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Research Article

A STUDY ON DIFFERENT TYPES OF MEDIAS USED FOR SCREENING OF CAFFEINE UTILIZING ORGANISMS

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ABSTRACT

Caffeine (1,3,7-trimethylxanthine) is one of the major product generated from coffee and tea processing plants. In recent years biodegradation emerged as promising approach to solve various environmental related problems and primarily depends on the strain employed to answer particular problem. In the present context Decaffeination can be cited as an example to appreciate the role of microbes in degradation process. As media plays an important role in the growth of organisms, an attempt was made to find out the media best suited and favorable for the growth of organisms capable of utilizing or degrading caffeine using simple screening techniques. Different types of minimal medias were used for screening of caffeine utilizing organisms. According to the literature available four media compositions were considered. The concentrations of caffeine varied in each of the media composition. A comparative study was done to know the media best suited for the growth. Amongst them the MMSC-II media was found to confer better results with good growth of the organisms.

INTRODUCTION

Caffeine (1, 3, 7-trimethylxanthine) is a commercially important purine alkaloid synthesized by plants. The purine alkaloid caffeine is found in more than sixty plant species, with significant levels in coffee beans, tea, cocoa, etc (Mazzafera, 1991; Suzuki *et al.,* 1992; Sylvain, 1967). Other two important alkaloids of the xanthine derivative group are theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethylxanthine). It is an active psychostimulant, (Nehlig, A; Daval JL, Debry G, 1992). which increases alertness and sustains concentration by overcoming fatigue. Environmentally, caffeine has been suggested as a chemical indicator of ecosystem since it is difficultly metabolized (Ogunseitan, 2002). Moreover, caffeine is also one of the major agroindustrial wastes generated from the coffee and tea processing plants and these wastes are often released into the water bodies. Therefore, decaffeination of waste is very necessary from the point of view of environmental conservation. (Sneha Nayak, M J Harshitha, *et.al.,* 2011)

The conventional methods of caffeine removal are water decaffeination, solvent extraction and supercritical carbon dioxide extraction (Dixon and Johnston, 1997). An enzymatic degradation of caffeine could be desirable as the decaffeinated coffee and tea pulp are rich in nutritional compounds such as carbohydrates and proteins, and thus have a good bioconversion potential (Pandey, A., Soccol, C.R., *et.al*., 2000). Generally, Bacterial strains of Serratia (Mazzafera et al., 1994) and Pseudomonas (Asano, Y., Komeda, T., Yamada, H., 1994, Blecher, R., Lingens, F., 1977, Yamoka-Yano, D.M., Mazzafera, P., 1998.) are known to degrade caffeine.

Caffeine is toxic to many microorganisms, while some microorganisms have the ability to grow in the presence of caffeine and to degrade it. In literature, strains belonging to *Pseudomonas, Serratia, Klebsiella, Rhodococcus, Alcalignes, Aspergillus, Penicillium, Fusarium and Stemphylium* have been reported to be able to degrade caffeine. (Hakil M, Voisinet F, *et.al.,* 1999, Roussos S, Aquiahuatla M, *et.al.,* 1995, Mohapatra B R, Harris N, *et.al.,* 2006).

Bioremediation is the use of bacteria, enzymes, or fungi to convert the toxic compound into a non-toxic compound in the environments. Bioremediation of coffee waste sometimes called biodegradation of coffee waste is an improvement method that contains any determined use of bacteria degrades toxic coffee waste in the natural environment to ecofriendly products. Bioremediation is aimed to decrease the toxic substances in an area by the use of bacteria, fungi, animals and plants to immobilise or degrade the toxic substances. (Salihu Ibrahim, Mohd Yunus Shukor, *et.al.,*2014).

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Comparatively, decaffeination through microbial degradation is more beneficial and attractive than other methods since it can be conducted safely and at a low budgetary requirement (Fang-Yuan Fan, Yan Xu, *et.al.,*2011).

Though various techniques are employed for reducing or removal caffeine content degradation by microorganisms is considered to give better and excellent results because of their ability to use and grow on variety of substrates and their adaptability to different environmental conditions. The present study focuses on the use of the media best suited for the growth of caffeine utilizing or degrading organisms. The organism that can degrade or utilize caffeine at its best was selected by their growth characteristics on different media. The ability to degrade caffeine by the isolates was highly influenced by the content of percentage of caffeine used in the media.

MATERIALS AND METHODS

Sample Collection

The sample was collected according to the standard sample collection technique. Soil collection point chosen for the experimental analysis were the nearby coffee shops so as to reach out for the organism which has a capability to degrade and utilize caffeine.

Isolation of Organism

10 fold serial dilution technique was employed and 0.1 ml of innoculum was plated onto nutrient agar medim and incubated at 37^{o}C for 24 hours. These colonies were subcultured and used for further analysis.

Preparation of Minimal Media

A standard procedure from the selected reviews was followed for the selection of media in which the media contained caffeine as the major carbon source. Four different medias were used from the literature available, and the comparative study of these medias were done and they were named as minimal media+sucrose+caffeine I (MMSC-I), minimal media+sucrose +caffeine II (MMSC-II), minimal media+caffeine III (MMC-III), minimal media+caffeine IV (MMSC-IV).
The compositions of the four medias were as follows –

Minimal media+sucrose+caffeine I (MMSC-I): Na_2HPO_4 -0.12 gm/l, KH_2PO_4-1.3 gm/l, $CaCl_2$- 0.3 gm/l, $MgSO_4.7H_2O$–0.3 gm/l, Caffeine–1.2 gm/l, Sucrose–5 gm/l, Agar –20 gm/l, pH – 5.5

Minimal media+sucrose +caffeine II (MMSC-II): KH_2PO_4–3 gm/l, Na_2HPO_4 –6 gm/l, NaCl–5 gm/l, NH_4Cl – 2 gm/l, Sucrose–8 gm/l, Caffeine -3.5 gm/l, $MgSO_4$–0.1 gm/l, Agar – 20 gm/l, pH – 6.7

Minimal media+caffeine III (MMC-III): Na_2HPO_4 – 7.8 gm/l, KH_2PO_4– 3 gm/l, NaCl – 0.58 gm/l, Caffeine – 1.0 gm/l, Agar– 20 gm/l, pH – 5.5

Minimal media+caffeine IV(MMC-IV): K_2HPO_4 – 0.8 gm/l, KH_2PO_4– 0.2 gm/l, $MgSO_4.7H_2o$ – 0.2 gm/l, $CaCl_2.2H_2O$ – 0.1 gm/l, $FeSO_4.7H_2O$ – 0.005 gm/l, Caffeine – 5 gm/l, Agar – 20 gm/l, pH – 6.0

Sterilization Parameters

The sterilization parameters were set as 121^{o}c at 15 lbs for 15 minutes. The carbohydrates were sterilized separately and then added into the medium before plating.

Selection of Medium

Bacterial isolates were streaked onto the above said media plates and were incubated first for a period of 24 hours and later extended for a period of 48 hours. The media which supported the good growth of isolates was selected for further procedures.

RESULTS

Isolation of Organism

Initially 8 colonies were isolated after the specified incubation period. These colonies were subcultured and preserved for further analysis.

Selection of Medium

The 8 bacterial isolates were streaked onto the above said media plates i.e MMSC I, MMSC II, MMC III, MMC IV and kept for incubation for a period of 24 hours where a negligible or nil growth was observed on all the four medias used for the study and so the incubation was extended for a period of 48 hours.

Table- 1

Media	Time in Hrs	IS 1	IS 2	IS 3	IS 4	IS 5	IS6	IS 7	IS 8
MMSC I	24	-	-	-	-	-	-	-	-
	48	+	+	+	+	+	+	+	+
MMSC II	24	-	-	-	-	-	-	-	-
	48	+++	+++	+++	+++	+++	+++	+++	+++
MMC III	24	-	-	-	-	-	-	-	-
	48	-	-	-	-	-	-	-	-
MMC IV	24	-	-	-	-	-	-	-	-
	48	+	+	+	+	+	+	+	+

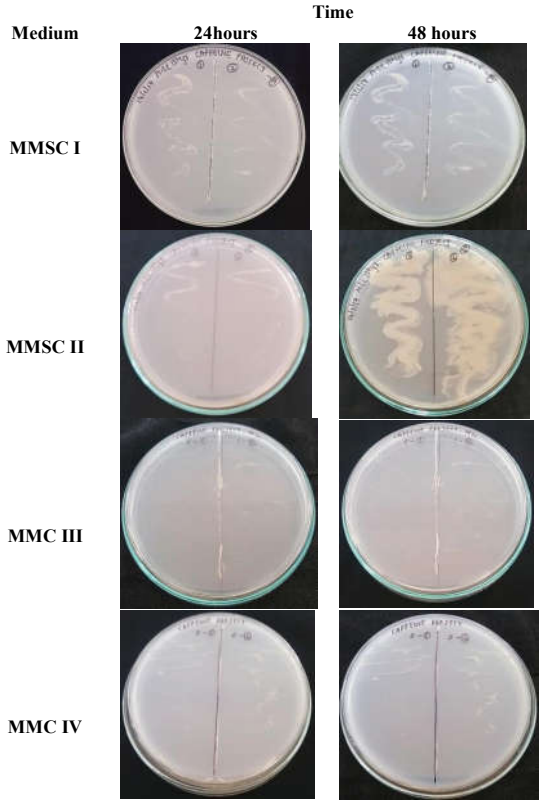

Figure 1

Out of the four, 3 medias i.e MMSC I, MMSC II, MMC IV supported the good growth of isolates after a period of 48 hours except for MMC III which didn't support the growth of organisms. A confluent growth was observed on MMSC II media. (Table-1)

DISCUSSION

Caffeine, a plant product has shown its occurrence in beverages like tea, coffee and soft drinks and cocoa **(**Hiroshi A. and Alan C., 2001). It is important to record the initial caffeine concentration in the fermentation medium since caffeine is toxic to microorganisms. Due to the existence of an external source of carbon/nitrogen, pH and caffeine concentration are important factors to be considered for microbial degradation of caffeine (Gokulakrishnan S, Chandraraj K, Gummadi SN, 2007).

The isolates were first obtained on nutrient agar and on the basis of their ability to utilize carbon, nitrogen sources; they were allowed to grow on different media with modified composition from the literature source available. The common property in all the media was caffeine. All the 8 isolated colonies were allowed to grow on MMSC I, MMSC II, MMC III, MMC IV medias. Profuse growth of the isolates was observed in MMSC-II media supplemented with sucrose and caffeine as a carbon source, NH_4Cl as nitrogen source. Caffeine utilization was low at 24hrs and high at 48hrs in the presence of sucrose. The cell growth was low when caffeine itself was used as carbon, nitrogen source when compared to the medium containing sucrose.

In the comparative study carried out MMSC II was found to be best suitable for the growth of caffeine utilizing microorganisms. This feature correlates with the literature cited (Gokulakrishnan S, Chandraraj K, Gummadi SN, 2007, Lakshmi V, Nilanjana Das, 2010). Henceforth selected for further experimental procedures. The microbial growth showed a distinct variation for 24 and 48 hrs time. The isolates exhibited scanty or nil growth at 24 hrs time interval, where as growth was more evident after a period of 48 hrs.

Henceforth the MMSC II media was selected as the isolates exhibited good growth characteristics. Maximum of the isolates have shown a good growth on MMSC II media with near neutral pH. Considering the nutrient availability, pH optima, the growth may be attributed to the presence of caffeine ammonium chloride and the presence of phosphates in the medium with sucrose being the catalytic factor. Thus comparative study of different medis clearly indicates that the MMSC-II media was found to confer better results with good growth of the organisms.

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