THE INFLUENCE OF COLLAGEN/THYMOL MATERIALS ON DEHYDROGENASE ACTIVITY AND ATP LEVEL OF PATHOGENS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 33]

Introduction

The overuse and misuse of antibiotics have led to the emergence of antibiotic-resistant bacteria. Antibiotic-resistant bacterial infections become one of the main worldwide health problems. Because of that, researchers are looking for new, more efficient solutions. Currently, plant-based substances are extensively tested. Thymol is one of the major phenolic compounds naturally occur in essential oil from *Thymus vulgaris* and *Origanum vulgare* [2,3]. Since the Food and Drug Administration recognized thymol as "generally safe", thymol become popular in medical, food and cosmetics field.

Materials and Methods

Materials based on collagen (Coll) with thymol (T) addition (0.25-4 mg thymol concentration) were prepared using the solvent casting method for better miscibility nonionic surfactant (NS) were added. Materials with different concentration of incorporated thymol were cut for 10 mm x 10 mm squares and sterilized using UV light. After sterilization, nutrient broth and cell culture (*Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*) were added to materials and incubated in 37ºC. After 90 min, part of the medium from each well was collected and transferred to sterile 96 well plates, where all next steps of analysis were performed. Dehydrogenase activity test was performed using CellTiter 96 AQ One Solution Cell Proliferation Assay. Absorbance was measured at 490 nm (GloMax Discover, Promega).

ATP level was analyzed using a luminescence BacTiter-Glo™ Microbial Cell Viability Assay. All steps were performed according to manufacturer guides.

Results and Discussion

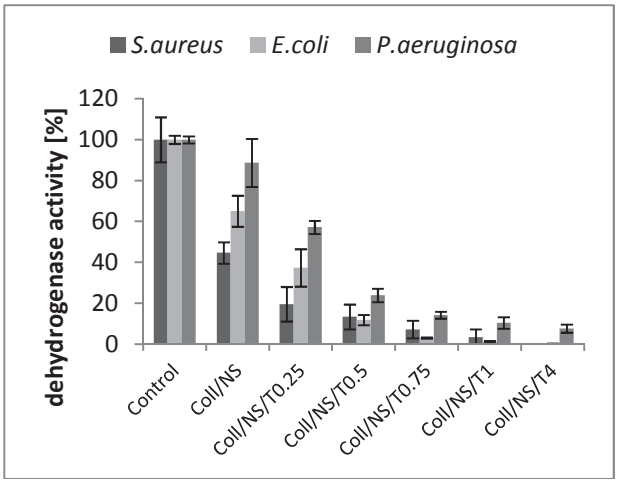

FIG. 1. Dehydrogenase activity of *S. aureus*, *E. coli* and *P. aeruginosa* after contact time with materials.

S. aureus, E. coli, and *P. aeruginosa* were chosen for this study. *S. aureus* is the most common bacteria involved in the initial stage of the infectious process [4]. *E. coli* and *P. aeruginosa* were found in chronic wounds [4]. dehydrogenase activity was inhibited between 11-55% and depends on bacteria strains. Thymol presented into materials, decrease the dehydrogenase activity. The most sensitive for thymol action were *S. aureus* and *E.coli*. Thymol addition in a concentration of 4 mg inhibited all dehydrogenase activity in those strains.

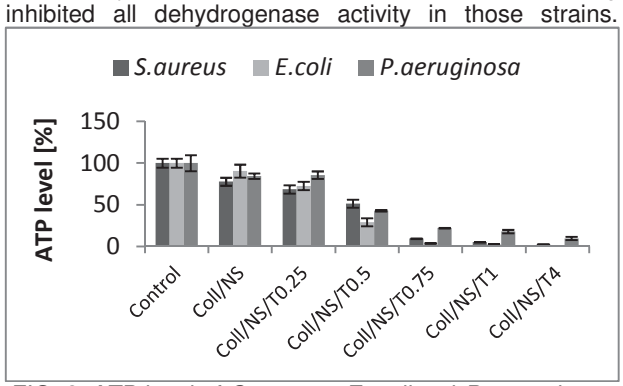

FIG. 2. ATP level of *S. aureus*, *E. coli* and *P. aeruginosa* cells after contact time with materials.

The adenosine triphosphate (ATP) is only produced into cells within phases of metabolic activity, it is not stored and degrades after cell death [5]. The ATP level measurements were collected in the form of relative light units (RLU). During this study, ATP standard curve was not utilized, therefore the ATP luminescent signal (RLU) could not be converted into units of ATP. However, the luminescent signal could still be used as an indication of viable cells being presented. The results are presented as a percentage value of ATP level treating positive control sample as 100%.

ATP is universal energy molecule and cells may generate ATP during oxidative phosphorylation or substrate level phosphorylation [6]. Therefore, dehydrogenase activity and ATP level are related. In our study, the relationship between thymol amount, dehydrogenase and ATP level was similar.

Conclusions

Thymol and some other phenolic compounds, which occurs in essential oils, are known with their ability to interacting with bacterial cells membrane by hydrogen bonds. Resulting in the disruption of cells membrane and causing the leakage of cellular components. Therefore, the inhibition of dehydrogenase activity and ATP level may be related to thymol mechanisms of antimicrobial action.

Acknowledgments

Financial support from the National Science Centre (NCN, Poland) Grant No 2018/31/N/ST8/01509 is gratefully acknowledged.

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