Soluble Antimicrobial Peptide Pyocin of *Pseudomonas aeruginosa* and its Therapeutics: A Review Article

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Abstract

Pyocinsis a bacteriocin produced by a group of Gram-ngative bacteria that belongs to *Pseudomonas* species. Pyocin is classified as a two distinct families of pyocins. (i) S-type pyocins (colicin-like bacteriocins), (ii) Tailocins (high-molecular weight bacteriocins that resemble phage tails).The structure of S-type pyocin is similar to that of colicin except that many S-type pyocins have three domains. Under normal conditions, the expression of prtN is repressed by PrtR. Upon exposure to stress conditions, such as DNA damage by chemicals or ultraviolet irradiation, an activated RecA triggers autoproteolytic cleavage of PrtR, which abrogates prtN repression and leads to pyocinproduction. The outer membrane receptors for three pyocins have been identified. These are FpvAI, FpvAII and FptA, all of which are involved in the uptake of iron-siderophore complexes. Before being translocated through the membrane and killing their target.This is becoming increasingly important as microbial imbalances in the natural gut flora have been suggested to play a role in a range of chronic diseases such as inflammatory bowel disease, diabetes, obesity and rheumatoid arthritis.Pyocin has now been shown to have antimicrobial activity againstbacteria in a biofilm is a limiting factor in the successful treatment of a range of chronic infections.

***Key Words**: P. aeruginosa, Bacteriocin, S-type pyocin , Structure, Genetics, therapeutics.*

Pyocin

Pyocins are ribosomally synthesized bacteriocins that appear to comprise of a heterogeneous group of substances ranging in size from a small low molecular weight protein to a high molecular weight protein with complicated structure and composition, but the part responsible for killing activity seems to be protein invariably[1].

P. aeruginosa is the quintessence of microbiological arms depot, living in all settings from aquatic to terrestrial, from dirt to distilled water, from plants to people. To protect itself against other fungus and distantly related bacteria, it creates a wide spectrum of secondary metabolites[2]. In order to compete with other Pseudomonads and closely related bacterial species for shared habitats [3], *P. aeruginosa*

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produces a wide spectrum of bacteriocins known as pyocins, which are produced by all strains.*P. aeruginosa* produces two distinct families of pyocins. (i) S-type pyocins (colicin-like bacteriocins), (ii) Tailocins (high-molecular weight bacteriocins that resemble phage tails) [4].

The differ by their morphology and mode of killing. Their bactericidal activities are strain specific and have been used as a typing tool for *P. aeruginosa* strains, along with other typing schemes such as serotyping and phage typing[5],secreted by over 90% of *P.aeruginosa* strains [6]. Which are used by bacteria to compete for resources by killing competitors, usually of the same bacterial species [7], Which have shown action against embryonal ovarian cancer, human hepatocellular carcinoma, and cervical adenocarcinoma[8].

Several authors study pyocins, characterized of the diversity of R- and F- pyocins and bacteriophages generated by diverse *P. aeruginosa* strains so as to identify pyocins of therapeutic value [9],Structure and Analysis of R1 and R2 Pyocin Receptor-Binding Fibers[10]. Susceptibility to R-pyocins of Pseudomonas aeruginosa clinical isolates from cystic fibrosis patients[11,12] suggest that the combined effects of dispersal limitation among sites and competitive exclusion within them maintain diversity in pyocin inhibition and susceptibility phenotypes, and that additional processes such as local adaptation and effects of phylogenetic distance could further contribute to spatial variability. Pyocins produced no adverse effects when injected alone into mice and showed good in vitro antipseudomonal activity. In an invertebrate model of sepsis using *Galleria mellonella*, both pyocins significantly prolonged survival[13].Role pyocin in protecting *p.aeruginosa* and their resistance to antibiotics[14].

History of pyocins

In 1952, researchers in Paris, Jacob discovered bacteriocin normally produced by *P.pyocyanea (the alternative species epithet for Pseudomonas aeruginosa)*. Japanese teamin 1960 found pyocin of the type [15]. In the late pyocin R type describe produced by *P. aeruginosa* strain R [16].

Finally, a third type of pyocin was described in 1970, called S [17].It has been suggested by some researchers that the gene coding for pyocin R2 originated from a common ancestor of phage P2 and pyocin F2 originated from of phage lambda inserted the bacteria's chromosome via transduction [18].The S type (soluble) pyocins, including S1, S2, S3, and AP41, possess the structures and the modes of action similar to those of colicins, bacteriocins produced by *Escherichia coli* [19].

Types of Pyocins

The majority of findings related to natural pyocin synthesis have come from research of *P. aeruginosa* strain PAO1. It is possible to classify (on the basis of their structure and mode of action). A single strain of P. aeruginosa, on the other hand, can create many types of pyocin at the same time. More than 90% of *P.aeruginosa* strains generate R- and F-type pyocins, while 70% of *P.aeruginosa* strains may create at least one S-type pyocin. Pyocins of the R/F type have a large molecular weight. Pyocins of the S-type have a lower molecular weight[20].

R-type (rod-like) is a protein particle which morphologically resembles a bacteriophage of the Myoviridae family, the particle is composed of a core, sheath, baseplate and tail fibers. They induce a depolarization of the cytoplasmic membrane in relation with pore formation[22] .

F-type (flexible and non-contractible) pyocins also resemble phage tails, but with a flexible and non-contractile rod-like structure. R- and F-type pyocins are particles evolutionary related to bacteriophage tails, hence representing [23].

S-type (soluble) pyocins are Colicin-like bacteriocins (CLBs) protease-and heat-sensitive[24].

More recently, a fourth type of pyocin, the **M-type**pyocins are lipid II-degrading bacteriocins that share homology with colicin M [25], and fifth typeThe lectin-like bacteriocins**(L-pyocins; Llb)** are comprised of one or two monocot mannose-binding lectin domains (MMBL) and may kill at the OM surface by blocking the function of BamA, a protein of the β-barrel assembly machinery[25].[26]Discovered a new pyocin called **pyocin G** (PyoG),that PyoG has broad killing activity against a collection of clinical *P. aeruginosa* isolates and is active in a *Galleria mellonella* infection model.

Pyocins S

One class of molecule that readily translocates across the impervious outer membrane of *P. aeruginosa* to deliver a cytotoxin is the S-type pyocins, which are 40- to 90-kDa protein bacteriocins made by *P. aeruginosa*[27]. These antibacterial are secreted as binary protein complexes consisting of a large protein that harbors the killing function and a smaller immunity protein that remains tightly bound to the cytotoxic domain of the former[28].

Structure of pyocins S

The structure of S-type pyocin is similar to that of colicin except that many S-type pyocins have three domains [29] domain I is N-terminal that recognizes the cell surface receptor, domain II has unknown function and domain III translocate and penetrates pyocin, C terminal domain carries out the killing activity [30].

One pyocin structure has been solved to date. The crystal structure of pyocin M shares structural similarities with colicin M, namely a short N-terminal T-domain, followed by a central globular -helical R-domain anda C-domain incorporating a half-barrel fold[18]. Early characterization of pyocins S2 and AP41, using analytical ultracentrifugation and gel filtration to estimate their molecular weights and shapes, suggested that these pyocins have elongated structures like those of colicins E3 and Ia. Like colicins, pyocins are predict be structurally diverse. Therefore, further structural characterization of pyocins would be extremely useful[31].

Pyocins Stranslocation

Pyocins are translocated across the outer membrane. The exact mechanism remains unknown though the use of the ferrisiderophore receptors suggests that S-type pyocins are translocated in a similar way as the pyoverdines and pyochelin, energized by the TonB system [32].

Little is known about the translocation of pyocins into sensitive cells. Pyocin AP41 is predicted to utilise the Tol system[33], similar to many colicins, as introduction of the tolQRA genes in a pyocin AP41 tolerant mutant restored killing by AP41. Pyocins S2, S3, S4 and S5 are predicted to utilise the TonB system for translocation due to their binding to TBDTs. However, colicins A and E1-E9 bind the TBDT BtuB and use the Tol system for translocation, so this assumption may not be valid[34].

PyocinsS receptors

The outer membrane receptors for three pyocins have been identified. These are FpvAI, FpvAII and FptA, all of which are involved in the uptake of iron-siderophore complexes [35]. [36]Before being translocated through the membrane and killing their target. the order of the receptor recognition domain and translocation domain is generally reversed: the N-terminal domain is involved in recognition of the cell surface receptor[10], domain II (not present in pyocin S1) has an unknown function and is dispensable for killing activity, the third domain is responsible for pyocin translocation and penetration,and the C-terminal domain carries the lethal activity[36].

Pyocin S killer protein

Soluble pyocins have an N-terminal receptor binding domain, a translocation domain, and a C-terminal killing domain[37]. The majority of S-type pyocins (AP41, S1, S2, and S3) cause cell death by DNA breakdown due to an endonuclease C-terminal domain [35], while pyocin S4 is predicted to have tRNase activityand S5 is predicted to have pore-forming activity[38].This motif constitutes the core of the catalytic site of the endonuclease and can chelate a single metal ion, required for hydrolysis of the dsDNA strand[39].

Self-Immunity of pyocins S type

This entry represents the DNase domain found in some colicin/pyocinbacteriocins, including colicin E2, E7, E9 and pyocin S1 and S2. In colicin E7, this domain has been described as a novel alpha/beta fold containing a Zn2+ ion binding site [40].Bacteriocin production can be lethal to the producer strain if specific protection mechanisms are not employed, leading to the employment of self-immunity mechanisms [41]. Immunity proteins from pyocins S1, S2 and AP41 share homology (approximately 44% to 99%) as they protect homologous H-N-H nuclease domains, whereas the pyocin S3 immunity protein shares little homology. Pyocins S1 and S6 are almost identical in their R- and T-domains, with their C-domains differing to alter nuclease activity[26]. The other RNasepyocin is pyocin S4, which has an almost identical R-domain to pyocin S2. The C-domain of pyocin S4 is similar to the tRNasecolicin E5 (31% amino acid identity), while sharing little homology with the tRNasecolicin D1. The only pore-forming pyocin identified to date is pyocin S5, which shares greater than 75% amino acid identity between amino acids 217-307 with the unknown domain of pyocin S2 (amino acids 207- 312) and less than 30% homology in the other domains. The C-domain shares 30% amino acid identity with that of colicinIa and is predicted to be structurally homologous to colicinsIa, B, S4 and N [42]. Pyocin S5, like the pore-forming colicins, does not form a complex with its cognate immunity protein prior to release [43]. The putative S immunity (SI) protein confers resistance to pyocin S in *P. aeruginosa*. Figure(1) show that SI prevents cellular lysis in cells expressing SI protein. This pave the possibility of learning more about its involvement in cellular adaptability

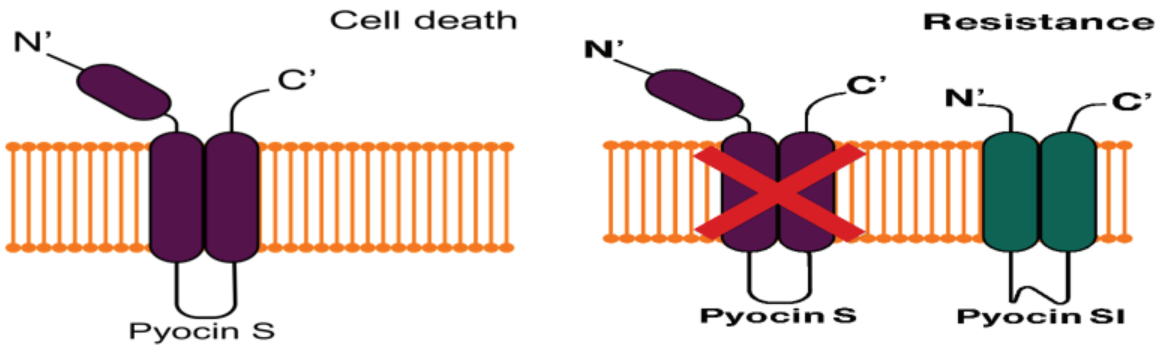

Figure 2.SI immunity protein resistance to pyocin S. Adapted from [44].

Regulation of pyocins synthesis

Pyocin production is highly energy cost and the release of pyocin is through cell lysis[45]. The synthesis of pyocin involves genes recA, prtR and prtN and proteins RecA, PrtR and PrtN. The gene recA encodes a protein

RecA that is responsible for the repair of damaged DNA, gene prtR encodes protein PrtR which is a repressor of gene expression and gene prtN encodes a protein PrtN which is an activator[46]. During normal conditions or in the absence of a mutagen, the recA gene produces very small amounts of RecA protein [47]. PtrN is a transcriptional activator that binds to the P boxes located approximately 60-100 bp upstream of the ribosome-binding site.The P-box consists of a10-12 nt consensus sequence - ATTGnn(n)GT-nn(n). PtrR is a transcriptional repressor protein, which binds to PtrN, preventing its binding to the P-box. Under conditions of stress, such as DNA damage RecA cleaves PtrR, releasing PtrN. The binding of PtrN to the P box induces transcription of pyocin genes[48].

Genetics of PyocinsS

The genes for bacteriocins are encoded on chromosomes, plasmids, and/or mobile elements such as transposons. Pyocins were reported to be chromosomally located [49]. Bacteriocins genes encoding are often found in clusters, which include a toxin, immunity, and lysis genes. The immunity gene produces a protein that protects against the toxin, while the lysis gene produces a protein that helps in the removal of the toxin from the cell, which also results in cell death[50].The immunity protein and killing proteins are co-transcribed at a similar rate to prevent DNA degradation within the producing cell.

P.aeruginosa PAO1 produces multiple S-type pyocins, which contains S2, S4, and S5, containing only a short region with high homology to pyocin S2, in addition to R2 and F2[51]. S-type pyocin loci are found scattered in the genome, three loci encode S-type pyocin complexes: PA1150-1151 (pyocin S2 and immunity gene), pyocin S4 (PA3866 plus non-annotated immunity gene), and PAO984-0985 (immunity gene and pyocin S5 gene). unlike the tail-like pyocin loci that are always often present between trpE (PA0609) and trpG (PA0649) on the chromosome[52].

The typical S-pyocin operon spans an approximately 2 kb region of DNA and includes two genes: the toxin gene and the immunity gene. Operon of pyocins S contains two open reading frames (ORF). The first ORF encodes large protein, while the small component is encoded by the second ORF [53].

Pyocins S as therapeutics

Bacteriocins from Gram-negative bacteria, and the pyocins of *P. aeruginosa* in particular are suited to therapeutic development for a number of reasons. They are a source of readymade antibiotics that are extremely potent (as low as pM affinity) and are amenable to protein engineering[54]. The modular composition of pyocins means that chimeric pyocin proteins can be constructed that contain R-, T- and C-domains from different pyocins, broadening the number of therapeutic candidates. This technique was used with pyocins S2 (T- and C-domains) and S5 (central R-domain) to identify the R-domain of pyocin S5 and was also demonstrated with pyocins S1 and AP41 [55]. The modular composition and conserved toxin activity of pyocins and colicins means that active pyocin/colicin chimeras can also be constructed [56]. This is becoming increasingly important as microbial imbalances in the natural gut flora have been suggested to play a role in a range of chronic diseases such as inflammatory bowel disease, diabetes, obesity and rheumatoid arthritis. The inability of antibiotics to kill bacteria in a biofilm is a limiting factor in the successful treatment of a range of chronic infections, including *P. aeruginosa* infection in the CF lung [57].

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