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TRANSFORMATION STUDIES OF CAFFEINE DEGRADING PLASMID FROM *BREVIBACTERIUM* SPECIES

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ABSTRACT

The study of caffeine degrading plasmid from *Brevibacterium,* isolated and maintained in our laboratory from the coffee samples obtained from West Karnataka was undertaken.A plasmid of about 2000 bp was isolated from *Brevibacterium* . The isolated plasmid was used to transform *Escherichia coli* DH5α and the transformed colonies were inoculated in 1 to 10 g/L of caffeine containing minimal media to investigate whether the plasmid was involved in biodegradation of caffeine. It was observed that the plasmid biodegraded caffeine up to 8 g/L in minimal media, whereas non-transformed colonies could tolerate only up to 1 g/L caffeine. Growth curves obtained in the minimal media showed that transformed cells of *Escherichia coli* DH5α have greater ability to tolerate and degrade caffeine as compared to non-transformed cells.

KEYWORDS: Gram positive Bacteria, caffeine degrading plasmid, transformation, *Escherichia coli* DH5α .

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INTRODUCTION

Caffeine (1, 3, 7-trimethylxanthine) is a purine alkaloid naturally occurring in coffee and cocoa beans, cola nuts, and tea leaves. Excessive consumption of caffeine through beverages results in a number of health problems like adrenal stimulation, irregular muscular activity, cardiac arrhythmias, osteoporosis and increased heart output (Schuh *et al*. 1997). Excess caffeine is reported to cause mutation, inhibition of DNA repairs and inhibition of adenosine monophosphodiesterase (Blecher *et al*. 1977). Due to the known adverse effects of caffeine in the widely consumed beverages like coffee and tea, a caffeine free product is desirable. Apart from health effects, caffeine degradation is important from environment point of view also. Disposal of coffee processing industrial waste into lakes render drinking water non-potable (Buerge *et al.* 2003) and studies on removal of many toxic ions from coffee spent is attempted [23]. The presence of caffeine in soil also affects soil fertility as it inhibits seed germination and growth of seedlings (Friedman *et al*. 1983; Batish *et al*. 2008). Though the coffee pulp and husk are rich in carbohydrates and proteins, the presence of antinutritional factors such as caffeine, polyphenols, and tannins restricted its use as animal feed (Mazzafera *et al.* 1994). The Conventional methods of decaffeination usually involve the use of decaffeinating agents such as methylene chloride, ethylacetate, charcoal, triglycerides and supercritical CO_2. These conventional methods are expensive, toxic and non-specific to caffeine. In this regard biodecaffeination using microbes has been considered more suitable than currently used chemical methods. The Gram positive bacteria, *Brevibacterium,* isolated in our laboratory were found to be capable of degrading high concentration of caffeine in our previous investigations. The present study was undertaken to check whether the plasmid isolated from the isolate can bring about transformation of *Escherichia coli* DH5α strain in such a way that transformed bacteria can grow better on caffeine containing media.

MATERIALS AND METHODS

Transformation Experiment

Method of Mandel and Higa and Cohen *et al* with minor modification was used for transformation experiments. *E. coli* DH5α obtained from Bangalore Genei was streaked and maintained on LB (Luria Bertini) agar plate. Then a single colony was inoculated into 5 mL of LB broth and was incubated at 37°C overnight on shaker incubator maintained at 120 rpm. When the OD of the broth reached 0.6, 1 mL of cooled culture was centrifuged at 5000 at 4°C. Supernatant was decanted and the cell pellet was resuspended in autoclaved 0.1 *M* $CaCl_2$ by gently inverting the eppendorf tubes. Re-suspended cells were kept on ice for 10 min and centrifuged at 5000 rpm for 10 min. Supernatant was decanted, 0.5 mL of 0.1 *M* CaCl2 was added to the cell pellet and re-suspended by inverting the eppendorf tube gently. The competent cells obtained were added to the vial containing isolated plasmid and the tube was inverted gently several times to mix the plasmid with competent cells. The tube containing competent cells with plasmid is kept on ice bath for 20 min and then transferred to a water bath maintained at 42°C for 90 sec. 500 µL of LB broth was added to the tube, mixed gently again and kept in an incubator at 37°C for 1 h (Sneha Nayak et al.,2012)

Screening of Transformed and Non-transformed E. coli DH5α for caffeine degradation

LB agar plates and minimal media plates containing different concentration of caffeine (1-10 g/L) and appropriate controls were maintained through the study. 10 µL of transformed and non transformed *E. coli* DH5α cells were spread on the plates. Culture plates were kept in an incubator at 37°C and growth of bacterium on control (with and without caffeine) and experimental were compared after 24 h. Colonies on each plate were counted by using colony counter and difference in the number of colonies at different concentration of caffeine (1-10 g/L) was taken an index for transformed and non-

transformed *E. coli* DH5α(Sneha Nayak et al.,2012)

Growth Curve of Transformed and Non-transformed E. coli DH5α

Following the transformation experiment, shake flask culture studies were carried out in minimal media containing different concentrations of caffeine (1-10 g/L) for the transformed *E. coli* DH5α cells in order to obtain the growth curve. The growth curves, thus obtained were compared with the non transformed *E. coli* DH5α and *Brevibacterium* sp. (Sneha Nayak et al.,2012)

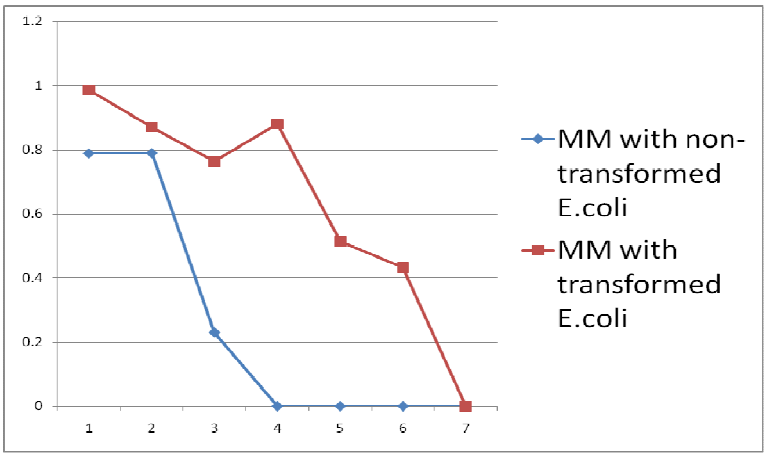
Biomass determination

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).

RESULTS AND DISCUSSION

In the present study, a plasmid of approximately 2000 bp was isolated from the *Brevibacterium* sp. This plasmid was used for transformation in *E. coli* DH5α bacterium. Non transformed *E. coli* DH5α cells could withstand up to 1 g/L caffeine in minimal media with a very slow growth rate. However, the transformation brought about in *E. coli* DH5 α by plasmid isolated from the *Brevibacterium* sp. showed that transformants could withstand up to 8 g/L caffeine concentration in the minimal medium.

Figure 1
Comparative study of the growth curves for non-transformed (a) and transformed (b) E. coli DH5 α in minimal media



X axis Caffeine concentration and Y axis Biomass concentration

TABLE 1
Comparative study of biomass of Brevibacterium sp. isolated from coffee pulp and transformed and non-transformed E. coli DH5 α

Slope values (biomass at different concentrations of caffeine)								
	1g/L	2g/L	3g/L	4g/L	5g/L	8g/L	10g/L	WCWO
MM with Sucrose *Brevibacterium*	4.082	4.006	3.987	3.876	4.102	2.908	1.167	1.654
MM with non-transformed *E.coli*	0.789	0.79	0.23	~0	~0	~0	~0	0.087
MM with transformed *E.coli*	0.986	0.87	0.763	0.879	0.513	0.432	~0	1.008

WCWO, Without caffeine with organism; MM, Minimal media

Screening for Transformed and Non-transformed E. coli DH5 α for caffeine degradation.

The transformation in *E. coli* DH5 α by the plasmid isolated from the *Brevibacterium* sp. showed that transformants could withstand up to 8 g/L caffeine concentration in the minimal medium. Growth rate of transformed was affected only beyond 8g whereas non-transformed *E. coli* DH5α in minimal media was significantly affected beyond 3 g/L concentration of caffeine.These experiments prove that the plasmid is used for caffeine metabolism. From the comparative study of growth of *Brevibacterium* sp. in minimal medium with glucose or sucrose as carbohydrate source and different concentrations of caffeine (1-10 g/L), it is evident that the growth was almost the same for 1 g/L concentration of caffeine in both the medium, whereas the growth increased in sucrose containing minimal medium. Moreover, when the concentration of caffeine exceeded beyond 3 g/L in minimal medium of non transformed cells, there was hardly any growth. Further, transformed and non-transformed DH5α *E. coli* showed a lower growth rate in minimal media beyond 2 g/L caffeine concentration and up to 4 g/L as compared to *Brevibacterium* sp. Transformed *E. coli* DH5α showed lower growth rate in minimal medium beyond 3 g/L caffeine concentration and up to 8g/L as compared to *Brevibacterium* sp. The doubling time for *Brevibacterium* sp. For the control medium was 0.6 h without caffeine, but with sucrose as the carbohydrate source. It was also observed that, with the increase in concentration of caffeine in the medium, the doubling time also increased, which was significantly higher for non-transformed *E. coli* DH5α. There was also a significant difference in doubling time for transformed and non-transformed DH5α *E.coli* in the control minimal medium without caffeine.

Growth rate of transformed and non-transformed

E. coli DH5α in minimal media was significantly affected beyond 4 g/L concentration of caffeine (Fig 1). Further, transformed and non-transformed DH5α *E. coli* showed a lower growth rate in minimal media beyond 4 g/L caffeine concentration and up to 8 g/L as compared to *Brevibacterium* sp. The caffeine containing products or spent let out from the industries have many adverse effects on the environment and soil fertility. Hence biodecaffeination can be used effectively for solid waste management, which can then be used as animal feed (Mazzefera *et al.* 2002).This can very well be achieved through the exploitation of naturally occurring organisms and genetically engineering them to enhance their capacity of caffeinedegradation.

CONCLUSION

The Study of caffeine degrading plasmid and its transformation experiments is of good use to understand the behavior of plasmid and to manipulate it for further experiments on caffeine degradation.

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