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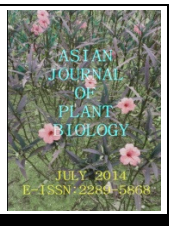

Bacterial Degradation of Caffeine: A Review

Salihu Ibrahim[1], Mohd Yunus Shukor[1], Mohd Arif Syed[1], Nor Arina Ab Rahman[1], Khalilah Abdul Khalil[2], Ariff Khalid[3] and Siti Aqlima Ahmad[1]*

[1]Department of Biochemistry, Faculty of Biotechnology and Bio-molecular Sciences, Universiti Putra Malaysia, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Department of Biomolecular Sciences, Faculty of Applied Sciences, Universiti Teknologi MARA, Sec. 2, 40150 Shah Alam, Selangor, Malaysia.
[3]Biomedical Science Program, Faculty of Biomedicine and Health, Asia Metropolitan University, 43200 Cheras, Selangor, Malaysia

Corresponding Author: Siti Aqlima Ahmad; Email: aqlimaahmad@gmail.com, aqlima@upm.edu.my

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Caffeine (1,3,7-trimethylxanthine) is an important naturally occurring, commercially purine alkaloid which can be degraded by bacteria. It is a stimulant central nervous system and also has negative withdrawal effects and is present in different varieties of plants such as coffee plant, tea leaves, colanut, cocoa beans and other plant. It is also present in soft drinks and is being used extensively in human consumption and has in addition some therapeutic uses but in minimal amount. Evidence has proved the harmful effects of caffeine thus opening a path in the field of caffeine biodegradation. Biodegradation by bacteria is considered to be the most efficient technique in degrading caffeine within the environment. Even though there are available methods for the removal of caffeine using conventional methods such as water, supercritical and solvent decaffeination but they are lack of accuracy/specificity for the removal of caffeine and in addition to the existing caffeine which sometime remains. Microbial degradation of caffeine provides a safe and cheaper alternative compared to chemical and physical methods. Microbial candidates for caffeine biodegradation are actively being isolated globally. Caffeine degradation can occur in both aerobically and an-aerobically depending on the contaminants. Organisms such as *Pseudomonas*, *Alcaligenes*, *Aspergillus*, *Serratia, Penicillium, Klebsiella*, *Stemphylium, Rhizopus, Rhodococcus, Brevibacterium*, *Bacillus* sp., and *Phanerochaete* strains have been reported to have the ability to degrade caffeine.

INTRODUCTION

Caffeine is a white crystalline alkaloid of xanthine, which is bitter, odourless and amorphous in its pure state that acts as a drug activator with an empirical formula of $C_8H_{10}N_4O_2$, half life of 5 h and molecular weight of 194.2 g/mol. It is a molecule in which its methyl groups are hydrophobic with hydrophilic parent chain. “Kaffe” and “café” are the words that caffeine was coined from each meaning coffee which are German and French words respectively. The consumption of caffeine in the form of foods and beverages was in practice long before its isolation in pure form in 1891 by the German chemist Friedrich Ferdinand [1]. Caffeine plays a major role in defence mechanism against pest, herbivores and other organism in plant which have pronouncing effects on living organism’s metabolism. Caffeine has numerous importances in commercial industry due to its popular applications in pharmaceutical preparation and beverages such as tea, coffee, and soft drinks. It is found in different amounts in the fruits, seeds, leaves of some plants species such as theobroma, camellia, coffee, where it kills and destroys some insects feeding on the plants, as well as promoting the pollinators memory. Monomethylxanthines and dimethylxanthines such as 1-methylxanthine, 3-methylxanthine, 7-methylxanthine, 3,7-dimethylxanthine, 1,3-dimethylxanthine, 1,7-dimethylxanthine, and xanthine forms a key group of compounds with the purine basic structure [2]. Figure 1 shows the basic carbon skeletal structure of caffeine and other related methyl xanthines.

At higher concentration, caffeine is toxic to saprophytic microorganisms that are concerned in the important biotransformation within environment, which causes disorderliness in environmental stability. Coffee waste disposal signifies vast pollution problem in the producer countries. The attempt to make use of the coffee pulp as an animal feed source

has been made from the economic to the environmental view point. For this reason, the removal of anti-nutritional parts such as caffeine, becomes essential [3]. The use of bacteria for decaffeination purpose has been more advantageous than other physical and chemical techniques that presently employed.

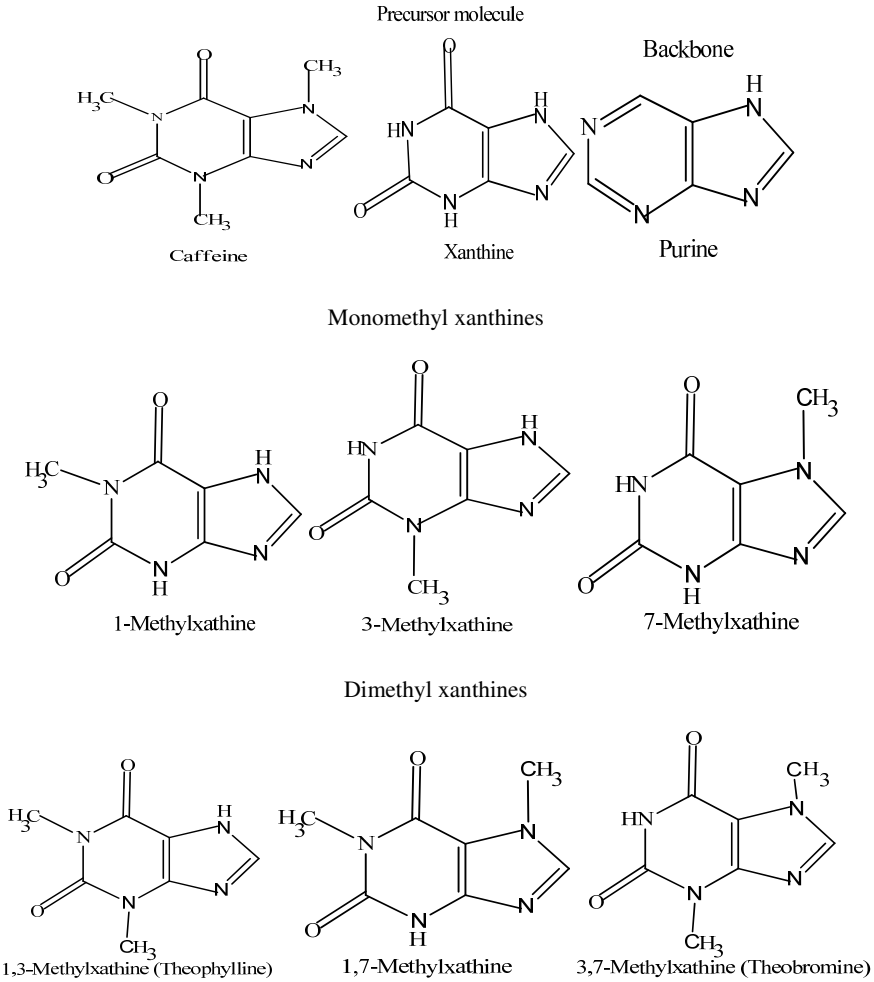

Figure 1: The basic carbon skeletal structure of caffeine and other related methyl xanthines.

Mazzafera (2002), and Hakil et al. (1999) isolated quite a few numbers of bacteria capable of utilising caffeine as a sole source of carbon and nitrogen and these serve as a possible goal for biodecaffeination [4,5].

Caffeine degradation pathway intermediates are as valuable as caffeine and have numerous applications. Thus, caffeine degradation by bacteria not only becomes important in overcoming environmental concerns but can also serve as a recovery method for other important commercial products [3]. Tea and coffee industries produced caffeine by solid wastes which is one of the main poisonous compounds, i.e. tea waste, husk and coffee pulp [4, 6]. Even though these industrial wastes are enriched with macromolecules such as proteins and carbohydrates, but due to the existence of caffeine and some other poisonous compounds, it cannot be used as animal feed [7, 8]. It is renowned that caffeine is toxic to germinating seeds, microorganisms, and marine organisms [9,10]. Hence, an extreme care must be taken to degrade caffeine from industrial and human waste products before they can be channelled into the aquatic environment and soil [10–13]. Because of that, biodecaffeination by bacteria is significant in environmental and medical scene in general [14, 15]. In recent years, numerous works have been reported on caffeine biodegradation by bacteria which indicates a general trend of bioremediation of caffeine that is now becoming a global agenda. Hence, this review is aimed on reporting various degradation works of caffeine by bacteria.

Sources and Consumption of Caffeine

Caffeine being as a plant alkaloid was found in the leaves and fruits of many types of plants plant species, where it acts as a

normal pesticide that kills and paralyses certain insects feeding on them [16,17]. Naturally, caffeine and other related purine alkaloids are produced by at least 13 different orders of plant kingdom (nearly 100 species), including *Camellia sinensis* (tea), *Coffea arabica* (coffee), *Theobroma cacao* (cacao, or cocoa), *Cola nitida* (Cola), *Paullinia cupana* (guarana), and *Ilex paraguariensis* (yerba mate) [9, 16, 18], which are the highly cultivated source of caffeine. Amongst these caffeine producing plants, most studies have been done with species belongs to the genera *Coffee* and *Camellia* [19, 20]. Tea and coffee plants are the main sources of caffeine and other related compound like theobromine and theophylline are formed by a large number of plant varieties belong to various classes, families, and orders. Other less regularly used sources of caffeine consist of the guarana, yerba mate plants [21], are in some cases used in the caffeinated beverages (cola drinks) and teas preparation. G*uaranine and mateine* are the two other possibility names of caffeine whichwere derived from *guaranine* and *mateine* plants. For instance, in the seed of guarana caffeine contains mainly exists in testa (1.6%) and leaves (4.3%).

Some other non-alcoholic drinks also contain caffeine. Tea, coffee, yerba mate, guarana, yaupon, yoco, colanuts and cocoa are the foods and beverages that contain caffeine [22]. The caffeine quantity found in these stuffs generally differs. Based on dry weight, there are about 4-7% that has been found in guarana. Cacao beans contain approximately 0.03% caffeine, cola nuts 1.5%, coffee beans 1.1-2.2%, and tea leaves 3-5% caffeine [9, 23–25]. It has been estimated that worldwide consumption of caffeine are at 120,000 tonnes per year, making it the world's most prevalent psychoactive substance. This amounts to one serving of a caffeinated drink foran individual each day. Andersson et al. (2004) reported that the average caffeine content in filter coffee, percolated coffee and instant coffee was found to be 103, 84 and 53mg/cup respectively [26]. Excessive caffeine consumption via beverages does not leads to the withdrawal and devotion effects such as drowsiness, vomiting, headache and nausea [27], but it is related to a number of health problems such as the increased cardaic output, cardiac arrhythmias [28, 29], irregular muscular activity, and adrenal stimulation [5, 30, 31]. Excessive caffeine consumption results to osteoporosis [8], inhibition of DNA repairs and inhibition of adenosine mono-phosphodiesteras e [4], mutation, and causes malformation of foetus during pregnancy which may reduce fertility rates [8, 32, 33]. Table 1 shows some plants containing caffeine.

Bioremediation of Coffee Waste

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 to biotransform/degrades toxic coffee waste in the natural environment to environmental-friendly substances. 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. The basic idea of coffee waste biotransformation has contributed a great deal in the field of biodegradation. In bioremediation, emotional response has been popularly used for food substances added value [20], for instance, decaffeinated coffee had used coffee beans and *Pseudomonas alcaligenes* MTCC 5264 as raw materials and microorganisms for biotransformation.

Catabolism of Caffeine

Caffeine was formed in immature fruits and young leaves. However, it is very difficult to remove the three methyl groups in caffeine metabolism, which resulting in xanthine formation. Since afterwards, it has been reported a number of tracer experiments using ^{14}C-labelled purine alkaloids [35–38] which shown that the major metabolic pathway of caffeine is caffeine – theophylline - 3-methyxanthine – xanthine – uric acid – allantion – allantoic acid - purine catabolic pathway and is metabolised to CO_2 and NH_4 [18, 20, 39, 40]. Figure 2 shows the general catabolism of caffeine.

Table 1: Plants containing caffeine [34].

Family	Species	Number of species	Number of genera
Annonaceae	*Annola cherimola*	1	1
Celastraceac	*Maytenus sp.*	1	1
Combretacaec	*Combretum jaquinii, Combretum loeflingii*	1	2
Guayakí yerba mate (loose leaf)	6 g	85	approx. 358
Dillenaiceac	*Davilla rugosa*	1	1
Geraniaceae	*Erodium cicutarium*	1	1
Phytolaccaceae	*Gallesia gorazema*	1	1

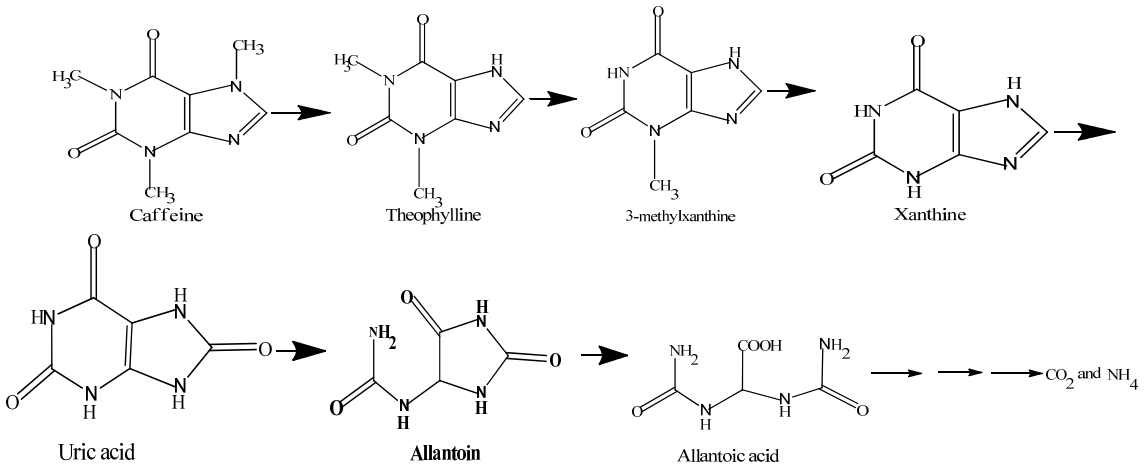

Figure 2: General catabolism of caffeine by bacteria

Degradation of Caffeine using Microbial Method

Until 1970s, it was believed that caffeine is toxic to bacteria and no studies on degradation of caffeine by microorganisms were reported until 1970 [7]. Enzymology and biochemistry of caffeine degradation in bacterial cells has been well studied within the microbial sovereignty [7, 41–44]. At concentration of caffeine above 0.0025 g/mL, it has been found to inhibit the growth of many bacterial species in the growth medium. When caffeine is added to anti-microbial agents such as chloramphenicol, some synergistic effect has been observed [44]. In the early 1970, caffeine degradation by microorganisms was first reported [45]. Since then, development has been done on using caffeine assource for microbial growth [47–49].

Caffeine Degradation by Bacteria

Caffeine degradation has been well studied in bacterial systems, primarily from the genus *Pseudomonas*, and others such as *Serratia*, *Rhodococcus* and *Klebsiella* species. Unlike any other higher organisms, bacteria can utilise caffeine as a sole source of nitrogen, carbon and energy for growth [20, 31, 43, 44, 50, 51]. A few studies have shown the mutagenic effect of caffeine through DNA repair inhibition in bacteria [2, 52, 53]. It was also known that 0.1% caffeine concentration reversibly inhibits protein synthesis in the bacteria. Though the report states that protein synthesis inhibition is post translational because caffeine does not affect RNA translation [53], high concentrations are essential for bactericide action, which regarded caffeine as toxic for bacteria [23]. SundarRaj and Dhala (1965) reported that some microorganisms have the capacity to grow in the presence of caffeine and survive depending on their ability to degrade the alkaloid [44]. Actually, it is uncommon to find bacterial strains that are resistant to caffeine [48]. Some microorganisms, for instance *Klebsiella pneumoniae*, can use purines as nitrogen or carbon sources [47]. Caffeine degradation by bacteria (*Pseudomonas*) begins with the conversion of caffeine to paraxanthine and theobromine

parallely by demethylases. There is many demethylationthat forms xanthine using 7-methyl xanthine as the intermediate. Evidence has showed for xanthine oxidation, mono and dimethyl xanthines to their respective uric acid which will enter the purine catabolic pathway [30, 55] (Fig. 3a). Caffeine catabolic pathway in *Serratia marcescens* is analogous to *Pseudomonas* sp. except for the methyl uric acid formation intermediate [55]. The information about pathway and different enzymes involved in caffeine degradation could help in developing an enzymatic process for the removal of caffeine. In literature, few reports have shown and described about the isolation of bacteria strains from soil with the ability to degrade caffeine [56, 57]. *Pseudomonas* and *Serratia* genus are the bacterial strains capable of degrading caffeine. It has been found that high concentration of caffeine which is greater than 2.5 g/L in the growth medium inhibited the growth of many bacterial species. Attempts were made with the help of inhibitors for the bio-production of caffeine catabolic intermediates.

In 1993, Asano and his colleagues reported theobromine production using *Pseudomonas* strain for the first time. Theobromine was accumulated in the presence of 1 mM of Zn^{2+}at different levels ranging from 5 g/L and above, The most suitable carbon and nitrogen sources found are fructose and tryptone [2, 30]. In the presence of 0.04% of Fe^{2+}, theobromine production increase has been observed in the medium by 10 folds, suggesting that Fe^{2+} may act as a co-factor or improve the production of demethylating enzymes. Caffeine is directly oxidised to 1,3,7-trimethyluric acid and consequently to uric acid, in another minor

pathway observed in microbial species consisting of *Alcaligenes, Klebsiella, and Rhodococcus* [31, 58, 59] (Fig. 3b).

It has been reported that *Pseudomonas*, *Alcaligenes*, *Aspergillus*, *Serratia, Penicillium, Klebsiella*, *Stemphylium, Rhizopus, Rhodococcus and Phanerochaete bacterial* strains have the ability to degrade caffeine [5, 30, 31, 47, 49, 55, 56, 60, 61]. Decaffeination by bacteria (*Pseudomonas*) has the highest growing rate capable of degrading caffeine at a rate of 90.00 mg/hr [48] and increased at 5.0 g/L of caffeine. However, research has shown that biodecaffeination is dependent on external nitrogen sources [5]. High caffeine concentrations in food

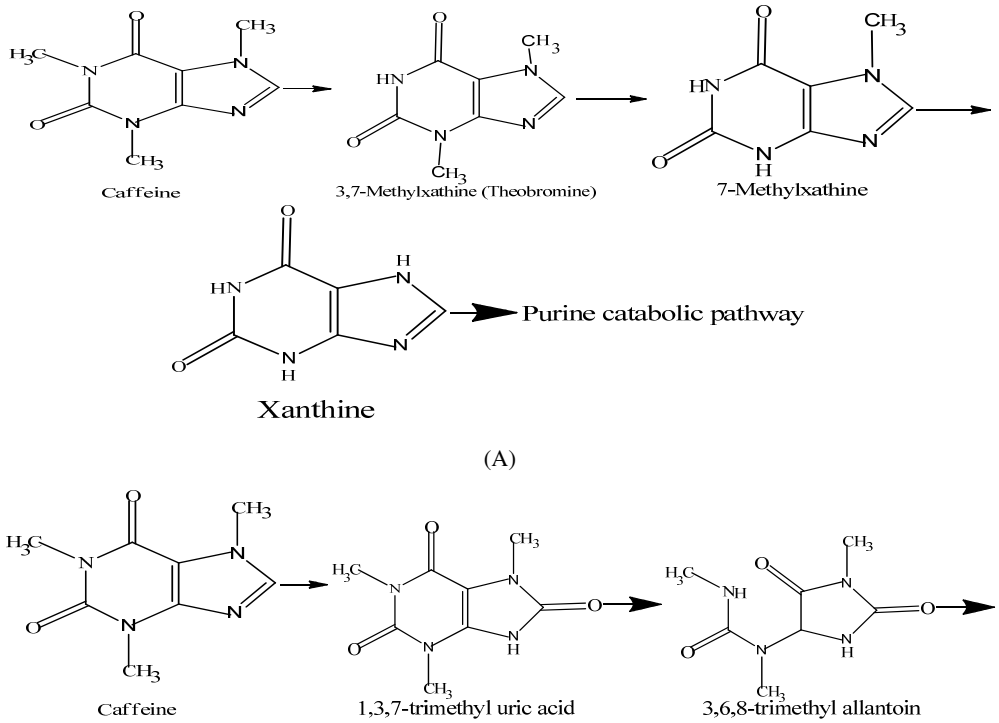

Figure 3(A) Catabolism of caffeine in bacteria, which results in theobromine as the first metabolite formed. The conversion of caffeine to methylxanthines to xanthine is mostly by the action of demethylase enzymes (B) Catabolism of caffeine in bacteria species like *Alcaligenes, Klebsiella* and*Rhodococcus* caffeine is directly oxidised to methlyuric acids primarily by caffeine oxidase enzymes, and this can be considered as a minor pathway of degradation.

products and liquid waste released from an industrial plant (10 g/L caffeine), nuclear fusion/fission power station, factory, and other sewage system. In view of that, the developmental process of biodecaffeination strain should be able to resist and degrade large caffeine concentration at a high speed.

Bacteria have the ability to degrade the content of caffeine in caffeine bearing (coffee waste) plants. Kumar et al. (2004) reported that the leaf surface plays an important role in *Agrobacterium* infection in tea plants [61]. A technique for tea leaves producing less caffeine content by growing caffeine-degrading bacteria on the leaf surface has been proposed. Ramarethinam and Rajalakshmi, (2004) found in situ lowering of caffeine in tea leaves without affecting the quality of the other tea components when tea plants were sprayed with a suspension of *Bacillus licheniformis* [62]. Under anaerobic conditions, metabolism of caffeine by microorganism has been observed in limited studies on the medical remedy of caffeine-containing liquid waste released from an industrial plant, nuclear power, factory and other waste matter system [60]. Pre-anaerobic fermentation of coffee pulp has been observed to have caffeine proteins which aimed at yielding decaffeinated coffee. Synthesis of caffeine from coffee waste consists the series of methylation at position *N*-1, *N*-3 and *N*-7 of xanthosine ring which transformed producing about 13–63% reduction in 14 weeks. In contrast, aerobic fermentation results in complete (100%) caffeine degradation were collected within 2 weeks [63].

Degradation Caffeine (Coffee Waste) by Enzymatic Method

Coffee waste anabolic proteins are the most non-spontaneous by various *N*-methyl transferases (NMT) viz., 1-methyl transferases, 3-methyl transferases and 7-methyl transferases [33, 44]. Synthesis of caffeine from coffee plant can be terminated if the synthesis of xanthosine in coffee plant is inhibited because caffeine is synthesised from xanthosine. Low caffeine coffee and tea plants can be yield by inhibiting the enzyme inosine monophosphate dehydrogenase using ribavarin inhibitor [64]. Currently, cDNA methyl transferase (theobromine synthase), a

protein which generates theobromine was isolated. The suppression of caffeine synthase gene and theobromine synthase genes in a coffee plant using RNA interference method have resulted in a plant with 50% lesser caffeine [65]. Harborne, (1993) proposed a chemical defence theory that caffeine may protects its flower buds, fruits, and young leaves from predators such as larvae [66]. It has also proposed an allelopathic theory that caffeine may stop the growth of other plants species in its environment [15]. Therefore, focusing on decaffeinated plants with the information in mind that the genes contained in the production of caffeine may not be useful for enzymatic and microbial caffeine degradation source when required. Caffeine is known to be degraded through theophylline to carbon dioxide and ammonia, a pathway similar to fungal metabolism. It is very important that all the enzymes involved are identical as those microorganisms. The prospect of using caffeine degrading enzymes from tea and coffee plants offer the benefit of abundant fresh leaf material for enzyme extraction and isolation. Oxidases and demethylases are the enzymes involved in caffeine biodegradation [15, 68]. Unfortunately, attempts were made by Yamoka-Yano and Mazzafera to purify demethylase enzymes but found that the purified enzyme was labile and it swiftly lost its activity [2, 68]. Experiment has shown that the use of freeze drying and cryoprotectants to lower down moisture contents enhance the enzymes stability. Generally, caffeine-degrading enzymes are labile and more researches are needed to enhanced caffeine demethylase enzyme stability.

Caffeine is directly oxidised by caffeine oxidase at the C-8 position in a mixed culture consortium belongs to *Rhodococcus* sp, *Alcaligenes* and *Klebsiella* sp., leading to the formation of 1,3,7-trimethyluric acid and this process does not have demethylation steps. Only the partial characterisation of this enzyme is available [20, 31, 51]. The oxidative caffeine degradation to trimethyluric acid (single step) appears to be proficient for the development of enzymatic caffeine degradation. Furthermore, 1,3,7-trimethyluric acid is consequently converted into uric acid. Researches on the stability of enzyme, cloning and over expression of enzyme in an appropriate vector will lead to an improvement of biotechnological method for degradation of caffeine.

Table 2: Comparison of caffeine degradation rates in various bacteria.

Bacteria	Initial caffeine concentration (g/l)	Carbon source (g/l)	Caffeine degradation (%)	Temper-ature	pH	Reference
Klebsiella and *rhodococcus*	0.5	glucose (1)	100% in 10h	-	-	[58]
Serratia marcescens	0.6	-	100% in 72h	-		[56]
Pseudomonas putida	5	sucrose (30.1)	67.2 in 48h	-	-	[49]
Pseudomonas stutzeri gr 21 zf	1.2	sucrose (5)	80.1% in 48h	30 °c	7.0	[70]
Pseudomonas sp. gsc 1182	5	sucrose (5)	80% in 48 h	30 °c	6.0	[71]
Pseudomonas alcaligenes cfr 1708	1.0	-	-	30 °c	7.0-8.0	[32]
Pseudomonas sp. immobilised in agar-agar matrix	10	-	-	-	-	[72]
Brevibacterium sp.	1-8	glucose and sucrose	-	-	-	[73]

Caffeine *N*-demethylation in bacteria was not well-known until recently when two individual caffeine *N*-demethylase enzymes, namely NdmA and NdmB from *Pseudomonas* CBB5 were isolated and characterised [41]. The first report revealed that a single broad-specific demethylase converted caffeine into xanthine [40]; though, due to the incapability to decide the demthylase further, the question of different monooxygenases catalysing position-specific *N*-demethylations was left open [20, 41, 69]. The cloning of *N*-demethylases gene successful leads to characterisation of two highly specific novel *N*-demethylases (NdmA and NdmB) from *Pseudomonas putida* CBB5 [41].

These proteins were categorised as Rieske non-heme iron monooxygenases that catalysed *N*-1 and *N*-3-specific caffeine *N*-demethylation which is related to mono and dimethylxanthines, respectively, to generate 7-methylxanthine. It was also reported that both proteins (NdmA and NdmB) were dependent on a third redox-centre dense Rieske reductase called NdmD, for electron transfer from NADH. While NdmB-NdmD pair catalysed *N*-3 theobromine demethylation, 3-methylxanthine, caffeine, and theophylline to 7-methylxanthine, xanthine, paraxanthine, and 1-methylxanthine respectively. NdmA-NdmD pair catalysed *N*-1 caffeine demethylation, theophylline, paraxanthine, and 1-methylxanthine to theobromine, 3-methylxanthine, 7-methylxanthine, and xanthine, respectively. A third N-demethylase, NdmC, was also partially characterised specifically catalysed *N*-7 demethylation of 7-methylxantine to xanthine. Theophylline production from caffeine was not active on NdmC; this clarified the different degradation pathways for the two compounds in CBB5 [20].

Anaerobic Degradation of Caffeine

Caffeine degradation under anaerobic conditions by microorganisms has been reported in few studies of caffeine-containing sewage treatment. Anaerobic caffeine degradation fermentation results in about 13-63% caffeine reduction in 100 days [2]. In contrast, aerobic fermentation shows 100% caffeine degradation within 14 days [63]. Further studies to enhance degradation of caffeine by this natural fermentation are required. Table 2 shows comparison of caffeine degradation rates in various bacteria.

Factors Affecting Caffeine Degradation

Caffeine degradation by microorganisms (bacteria) is affected by many factors such as temperature, pH, high caffeine concentration, additional carbon and nitrogen source. Caffeine degradation plays a vital role in controlling the rate of enzymes production, degradation of caffeine, which involved in

proliferation of caffeine-degrading microbial cells population and caffeine metabolism in caffeine-rich environment [8]. Also, a number of factors have to be taken into consideration in developing a caffeine degradation method. One of them is temperature, which is an important factor in the decaffeination of hot beverages using biological method [69].

Effects of Caffeine on Microorganism

Caffeine has many effects on different microorganism which varies from one microorganism to another. At low concentration of about 10^{-2} M, caffeine inhibits phosphodiesterase which causes an inhibition or a delay of cell division resulting in an increase in intracellular cAMP levels, which antagonises adenosine receptors and affects intracellular calcium levels [70]. Phosphorylase and others glycolytic metabolic pathway enzymes and nucleic acid metabolic enzymes were not affected by caffeine such enzymes include glucose-6-phosphate dehydrogenase,phosphoglucomutase, hexokinase, nucleoside Phosphorylase, xanthine oxidase, and deoxyribonucleases. Harm, (1967) reported that at a relatively low concentration, caffeine effects on microorganisms shows an antimicrobial effect, resulting in the death and growth inhibition of bacterial strains like *E. coli* and other bacterial strains especially on addition of caffeine at logarithmic phase of growth [76, 77]. Caffeine also inhibits the ultra violet wounds repair in the host-cell-reactivating (hcr^+) strain in *E.coli*. Ultra violet resistant strain of *E.coli* was not infected by caffeine at a concentration of 8mM which suggested that caffeine does not affects recombination repair but inhibits excision repair [8]. In addition, Sandlie et al. (1980) found out that caffeine inhibits DNA synthesis by inhibiting thymidine kinase which sequentially deactivates thymidine incorporation into the DNA and weakens the synthesis of RNA by blocking the uptake of uridine and synthesis of protein by hindering of one of the branched chain keto acids (leucine, valine, isoleucine) [78, 54]. Studies also revealed that caffeine improves the inhibitory effect of some antimicrobial agent such as furazolidone against vibrios and penicillin against *S. aureus*. The effect of caffeine and other related methylxanthines such as 3,7-dimethylxanthine, 1,3-dimethylxanthine, 3-methylxanthine, 7-methylxanthine and 1-methylxanthine on a broad range on Gram-negative and Gram-positive bacteria was studied [8]. It was found that the inhibitory effect is bacteriostatic in sub-lethal doses and bactericidal at high concentrations. Caffeine also affects lactose fermentation and the synthesis of indole by *E. coli* which and these effects are temporary.

The mutagenic effects of caffeine in microorganism (bacteria) is well documented, while its competence to create a changes in genetic codon of nucleotide in higher living organism is not properly documented as there were many contradictory reports on the effects in fruit fly. It provide a nearly definitive situation of extrapolation dangers of an outcome from bacteria to mammals and considering the harm of rod shape structure genes as alarm of changes in the genetic codon.

Biochemical Aspect of Caffeine Degradation

The biochemical aspect of caffeine degradation was not discovered until in the middle of 20^{th} century due to the reports of caffeine toxicity in an independent form or synergism with other antibiotics [52]. The heterocyclic structure of caffeine can be used as a sole source of nitrogen and carbon [39]. After the studies of Kurtzman and Schwimmer on the degradation of caffeine by microorganism, several findings have been reported on various exposure of metabolism of caffeine by microorganism [45].

Culture Conditions and Nutrients

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. It is important to record the initial caffeine concentration in the fermentation medium since caffeine is toxic to microorganisms [7, 53]. Dash and Gummadi, (2006, 2007) for instance, reported that the best microorganism for degradation of caffeine is *Pseudomonas putida* showing complete caffeine degradation in 24 h with caffeine concentration of 6400 mg/L while the inhibitory concentration of *Serratia marcescens* 1200 mg/L of caffeine [56, 69, 80]. Most of the organisms using caffeine as nitrogen source, with the existence of additional nitrogen source to degrade caffeine inhibition in *Pseudomonas* sp, can be logically viewed as a usual situation [71]. Although in contrast to that, in *Pseudomonas putida* no. 352, the existence of an external source of nitrogen does not alter the degradation of caffeine [14].

Applications of Caffeine Degradation

The investigation of caffeine-degrading bacteria begins almost 4 decades ago, and researches done in this field are limited. Very few studies have been reported to isolate the potential caffeine-degrading bacterial strains. More bacteria need to be isolated which could degrade caffeine. There is tendency that organisms which have the ability to grow successfully on tea and coffee plants can degrade caffeine. Apart from offering advantages in decaffeination, degradation of caffeine by microbial enzymes or bacteria is useful in manufacturing useful by-products. *Pseudomonas* species are the best microorganism for caffeine degradation. Decaffeination by bacteria exists mainly through the route of demethylation but in mammals, it occurs through oxidative route. Theophylline being an antagonist of adenosine has been shown to lessen the incidence of kidney malfunction [81]. Caffeine degradation by caffeine-degrading bacteria was reported by Asanoet al. (1993) as the theobromine production is used as an antitussive agent and also in the treatment of coronary heart disease, hypertension and angina pectoris [30, 82, 83]. With their report, caffeine-degrading isolated from soil samples *Pseudomonas* sp. no. 6 shows that Zn^{2+} can deactivate some enzymes which responsible for caffeine catabolism by inhibiting the conversion of theobromine to 7-dimethylxanthine, industrial theobromine production from caffeine (coffee waste) for commercial use has not gone far particularly with *P. putida* no. 352 which showed that conversion of theobromine from caffeine is about 20 g/L yielding about 92% among almost 1000 microorganisms of soil isolates and stock cultures [84]. In addition, it have already been reported that caffeine degradation of coffee pulp either individually or in mixture can also be used in the production of useful by-products, such as production of different types of edible fungus like *Flammulina velutipes*, *Pleurotus* sp. and *Lentinula edodes* using different residues like leaves and coffee husk [85–87].

In addition to the harmful caffeine degradation, solid-state fermentation of coffee pulp can be used in the manufacturing of valuable by-product. In solid-state fermentation, production of plant hormones such as gibberellins has also been achieved using

coffee as a carbon source. Machado et al. (2002) reported at optimised conditions, 0.4925 g/kg of dry substrate gibberlic acid has been produced using one strain of *Fusarium moniliforme* and five strains of *Gibberella fujikuroi* by solid-state fermentation of coffee husk [88]. Gibberlic acid (GA3) production has reached more than 10 g/kg of dry coffee husk while the other was the only substrate of fermentation [89, 90]. In north-shell caffeine degradation by microorganism has opened channels for industrial and clinical production of valuable commercial goods.

Conclusion

Caffeine degradation by microorganisms provides an attractive alternative to the conventional methods of decaffeination; it has an important ability to the environment in the removal of caffeine in caffeinated beverages, soil samples, food products, and water waste biologically. Clinically useful by-products of caffeine metabolism are theophylline, theobromine and other related xanthine oxidases such as 7-methylxanthine, 1-methylxanthine, 3-methylxanthine and demethylase which are the protein responsible for the degradation of caffeine, an effective, stable and specific enzyme immobilisation system that could bring about the production of the above-named by-products will be of high commercial significance. Even though there are some microorganisms that are capable of degrading caffeine successfully, it is not advisable to use of immobilised cells on foodstuff commodities that need to be decaffeinated. Hence harmless enzyme can be used for this purpose which can bring about a successful decaffeination. Biodecaffeination can in addition to be used especially as feed for animal in the treatment of solid caffeine waste like pulp, soil samples, liquid waste and husk.

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