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T3-P-19 Defensive secretions of Millipedes *Megaphyllum unilineatum* (C. L. Koch, 1838), *Pachyiulus hungaricus* (Karsch, 1881) and *Cylindroiulus boleti* (C. L. Koch, 1847) (Diplopoda, Julida) as antimicrobial agents in the inhibition of biofilms of *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus*

Jelena Đorđević[70], Jelena Milovanović[72], Bojan Ilić[72], Aleksandra Stevanović[71], Anastasija Malešević[72], Slobodan Makarov[72], Branka Vuković-Gačić[72]

KEYWORDS: biofilm inhibition; antimicrobial agents; millipedes; *Pseudomonas aeruginosa* PAO1; *Staphylococcus aureus*

INTRODUCTION:

In recent years, the emphasis of the scientific community has been placed on the invention of new antimicrobial agents due to the increasing resistance of bacteria to antibiotics. However, serious global health concern is focused on bacterial biofilms, a complex structure of a microbiome made up of colonies of bacteria or individual bacterial cells in a group, attached to a surface. Bacterial biofilms are highly resistant to antimicrobial agents and grow on the surfaces of medical implants such as sutures, catheters, and dental implants. Given that plants and animals are a valuable source of natural biologically active products, they are a good basis for finding new antimicrobial and antibiofilm agents. Bacterial strains of *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* are known for biofilm production and cause opportunistic and chronic infections in humans, some of which are due to biofilm production. Due to their characteristic way of life, millipedes (Diplopoda) are characterized by a diverse and complex defense against predators, which includes the secretion of various chemical compounds that are toxic, repellent, or tasteless to predators. Analyzes have shown that millipedes produce chemical compounds such as phenols, alkaloids, quinones, terpenoids, cyanogenic compounds, and fatty acid esters, which showed antimicrobial activity, among other. Representatives of the order Julida, which are frequent in Republic of Serbia, produce defense secretions that are chemically very complex (the most complex within Diplopoda) and exhibit antimicrobial, antioxidant, and neurodegenerative potential, so they represent a good basis for the invention of new antibiofilm agents.

OBJECTIVES:

Objectives are to determine the inhibition of biofilm formation and degradation of the formed biofilm of *P. aeruginosa* PAO1 and *S. aureus* by defense secretions of selected millipede species from the family Julidae as well as to determine their antimicrobial activity.

METHOD / DESIGN:

Biofilm formation was quantified by the crystal violet staining method, while antimicrobial activity was examined using the broth dilution minimum inhibitory concentration (MIC) test.

RESULTS:

Defensive secretions of *Megaphyllum unilineatum* (MUN), *Pachyiulus hungaricus* (PHU), and *Cylindroiulus boleti* (CBO) showed

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antimicrobial activity against *S. aureus* with MIC values of 0.03, 0.06, and 0.06 mg/mL, respectively. On *P. aeruginosa* PAO1, defense secretions did not show antimicrobial activity even at the highest tested concentration of 1 mg/mL for MUN while for PHU and CBO the MIC was 1 mg/mL, which is most likely due to the high resistance of this bacterial strain. The antibiofilm effect was observed in all tested defense secretions and was more pronounced against *S. aureus* than against *P. aeruginosa* PAO1. The strongest biofilm inhibition of *S. aureus* was at the highest tested concentrations (2 × MIC) with percentages of inhibition of CBO: 88.6%, PHU: 73.7%, and MUN: 67.2%. Degradation of already formed *S. aureus* biofilm was shown at lower tested defensive secretions concentrations (MIC/4), about 40% of biofilm degradation for MUN and PHU and about 30% for CBO. The strongest inhibition of *P. aeruginosa* PAO1 biofilm formation was observed at the highest tested concentrations of defensive secretions, 1 and 0.5 mg/mL for PHU (82 and 54%), and CBO (64.3 and 38.5%) while MUN had the strongest activity at the lowest tested concentration of 0.06 mg/mL (34.3%). All examined defense secretions had similar degradation activity of *P. aeruginosa* PAO1 biofilm with stronger activity at lower tested concentrations (about 30%). Defensive secretions of MUN and PHU extracted in DMSO solvent showed a stronger antibiofilm effect compared to the same ethanol extracts.

CONCLUSIONS:
The defense secretions of MBO, PHU, and CBO show a good basis for further investigations of their use as antimicrobial agents, especially against *S. aureus*.

T3-P-20 Effect of metformin on AMPK/Akt/mTOR pathway against butyrate-resistant colorectal cancer spheroid cells

Kesara Nittayaboon, Surasak Sangkhathat, Raphatphorn Navakanitworakul[73]

KEYWORDS: Metformin; Butyrate-resistant cells; Colorectal cancer; Spheroid cells.

INTRODUCTION: Metformin, the anti-diabetic drug, has been studied as anti-cancer drug in various types of cancer, such as cervical, breast, prostate, and colorectal cancer. The meta-analysis study has been shown that metformin is associated with decreasing cancer incidence and mortality rate. However, there is no study on the effect of metformin on butyrate-resistant colorectal cancer cells. Normally, butyrate is an anti-cancer agent produced by colonic microbiota. However, the microbiota study reveals that *F. nucleatum*, a butyrate producing and inflammatory stimulator bacteria, was increased in colorectal cancer patients. The resistant-to-butyrate cell show a chemotherapy resistant phenotype which related to treatment failure and cancer recurrence.

OBJECTIVES: The aim of this study was evaluated the effect of metformin on butyrate-resistant colorectal cancer spheroid cells.

METHOD / DESIGN: HCT-116, and PMFko-14 colorectal cancer cells were induced with butyrate reagent at the final concentration of 3.2 mM. The resistant properties were determined by cell viability at IC50 of parental and resistant cells. Influx and efflux transporters were evaluated by qRT-PCR. The spheroids of parental and resistant cell were generated, then the effect of metformin was studied. Live/dead and caspase assays were used to examine the inhibitory effect of metformin on spheroid cells. Finally, molecular mechanisms of metformin were investigated by Immunoblotting analysis.

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