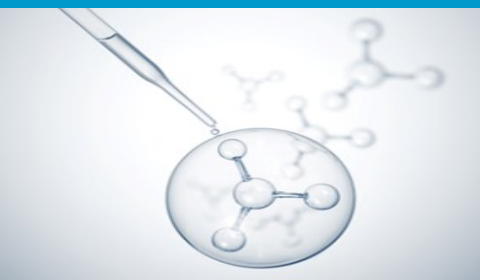


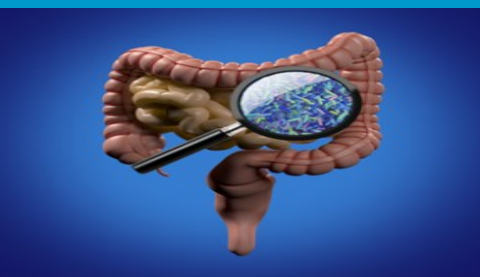
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COMPETITIVE STUDIES OF FOUR MICROCOCCIN P1 PRODUCER STAPHYLOCOCCAL STRAINS AGAINST A METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAIN

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Introduction: Bacteria live in communities with complex interactions both including mutualistic and competitive dynamics. Bacteriocins are antimicrobial peptides produced and secreted in natural microbial communities and they have an important role in niche competition¹.

Objective: In this respect, the main objective of this work was to evaluate the potential use of four commensal bacteriocin-producer (BP) staphylococcal strains against a multidrug-resistant (MDR) relevant pathogen, a methicillin-resistant *Staphylococcus aureus* (MRSA, CC398) strain.

Methodology: Four BP staphylococcal strains from different species and origins (one *S. aureus*-water, one *S. hominis*-water, and two *S. sciuri*-meat derived food) were selected from a previous study² for competition studies. The bacteriocins produced by these strains were determined by mass-spectrometry and *whole-genome-sequencing* (WGS). Competition studies were carried out with the BP strains (clindamycin-susceptible) against a MDR, MRSA-indicator strain (clindamycin-resistant); non-BP strains (of the same species) were used as negative controls. Fresh culture of competitors adjusted to 1·10⁸ CFU/mL were mixed at 1:1 ratio and 10µL was spotted in triplicate on basic medium (BM) agar. Samples were taken at 0h, 24h, 48h, and 72h and serial dilutions were plated on BM with/without clindamycin for the indicator selection. Bacterial ratios of MRSA and the respective competitor BP strains were calculated.

Results and discussion: The presence of micrococcin P1 was identified in the four BP strains by mass-spectrometry and WGS. Nevertheless, differences were observed in the four bacteriocin-gene-clusters, even in the structural gene. The two *S. sciuri* BP strains showed high inhibition against the MRSA-indicator strain at 24h of competition (>99% of BP-grown) and this effect was maintained at 48h and 72h. The BP *S. aureus* strain (methicillin-susceptible/CC130) also inhibited the MRSA-indicator strain at 24, 48 and 72h (BP-growth: 89%, 73%, 100%, respectively), although the kill effect was lower than for *S. sciuri* BP strains. Relevant inhibition effect was only shown after 48h for the *S. hominis* BP strain (52% of BP at 72h). Competition assays carried out with non-BP strains, revealed a prevalence growth of the MRSA-indicator in relation with the BP strains.

Conclusion: Three of the micrococcin P1 producer strains of the *S. sciuri* and *S. aureus* species were able to avoid the growth of the MRSA-indicator strain in competitive studies, being this effect evident at 24h, 48h and 72h of incubation. These preliminary studies indicate the interest of these BP strains as potential modulators to control the growth of MDR bacteria, of great interest in biomedical and food-industry applications.

¹Fedorec, A.J.H., Karkaria, B.D., Sulu, M. et al., Nat Commun. 12:1977, 2021.

²Fernández-Fernández, R., Lozano, C., Eguizábal, P. et al., Front. Microbiol. 13:870510, 2022.