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Microbial Interactions 2019: Assessment of predatory traits of *Bdellovibrio bacteriovorus* and *Agromyces ramosus* on clinical pathogenes *Klebsiella pneumonie*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*- Ahmet Volkan Kurtoglu- Bezmialem Vakıf University

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Introduction: Misuse of antibiotics has caused increase in drug-resistance among bacteria and this forced the researchers to find new strategies and tools to encounter the growing threat of public health at a global scale. And predatory bacteria are one of these options. *Bdellovibrio bacteriovorus* and *Agromyces ramosus* species are two examples of such predatory bacteria with traits proven on several clinical pathogens. When studying resistance patterns, they employed a multi-omics approach to examine colistin resistance mechanisms. Specifically, colistin resistance was induced in an A. *baumannii* clinical isolate and then whole genome sequencing (WGS), transcriptome and real-time quantitative PCR analysis, proteomics analysis, and growth rate studies were performed on the isolate. It was found that the growth rate of the mutant isolate was slower than that of the original strain. In addition, WGS showed the presence of ISAba1 upstream of lpxC in the mutant strain but not in the original isolate. Transcriptome and real-time quantitative PCR analysis revealed that 137 genes showed significant differential expression following induced resistance. Finally, proteomic analysis showed that while the expression of the AdeABC efflux pump was upregulated, certain biochemical pathways were downregulated in the mutant strain as compared to the original isolate

Aims: The aim of the project was to assess the predatory abilities of predator bacteria *Agromyces ramosus* and *Bdellovibrio bacteriovorus*, against clinical pathogens, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, isolated in Bezmialem Clinical Microbiology Laboratories.

Method: Assay for B. *bacteriovorus*: For creating the control group, each pathogen K. i, A. *baumannii* and P. *aeruginosa*, was cultivated one night before, and prepared in falcon tubes at OD (600)=1 in 10 ml HepesCa++Mg++ and incubated for 2 days. Bacteria were counted as CFU on LB agars before and after incubation by serial dilutions. Likewise, one sample of each pathogen was prepared in tubes at OD (600=1) in 10 ml HepesCa++Mg++. Each tube was mixed with 300µlof *Bdellovibrio bacteriovorus* which had been prepared in HepesCa++Mg++ with using E. *coli* as prey. Prepared B. *bacteriovorus* co-cultures also incubated for 2 days. After 2 days, pathogen bacteria numbers were counted as CFU on LB agars before and after incubation by serial dilutions.

Assay for A. *ramosus*: For control group, one sample of each pathogen, K. *pneumoniae*, A. *baumannii* and P. *aeruginosa*, were cultivated in for 24 hr before, and prepared in falcon tubes at McFarland=0.5 in 10 ml Brain Heart Infusion buffer (BHI), and incubated for 2 days. Bacteria were counted as CFU on BHI agars before and after incubation by serial dilutions. Similarly, each pathogen was diluted to McFarland=0.5 in 10 ml BHI buffer and mixed with 2ml A. *ramosus* diluted to McFarland=0.5 in BHI buffer. A. *ramosus* co-cultures were also incubated for 2 days. After 2 days, the pathogens were counted as CFU on BHI agars before and after incubation by serial dilutions.

Result: B. *bacteriovorus* reduced all tested pathogen cell densities significantly ($p<0.05$). A. *ramosus* was significantly effective against *Acinetobacter baumannii* isolates ($p<0.005$). However, no such effect was noticed against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Conclusion: According to results, B. *bacteriovorus* could be considered as a predatory agent against *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* alternative to antibiotics. Further research on the applicability of these predatory agents in actual clinical settings is needed. A. *ramosus* could potentially be a future antimicrobial agent against *Acinetobacter baumannii* infections. Further research is needed to establish the actual conditions of such application. Also, the reason behind the lack of observation for the predation of A. ramosus against K. *pneumoniae* and P. *aeruginosa* remains to be established.

Overall, our study demonstrates that predatory bacterias *Bdellovibrio bacteriovorus* and *Agromyces ramosus* species could be one alternative to the antibiotics which are increasingly becoming useless in the war against multidrug resistant bacteria and this study indicates that this option deserves further investigations.

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