In silico study of the polymyxin resistance in the genomes of Pseudomonas aeruginosa

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Abstract: In recent years, the clinical and scientific interest in antibiotics known as polymyxin has increased greatly due to the large number of reports of multiresistant Gram-negative bacteria, among them *P. aeruginosa*. This work aimed to investigate proteins responsible for resistance to polymyxins encoded in *P. aeruginosa* genomes using in silico tools. To do so, *E. coli MCR1* protein was used as bait. Sequences with similarities to *MCR1* encoded in *P. aeruginosa* were determined. These proteins are between 465 and 521 amino acids in length. Molecular masses between 52.06 - 57.58 kDa, isoelectric point between 5.83 to 8.06, instability index between 60.33 to 66.42, aliphatic index between 99.980 to 107.39 and the hydropathy index between -0.038 to 0.037. These proteins belong to the DUF1705 superfamily with a Bit-score between 592,806 and 608,599. In conclusion the results evidenced the high degree of similarity between EpTAs including amino acids number, molecular mass, isoelectric point, instability index, aliphatic and hydrophobicity index, as well as secondary structures and protein domain with other proteins that confer resistance to polymyxins present in Gram-negative bacterial species of clinical interest. However, further studies are needed to identify the actual contribution of *EptAs* in *P. aeruginosa* species.

Keywords: Computational Biology; Bacterial Resistance; Gram-negative bacteria

Introduction

The clinical and scientific interest in antibiotics known as polymyxin has increased greatly due to the large number of reports of multiresistant Gram-negative bacteria.

Among the hypotheses about the mechanism of resistance acquired by the P. aeruginosa microorganism, the polymyxins are: (I) adaptive mechanism, being a direct consequence of the gradual adaptation to the presence of this antimicrobial associated with the culture medium used in clinical [1] or experimental diagnosis. Another possibility is interference during active transport through the membrane especially in the lipidic portion A of the endotoxin present in the lipopolysaccharide composition [2], resulting in loss of OMPs (*Outer membrane proteins*) or a reduction in interaction between polymyxin and envelope [3]; (II) genetic mutation mechanism, is associated to the increase of H1 (*H1- T6SS*) protein levels, cation substitution minimizing the Mg^{+2} concentration and increasing that of Ca^{+2} present in the membrane, reducing possible electrostatic interactions with polymyxin [4].

In 2010, it was identified that *H1-T6SS* in *P. aeruginosa* has three effector proteins, namely *Tse1-3* (type VI secretion exporters 1-3), where *Tse1* and *Tse3* have the ability to cleave peptideoglycan associated with the bacterial envelope [4], and this function of *H1-T6SS* is directly linked to antibiotic resistance in biofilms [5].

Other possible causes of resistance to polymyxins may be the induction of the *pmrCAB* operon. These genes comprise a three-component system: response regulator (*pmrA*), histidine kinase sensor (*pmrB*), and the protein that adds phosphoethanolamine to lipid A (*pmrC*) encoding the protein that adds phosphoethanolamine to lipid A. Thus, resistance to polymyxins may be related to increased expression of the *pmrC* gene [6].

In this context, *P. aeruginosa*, a gram-negative bacterium, has attracted a great deal of attention in the last decade due to its capacity to cause serious infections in Brazilian hospitals.

For this reason, this work aims to study the physicochemical properties of EptA proteins expressed in P. aeruginosa genomes deposited at the National Center for Biotechnology Information - NCBI (https://www.ncbi.nlm.nih.gov/) , in order to improve the understanding of the mechanisms of resistance to polymyxins developed by this bacterial species.

Results and Discussion

By performing searches on the Protein Blast server (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for sequences related to the E. coli mcr-1 gene in known genomes belonging to the genus Pseudomonas, 100 protein sequences , 80 species were associated with P. aeruginosa (Table 1).

These proteins are between 465 and 521 amino acids in length. Molecular masses between 52.06 - 57.58 kDa, isoelectric point between 5.83 to 8.06, instability index between 60.33 to 66.42, aliphatic index between 99.980 to 107.39 and the hydropathy index between -0.038 to 0.037. These proteins belong to the DUF1705 superfamily with a Bit-score between 592,806 and 608,599. In conclusion the results evidenced the high degree of similarity between EpTAs including amino acids number, molecular mass, isoelectric point, instability index, aliphatic and hydrophobicity index, as well as secondary structures and protein domain with other proteins that confer resistance to polymyxins present in Gram-negative bacterial species of clinical interest (Table 2 and 3).

Considering the cladogram, it is possible to identify the mode of acquisition of antimicrobial resistance polymyxins. *N. meningitidis* appears to be the species that initially developed resistance to polymyxins, followed by enterobacteria *K. pneumoniae, E. coli* and *S. enterica*, and then by *S. plymuthica* bacteria. Finally, *P. aeruginosa* through *EtpA* proteins began to resist the action of polymyxins (fig.1).

Conclusions

In conclusion the results evidenced the high degree of similarity between the *EptAs* proteins of *P. aeruginosa* and the *E. coli MCR-1* proteins, *MCR 1.9* of *K. pneumoniae* and the *EptAs* of *S. enterica, N. meningitidis* and *S. plymuthica*, based on their physicochemical properties, including composition and number of amino acids, molecular mass, isoelectric point, instability, aliphatic and hydrophobicity indexes, as well as the secondary structures and protein domain shown by these molecules.

However, further studies are needed to identify the actual biochemical contribution of *EptAs* in *P. aeruginosa* species.

Table 1 - List of genomes of the Gram-negative bacterium Pseudomonas aeruginosa deposited at the National Center for Biotechnology Information - NCBI (https://www.ncbi.nlm.nih.gov/).

Linhagem de *Pseudomonas aeruginosa*	
P. aeruginosa (*taxid*:287)	*P. aeruginosa* OS42 (*taxid*:1402581)
P. aeruginosa group (*taxid*:136841)	*P. aeruginosa* BWHPSA028 (*taxid*:1402528)
P. aeruginosa PAO1 (*taxid*:208964)	*P. aeruginosa* BL12 (*taxid*:1402553)
P. aeruginosa PAK (*taxid*:1009714)	*P. aeruginosa* PA7 (*taxid*:381754)
P. aeruginosa DSM 50071 (*taxid*:1123015)	*P. aeruginosa* BL04 (*taxid*:1402545)
P. aeruginosa E2 (*taxid*:1163395)	*P. aeruginosa* ATCC 25324 (*taxid*:1163393)
P. aeruginosa str. PA 17 (*taxid*:1333546)	*P. aeruginosa* VRFPA07 (*taxid*:1431713)
P. aeruginosa PA38182 (*taxid*:1407059)	*P. aeruginosa* UCBPP-PA14 (*taxid*:208963)
P. aeruginosa str. Stone 130 (*taxid*:1125697)	*P. aeruginosa* BWHPSA037 (*taxid*:1402529)

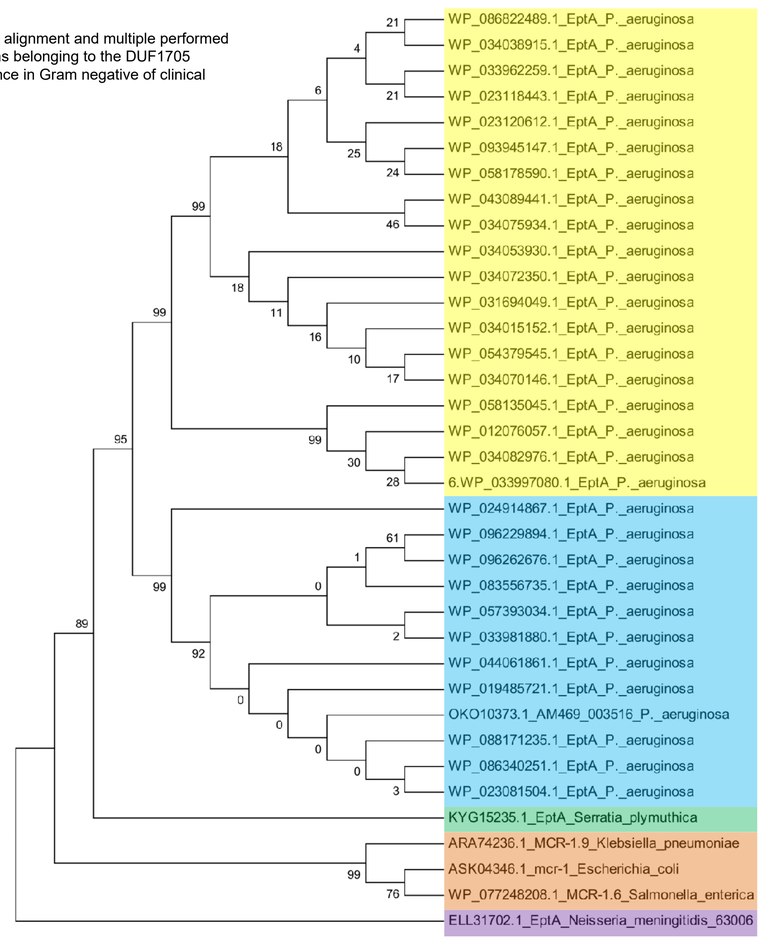
Table 2 - Classification of P. aeruginosa EptA isoforms based on their Aliphatic Index

Número de acesso	IA	GRAVY	Número de acesso	IA	GRAVY
WP_086340251,1	99,98	0,060	WP_093945147,1	104,94	0,075
WP_024914867,1	100,17	0,052	WP_034038915,1	105,14	0,067
WP_083556735,1	100,57	0,039	WP_034053930,1	105,33	0,083
WP_096229894,1	100,60	0,043	WP_043089441,1	105,50	0,065
WP_057393034,1	100,60	0,051	WP_034075934,1	105,50	0,071
WP_023081504,1	100,60	0,050	WP_023118443,1	105,50	0,072
WP_096262676,1	100,60	0,050	WP_054379545,1	105,52	0,082
WP_033981880,1	100,82	0,049	WP_058178590,1	105,52	0,082
OKO10373,1	100,82	0,049	WP_031694049,1	105,52	0,082
WP_019485721,1	100,82	0,049	WP_033962259,1	105,69	0,076
WP_044061861,1	101,01	0,045	WP_034072350,1	105,71	0,091
WP_088171235,1	101,44	0,038	WP_034070146,1	105,71	0,082
WP_058135045,1	102,48	0,037	WP_023120612,1	105,89	0,086
WP_034082976,1	102,67	0,042	WP_034015152,1	107,39	0,113
WP_012076057,1	103,22	0,048	WP_086822489,1	107,39	0,107
WP_033997080,1	103,42	0,052			

Table 3 - Identification of *EptAs* amino acid sequences encoded by the bacterium *P. aeruginosa* based on access from the National Center for Biotechnology Information - NCBI (https://www.ncbi.nlm.nih.gov/).

Identidade	PSSM-ID	Início	Fim	*Bit-score*	Acesso	Superfamilia
WP_012076057.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	520	608.599	cl26815	DUF1705
WP_058135045.1 Fosfoetanolamina transferidora de lipídio A EptA [*P.aeruginosa*]	331636	9	520	606.673	cl26815	DUF1705
WP_034082976.1 Fosfoetanolamina transferidora de lipídio A EptA [*P.aeruginosa*]	331636	9	520	594.732	cl26815	DUF1705
WP_033997080.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	520	594.346	cl26815	DUF1705
WP_034015152.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	479	551.204	cl26815	DUF1705
WP_044061861.1 Fosfoetanolamina transferase [*P.aeruginosa*]	331636	1	465	560.834	cl26815	DUF1705
WP_083556735.1 Fosfoetanolamina transferidora de lipídio A [*P. aeruginosa*]	331636	1	471	562.76	cl26815	DUF1705
WP_096229894.1 Fosfoetanolamina transferidora de lipídio A [*P. aeruginosa*]	331636	1	465	558.523	cl26815	DUF1705
WP_086340251.1 Fosfoetanolamina transferidora de lipídio A [*P. aeruginosa*]	331636	1	465	558.908	cl26815	DUF1705
WP_088171235.1 Fosfoetanolamina transferidora de lipídio A [*P. aeruginosa*]	331636	1	465	560.064	cl26815	DUF1705
WP_086822489.1 Fosfoetanolamina transferidora de lipídio A [*P. aeruginosa*]	331636	9	479	537.722	cl26815	DUF1705
WP_057393034.1 Fosfoetanolamina transferase [*P. aeruginosa*]	331636	1	465	559.293	cl26815	DUF1705
WP_033981880.1 Fosfoetanolamina transferase [*P. aeruginosa*]	331636	1	465	558.523	cl26815	DUF1705
OKO10373.1 Proteína hipotética AM469_003516 [*P. aeruginosa*]	331636	1	465	558.138	cl26815	DUF1705
WP_019485721.1 Fosfoetanolamina transferase [*P. aeruginosa*]	331636	1	465	560.064	cl26815	DUF1705
WP_034053930.1 Fosfoetanolamina transferidora de lipídio A EptA [*P.aeruginosa*]	331636	9	518	591.65	cl26815	DUF1705
WP_043089441.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	518	593.576	cl26815	DUF1705
WP_034075934.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	518	593.576	cl26815	DUF1705
WP_033962259.1 Fosfoetanolamina transferidora de lipídio A EptA [*P.aeruginosa*]	331636	9	518	593.961	cl26815	DUF1705
WP_023081504.1 Fosfoetanolamina transferase [*P. aeruginosa*]	331636	1	465	559.678	cl26815	DUF1705
WP_054379545.1 Fosfoetanolamina transferidora de lipídio A EptA [*P.aeruginosa*]	331636	9	518	593.576	cl26815	DUF1705
WP_034038915.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	518	593.191	cl26815	DUF1705
WP_034072350.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	518	596.658	cl26815	DUF1705
WP_034070146.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	518	591.65	cl26815	DUF1705
WP_093945147.1 Fosfoetanolamina transferidora de lipídio A [*P. aeruginosa*]	331636	9	518	593.961	cl26815	DUF1705
WP_024914867.1 Fosfoetanolamina transferase [*P.aeruginosa*]	331636	1	465	561.219	cl26815	DUF1705
WP_023120612.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	518	593.191	cl26815	DUF1705
WP_096262676.1 Fosfoetanolamina transferidora de lipídio A [*P. aeruginosa*]	331636	1	465	559.678	cl26815	DUF1705
WP_058178590.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	518	595.117	cl26815	DUF1705
WP_023118443.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	518	593.961	cl26815	DUF1705
WP_031694049.1 Fosfoetanolamina transferidora de lipídio A EptA [*P. aeruginosa*]	331636	9	518	592.806	cl26815	DUF1705

Figure 1 - Cladogram generated by the Neighbor-Joining method employed alignment and multiple performed with Molecular Evolutionary Genetics Analysis software - MEGA 6.0. Proteins belonging to the DUF1705 superfamily of P. aeruginosa and proteins responsible for polymyxin resistance in Gram negative of clinical interest. Node values = bootstrap test (1,000 pseudo replicas).



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