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 arabica, 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 habituses such as soil under coffee cultivation and human domestic waste water (Yamaoka-Yano and Mazzafara, 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 dermatophyoses (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 °C 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 d 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 sterilized Petri dishes. A disk (9 mm) of old grown fungi (at 28ºC for 1 week) was inoculated in 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 carbon and nitrogen by \( Serratia marcescens \), paraxanthin and or theobromine were released in liquid medium (Mazzaferra \textit{et al.}, 1996). Fungi strains with the highest ability to degrade caffeine were identified as \textit{Aspergillus} and \textit{Penicillium} (Mazzaferra, 2002). Hence, seven of twenty strains of \textit{Aspergillus} and \textit{Penicillium} genuses were able to grow on media containing caffeine (Hakil \textit{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
Colonies of *Rhodococcus* own affinity in can of incubation for dermatophytes growth. The caffein was degraded by *Y. vermucosum*. Caffeine was found in fungi of organism (Berthouzoz et al., 1999). Enzymatic activities are considered the most important activity in the degradation of caffeine as a nitrogen source (Roussos et al., 1998). Through the degradation in liver, theobromine is diminished in the presence of theophylline (Mazzaferr et al., 1998). 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 (Mazzaferr 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 (Berthouzoz 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 *Monacrosporum 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.

REFERENCES


