipeptides (LDPs), exemplified with ramoplanin. The latter is produced by a soil-dwelling «rare» actinobacterium *Actinoplanes ramo-*

planinifer ATCC 33076. Mode of action of this antibiotic is considered promising for the treatment of infections caused by Gram-positive MDR pathogens. Ramoplanin inhibits cell wall biosynthesis by binding to the lipid II and consequential inhibition of transglycosylation reactions [31]. The clinical development of ramoplanin was initially hampered due to its low local tolerability when injected intravenously. More recently, this LDP has been evaluated to treat *Clostridioides difficile* infections since ramoplanin is not-absorbable and achieves high colonic concentrations [25].

Today, except for ramoplanin, chemical structures for only two ramoplanin-like LDPs are known: enduracidin, which is produced by *Streptomyces fungicidicus* ATCC 21013 [8], and the recently discovered chersinamycin, produced by *Micromonospora chersina* DSM 44151 [23]. In addition, five more biosynthetic gene clusters (BGCs) of potential LDPs have recently been described *in silico* [23]. Despite antimicrobial potential of LDPs, corresponding BGCs remain poorly explored. To date, few aspects of ramoplanin and enduracidin biosynthesis, such as non-ribosomal synthesis of aglycone [10], mannosylation [4], halogenation [15], pathway-specific regulation [5] (only for enduracidin), and some others, have already been studied experimentally. However, the mechanisms that can potentially ensure the producer's resistance to its own secondary metabolite have escaped comprehensive elucidation.

Although extracellular lipid II is the most probable target of LDPs, evidence exists that LDPs can also bind lipid I, inhibiting intracellular lipid II biosynthesis [31]. Hence, intracellular accumulation of LDPs might be an issue for the producing culture. Transmembrane transporters encoded within LDP BGCs might contribute to the self-resistance of LDP producers. Some transport proteins have been associated with bacterial self-resistance to synthesized product, such as DrrA and DrrB in daunorubicin and doxorubicin producer *Streptomyces peucetius* ATCC 27952 [13]. DrrA and DrrB interact to generate an ATP-dependent efflux pump that transports daunorubicin and doxorubicin out of the cell, thereby conferring resistance [13]. With overexpression of *arrA* and *arrB*, doxorubicin synthesis was increased by a factor of 2,2 [18].

Recently, the features of the distribution, structure and phylogeny of ABC transporters in GPA and related peptide antibiotics BGCs were investigated [35]. Notably, these transporters share similarities in their amino acid composition, and categorization as MdlB(MsbA)-like, characterized by a six-helix N-terminal transmembrane domain [35]. Despite their widespread presence, experimental investigations into these transporters have primarily focused on *tba* gene from the BGC of balhimycin in *Amycolatopsis balhimycina* DSM 5908. This protein likely functions as a homodimer, and the knockout of the corresponding gene leads to an increased intracellular and decreased extracellular concentration of balhimycin [21].

The ramoplanin BGC was first described in 2005 [24], and after studies of mannosylation and halogenation of ramoplanin aglycone in 2016 [16, 17], no *in silico* or *in vitro* studies of the functions of the genes responsible for the synthesis of this antibiotic has been performed. Modern bioinformatics analysis benefits from a broad and constantly growing toolbox of data analysis resources and algorithmic approaches. Therefore, the purpose of this work was to investigate the properties of genes and encoded transport proteins in ramoplanin and related LDP BGCs using contemporary *in silico* methods. Such an evaluation can reveal new peculiarities of transporters encoded in ramoplanin and related LDP BGCs. Also, in this work, we offer the detailed description of all genes that code for transport proteins in the ramoplanin BGC, a comparison of the domain structures of their products, and distribution among other LDP BGCs.

Methods

BGC analysis. For nucleotide and amino acid sequence analysis, the nucleotide sequence of ramoplanin BGC (DD002243) [24] was used. All other LDP BGCs were obtained from genome sequences deposited in GenBank or the MIBiG repository under accession numbers:

VFOE01000001 (*Streptomyces* sp. SLBN-134 Ga0314649_11), BGC0000341 (*S. fungicidicus* ATCC 21013), NZ_KB913037 (*Amycolatopsis balhimycina* FH 1894), CP016174 (*Amycolatopsis orientalis* B-37), LT629775 (*Streptomyces* sp. TLI_053), NZ_FMIB01000002 (*M. chersina* DSM 44151). Analysis of BGCs was carried out using the AntiSMASH 6.0.1 [1] and Geneious 4.8.5 [14] software.

Search for homologous proteins. A search for homologues of all genes encoding transport proteins of the ramoplanin BGC was conducted using the MIBiG repository [20] and the Protein BLAST [27]. The search for homologous proteins encoded in known LDP BGCs was performed using the built-in algorithm in the Geneious 4.8.5 software.

Predicting the domain organization of exporter proteins and modeling their location in the cell membrane. The presence of conservative domains was determined using CD-search algorithm from NCBI [17]. The transmembrane α-helices within the amino acid sequences of ABC transporters were identified using the TMHMM 2.0 software [16]. Subsequently, the two-dimensional topology of these transporters relative to the cytoplasmic membrane was reconstructed using TMRPres2D [29].

Modeling of the tertiary and quaternary structure of the ABC transporter Ramo8. The tertiary structure of Ramo8 was modeled based on of the experimentally determined crystal structure of the ABC transporter 9ACTN from *Streptomyces* sp SLBN-134 using the Swiss-mode server [32]. To model the quaternary structure – the Ramo8 homodimer – AlphaFold2-based prediction and visualization of secondary and 3D structures of proteins were used [26–28]. The best model according to AlphaFold2, was visualized using Mol* Viewer [28].

Results and Discussion

We began by *in silico* searching for genes encoding transport proteins in ramoplanin BGC. In addition to the previously identified *in silico* putative transport protein genes – *ramo1*, *ramo2*, *ramo8*, *ramo23*, and *ramo31* [6, 7, 17] – we discovered one more gene with a similar function in ramoplanin BGC, namely, *ramo3*. The literature lacks detailed functional characterization of these genes; however, given the domain structure of their products and similarity to ABC transporters, it is likely that they play a role in ramoplanin export. With these prerequisites, we will further describe the above-mentioned probable transporter genes, the domain structures of their encoded proteins, the homologous proteins found in MIBiG and using BLAST search, and, in particular, homologues found in other LDP BGCs using Geneiuos 4.8.5. This information, along with amino acid sequence identity (a.s.i,) to transporter proteins from ramoplanin BGC, is summarized in Table 1 and Table 2.

The *ramo1* gene is 1002 bp long (the product is 333 aa). The protein encoded by this gene contains the ABC-2 transporter permease domain (75-198 aa, Fig. 1). The most similar described protein in the MIBiG database is the transmembrane transport protein from the BGC of dynemicin in *M. chersina* (64 % a.s.i., 331 aa), and the closest homologue we found using the BLAST algorithm is the permease subunit of the ABC transporter in *S. vitiensis* (WP_018215178, 81 % a.s.i., 336 aa). According to the AntiSMASH analysis of *S. vitiensis* genome sequence (GenBank NZ_KB900388), no secondary metabolite BGCs are detected in this region (Table 2).

We found six similar gene products in the BGCs of LDP: TQL19422 (50,3 % a.s.i.) from *Streptomyces* sp. SLBN-134 Ga0314649_11, ABD65951 (50,0 % a.s.i.) from *S. fungicidicus* ATCC 21013, ctg1_5219 (48,8 % a.s.i.) from *Am. balhimycina* FH 1894, WP_037306103 (48,2 % a.s.i.) from *Am. orientalis* DSM 40040/KCTC 9412, ANN17109 (47,6 % a.s.i.) from *Am. orientalis* B-37, and SDT44233 (43,5 % a.s.i.) from *Streptomyces* sp. TLI_053. It is worth noting that no such protein was discovered in chersinamycin BGC (Table 1).

Table 1

Homologues of ramoplanin BGC transporter proteins encoded in other LDP BGCs

Organism with LDP BGC	Ramo1 (Permease subunit of the ABC2-family transporter)	Ramo2 (ATP-binding subunit of the ABC transporter)	Ramo3 (Permease subunit of the ABC2-family transporter)	Ramo8 (An ATP-binding ABC transporter protein containing both ATPase and transmembrane parts)	Ramo23 (ATP-binding subunit of the ABC transporter)	Ramo31 (Proton membrane antiporter)
A. ramoplaninifer ATCC 33076	Ramo3 (54.0% a.s.i.)	Ramo23 (59.9% a.s.i.)	Ramo1 (54.0% a.s.i.)	Not found	Ramo2 (59.9% a.s.i.)	Not found
M. chersina DSM 44151	Not found	WP_091305522 (73,8% a.s.i.)	Not found	WP_091321314 (77,3% a.s.i.)	WP_091305522 (61,1% a.s.i.)	WP_091305532 (34,8% a.s.i.)
S. fungicidicus ATCC 21013	ABD65951 (50,0% a.s.i.)	ABD65952 (71,3% a.s.i.)	ABD65951 (56,0% a.s.i.)	ABD65953 (72,5% a.s.i.)	ABD65952 (55,6% a.s.i.)	Not found
Streptomyces sp. SLBN-134 Ga0314649_11	TQL19422 (50,3% a.s.i.)	TQL19421 (71,0% a.s.i.)	TQL19422 (56,3% a.s.i.)	TQL19420 (72,5% a.s.i.)	TQL19421 (55,6% a.s.i.)	Not found
Streptomyces sp. TLI_053	SDT44233 (43,5% a.s.i.)	SDT44257 (67,1% a.s.i.)	SDT44233 (44,0% a.s.i.)	SDT44201 (63,3% a.s.i.)	SDT44257 (54,6% a.s.i.)	Not found
Am. balhimycina FH 1894	ctg1_5219 (48,8% a.s.i.)	ctg1_5220 (71,4% a.s.i.)	ctg1_5219 (58,2% a.s.i.)	ctg1_5221 (74,4% a.s.i.)	ctg1_5220 (55,3% a.s.i.)	Not found
Am. orientalis DSM 40040/ KCTC 9412	WP_037306103 (48,2% a.s.i.)	WP_051173842 (71,3% a.s.i.)	WP_037306103 (58,3% a.s.i.)	WP_037306101 (73,7% a.s.i.)	WP_051173842 (54,8% a.s.i.)	Not found
Am. orientalis B-37	ANN17109 (47,6% a.s.i.)	ANN17110 (71,0% a.s.i.)	ANN17109 (57,7% a.s.i.)	ANN21821 (73,7% a.s.i.)	ANN17110 (54,4% a.s.i.)	Not found

Table 2

Homologues of ramoplanin BGC transporter proteins found in MIBiG repository and using the BLAST search

Protein encoded in ramoplanin BGC	Homologue from MIBiG	MIBiG BGC, organism	Homologue found using BLAST	GenBank accession number, organism
Ramo1	ACB47084 (64% a.s.i.)	dynemicin, BGC0001060, *M. chersina*	WP_018215178 (81% a.s.i.)	NZ_KB900388, *Salinispora vitiensis*
Ramo2	ACB47083 (77% a.s.i.)	dynemicin, BGC0001060, *M. chersina*	WP_018215179 (89% a.s.i.)	NZ_KB900388, *S. vitiensis*
Ramo3	ACB47082 (71% a.s.i.)	dynemicin, BGC0001060, *M. chersina*	WP_223874070 (83% a.s.i.)	NZ_KB900388, *S. vitiensis*
Ramo8	ABD65953 (72,5% a.s.i.)	enduracidin, BGC0000341, *S. fungicidicus*	WP_107078706 (78% a.s.i.)	NZ_MUYZ00000000, *Micromonospora* sp. MH33
Ramo23	ACB47083 (59% a.s.i.)	dynemicin, BGC0001060, *M. chersina*	WP_254341048 (60% a.s.i.)	NZ_JANAVK000000000, *Micromonospora* sp. A3M-1-15
Ramo31	ADU86006 (82% a.s.i.)	tiacumicin B, BGC0000165, *Dactylosporangium aurantiacum* subsp. *hamdenensis*	WP_203777073 (81% a.s.i.)	NZ_BOMI01000186, *Actinoplanes deccanensis*

Proteins of this type are typically one of the components of transport complexes of the ABC-2 type, which facilitate ATP-mediated transport of one or more diverse substrates. Well-known examples of such proteins include CcmB, responsible for transporting haeme in *Escherichia coli* and *Mycobacterium tuberculosis*, or DrrB in the doxorubicin producer *S. peucetius* [3].

Based on previous reports, *ramo2* is predicted to code for an ATP-binding subunit of the ABC transporter complex [10]. The 915 bp coding sequence of this gene translates into a 304 aa polypeptide. In the MIBiG database, the most similar protein is also from the BGC of dynemicin, namely ACB47083 (77 % a.s.i., 305 aa). According to BLAST results, a similar protein is present in the genome of *S. vitiensis* (WP_018215179, 89 % a.s.i., 304 aa). The gene encoding this protein in *S. vitiensis* genome is located alongside the gene encoding Ramo1 homologue and is also not a part of any secondary metabolite BGC. Homologues are present in all LDP BGCs (Table 1).

The ATP-binding subunit of the ABC transporter protein has no transmembrane regions and operates by a mechanism that enables movement across membranes of almost any type of molecule, from large polypeptides to small ions. It can use a large number of other proteins as mediators [9]. Thus, a particular ABC-ATPase evolved specifically to function in complex with its cognate membrane protein. Together, they form a transport pathway necessary for the transport of a specific type of molecule, or in the case of some ABC transporters, multiple types of molecules. Specific transport molecules possess recognition motifs that allow them to bind selectively to their cognate transporters. Binding triggers conformational changes in the transporter, activating the ATPase activity and initiating the appropriate transport pathway for the bound substrate [11]. Ramo2 is most likely an ABC-ATPase capable of establishing one transport mechanism with a membrane transport protein; however, the precise function of such a protein in ramoplanin biosynthesis remains unknown.

We discovered a previously unidentified gene encoding an ABC transporter within ramoplanin BGC – *ramo3*. The nucleotide sequence of *ramo3* (1011 bp) codes for a protein with 54 % a.s.i. to Ramo1. Accordingly, the products of these genes both have six transmembrane α-helices and the ABC-2 transporter permease domain (Fig. 1, the ABC-2 domain is marked in green).

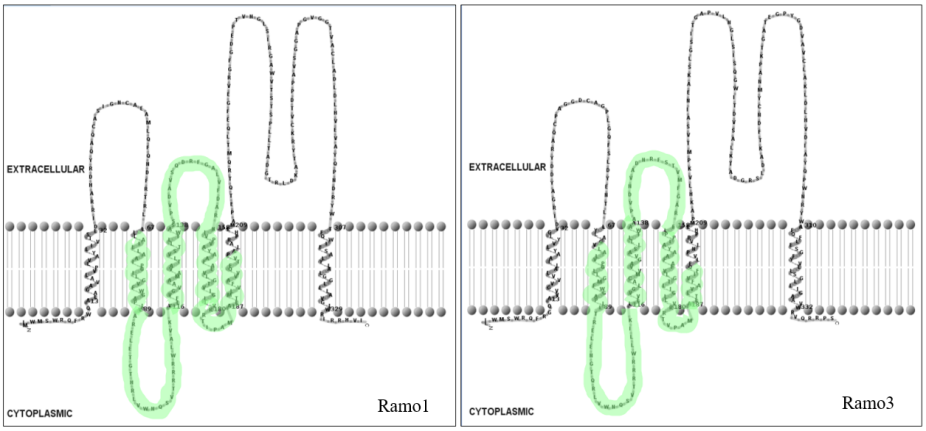

Fig. 1. Secondary structure of ABC transporters Ramo1 (336 aa) and Ramo3 (321 aa). Prediction of transmembrane α-helices and placement of proteins in the cell membrane was carried out as described in Methods section. The ABC-2 domain of Ramo1 (75-198 aa) and Ramo3 (70-201 aa) is marked in green

As summarized in Table 2, the closest hit in the MIBiG database is the protein from the dynemicin BGC ACB47082 (71 % a.s.i., 339 aa). The closest homologue found using the BLAST algorithm is the protein WP_223874070 in *S. vitiensis* (83 % a.s.i., 336 aa), located not in secondary metabolite BGC. Analyzing Table 1, we can conclude that except for chersinamycin, all the other LDP BGCs contain only one homologue to both Ramo1 and Ramo3. Six similar gene products in the BGCs of LDP with a.s.i. percentage are listed: WP_037306103 (58,3 % a.s.i.) from *Am. orientalis* DSM 40040/KCTC 9412, ctg1_5219 (58,2 % a.s.i.) from *Am. balhimycina* FH 1894, ANN17109 (57,7 % a.s.i.) from *Am. orientalis* B-37, TQL19422 (56,3 % a.s.i.) from *Streptomyces* sp. SLBN-134 Ga0314649_11, ABD65951 (56,0 % a.s.i.) from *S. fungicidicus* ATCC 21013, and SDT44233 (44,0 % a.s.i.) from *Streptomyces* sp. TLI_053.

The *ramo8* gene (1923 bp long) codes for the most similar protein to the typical ABC transporters present in GPA BGCs. This gene product contains the MdlB (MsbA) superfamily domain, characterizing this protein as an ATP-binding ABC transporter containing both ATPase and transmembrane parts. The presence of this domain characterizes this protein as a probable component of the antibiotic transport system [35]. Genes encoding exporter proteins with the described structure are present in all sequenced glycopeptide BGC [6].

As seen in Table 2, in the MIBiG database, the greatest similarity of Ramo8 was found with the ABC transporter present in the BGC of enduracidin (ABD65953, 651 aa, 72,5 % a.s.i.). The most similar ABC transporter found using BLAST belongs to *Micromonospora* sp. MH33 (WP_107078706, 642 aa, 78 % a.s.i.). In this region of the *Micromonospora* genome, AntiSMASH annotates possible BGC for type III polyketide synthase metabolite. Homologous proteins are also present in all other LDP BGCs (Table 1): WP_091321314 (77,3 % a.s.i.) from *M. chersina* DSM 44151, WP_037306101 (73,7 % a.s.i.) from *Am. orientalis* DSM 40040/KCTC 9412, ANN21821 (73,7 % a.s.i.) from *Am. orientalis* B-37, ctg1_5221 (74,4 % a.s.i.) from *Am. balhimycina* FH 1894, TQL19420 (72,5 % a.s.i.) from *Streptomyces* sp. SLBN-134 Ga0314649_11, and SDT44201 (63,3 % ide a.s.i.) from *Streptomyces* sp. TLI_053. For comparison, we also chose previously described [35] ABC transporter encoded in teicoplanin BGC Tei4*, which has 54,3 % a.s.i. to Ramo8.

The secondary structure of Ramo8, ABD65953, WP_091321314, PSK62646, and Tei4* ABC transporters with color-coded functional motifs is shown in Fig. 2. The C-terminal ATPase domains have a complete set of motifs [33] necessary for their functioning, these include the Walker motif A (P-loop), the Q-loop, the Walker motif B, the D-loop, the H-loop, and the signature motif of the ABC transporter [35].

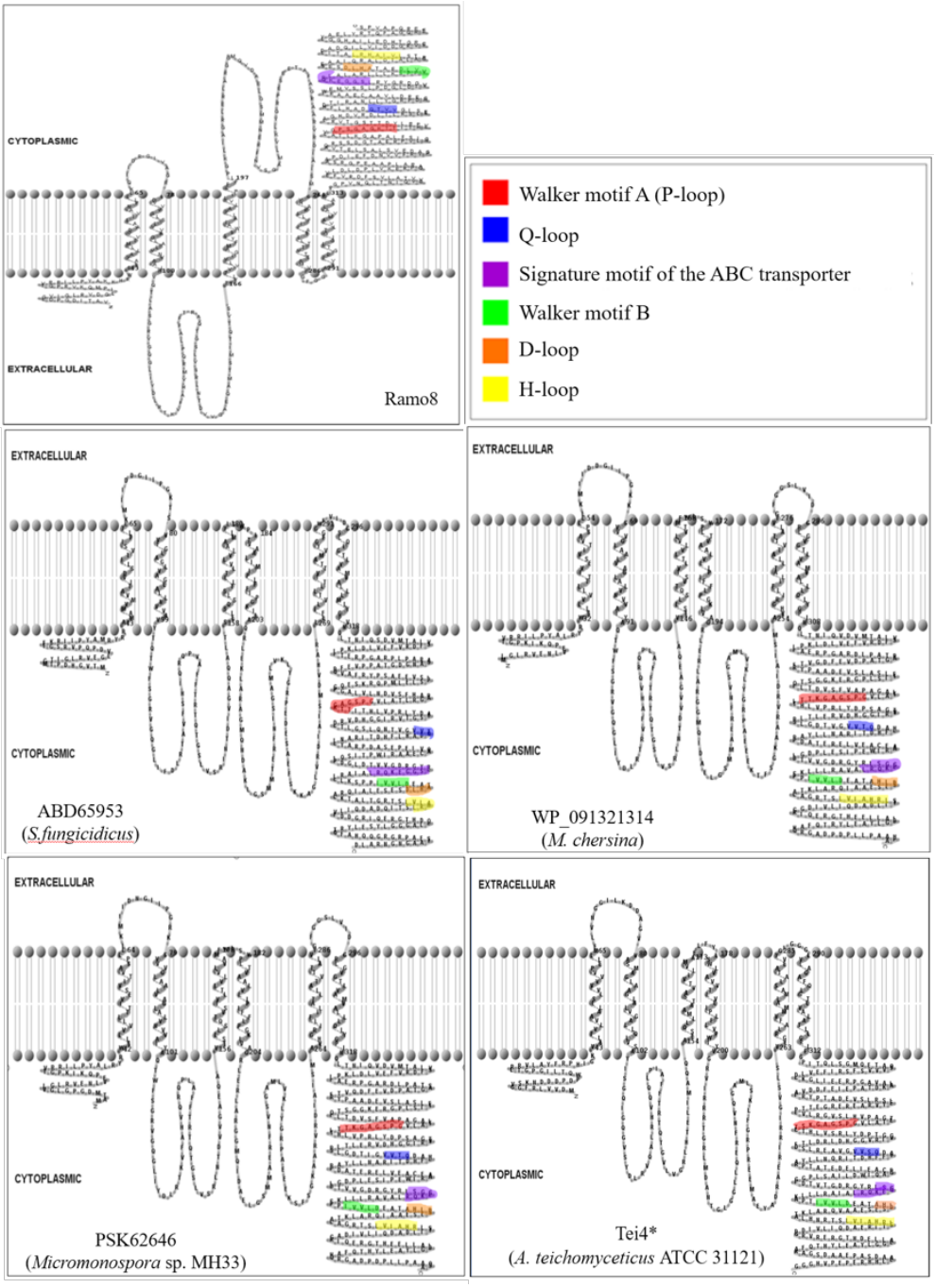

Fig. 2. Secondary structure of ABC transporters Ramo8, ABD65953, WP_091321314, PSK62646, and Tei4*. Prediction of transmembrane α-helices and placement of proteins in the cell membrane was carried out as described in Methods section. Colors indicate C-terminal functional motifs (see the legend to the figure)

It is interesting to note that almost all ABC transporters encoded in the glycopeptide antibiotics BGCs have an N-terminal transmembrane domain with six α-helices, in contrast to the ABC transporter encoded in the ramoplanin BGC, which, according to the prediction of the secondary structure, has a transmembrane domain consisting of five α-helices (the region corresponding to the fourth transmembrane α-helix is missing). Corresponding ABC transporters encoded in chersinamycin and enduracidin BGCs have six α-helices. This arrangement of α-helices in Ramo8 can be explained by the inaccuracy of the construction of the secondary structure model. When constructing the 3D model of Ramo8, the third and fourth α-helices are formed but do not cross the membrane completely (Fig. 3). They can form a kind of «anchor», which may be important for stabilizing the protein structure in the membrane.

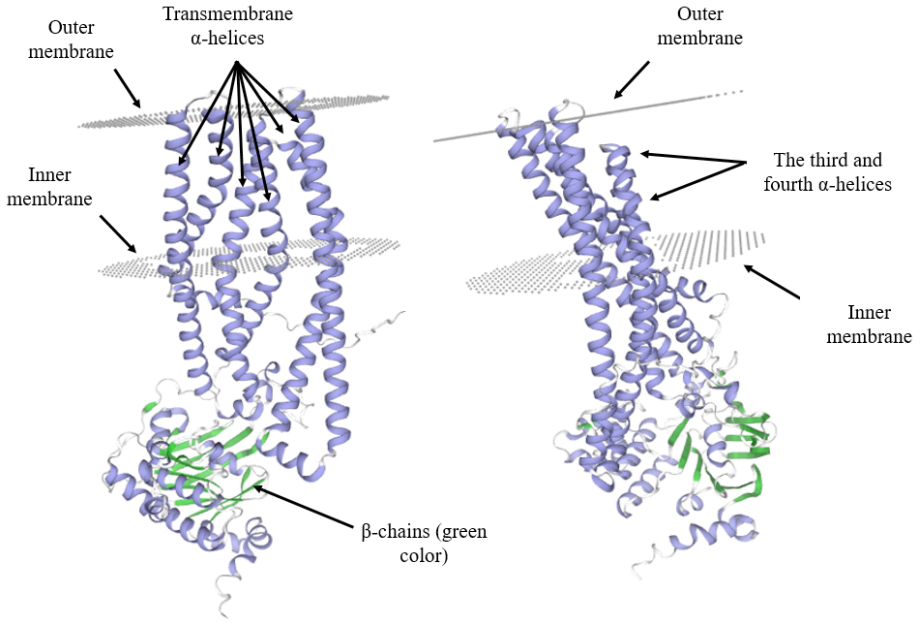

Fig. 3. Model of the tertiary structure of the ABC transporter Ramo8. The tertiary structure of Ramo8 was modeled based on the experimentally established crystal structure of the ABC transporter 9ACTN from *Streptomyces* sp SLBN-134 using the Swiss-mode server [32]. Elements of secondary structures are highlighted in colors

Based on the observed homodimer behavior of many ABC transporters [21], we constructed a homodimer model for Ramo8. AlphaFold2 analysis reveals a highly plausible homodimeric architecture for Ramo8, characterized by precise positioning of all transmembrane α-helices and critical components within the C-terminal ATPase domain active site (Fig. 4). The quaternary structure of Ramo8, as illustrated in Fig. 4, conforms to the characteristic features of ABC exporters. They are homodimers, each transmembrane domain contains six transmembrane α-helices [36].

The *ramo23* (930 bp) gene codes for the ATP-binding subunit of the ABC transporter, as discovered previously *in silico* [23]. The product of the *ramo23* gene contains the CcmA domain, which is also observed in Ramo2. As summarized in Table 2, the most similar described protein in the MIBiG database is the ATP-binding subunit of the ABC transporter encoded in the BGC of dynemicin in *M. chersina* (59 % a.s.i.). The closest homologue found by the BLAST algorithm is the ATP-binding subunit of the ABC transporter in *Micromonospora* sp. A3M-1-15 (WP_254341048, 60 % a.s.i.). The gene for this transporter is not located in any secondary

metabolite BGC. The exact functions of these transporter proteins are not known, but it is most likely that they participate in the transport of substances through the membrane in a complex with a membrane protein.

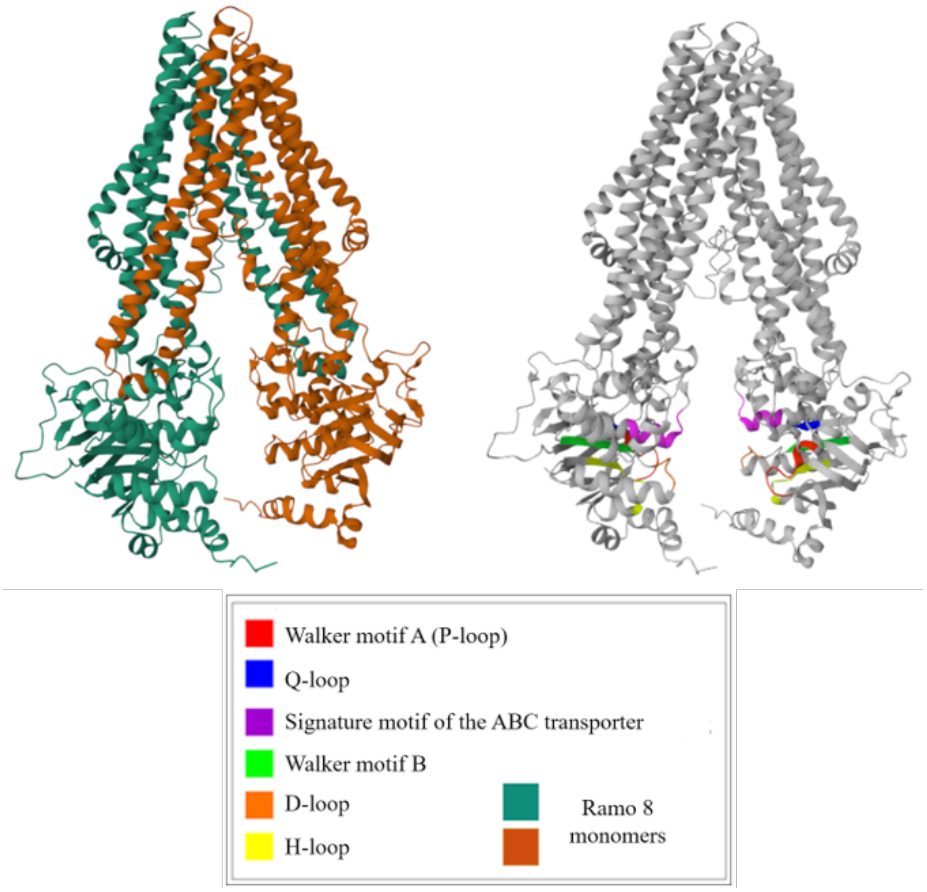

Fig. 4. Model of the quaternary structure of the ABC transporter Ramo8. The model was built using the AlphaFold2 service [12]. Structural elements are highlighted in colors (see the legend to the figure)

The Ramo23 shares 59,9 % a.s.i. to Ramo2. Correspondingly, in each LDP BGCs, there is one gene encoding homologue of both Ramo23 and Ramo2.

The last gene encoding a transport protein in the ramoplanin BGC is *ramo31* (1290 bp). The *in silico* predicted product of this gene is a proton membrane antiporter. The most similar described protein in the MIBiG database is a membrane antiporter protein encoded in tiacumicin B BGC in *D. aurantiacum* subsp. *hamdenensis* (82 % a.s.i.), and the closest homologue found by the BLAST algorithm is a proton antiporter in *A. deccanensis* (81 % a.s.i.), which gene is located in tiacumicin-like BGC (see Table 2).

Ramo31 contains the domain of the PLN03159 superfamily, characterized by the CD-search algorithm as a cation-proton antiporter that performs the functions of maintaining cation homeostasis and cell pH. Among all LDP BGCs, only chersinamycin BGC encodes a homologous protein WP_091305532 with 34,8 % a.s.i. to Ramo31 (Table 1). The secondary structures of mentioned proteins are shown in Fig. 5.

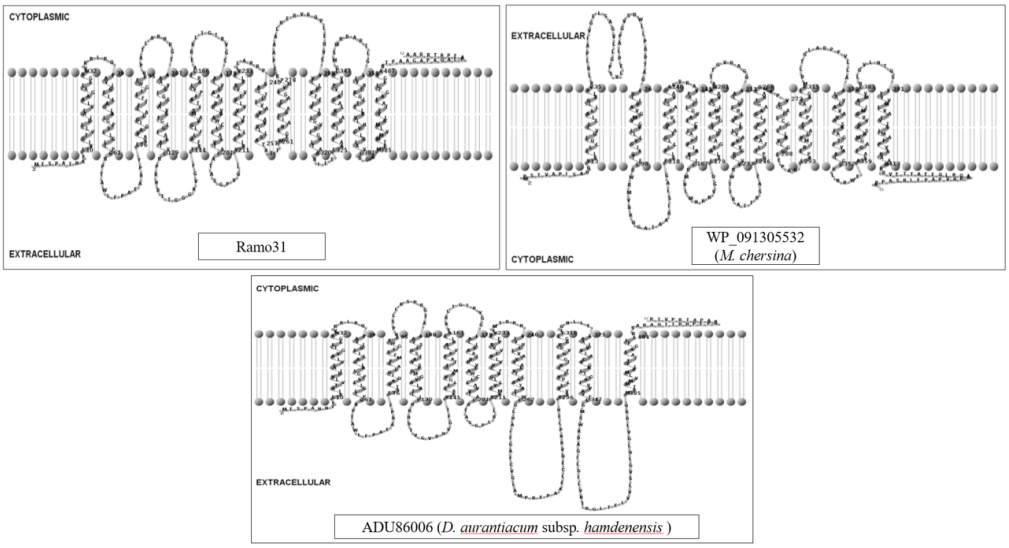

Fig. 5. Secondary structure of antiporter proteins Ramo31 (429 aa), WP_091305532 (*M. chersina*, 453 aa), ADU86006. (*D. aurantiacum* subsp. *hamdenensis*, 428 aa). The prediction of transmembrane α-helices and the localization of proteins in the cell membrane was performed as described in Materials section

The structure of the above-mentioned proteins is partially different. Ramo31 has 13 transmembrane α-helices, WP_091305532 has 12 transmembrane α-helices, and ADU86006 has 11 transmembrane α-helices. In addition to the PLN03159 superfamily domain, Ramo31 also contains KefB domains (typical for the potassium ion transport system described in *E. coli*, involved in the transport and metabolism of inorganic ions) and the Na^+-H^+ exchanger domain cl01133. The canonical *E. coli* KefB protein has a structure similar to Ramo31, with 13 transmembrane α-helices, but is characterized by the presence of the PRK03659 superfamily domain instead of PLN03159. The PRK03659 domain is typical for proteins responsible for potassium exchange and is regulated by glutathione adducts, leading to transient acidification of the cytoplasm [2].

The *ramo31* gene product appears to be involved in cation transport and the regulation of homeostasis and pH. However, whether this protein is directly related to ramoplanin biosynthesis and whether its inclusion in the ramoplanin BGC is warranted remains to be determined.

Analysis of LDP BGCs reveals a single gene encoding a protein homologous to *ramo1* and *ramo3* products in all cases, except for chersinamycin BGC, which does encode a similar protein at all (Fig. 6).

From Fig. 6 we can also conclude that there is only one corresponding protein homologous to the *ramo2* and *ramo23* gene products in all LDP BGCs. All BGCs, except for chersinamycin, also lack a homologue of the proton membrane antiporter encoded by the *ramo31* gene.

It is intriguing to consider that some gene products in the ramoplanin biosynthetic pathway might not be directly involved in the final product export, but their contributions to other vital processes are likely significant.

In all LDP BGCs, except for ramoplanin BGC, only one gene encoding ATP-binding subunit and one gene for permease subunit of the ABC transporter are present. In these BGCs, the corresponding genes are located side by side, and corresponding proteins likely form one transport system, although the substrate of this system in unclear. This is why it is difficult to explain

the presence of the two genes for ATP-binding subunit and two for permease subunit in ramoplanin in BGC. It is possible that one functional pair is not essential for antibiotic export.

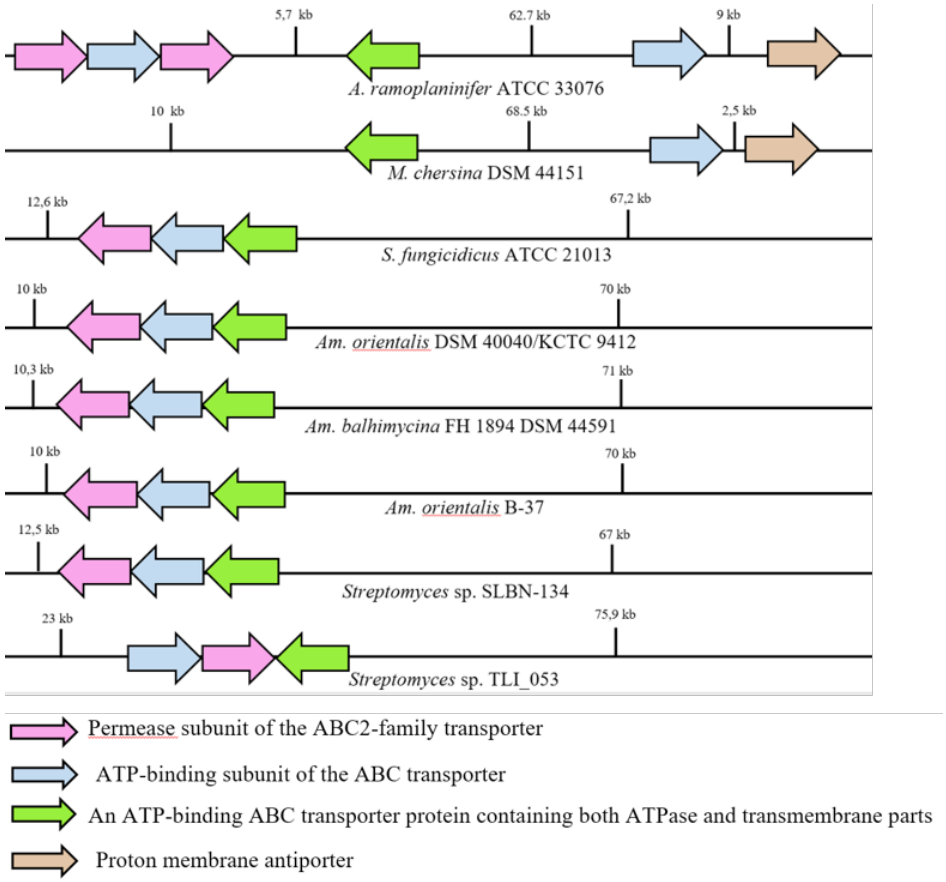

Fig. 6. Schematic arrangement of genes encoding transport proteins in LDP BGCs. Different types of transport proteins are indicated by colors (see the legend to the figure)

The precise explanation for this phenomenon is yet to be determined, but the chersinamycin BGC lacks the gene encoding the permease subunit, although it possesses a gene for the ATP-binding subunit of the ABC transporter. We did not find a corresponding gene for the permease subunit of the ABC transporter in the genomic region near chersinamycin BGC either.

The Ramo31 homologue is present only in chersinamycin BGC with low percentage of a.s.i. (34,8 %). Also, considering the probable function and location of the genes encoding these transport proteins on the edges of both BGCs, we can assume that they do not participate in the export of the corresponding antibiotics.

On the contrary, the observed features of Ramo8, including its potential for homodimerization, suggest it plays a crucial role in ramoplanin transport, likely acting as a key component of the antibiotic's export system across the cell membrane.

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ПОРІВНЯЛЬНИЙ *IN SILICO* АНАЛІЗ БІЛКІВ-ТРАНСПОРТЕРІВ, ЗАКОДОВАНИХ У КЛАСТЕРАХ БІОСИНТЕТИЧНИХ ГЕНІВ РАМОПЛАНІНУ ТА СПОРІДНЕНИХ АНТИБІОТИКІВ

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Глікопептидні антибіотики (ГПА), такі як тейкопланін і ванкоміцин, є одними з препаратів першої лінії для лікування інфекцій, спричинених грампозитивними мікроорганізмами, стійкими до різних лікарських засобів. ГПА пов'язані з ліподепсипептидами (ЛДП), ще одним важливим класом антибіотиків, здатних зв'язувати ліпід II. Основними сполуками, що належать до групи ЛДП, є рамопланін (ключовий представник), ендурацидин і черсинаміцин. Для цих антибіотиків відомі кластери біосинтетичних генів (КБГ), що кодують їхній біосинтез. Нещодавно було описано п'ять додаткових КБГ, які кодують імовірні ЛДП. КБГ ЛДП недостатньо досліджені; одним із аспектів, які потребують подальшого вивчення, є білки-транспортери, закодовані в межах цих КБГ. Ці білки, скоріш за все, беруть участь в експорті антибіотиків із клітини, а також у забезпеченні стійкості продуцента до власного вторинного метаболіту. У цій роботі ми провели *in silico* аналіз генів, які кодують транспортери, в межах КБГ рамопланіну й інших ЛДП. Ми дослідили доменну архітектуру цих транспортних білків, виявили їхні гомологи в КБГ, депоновані у репозиторії MIBiG та за його межами, створили моделі вторинних і третинних структур, і порівняли розташування транспортних генів у КБГ ЛДП. Нам вдалося ідентифікувати раніше неохарактеризований ген, що кодує ABC-транспортер у КБГ рамопланіну – *ramo3*. Ramo1 і Ramo3 у КБГ рамопланіну є паралогами, що кодують пермеазну субодиницю ABC-транспортера. У всіх інших КБГ ЛДП, окрім КБГ черсинаміцину, ми знайшли тільки один відповідний гомолог, який кодує цей тип білка. Подібним чином ми виявили, що Ramo2 і Ramo23 також є гомологічними білками, які, найімовірніше, є АТФ-зв'язуючими субодиницями ABC-транспортера; Ramo2 і Ramo23 мають лише по одному гомологу в інших КБГ ЛДП. Ми описали Ramo8 як АТФ-зв'язуючий ABC-транспортер, що містить як АТФазну, так і трансмембранну частини, і виявляє схожість до транспортерів, що кодуються в КБГ ГПА. Для Ramo8 ми змоделювали третинну структуру мономера, а також четвертинну структуру гомодимера цього білка. Також аналіз *in silico* виявив, що Ramo31 є протонним мембранним антипортером, віддалений гомолог якого закодований лише в КБГ черсинаміцину та, скоріш за все, цей білок не пов'язаний із біосинтезом рамопланіну.

Ключові слова: кластери біосинтетичних генів, рамопланін, мембранні транспортні білки, вторинні метаболіти, ґрунтові мікроорганізми