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RESEARCH ARTICLE

EFFECT OF CAFFEINE ON MORPHOLOGICAL CHARACTERISTICS AND BIOMASS CONCENTRATION OF VARIOUS MICROORGANISMS

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ABSTRACT

The study was undertaken to investigate the effect of caffeine (1, 3, 7- trimethylxanthine) on growth, morphology, viability of bacterial, yeast and fungal strains in minimal media with caffeine and without caffeine. Growth, morphology, cell viability of the bacterial, yeast and fungal strains were studied in caffeine medium, minimal medium without caffeine and in minimal medium. The growth and viability of bacterial, fungal and yeast strains was greatly reduced or increased upon addition of caffeine at log phase of growth. This study shows the effects of caffeine on morphology and biomass concentration of various microorganisms including yeasts, fungi and bacterial strains.

INTRODUCTION

Caffeine (1,3,7- trimethylxanthine) is a commercially important purine alkaloid synthesized by plants. Coffee pulp is rich in carbohydrates, protein, minerals and appreciate amount of pectin, tannin which focus the rapid growth of micro-organisms. The most common sources for caffeine are coffee, tea, soft drinks and chocolate with approximately 80% of daily caffeine consumption coming from coffee. The amount of caffeine in various products depends on the serving size, type of product and plant variety, as well as preparation method. Several studies were carried out to investigate the use of purines, including caffeine, as a source of energy for microorganism growth[1]

Effect of Caffeine on various Organisms

Effects of caffeine on Animals

Excessive consumption of caffeine through beverges results in a number of health problems like adrenal stimulation, irregular muscular activity, cardiac arrhythmias, osteoporosis and increased heart output[2] and during pregnancy causes malformation of foetus [3]. Once ingested, caffeine is rapidly absorbed through the stomach and small intestine into the bloodstream where it takes 15 to 45 minutes to reach its highest levels depending on how much was ingested as well as on the source of the caffeine. The feeling of increased energy may experience from caffeine is likely due to a decreased perception of fatigue caused by caffeine's action as a central nervous system stimulant. Instant coffee at concentration of 4% was toxic to 75% of flies while home-brew coffee at 3% was toxic to 90% of the flies.

Effects of Caffeine on Microorganisms

Caffeine is known to exert numerous physiological effects on different organisms at micro molar concentrations. The most significant effects being inhibition of phosphodiesterase resulting in increase in intracellular cAMP levels; effect on intracellular calcium levels and antagonism of adenosine receptors[4]. Caffeine has also been reported to be an antimicrobial agent most effective against *E. coli* [5] and this is attributed to the effect of caffeine on DNA and protein synthesis in *E. coli.* Reports also indicate that caffeine

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enhances the inhibitory effect of certain antibacterial agents like penicillin and tetracycline against *Staphylococcus aureus* and of furazolidone against vibrios [6]

MATERIALS AND METHODS

Microoorganism and Media

Yeast, staphylococcus, Bacillus, E.coli, Rhizopus previously isolated in our laboratory was maintained on nutrient agar medium which had the following composition (g L^{-1}): beef extract 1; yeast extract 2; peptone, 5; NaCl 5 and agar 25 and Rhizopus on Sabourad dextrose agar and was sub cultured every two weeks. ATCC cultures of staphylococcus, Bacillus and E.coli was also maintained on nutrient agar medium of the above same composition.

Analysis of Growth of Bacterial Strains

$OD_{600\ nm}$ was measured for samples taken at regular intervals. Broth culture for the bacterial strains was measured and plotted against time for getting the growth profile. Broth culture for the bacterial strains was calculated from the OD_{600nm} values as per the following: for *E. coli, S. aureus, Bacillus spp* using the spectrophotometer.

Analytical method used for measuring and analyzing yeast growth

Yeast growth kinetics

Five ml of cultured medium sample was taken in every 12 hours time interval until the 36th hour. The resulting solution optical density was measured at 540 nm wave length. A blank solution was used for reference. The results were recorded and used in plotting growth curve of the yeast cells in different caffeine concentration.

Biomass estimation

At the end of the yeast growth, the biomass was harvested centrifuging the culture medium at 4500 rpm for 20 minutes. During centrifuging the supernatant and pellet was separated. The liquid part of the culture, supernatant, was utilized for the determination of the residual caffeine estimation. The remaining pellet was diluted with 5 ml distilled water and its optical density was measured at 540 nm.

Study of Cell Morphology

Gram staining was performed on cells before and after caffeine addition at log phase and morphology of the strains was observed under microscope (Carl Zeiss) at 100X magnification under oil immersion objective only for bacterial cells. Lactophenol cotton blue staining was performed on fungi Rhizopus before and after caffeine addition and morphology of the strains was observed under microscope at 40x magnification.

Cell Viability

Cell viability before and after caffeine addition during growth of bacterial cells was estimated by determining the Colony Forming Units (CFU) of culture using standard plate count technique. Briefly, samples were serial diluted in sterile Normal Saline Solution and 100 µL of diluted sample was plated onto nutrient agar plates. The plates were incubated at 37°C for 18 h and CFU were estimated as per the following formula:

CFU mL^{-1} = No. of colonies x 1/ Volume of culture x dilution factor

RESULTS AND DISCUSSION

Growth Profile of Bacterial Strains

The growth profile of different bacterial strains was analyzed in minimal medium (without caffeine), caffeine medium and minimal medium with caffeine added at the log phase of growth. *E. coli* achieved maximum growth in minimal medium with maximum cell growth. No growth was noticed for this strain in caffeine medium. When caffeine was added to actively growing cultures in minimal medium, there was a subsequent decrease in $OD_{600\ nm}$ values indicating that the cells failed to grow upon exposure to caffeine. The effect of caffeine on other bacterial species, experiments were performed with Gram positive bacteria viz. *Bacillus subtilis* and *Staphylococcus aureus.* All the above mentioned strains grow in limited concentrations in caffeine medium and achieved maximum growth in minimal medium.

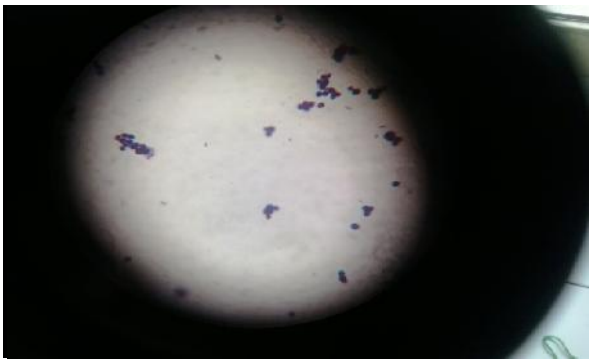

Fig 1 S.aureus without caffeine

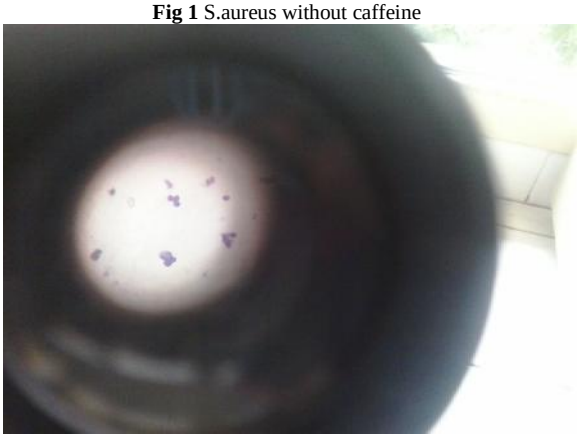

Fig 2 S.aureus with caffeine

Upon caffeine addition *Bacillus subtilis and Staphylococcus aureus* at minimum concentrations and the optical density value at 600nm is very low, but there is no growth in *E.coli* culture plates. Thus *E.coli* is very sensitive to the drug caffeine.

Morphology

Gram staining was performed to study the morphology of the bacterial strains before and after addition of caffeine to growing cultures. Interesting results were obtained upon microscopic examination at 100X magnification. The prominent morphological change was observed for *E. coli* which formed long filamentous structures upon addition of caffeine instead of the normal short rods and this was observed after 3 h of caffeine addition. The length of the filaments further increased with time and also a decrease in the number of cells was observed. The morphological change was observed for S.aureus which formed mostly diploid structure upon addition of caffeine instead of cluster of cocci and this was observed after 24hrs of caffeine addition (fig. 3.2.1 and 3.2.2). The diploid structure decreased with time and becomes a single cocci forms. The morphological change was observed for B.subtilis which formed short rods upon addition of caffeine instead of the normal long rods and this was observed after 24hrs of addition. It is related with time.

Cell Viability

The viability of the bacterial strains after addition of caffeine at log phase was measured by standard plate count technique. The Gram negative bacterial species *E. coli* were rendered completely non viable after exposure to caffeine. This was also observed for almost all the dilutions of the bacterial cultures and can be and regarded as the complete loss of viability. In case of Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* the viability was not much affected after caffeine addition, the bacterial count only decreased to$12\pm0.05x10^{8}$ cfu mL^{-1} from $16\pm0.3x10^{8}$ cfu mL^{-1} in case of *Bacillus subtilis* and to $8.5\pm0.05x10^{8}$ cfu mL^{-1} from $11.2\pm0.2x10^{8}$ cfu mL^{-1} in case of *Staphylococcus aureus* after caffeine exposure. For both the strains, the bacterial counts were same in minimal medium with caffeine added at log phase and in minimal medium without caffeine addition, indicating that caffeine addition does not affect cell viability in Gram positive bacteria as it does for Gram negative bacterial species. The values presented are the average of data obtained from our experiments.

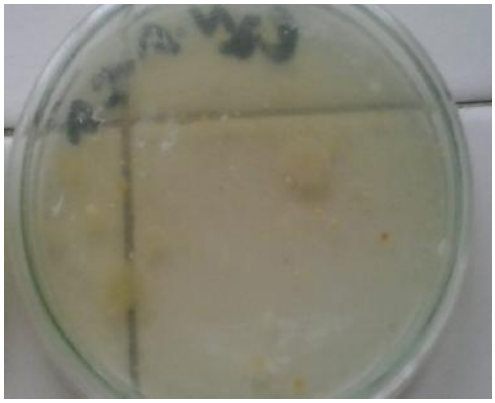

Figure 3 *S.aureus* – nutrient agar plates with caffeine

Growth of fungi (*Rhizopus spp*)

The growth profile of fungal strains was analyzed in minimal medium SDA (without caffeine), caffeine medium and minimal medium with caffeine. *Rhizopus* achieved maximum growth in minimal medium with maximum cell dry weight. No growth was noticed for this in caffeine medium. When caffeine was added to actively growing cultures in minimal medium, there was a subsequent decrease in the propugules which failed to grow upon exposure to caffeine.

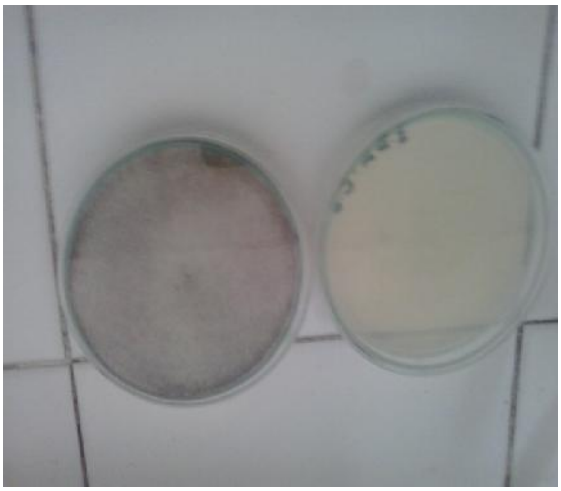

Figure 4 Rhizopus without caffeine and with caffeine (no fungal growth)

Growth of Yeast (*C.albicans*)

The growth of yeast was analyzed in minimal medium - nutrient agar (without caffeine), caffeine medium and minimal medium with caffeine. *C. albicans* achieved maximum growth in minimal medium with maximum cell dry weight. In minimal media with caffeine *C.albicans* achieved the same growth rate as in minimal media without caffeine. From this study it is known that yeast cells *C.albicans* may be resistant to the drug caffeine.

Morphology of yeast cells

Gram staining was performed to study the morphology of the yeast cells before and after addition of caffeine to growing cultures. Interesting results were obtained upon microscopic examination at 100X magnification. No considerable morphological change was observed under microscope before and after addition of caffeine.

CONCLUSION

Caffeine exerts either a minimal or lacurous effect on the growth and viability of almost all the strains tested. The morphology of some strains exhibited changes while others remain unaffected. Thus this study adds a support to the literature on effects of caffeine on various microbes.

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