Anti-bacterial Effect of Caffeine on *Escherichia coli* and *Pseudomonas fluorescens*

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The aim of this study was to investigate the direct effect of caffeine on Gram-negative bacteria cultures. For this purpose we have selected *Escherichia coli* as widely distributed in the environment and within human intestines, and *Pseudomonas fluorescens* as commonly found in a wide range of terrestrial and aquatic habitats.

Our investigations showed that caffeine affects the growth of *E. coli* DH5α and *Ps. fluorescens* 5443 bacteria. At high a caffeine concentration (1%) in the culture medium the growth of both bacteria was inhibited. The optical density of *E. coli* and *Ps. fluorescens* cultures decreased 2.7 and 3.1 times ($p < 0.001$), respectively, in comparison with the control samples, where these bacteria were growing in the culture medium without any caffeine. Caffeine concentration decrease influenced the growth of bacteria in a different way. The growth of *E. coli* was inhibited more significantly as compared to *Ps. fluorescens* at caffeine concentrations 0.1-0.5%.

Different concentrations of *E. coli* DH5α and *Ps. fluorescens* grew on a solid medium without or with 1% of caffeine. It was shown that caffeine present in the solid medium suppressed the growth of *E. coli* and *Ps. fluorescens* bacteria 6.4 and 5.3 times ($p < 0.05$), respectively.

Our findings show an antibacterial effect of caffeine. In addition to it, the sensitivity of different bacteria to caffeine can vary greatly depending on caffeine concentration. Further, *E.coli* bacteria are more sensitive to caffeine in comparison to *Ps. fluorescens* bacteria.

Key words: caffeine, *E. coli*, *Ps. fluorescens*

INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) at submillimolar concentrations exerts a wide variety of physiological effects on different organisms (1). Caffeine has long been known to have numerous actions (2), including (*i*) inhibition of phosphodiesterases, thereby increasing intracellular cAMP; (*ii*) direct effects on intracellular calcium concentrations; (*iii*) indirect effects on intracellular calcium concentrations via membrane hyperpolarization; and (*iv*) antagonism of adenosine receptors. Caffeine also influences multiple pathways involved in the cellular response to DNA damage. It reduces DNA damage-induced cell cycle arrested in G_1, S, and G_2/M, abolishing the G_2/M checkpoint by inhibiting ATM (ataxia-telangiectasia-mutated) kinase activity (3, 4). Caffeine potentiates the lethal effects of ionizing radiation, which could be useful for cancer therapy (5). It also plays an important role in the development of immune resistance against bacterial invaders by increasing the concentration of some immunocompetent cells and reinforcing the activity of lysozyme (6, 7). The antimicrobial activity of lysozyme is known to be directed against certain Gram-positive bacteria and to a lesser degree *in vitro* against Gram-negative bacteria (8, 9).

To expand our knowledge about the influence of caffeine on cells, some immune parameters and an-

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timicrobial activity of lysozyme, we decided to investigate the direct effect of caffeine on Gram-negative bacteria culture. For this purpose we have selected *Escherichia coli* DH5α as a model strain frequently used in microbiological experiments, and *Pseudomonas fluorescens* 5443 for comparison and estimation of caffeine effect.

E. coli bacteria are widely distributed in the environment. The presence of *E. coli* and other species of bacteria within our intestines is necessary for us to develop and operate properly and to remain healthy, as along with other species of bacteria, *E. coli* provide us with many necessary vitamins (10). However, some of these different strains of bacteria (there may be several within given species) can be harmful to human (11).

Ps. fluorescens encompasses a diverse group of bacteria that are commonly found in a wide range of terrestrial and aquatic habitats. There are over 200 species of *Pseudomonas* bacteria. Some of them are pathogenic to plants, animals and human (12). However, only three species are known to be pathogenic for man; they are resistant to most antibiotics (13). Their main targets are immunocompromised individuals, burn victims, and individuals on respirators or with indwelling catheters.

The aim of this study was to investigate the direct effect of caffeine on bacteria (*E. coli* and *Ps. fluorescens*). Our finding could be taken into account in medical practice.

MATERIALS AND METHODS

E. coli DH5α and *Ps. fluorescens* 5443 bacteria strains were obtained from the Institute of Biochemistry. For estimation and confirmation of the results, bacteria were grown in a liquid and on a solid medium. Two different estimation methods were used and the experiment were repeated three times. These bacteria were growing in Nutrient Broth (NB) culture medium.

To start the growth, 2 ml of overnight cultured *E. coli* and *Ps. fluorescens* stocks were added to 100 ml of NB medium containing 0.12 % of glucose with 0.01%, 0.1%, 0.5%, 1% or without (in control flask) caffeine (Sigma, St. Louis, USA), respectively. The bacteria were aerobically cultured in shaking flasks closed with cotton stoppers on a rotary shaker at 30 °C for 24 hours. Optical density (OD) measurements at 600 nm were used to monitor the concentration of bacteria.

The same bacteria strains also were growing on a solid NB containing 0.12% of glucose, 2% agar (control plates) medium and on the same solid medium with 1% of caffeine (experimental plates). For this experiment different dilutions of *E. coli* and *Ps. fluorescens* bacteria (10, 100, 1000, 10000, 100000, 1000000) were prepared in physiological solution, and 20 µl of each bacteria solution was poured into control and experimental plates. Bacteria were growing at 30 °C for 48 hours. Afterwards the plates were visually estimated and bacteria colonies counted. The pictures were taken by an Olympus C2020Z digital camera.

The data obtained in all tests were compared with the control. Sigma Plot for Windows (8th demo version) Student's t-test was used for determination of the significance ($p < 0.05$) and evaluation of the experiments.

RESULTS

Our investigations show that caffeine affects *E. coli* DH5α and *Ps. fluorescens* 5443 strains. At a high caffeine concentration in the culture medium (1%) both bacteria were growing at a comparable rate. In the flask with *E. coli,* the optical density decreased 2.7, while in the flask with *Ps. fluorescens* 3.1 times ($p < 0.001$) in comparison with the control. The growth of *Ps. fluorescens* was practically not influenced by lower than 1% caffeine concentrations, the optical density increased at 0.5% caffeine up to 3.54 OD units and remained very similar at lower caffeine concentrations if compared with control media (Fig. 1). At 1% and 0.5% caffeine concentrations, the optical density of *E. coli* was very similar and approximately 2.7 times lower ($p < 0.01$) if compared with control samples. We also observed

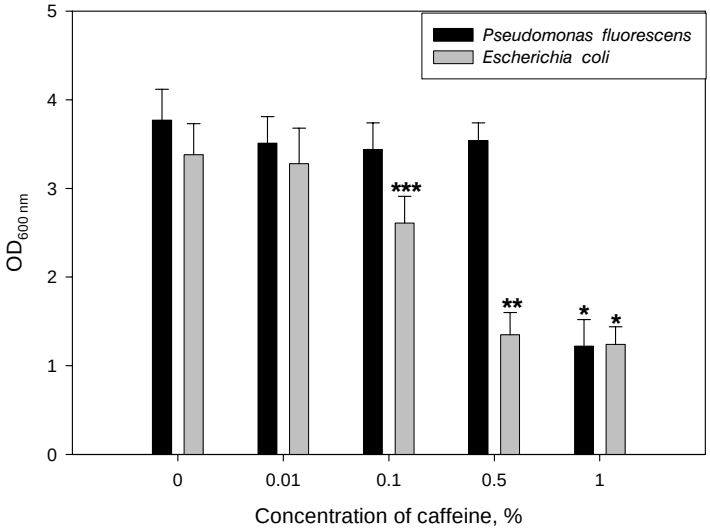


Fig. 1. *E. coli* and *Ps. fluorescens* concentration dependence upon different caffeine concentration in the culture medium. * $p < 0.001$, ** $p < 0.01$, *** $p < 0.05$ compared to the control samples

that the concentration and optical density of *E. coli* bacteria increased if caffeine concentration decreased, and at 0.01% caffeine in culture medium the optical density became similar to the control (Fig. 1).

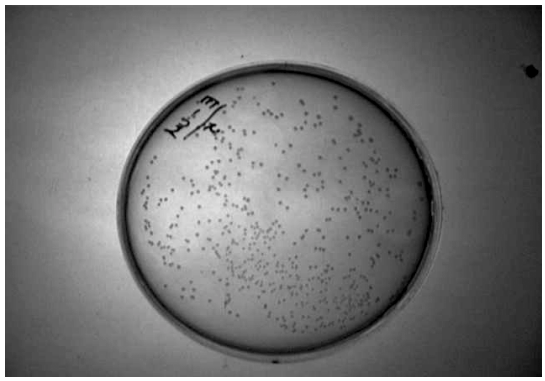
Different amounts of *E. coli* and *Ps. fluorescens* were grown on solid medium without or with 1% of caffeine. The largest and distinct bacteria colony was observed at a 100000 time dilution. The visual estimation and bacteria colony counting were performed at this dilution. In Fig. 2 we can see a smaller amount of *E. coli* bacteria colony on a solid medium with caffeine (plate B) in comparison with the control (plate A). In control plates we calculated 402 ± 75 bacteria colonies and in experimental plates with 1% of caffeine 63 ± 19 bacteria colonies. Thereby, caffeine in the solid medium suppresses the growth of bacteria colonies 6.4 times ($p < 0.05$).

Ps. fluorescens bacteria grow better on a solid medium with caffeine than *E. coli*, but in comparison with the control we observed a decreased amount of bacteria colonies (Fig. 2). In control plates (plate C) we counted 1708 ± 205 bacteria colonies and in experimental plates with 1% of caffeine (plate D) 324 ± 32 bacteria colonies. Hence, caffeine in the solid medium suppressed bacterial growth 5.27 times ($p < 0.05$).

DISCUSSION

Caffeine plays an important role in the development of immune resistance against bacterial invaders by increasing the concentration of some immunocompetent cells and reinforcing the lysozyme activity (6, 7). The results of this work demonstrate that caffeine has a direct antibacterial effect. We observed that the same caffeine concentration affects the growth of *E. coli* and *Ps. fluorescens* bacteria in a different way. Our findings show that *E. coli* bacteria are more sensitive to caffeine than *Ps. fluorescens.* A statistically significant caffeine effect on *E. coli* was observed at a 0.1% caffeine concentration; below this concentration caffeine had almost no effect on the bacterial growth, whereas a statistically significant effect of caffeine on *Ps. fluorescens* bacteria colony growth was observed only at a 1% concentration of caffeine. These data agree with the results published by other authors that depending on the concentration caffeine has a different effect not only on mammalian organisms (14, 15), but also on different bacteria. Rocha GM et al. (16) found that caffeine alone up to 0.02% had a low effect on mouse renal inner medullary collected duct cells. The results of other authors show that at caffeine concentrations from 0.01 to 0.2% the cytotoxic effect of ionizing radiation and other physical or chemical agents is strongly enhanced (4, 5).

A



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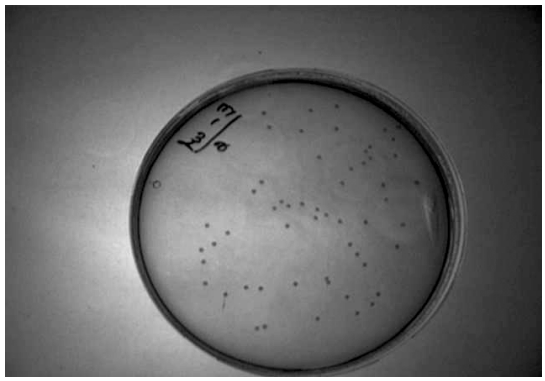

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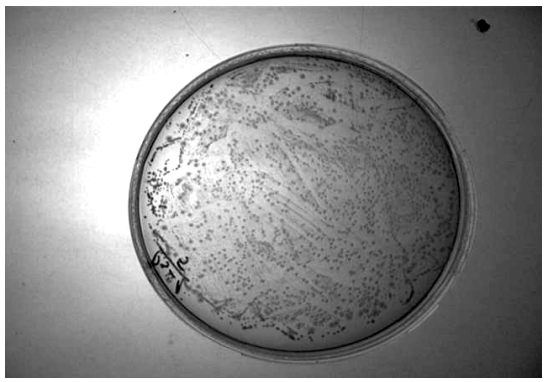

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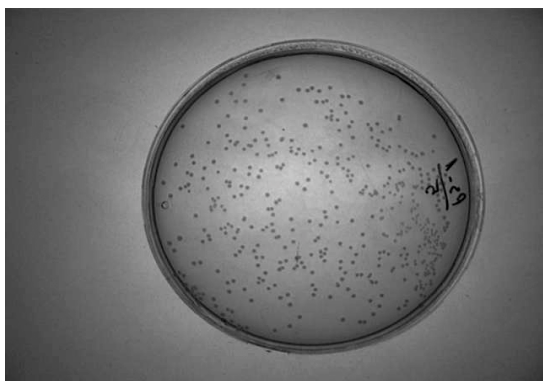

Fig. 2. *E. coli* growing on solid medium without caffeine (A) and with 1% of caffeine (B). *Ps. fluorescens* growing on solid medium without caffeine (C) and with 1% of caffeine (D)

The antibacterial effect of caffeine can have a positive or a negative impact on human health. Caffeine destroys the beneficial bacteria in the colon (17, 18). On the other hand, if some pathogenic bacteria prevail in the organism, caffeine intake can have a positive and curative effect.

CONCLUSIONS

Our findings show a clear antibacterial effect of caffeine. The sensitivity of different bacteria to caffeine can vary greatly depending on caffeine concentration. *E. coli* DH5α strain is more sensitive to caffeine than *Ps. fluorescens* 5443 strain. This effect could be taken into account in medical practice.

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ANTIBAKTERINIS KOFEINO POVEIKIS *E. COLI* IR *PS. FLUORESCENS* BAKTERIJOMS

S a n t r a u k a

Šio darbo tikslas buvo ištirti tiesioginį kofeino poveikį gramneigiamoms bakterijoms. Šiam tikslui pasirinkome plačiai aplinkoje ir žmogaus žarnyne paplitusias *Escherichia coli* bakterijas ir *Pseudomonas fluorescens,* įvairiai paplitusias tarp sausumos ir vandens gyvūnų.

Mūsų tyrimais, kofeinas veikia *E. coli* DH5α ir *Ps. fluorescens* 5443 bakterijų augimą. Didelė kofeino koncentracija (1%) slopina abiejų bakterijų augimą. *E. coli* ir *Ps. fluorescens* kultūrų optinis tankis sumažėjo atitinkamai 2,7 ir 3,1 karto ($p < 0,001$), lyginant su kontroliniais pavyzdžiais, kur šios bakterijos buvo augintos terpėje be kofeino. Mažėjant terpėje kofeino koncentracijai, bakterijų augimas skiriasi. *E. coli* yra jautresnė kofeinui 0,1–0,5% ribose.

Skirtingai praskiestos *E. coli* ir *Ps. fluorescens* bakterijos buvo auginamos ant kietos terpės be kofeino ir su 1% kofeino. *E. coli* ir *Ps. fluorescens* bakterijų augimas atitinkamai sumažėjo 6,4 ir 5,3 karto ($p < 0,01$).

Mūsų tyrimai patvirtina antibakterinį kofeino poveikį ir skirtingą bakterijų jautrumą augimo terpėje esančiam kofeinui. *E. coli* yra jautresnė kofeinui lyginant su *Ps. fluorescens*.

Raktažodžiai: kofeinas, *E. coli, Ps. Fluorescens*