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EFFECT OF CAFFEINE CONCENTRATION ON CAFFEINE DEGRADING BY BREVIBACTERIUM

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ABSTRACT

Caffeine is toxic to microorganisms and is an important parameter to be tested when employing microbes for biodecaffeination. Higher initial concentrations of caffeine is said to affect the whole process of biodecaffeination. Therefore there is a need to estimate the threshold level of the microbe to withstand caffeine. This paper deals with the effect of caffeine concentration on Brevibacterium isolated and maintained in our laboratory. The strain found to be promising withstanding more than 3grams of initial caffeine concentration after which the degradation ability is found to decrease.

1.Introduction

Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6 dione), a purine alkaloid, a key component of coffee, tea and other beverages is consumed throughout the world which when consumed in a higher amount is known to affect the system in various ways. Hence decaffeination is a sought after field for the past decades and conventional decaffeination techniques is said to affect the flavor and taste of coffee and also has the disadvantage of chemical usage.

Biodecaffeination is a technique of employing microbes to degrade caffeine. The technique proves not to alter the flavor and taste of coffee.

The important parameters for development of technological processes are rate of caffeine degradation and initial caffeine concentration. Since caffeine is toxic to microorganisms (Sundarraj et. al., 1965; Putrament et. al.,1972; Kihlman 1974), the initial concentration of caffeine in fermentation is crucial. In *S. marcescens*, the critical inhibitory concentration has been found to be 1.2 mg/ml (Mazzafera et. al., 1994a).

2. Materials and Methods

2.1 The Microbe

The Culture is isolated and maintained in the laboratory of JBAS College for Women. Pure cultures were maintained on nutrient agar medium at 4ºC and were sub-cultured at an interval of every 2 week.

2.2 Bacterial Identification

Various morphological, physiological and biochemical tests were performed to identify the bacteria with reference to Bergey's Manual of Systemic Bacteriology.

2.3Amplification of the caffeine tolerant bacteria

Solid screening medium (SSM) for isolating the caffeine-tolerant bacteria was prepared by mixing the mineral solution with caffeine (2.5 g/ L) and agar (1.5%) and autoclaved at 121°C for 10 min. Solid purifying medium (SPM) was also prepared as SSM except different concentrations of caffeine (1 to 10 g/L) was supplemented. Liquid amplifying medium (LAM) was obtained after addition of caffeine (0.5 g /L) and sucrose/glucose (5.0 g /L) in the mineral solution and disinfection.

2.4 Effect of Caffeine concentration on caffeine degradation by *Brevibacterium*

The optimum caffeine concentration for biodecaffeination was checked by incubating the organism in CLM containing caffeine in the concentration range of 0.5 g.L-1 to 10 g.L-1.

2.5Sample Analysis

The media was sterilized and a loopful of actively growing culture was inoculated into the medium and incubated on a shaker at 150rpm at a temperature of 30± 2oC for 96 hours. All the above processed samples were drawn at 12 hours intervals and the growth was recorded as an increase in the biomass by weight. Caffeine degradation was followed by HPLC analysis of the residual caffeine present in the medium

2.5.1 Biomass determination

The cell pellets after centrifugation of the culture samples were washed twice with deionized water and O.D 600 nm was measured. For cell dry weight (O.D600 nm of 0.5 corresponds to 0.379 g dry weight /100ml according to standard curve).

2.5.2 Estimation of methylxanthines by high performance liquid Chromatography (HPLC)

HPLC analysis of caffeine was performed in a Shimadzu LC 10 A- HPLC System, and the Methylxanthine compounds were separated on a C18 ODS-Luna column under isocratic conditions with 15 % acetonitrile in water at a flow rate of 1.0 ml/min. Compounds eluting from the column were detected at 273 nm, and the peak areas were compared with those obtained with standards of known concentration.

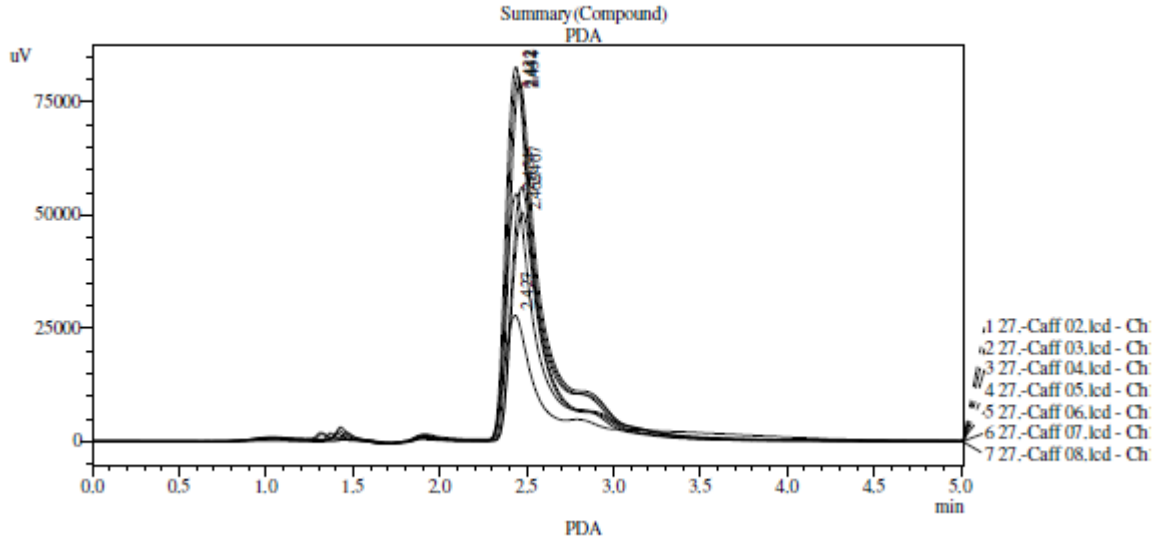
3. Results and Discussion

Brevibacterium exhibited high biomass of 3.289 g.L-1 and 90.85 % of the initial caffeine was degraded in liquid medium containing 1.0 g.L-1 of caffeine(Table 1)

Table 1. ***EFFECT OF CAFFEINE CONCENTRATION ON THE CAFFEINE DEGRADING BREVIBACTERIUM***

EFFECT OF CAFFEINE CONCENTRATION ON THE CAFFEINE DEGRADING BREVIBACTERIUM		
CAFFEINE CONCENTRATION(g/L)	*BIOMASS CONCENTRATION (g/L)*	*CAFFEINE DEGRADATION (%)*

1	*3.289*	*90.85*
2	*2.543*	*95.23*
3	*2.098*	*94.65*
5	*0.169*	*70.83*
8	*0.058*	*64.84*
10	*0.043*	*35.89*



As the caffeine concentration increased to 2.0 g.L-1 , there was a slight decrease in the growth but more than 95 % of the initial caffeine was degraded within 96 hrs of incubation. At the beginning of the incubation period, a slight growth inhibition of growth and caffeine degradation was observed at 2.0 g.L-1 of caffeine, which was probably due to the initial toxic effect of caffeine. The organism could however degrade more than 95% of the caffeine within 96 hrs and this trend was observed till a concentration of 2.0 g.L-1.Initial concentration of caffeine above 2.0 g.L-1 exhibited slight decrease in caffeine degradation.

Although caffeine is known to be toxic, *Brevibacterium* appears to have adapted itself to survive the high concentrations of caffeine by expressing enzymes capable of degrading the caffeine. The low efficiency of caffeine degradation is due to the inhibitory effect of caffeine on the enzymes.

4.Conclusion

The isolate *Brevibacterium MTCC 10313* being reported is an efficient caffeine degrader, which may be useful in the development of an environmental friendly biodecaffeination process. Caffeine degradation efficiency was found to be greatly influenced by initial concentration of caffeine.

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