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The Ability of Dermatophytes to Utilize Methylxanthine as Sole Source of Carbon and Nitrogen

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Abstract: Methylxanthine contains common compounds that widely consumed by human through their present in food, drink and beverages or through involving in pharmaceutical drugs. Dermatophytes were selected to investigate for their ability to utilize three main compounds of methylxanthine as sole source of carbon and nitrogen. *Trichophyton mentagrophytes* and *Epidermophyton floccosum* were grown on three types of media: Medium A lacks of carbon source, medium B lacks of nitrogen source and medium C lacks of carbon and nitrogen sources. Two isolated species of dermatophytes revealed well growth on medium A and less on media B and C. Dermatophytes showed ability to use methylxanthine as sole source of carbon and nitrogen with efficiency to use carbon than nitrogen.

Key words: Caffeine, dermatophytes, methylxanthine, theophylline and theobromine

INTRODUCTION

Methylxanthine is an important group of purine alkaloids. It consists of three main compounds; caffeine, theophylline and theobromine (Scheindlin, 2007). Their chemical structures are composed of nitrogen and carbon within heterocyclic structure. Thus, methylxanthine can be considered enrichment source of nitrogen and carbon and degradation of its compounds can permit the recycling of their elements into central metabolic pools of environment (Zrenner *et al*., 2006).

In plants-containing methylxanthine which are including *Coffea arbacia*, *Cola nitida*, *Camellia sinensis*, catabolism of caffeine produces theophylline, theophylline converts to 3- methylxanthine and theobromine converts to caffeine (Ashihara *et al*., 1996). This degradation process is related to seasonal variation (Mohanpuria *et al*., 2009).

Generally, caffeine degrading microbes have been isolated from natural habitudes such as soil under coffee cultivation and human domestic waste water (Yamaoka-Yano and Mazzafera, 1998; Mohapatra *et al*., 2006). Microorganisms, especially bacteria and fungi, play an important role in degradation of methylxanthine to their simple metabolites. In contrast with bacteria, a few studies illustrated the ability of fungi to degrade methylxanthine. Most of them are focused on the activity of fungi to catalyze caffeine than other methylxanthine which may be related to highest available amounts of caffeine in nature compared with other members of methylxanthine group. However, some fungi have the ability to degrade methylxanthine while other are not based on specific enzymes that fungi may have to perform this process. The fungus *Pleurotus florida* can grow on coffee wastes contain 3% caffeine and after fungal growth the percentage of caffeine decreased to 0.4% (Murthy and Manonmani, 2008). *Rhizopus delemar* also showed ability to catalyze caffeine and theophylline by solid-state fermentation (SSF) in packed bed column bioreactor (Tsubouchi *et al*., 1985). Furthermore, most of *Aspergillus ochraceus* strains showed a significant degree of degradation of caffeine when grown on green coffee medium (Tagliari *et al*., 2003).

On the other hand, many other species of fungi could not be able to use methylxanthine as sole source of carbon or nitrogen. *Fusarium moniliforme, Penicillium chrysogenum* and the yeast *Pichia butonii* are good examples for this inability when they could not utilize caffeine and theobromine as sole source of nitrogen, while other purine can be utilize by these fungi (Vega *et al*., 2003; Allam and Elzainy, 1969; Allam and Elzainy, 1971).

Dermatophytes consider the most common pathogenic fungi causing cutaneous disease known as dermatophytoses (Hainer, 2003). Infection by these fungi is distributed all over the world. They contain only three genera; *Trichophyton, Microsporum* and *Epidermophyton*. Keratinous substances present in skin, hair and nail are the prefer materials for nutrition of dermatophytes (Simpanya, 2000).

Determination the ability of dermatophytes to use methylxanthine as sole source of carbon and nitrogen was the aim of the present study.

MATERIALS AND METHODS

Organisms: Two strains of dermatophytes including *Trichophyton mentagrophytes* and *Epidermophyton floccosum* were clinical isolated from AL-Hussein general hospital of Karbala providence in February 2009. Skin scales of fungal lesion were cultured on Sabouraud's glucose agar of the following components; glucose 20 g, peptone 10 g, agar 15 g, chloramphenicol 0.05 g and 1000 ml of distilled water. Cultures were incubated at 28 ºC for two weeks. Grown fungi were diagnosed according to criteria recorded by Rippon (1988) and Emmons (1970).

Chemical agents: Theophylline, caffeine, and theobromine were purchased from HiMedia, Mumbai-India. Different concentrations of methylxanthine were used.

Media preparation: Standard medium used for ordinary culturing of fungi contain dextrose or glucose as carbon source and peptone as nitrogen source (Emmons, 1970). Thus, three types of media were prepared that have the following design: Medium A prepared from mixing peptone 10 g, agar 15 g with 1 L of distilled water; Medium B contains glucose 20 g, agar 15 g dissolved in 1 L of distilled water; and medium C that contains agar 15 g only in 1 L of distilled water.

Fungal growth assay: Colony diameter method employed by Kücüc and Kivan (2003) was used. Various concentrations of methylxanthine were mingled with melting prepared media. Then, poured in steriled Petri dishes. A disk (9 mm) of old grown fungi (at 28ºC for 1 week) was inoculated on the center of culture media. Plates were incubated at 28ºC for 1 week. Perpendicular colony diameters (mm) of grown strains were measured. Each experiment was repeated triplicate for statistical analysis.

Statistical analysis: Result data were statistically analyzed by using two-way variance of analysis (ANOVA) with less significant difference (L.S.D.) at $P < 0.05$.

RESULTS

Caffeine, theophylline and theobromine are the main members of methylxanthine were selected for their ability to supply dermatophytes by nitrogen and carbon when the absence of other sources.

In the absence of glucose in medium A, distinct growth was observed of two strains of dermatophytes. Lower concentrations of methylxanthine showed more effectiveness to enhance fungal growth. The growth of *T. mentagrophytes* regarded to be very well on medium A than those of *E. floccosum* (Fig. 1 and 2).

On media B and C, the density of fungal mycelia was very low and may reach to form tiny extended colonies (Fig. 3). Colony diameter of grown fungi was approximately the same as on medium A (Fig. 4 and 5). *E. floccosum* did not exhibit any growth on both B and C media compared with enlargement diameter of *T. mentagrophytes* colonies. However, lower concentrations of methylxanthine also encouraged *T. mentagrophytes* to grow well than at high doses of methylxanthine.

DISCUSSION

Although bacteria consider the most effective microorganisms of methylxanthine degradation that can utilize the metabolites of methylxanthine as main source of carbon and nitrogen, fungi also demonstrated to have such ability. When caffeine was used as sole source of

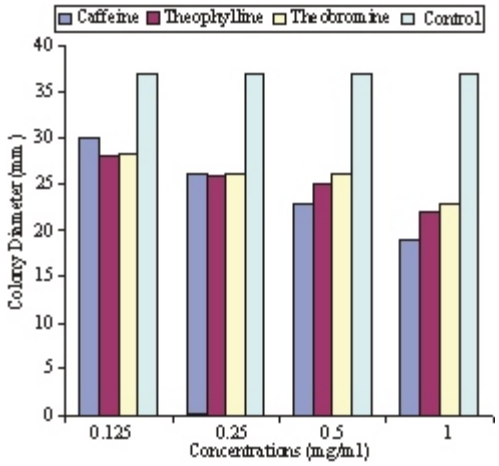

Fig. 1: Colonies diameter of grown *T. mentagrophytes* on medium A (no carbon source)

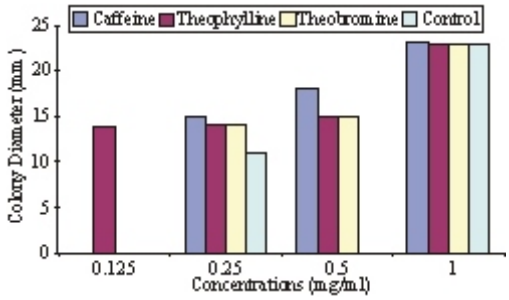

Fig. 2: Colonies diameter of grown *E. floccosum* on medium A (no carbon source)

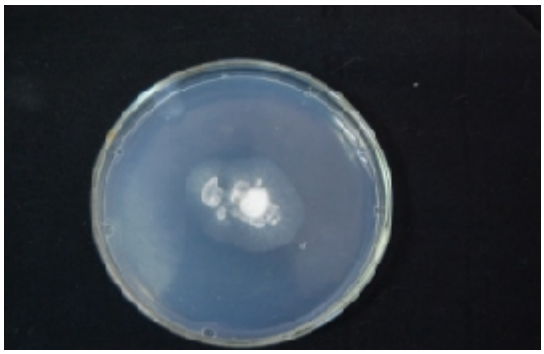

Fig. 3: Colony of *T. mentagrophytes* grown on medium B and C

carbon and nitrogen by *Serratia marcescens*, paraxanthin and or theobromine were released in liquid medium (Mazzafera *et al*., 1996). Fungi strains with the highest ability to degrade caffeine were identified as *Aspergillus* and *Penicillium* (Mazzafera, 2002). Hence, seven of twenty strains of *Aspergillus* and *Penicillium* genuses were able to grow on media containing caffeine (Hakil *et al*., 1998).

Growth of dermatophytes on media containing methylxanthine with lack of simple sugar as source of carbon found to be excellent and combined with normal

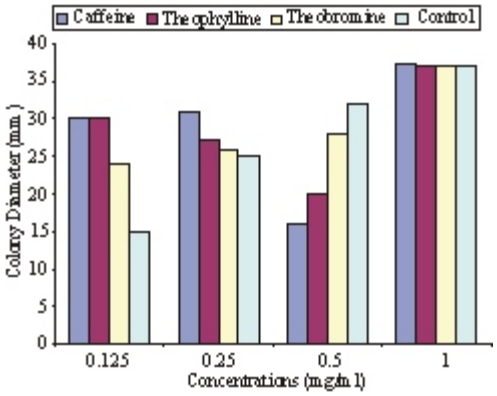

Fig. 4: Colonies diameter of grown *T. mentagrophytes* on medium B (no nitrogen source)

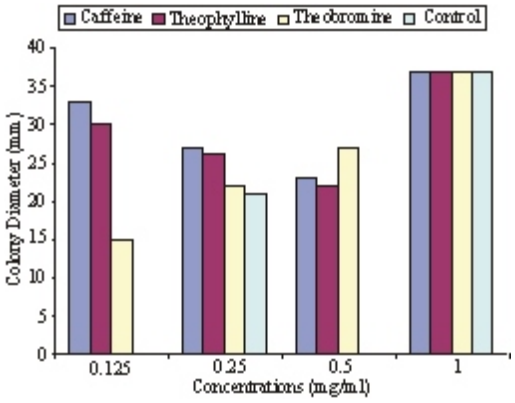

Fig. 5: Colonies diameter of grown *T. mentagrophytes* on medium C (no carbon and nitrogen source)

density of fungal colonies. Based on these results, all of three selected methylxanthine were consider suitable source of carbon with sufficient supplying during the long periods of incubation for dermatophytes growth. The efficiency of dermatophytes for obtaining nitrogen from methylxanthine degradation in the absence of peptone was less than when fungi used methylxanthine to obtain carbon. Thin density of *T. mentagrophytes* mycelia with absent of *E. floccosum* growth may consider an indicator for inefficiency of dermatophytes to utilize methylxanthine as nitrogen source with species-dependent manner. Thus, methylxanthine can be represented a good source of carbon than nitrogen for dermatophytes growth.

By contrast, many studies demonstrated that methylxanthine can be useful for fungi as a source of nitrogen when culturing media supplied with carbon source. Results in Hakill *et al.* (1999) study confirmed that caffeine can be used in solid-state fermentation as sole nitrogen source if sucrose is used as the carbon source. The degradation of caffeine by *Penicillium verrucosum* was complete (100%) at 72 h fermentation in the absence of any added nitrogen source (Roussos *et al.*, 1994).

Caffeine is the most important agent of methylxanthine group due to high consuming of it not only in pharmaceutical therapy, but in food, drink and beverages (Chou, 1992) Catabolism of caffeine in microorganisms commences via two possible mechanisms; demethylation and oxidation. Through the demethylation route, the major metabolite form in fungi is theophylline (Hakil *et al.*, 1998), while theobromine is the major metabolite in bacteria (Yamaoka-Yano and Mazzafera, 1999). Enzymatic activities are considered responsible of methylxanthine degradation.

In bacteria, the conversion of caffeine to its metabolites is primarily brought about by N-demethylase, caffeine oxidase and xanthine oxidase that are produced by several caffeine-degrading bacteria species such as *Pseudomonas putida* and species within the genera *Alcaligenes, Rhodococcus* and *Klebsiella* (Dash and Gummadi, 2006). Whereas, fungi caffeine in can degrade by caffeinases enzymes. These enzymes are methyl-releasing from caffeine molecule to give theophylline and methanol as products, then theophylline is transformed in 3-methylxanthine and xanthine (Aguilar *et al.*, 2008). Thus, the principle caffeine degradation products in fungi were theophylline and 3-methylxanthine (Tagliari *et al.*, 2003).

In present study, dermatophytes did not prefer any of methylxanthine than other to grow with. However, theophylline considers a complex compound for microorganism's nutrition and they could not be able to degrade it compared with other members of methylxanthine. Growth of *Serratia marcescens* was diminished in the present of theophylline (Mazzafera *et al.*, 1996). All of twenty strains of *Aspergillus* and *Penicillium* showed the efficiency to catalyze theobromine with inability to degrade theophylline (Hakil *et al.*, 1998).

In human body, caffeine and other methylxanthine are degradation in liver through the activity of cytochrom P450 (Berthou *et al.*, 1992). Sauer (Sauer, 1982) obtained indications that caffeine in yeast was degraded by cytochrom P450 suggesting that the catabolic pathway might be similar to animals. However, kinetic study showed that caffeine-degradation by *Aspergillus* sp. was related to the development of mold and its respiration (Brand *et al.*, 2002).

Methylxanthine could be inhibited fungal growth when they presence in high concentrations. Jayaratna *et al.* (2007) found that colony diameter of *Monacrosporium ambrosium* fungus grown on caffeine containing media was significantly less than on the control media. Furthermore, conidia germination of *Sporothrix schenckii* was prevented by caffeine (Rodriguez-Del-Valle *et al.*, 1984). Thus, the results of above studies supporting our finding, when low concentrations of methylxanthine encouraged the growth of dermatophytes more than at higher concentrations.

Additionally, development of a process involving microbial enzymatic degradation of caffeine to non-toxic compound is necessary to solve the problems of chemical extraction of caffeine in food products as well as treating the caffeine containing waste product (Gokulakrishnan *et al.*, 2005).

In conclusion, dermatophytes showed the ability to utilize methylxanthine as sole source of carbon and nitrogen. The significant supplying of carbon by methylxanthine to dermatophytes can be consider greatest than of nitrogen through dermatophytes were producing less mycelia density on medium lack of peptone.

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