

# Pyocin S2 Conjugates for the Treatment of PyoS2-Resistant MDR Clinical Isolates of *Pseudomonas aeruginosa*.

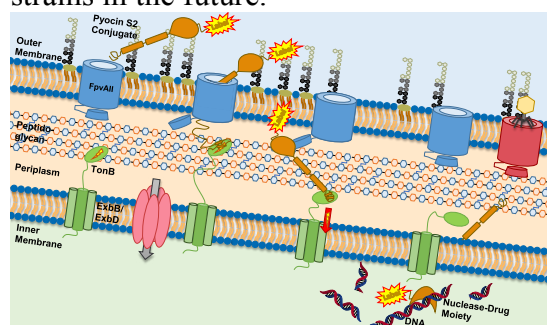
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The opportunistic human pathogen *Pseudomonas aeruginosa* is a Gram-negative bacterium that can be isolated from environmental sources, but is of great concern when found in a clinical context.[1] Widespread multidrug resistance (MDR) mechanisms against common antibiotics, reserve group antibiotics, and environmental toxins make *P. aeruginosa* difficult to treat and prompted the World Health Organization (WHO) in 2017 to list *P. aeruginosa* with priority 1 (“critical”) on a list of pathogens, for which new antibiotics or promising alternatives are urgently needed.[2] An often mentioned potential alternative are so-called bacteriocins; however, bacteriocins of Gram-negative bacteria are lacking proof of applicability so far.[3] Bacteriocins are toxic multi-domain proteins and are found in most bacterial Gram-negative and Gram-positive species. Other than most small molecule antibiotics, bacteriocins are narrow band antibiotics, mostly acting on closely related strains of the same genus and are evolutionary relevant for the fight for ecological niches. Selectivity and host specificity rely on a conserved uptake mechanism, which involves the outer membrane protein machinery of the bacterial target cell. To date, several bacteriocins have been found in *P. aeruginosa* □ called pyocins □ that kill other strains of *P. aeruginosa*. The producing strain itself is protected from the pore-forming or nuclease killing activity of pyocins by co-expression of immunity proteins that confer resistance to this particular strain.[4] Here we present preliminary proof that resistance against the DNase pyocin S2 (PyoS2) can be overcome by partial blockage of the interaction site of the PyoS2 cytotoxic domain and its cognate immunity protein ImS2. *In vitro* labeled toxin with a non-toxic fluorophore label was shown to kill the PyoS2-producing *P. aeruginosa* PAO1 and several PyoS2-resistant MDR clinical isolates of *P. aeruginosa*. We believe that our findings might contribute to bringing bacteriocins of Gram-negative strains closer to application as potential substitutes for antibiotics or as extension of our common antibiotics portfolio for treatment of clinically relevant MDR strains in the future.



**Fig. 1:** Schematic depiction of the translocation mechanism of pyocin S2 and its conjugates across the bacterial membrane of *Pseudomonas aeruginosa*. The outer membrane porin FpvAII serves as primary receptor and is hijacked by PyoS2 derivatives, the inner membrane TonB machinery energizes the import into the periplasm. Protease-assisted cleavage of pyoS2 leads to translocation into the cytoplasm, where the C-terminal cytotoxic domain of PyoS2 (DNase activity) causes cell death by degradation of genomic information.

## References

[1] Wolfgang, M. C. *et al.*, *PNAS* **2003**, *100*(14), 8484–8489.

[2] <https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed>

[3] Chikindas, M. L. *et al.*, *Curr. Opin. Biotechnol.* **2018**, *49*, 23–28.

[4] Michel-Briand, Y., & Baysse, C., *Biochimie* **2002**, *84*(5–6), 499–510.