CEJEB-2020-36

Detoxification of Caffeine by Halotolerent *Pseudomonas alcaligenes*-N58

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ABSTRACT

Caffeine is a bitter, white crystalline xanthine alkaloid that acts as a stimulant drug. Caffeine is found in varying quantities in the seeds, leaves, and fruit of some plants, where it acts as a natural pesticide that paralyzes and kills certain insects feeding on the plants. It is most commonly consumed by humans in infusions extracted from the seed of the coffee plant and the leaves of the tea bush, as well as from various foods and drinks containing products derived from the kola nut. Other sources include yerba maté, guarana berries, guayusa, and yaupon holly.

In humans, caffeine acts as a central nervous system stimulant, temporarily warding off drowsiness and restoring alertness. It is the world's most widely consumed psychoactive drug, but, unlike many other psychoactive substances, it is both legal and unregulated in nearly all parts of the world. Beverages containing caffeine, such as coffee, tea, soft drinks, and energy drinks, enjoyed with great popularity. Caffeine is toxic at sufficiently high doses, Caffeine overdose can result in a state of central nervous system over-stimulation called caffeine intoxication or colloquially the "caffeine jitters". The symptoms of caffeine intoxication are comparable to the symptoms of overdoses of other stimulants: they may include restlessness, fidgeting, anxiety, excitement, insomnia, flushing of the face, increased urination, gastrointestinal disturbance, muscle twitching, a rambling flow of thought and speech, irritability, irregular or rapid heartbeat, and psychomotor agitation. In cases of much larger overdoses, mania, depression, lapses in judgment, disorientation, disinhibition, delusions, hallucinations, or psychosis may occur, and rhabdomyolysis (the breakdown of skeletal muscle tissue) can be provoked.

By considering this in the present study a bacterium halotolerant Pseudomonas alcaligenes N-58 is isolated from saline soils of Kolhapur District of Maharashtra. The isolate Pseudomonas alcaligenes N-58 was identified by 16srRNA analysis. Detoxification study indicated that the isolate degrades caffeine completely into non-toxic products at 37OC, 120 rpm agitation, within 48 hours of inoculation.

The study indicated that the isolate Pseudomonas alcaligenes N-58 can be used in the detoxification of caffeine from the soft drinks and other caffeine-containing drinks.

Keywords:

Caffeine, *Pseudomonas alcaligenes* N-58, Detoxification, Saline soils.

INTRODUCTION

Caffeine is a bitter, white crystalline xanthine alkaloid that acts as a stimulant drug (Hiroshi and Alan 2001). Caffeine is found in varying quantities in the seeds, leaves, and fruit of some plants, where it acts as a natural pesticide that paralyzes and kills certain insects feeding on the plants. It is most commonly consumed by humans in infusions extracted from the seed of the coffee plant and the leaves of the tea bush, as well as from various foods and drinks containing products derived from the kola nut. Other sources include yerba maté, guarana berries, guayusa,

and the yaupon holly

In humans, caffeine acts as a central nervous system stimulant, temporarily warding off drowsiness and restoring alertness. It is the world's most widely consumed psychoactive drug, but, unlike many other psychoactive substances, it is both legal and unregulated in nearly all parts of the world (Hiroshi, et al, 2008). Beverages containing caffeine, such as coffee, tea, soft drinks, and energy drinks, enjoyed with great popularity. Caffeine is toxic at sufficiently high doses, Caffeine overdose can result in a state of central nervous system over-stimulation called caffeine intoxication or colloquially the "caffeine jitters". The symptoms of caffeine intoxication are comparable to the symptoms of overdoses of other stimulants: they may include restlessness, fidgeting, anxiety, excitement, insomnia, flushing of the face, increased urination, gastrointestinal disturbance, muscle twitching, a rambling flow of thought and speech, irritability, irregular or rapid heartbeat, and psychomotor agitation. In cases of much larger overdoses, mania, depression, lapses in judgment, disorientation, disinhibition, delusions, hallucinations, or psychosis may occur, and rhabdomyolysis (the breakdown of skeletal muscle tissue) can be provoked.

Adverse effects

Caffeine in Humans through - Beverages - tea, coffee, and soft drinks and cocoa

1. Toxicity in excess of consumption (Abebe et al, 2008).
2. High adrenal stimulation, Irregular muscular activity, Cardiac arrhythmia, High heart output
3. Mutations, restlessness, fidgeting, anxiety, excitement, insomnia, flushing of the face, increased urination, gastrointestinal disturbance, muscle twitching, a rambling flow of thought and speech, irritability, irregular or rapid heartbeat, and psychomotor agitation. In cases of much larger overdoses, mania, depression, lapses in judgment, disorientation, disinhibition, delusions, hallucinations, or psychosis may occur, and rhabdomyolysis (the breakdown of skeletal muscle tissue) can be provoked.

Earlier decaffeination techniques

Chemical: Water, solvent and supercritical fluid extraction - nonspecific and expensive use toxic solvents.

Microbial: More ‘green’ but not safe

Enzymatic techniques: More safe, Food sensory quality maintained

Decaffeination Advantages

Coffee Husk: Animal feed and manure.

Food industry: Reduce the risk of caffeine dependence & side-effects

By considering this in the present study a bacterium halotolerant Pseudomonas alcaligenes N-58 is isolated from saline soils of Kolhapur District of Maharashtra. The isolate Pseudomonas alcaligenes N-58 was identified by 16srRNA analysis. Detoxification study indicated that the isolate degrades caffeine completely into non-toxic products at 37OC, 120 rpm agitation, within 48 hours of inoculation.

MATERIALS AND METHODS

Screening and isolation

Soil sample → serially diluted → Caffeine enriched media (Lauryl Sulphate HiVeg Broth (30.0 g/l), anhydrous caffeine (0.3 g/l), sodium chloride (0.5 g/l) and coffee husk extract (0.5% w/v) → 48hrs at 37°C → Zones of caffeine degradation (Figure 1).

UV-visible spectroscopy

As per Abebe et al. (2008).Broth containing Caffeine → 24 hrs at 37°C → Two intervals – 24 hours and 48 hours → 12000rpm → Supernatant → UV-visible at 275nm (Figure 3).

Biochemical identification

Advanced Bacterial Identification Software (ABIS) → Probable species' presence.

16S rRNA sequencing

16S partial rRNA sequence → Sangers' dideoxy sequencing method → BioEdit Sequence Alignment Editor (Version 7.1.3.0) → Sequence → Basic Local Alignment Search Tool (BLAST) → GenBank Submission.

RESULTS AND DISCUSSION

Colony morphology

1. Smooth
2. Gram-negative
3. Slimy exudate

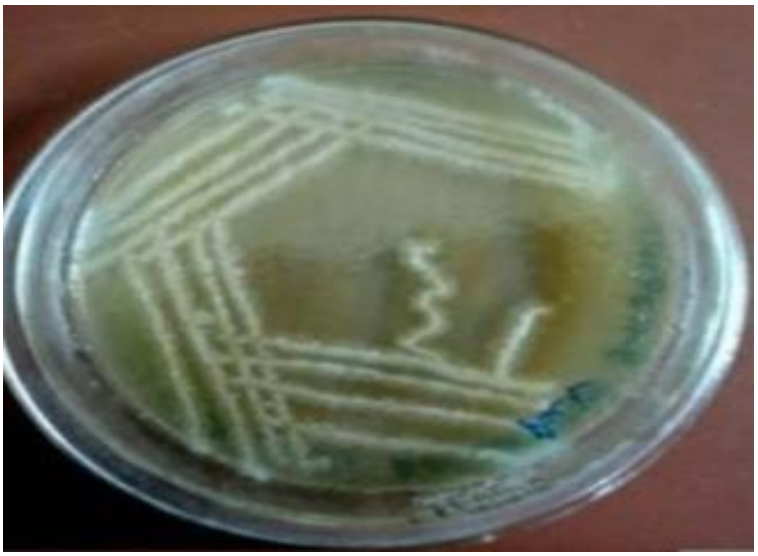

Figure 1: Figure showing bacterial colony morphology.

Sequence similarity identification

Caffeine dehydrogenase of *Pseudomonas sp. strain* CBB1 → Alignment with *Pseudomonas alcaligenes sequence* → Highest similarity protein: Chain A of the crystal structure of Amidohydrolase Pmi1525 (Target Efi-500319) from *Pseudomonas alcaligenes* Hi4320 → 43% similarity and 16% query coverage (Low) → multiple sequence alignment → secondary structure analysis → For higher efficiency.

Homology modeling

Homology modeling for various species showing the probability and accuracy of different species (Table 1).

Table 1: The caffeine dehydrogenase sequence of Pseudomonas sp. strain CBB1.

Probable Species	Probability (%)	Accuracy (%)
Pseudomonas putida	82	23
Citrobacter freundii	73	23
Proteus penneri	65	23
Citrobacter werkmanii	61	23

Secondary structure prediction and fold recognition

Homology Modeling- multiple sequence alignment - caffeine dehydrogenase of the *Pseudomonas alcaligenes* enzyme, which was similar to Yu et al. (2008) (Figure 3).

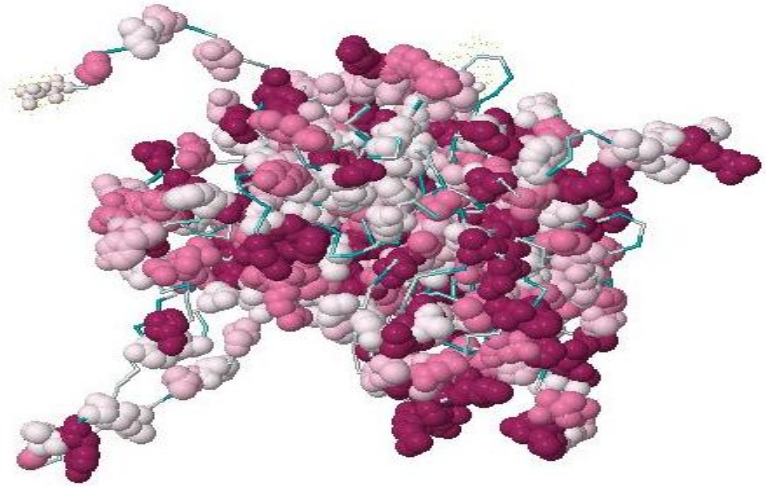

Figure 2: Secondary structure prediction.

Biochemical identification: Biochemical identification of various enzymes is shown in Table 2.

Table 2: Biochemical identification of various enzymes through Biochemical tests.

Biochemical Test	+/-

Motility	+
Catalase	+
Oxidase	-
Ornithine decarboxylase	-
Methyl Red	+
Indole	-
Voges- Proskauer	-
Hydrogen sulfide	+
Urea Hydrolysis	+
Maltose Fermentation	-
Gas from Glucose	+
Sucrose Utilisation	+

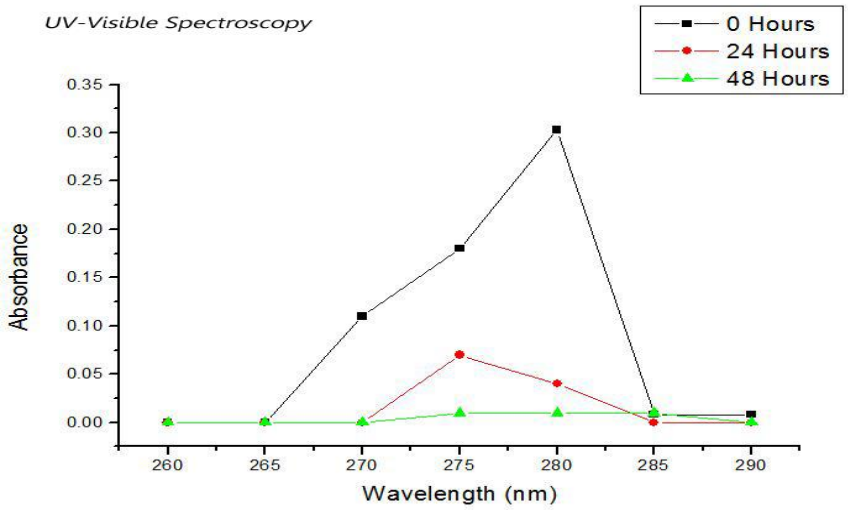

Figure 3: UV visible spectroscopy showing visibility at 275nm.

16S rRNA sequencing

1. 1363 base pairs
2. BLAST: 99% identical – Pseudomonas alcaligenes. Sussman et al,(1998).

Homology modeling: As per Sussman et al, 1998 (Table 3).

Table 3: Homology modeling as per Sussman et al. 1998.

Feature	Resolution
The resolution read from the PDB file	-1
1st generation packing quality	-3.813 (poor)
2nd generation packing quality	-4.361 (bad)
Ramachandran plot appearance	-4.122 (bad)
Chi-1/chi-2 rotamer normality	-4.347 (bad)
Backbone conformation	-6.217 (bad)

Inside/Outside distribution	1.205 (unusual)

Software used DSSP and PSIPRED: The software used for sequence analysis is DSSP and PSIPRED (Figure 4).

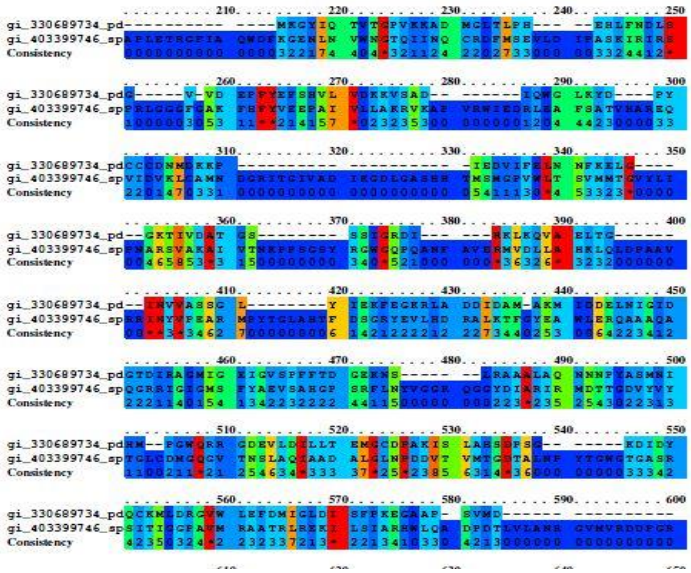

Figure 4: Conserved Region in sequence analysis.

Software used PRALINE: The software used for sequence analysis is PRALINE

- A preliminary study of caffeine degradation by Pseudomonas alcaligenes N-58 indicated that the isolate Pseudomonas alcaligenes N-58 can be used in the detoxification of caffeine from the soft drinks and other caffeine-containing drinks.
- Hence further studies on the organism to prove its caffeine degrading ability and on the enzyme to find out its exact nature and action would be a promising scope in the future.

ACKNOWLEDGMENT

The author(s) wishes to thank the Principal Dr.VB Helavi, for his constant encouragement and support for providing facilities to do research work.

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