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Degradation of Ethanol by Two Species of Dermatophytes: *Trichophyton mentagrophytes and Epidermophyton floccosum*

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Abstract: Ethanol is an important industrial product. It can be degraded by microorganisms to use as sole source of carbon. *Trichophyton mentagrophytes and Epidermophyton floccos*um are most known species of dermatophytes. Their ability to degrade ethanol was detected. Low concentrations of ethanol enhanced two tested species of dermatophytes to grow on culture medium faster in absence of carbon source. Activity of higher percentages of ethanol was also mention. In this study, dermatophytes showed capacity to degrade ethanol and used it as sole carbon source.

Key words: Ethanol · Dermatophytes · Degradation · *T. mentagrophytes* · *E. floccosum*

INTRODUCTION

Cultivation of fungi on different materials including plant debris, foods and some industrial products stimulates this organism to produce greatest biomass. Additionally, nutritional substances that consider a source of carbon and nitrogen must be available in sufficient amounts to complete life cycle of fungi. Carbohydrates, especially simple sugars, consider enrichment substances supplying fungi with carbon. In the absence of sugar, fungi seeking for another source of carbon for their persistence. Thus, availability of various types of alcohol could be utilized by fungi for obtaining carbon under aerobic and anaerobic condition. Neidig [1] found that eight species of fungi showed the ability to utilize alcohol as sole source of carbon. Several aromatic compounds and alcohol can also consider a sole source of carbon for growing *Penicillium sinplicissimum* through induction of enzymes activities [2].

Ethanol is the most common type of alcohol that is widely consuming by many industrial activities. Toxic effects of ethanol on fungi may convert to useful at specific concentrations through utilizing ethanol as sole carbon source. The efficiency of fungi to consume ethanol is variable based on enzymatic activities when some of them are able to degrade ethanol, while other is not. However, *Saccharomyces cerevisiae* is one of the more effective fungi to utilize ethanol as carbon source [3]. It revealed a wide rang of ethanol tolerance level (10%-20%) [4]. In addition to *S. cerevisiae*, Prinie-Fisker and Woetz [5] demonstrated that *Exophiala lecanii-corni* fungus is able to utilize ethanol as sole carbon and energy sources for its growth. Whereas, other fungi have inability or poorly usage of ethanol as with *Penicillium cinnabarinus* [6].

Furthermore, ethanol has many other effects on fungi. The transition from yeast-like cells to mycelia of *Aureobasidium pullulans* was induced by ethanol [7]. Meanwhile, ethanol increased survival time of *Mucor* species when they presence under high oxygen condition [8].

Dermatophytes are common known group of pathogenic fungi that contain three genera: *Trichophyton, Microsporum* and *Epidermophyton*. They cause cutaneous disease on human and animals skin [9]. These fungi need keratinous substances for their growth. Thus, their infection is limited within skin, hair and nail [10]. The ethanol has also effects on dermatophytes. It was increased the level of phospholipids in cells of *Microsporum gypseum* [11].

Dermatophytes were selected to be the main subject of present study through tested their ability to degrade ethanol and used it in absence of carbon source.

MATERIALS AND METHODS

Fungi Strains: *Trichophyton mentagrophytes* and *Epidermophyton floccosum* were clinical isolated from

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dermatology consultation of AL-Hussein general hospital of Karbala province in February 2009. Skin scales of fungal lesion from patients were cultured on Sabouraud's glucose agar has 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 the criteria recorded by Rippon [12] and Emmons [13].

Preparation of Media: Medium that applied to detect ethanol degradation by dermatophytes was prepared based on standard compositions of Sabouraud's glucose agar that mention previously in primary isolation of dermatophytes. Glucose was eliminated from this medium. Therefore, medium in this study contains the following compositions: peptone 10 g, agar 15 g and 1 L of distilled water.

Determination of Fungal Growth: Colony diameter method recorded by Kücüc and Kivan [14] was used. For obtaining various percentages, ethanol was diluted with melting prepared media. Then, poured of media in steriled Petri dishes. A disk (9 mm) of old grown fungi (at 28°C for 1 week) was inoculated on the center of culture media. Plates were incubated at 28°C 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

The ability of dermatophytes to grow on media containing various percentages of ethanol in absence of glucose was considered as indicator for ethanol degradation.

Primary experiments with highest percentages of ethanol (up to 15%) showed absolute absence of fungal growth. Thus, ethanol levels in culture media were decreased. Growth of two isolated dermatophytes exhibited increasing of colony diameter in presence of low percentages of ethanol. Therefore, lower levels of ethanol consider stimulating factor that allows dermatophytes to grow faster despite the absence of carbon source (Figure 1).

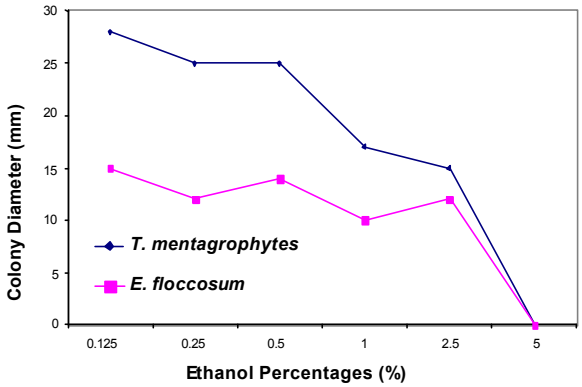

Fig. 1: Colony diameters of *T. mentagrophytes* and *E. floccosum* on medium containing different percentages of ethanol

Furthermore, texture of grown colonies of *T. mentagrophytes* and *E. floccosum* on ethanol media revealed perfect characters in combining with yielding mycelia that have normal hyphal density.

DISCUSSION

At present time, ethanol considers to be an important substance due to entering in production a wide rang of industrial products. Furthermore, it thought to be a promising alternative fuel in future [15].

Ethanol can produce by many microorganisms under anaerobic conditions through fermentation process. After production, ethanol utilizes as carbon and energy sources. While, degradation of ethanol under aerobic conditions can be performed by few types of fungi.

Concentrations of ethanol could be considered the most effective factor for degradation process. At high concentrations, availability of ethanol in medium can prevent fungal growth. This was distinctly noted when the growth of dermatophytes was completely diminished in the presence of high percentages of ethanol. This phenomenon was also recorded with the fungus *Baudoinia*. The growth of this fungus was strongly inhibited following exposure to high ethanol concentrations for shorter durations, while its germination increased at low concentrations [16].

At greater amounts, the main effects of ethanol in fungi is inhibited the activity of protease enzyme [6]. Whereas, low concentrations of ethanol estimated the activation of other enzymes, such as catalase [17] and peroxidase [18]. Dermatophytes have several types of protease enzymes [19,20]. Thus, growth of two isolated

strains based on this fact was inhibited in presence of high level of ethanol. However, degradation of ethanol by fungi is found to be under gene control. The study of ethanol degradation by *Aspergillus nidulans* demonstrated that ethanol is utilized by alc system [21].

Dermatophytes have the ability to secret many enzymes including a-naphthyl acetate esterase, acid phosphatase, lactate dehydrogenase, malate dehydrogenase, tetrazolium oxidase and catalase [22]. Furthermore, they also have other enzymes that responsible for glucose metabolism through hexosediphosphate and hexosemonophosphate pathways of carbohydrate dissimilation, as well as the tricarboxylic acid cycle to obtain carbon and energy [23] as studied in *T. mentagrophytes* [24]. In the absence of glucose, usage of ethanol as a major carbon source is likely performing through glyoxylate cycle that includes activation of isocitrate lyase (ICL) [16]. Glyoxylate cycle enzymes are usually appeared when microorganisms were grown on non-sugar substrate like ethanol [25]. However, Ewaze *et al.* [16] found that ethanol was catabolized into central metabolism via the activity of alcohol dehydrogenase and acetaldehyde dehydrogenase [16].

Two isolated strains of dermatophytes that grown on medium containing low percentages of ethanol produced normal colonies with perfect texture. While in other study, the mycelia of *Laccaria amethystea* could not sufficiently grow in the ethanol medium which meaning that ethanol can little utilized by this fungus as carbon source [25].

Although *T. mentagrophytes* and *E. floccosum* were cultivated on the same ethanol medium, they showed no difference in growth rate or their ability to degrade ethanol. They both grew in parallel manner in presence of various levels of ethanol.

In conclusion, *T. mentagrophytes* and *E. floccosum* have the ability to degrade ethanol and used its metabolites as sole source of carbon. Moreover, ethanol can be consider a good sources of carbon for dermatophytes when it presence in low concentrations.

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