



Biodecaffeination by endophytic *Pseudomonas* sp. isolated from *Coffee arabica* L

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Received on:08-04-2012; Revised on: 16-05-2012; Accepted on:24-06-2012

ABSTRACT

Bacterial endophytic flora from *Coffee arabica* L. was screened and evaluated for caffeine degrading experiments. Among the endophytes isolated bacterium belonging to *Pseudomonas* sp., exhibiting 98.61% caffeine degradation. The bacterium was capable of growing luxuriantly when caffeine was supplement as a sole source of carbon and nitrogen. Induced cells were capable of degrading caffeine. This is the first report on any endophytes being evaluated for caffeine degradation. Obtained results are promising enough and can be an alternative method for existing conventional methods employed in caffeine removal. Further study in this area will give an insight on the facile route in biodegradation of caffeine and also the possible role for these endophytic bacteria in the biology of the coffee plant.

Key words: Endophytes, *Coffee arabica* L., Caffeine degradation, *Pseudomonas* sp.

INTRODUCTION

Caffeine is a purine alkaloid and one of the key component in many popular drinks especially tea and coffee. It is naturally found in coffee, cocoa beans, cola nuts and tea leaves, and is described as a methylated xanthine alkaloid derivative. Caffeine is mildly stimulating and is used as a therapeutic agent. It is widely distributed in pharmaceutical preparations and beverages and has become a ubiquitous drug with a variety of biological effects [1]. Apart from its beneficial aspects habitual and prolonged consumption of caffeine is bound with various deleterious effects such as headache, fatigue, apathy, adrenal stimulation, irregular muscular activity, cardiac arrhythmias, osteoporosis[2,3,4,5,6]. Consumption of caffeine during pregnancy causes malformation of fetus and among teenagers it reduces fertility rates [7]. Hence research on caffeine degradation has gained momentum in recent years, there is an increasing demand for decaffeinated beverages. By far the conventional methods for caffeine removal are water decaffeination, solvent extraction and supercritical carbon dioxide extraction [8]. These methods are bound with their own limitations such as use of toxic solvents, expensive and time consuming etc. Consequently, besides technical removal of caffeine, these methods are not specific to only caffeine therefore other flavor and aroma compounds are affected resulting in poor quality caffeine beverages. Hence there is a great demand to develop a facile route of decaffeination with environmental prospective offering relatively cost effective techniques, which generally have a high public acceptance [9].Hence in recent years biotechnological process for caffeine removal has been subject of interest across the globe.

Various reports have exhibited several microorganisms isolated from soil of tea and coffee garden were known to degrade caffeine. Among which *Pseudomonas* species are reported to utilize high caffeine concentration as sole source of carbon and nitrogen [10,11,12,13]. Several studies have demonstrated that there might be enzymes, acting separately or as a complex which brings about specific demethylation in *Pseudomonas* sp. [14,15,16]. Whereas till date little is known about microorganisms associated with the

Coffee arabica L. Associations between plants and microorganisms are very complex and are the subject of an increasing number of studies in recent past as plants harbors untold number of microbes known as endophytes and epiphytes. Among which endophytic plethora inhabit a unique niches in host and are known to produce novel bioactive compounds of pharmaceutical and industrial importance. Endophytes are the microorganisms that reside within the living tissue of their host plants without substantively harming it [17].

These endophytes play a major role in plant defense mechanism and in their host life cycle. The present investigation gives a new vista of endophytic bacterium in caffeine degradation. This is a first report of any endophyte in caffeine degradation.

In the present study altogether thirty two bacterial endophytes were isolated from the stems, leaves and roots of *Coffee arabica* L. Both Gram positive and Gram negative bacteria were isolated, with a greater percentage (70%) being Gram negative. Among which seven strains were capable of utilizing caffeine. One strain belonging to *Pseudomonas* species was capable of growing and utilizing caffeine at higher concentration of 5g/l. Further caffeine degrading experiments were carried out with this bacterial endophyte which can be exploited as a valuable tool in facile route of caffeine degradation.

MATERIAL AND METHODS

Collection of plant material

Plant material such as stem, roots and leaves were collected during the growing season of *Coffee arabica* L. from 10 healthy mature plants per site at two different geographical locations from southern parts of India. Individual plant materials were collected aseptically 3 cm above the soil level, and the stalks were stripped of leaves, put into sterilized plastic bags and carried to laboratory.

Surface sterilization

Plant materials were individually washed in running tap water air dried and sections were cut with a sterile scalpel. These sections were surface sterilized by dipping in 3% sodium hypochlorite for 2 min, and rinsed once in 70% ethanol for 2 min and twice in sterile distilled water followed by drying on sterile tissue paper. The edges of each sampled tissue were cut off with a

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