THE CARBOHYDRATE ASSIMILATION PATTERN IN IRANIAN TYPICAL AND ATYPICAL STRAINS OF MICROSPORUM CANIS

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ABSTRACT - The values of fourteen carbohydrates assimilation patterns were investigated for identification of typical and atypical strains of Microsporum canis. Thirty-eight strains of typical and twenty-two strains of atypical Microsporum canis, Microsporum canis NCPF 352 and one Microsporum distans were included in this study. Statistical analysis of the results indicated that despite rapid variations within the pattern of carbohydrate utilization, no variation was observed between strain and race of carbohydrate assimilation. The results also revealed that galactose and melibiose were best utilized for assimilation by the typical and atypical strains of Microsporum canis. Production of abundant microconidia, macroconidia and chlamydospores by use of galactose and melibiose suggested that these two carbohydrates were effective in production of fluffy appearance in colonies examined. The Microsporum canis NCPF 352 strongly utilized glucose, melezitose and melibiose in addition to the two above-mentioned carbohydrates. Weak fructose assimilation was observed in Microsporum distans. Carbohydrate assimilation pattern is similar in different typical and atypical strains of Microsporum canis, but it could be regarded as a valuable and fast method of identification of Microsporum canis as well as a marker in epidemiological investigations. Arch Med Sci (2006) 11: 132-137

Key Words: Microsporum canis, Microsporum distans, carbohydrate assimilation

INTRODUCTION

Biochemical techniques such as enzymatic activities, SDS polyacrylamide gel electrophoresis (SDS-PAGE), carbohydrate assimilation, free hydrolys, assimilation of nitrogen compounds as sodium nitrate and also ammonium sulphate, carbon, tyrosin and genin hydrolys were frequently used in identification of microorganisms (1,2,3). Of those the carbohydrate assimilation test is a simple and established method for identification of yeasts. Nowadays, attempts are made to apply this method in identification of mycelial fungi. Since 1977, there has been substantial research in the growth of different dermatophytes in various carbohydrate environments. However, the results showed that the ability of isolates to assimilate some compounds was more related to the pathogenicity rather than their morphological characteristics, but in the other routine morphological features like identification of known isolates, carbohydrate assimilation test can be used as a aid in identification of such organisms (3). Mirmouche et al showed that combination of 5-glucose and 6-glucanin encouraged macroconidia production in Microsporum canis (4). This technique could identify not only the specific species and its related strains but also could provide a marker for global epidemiological investigation (5). Records in Iran indicate that the occurrence of Microsporum canis has been increased from one in 144 cases of tinea capitis in 1965 to 41.15% in 1988 (6), specially during the years of imposed Iraq-Iran war. As the Microsporum canis is a common known causal agent of tinea capitis in Iran on one hand and drug resistance of the typical or atypical strains of this species on the other hand, we decided to evaluate the efficacy of the pattern of carbohydrate assimilation in identification of the Iranian species and its related strains (7).

MATERIALS AND METHODS

Strains Used

60 Iranian human isolates of Microsporum canis (38 typical and 22 atypical strains), NCPF 352 and one
Iranian Microsporum distortum were used.

**Assimilation Method**

Growth rate was investigated in media with 14 different carbohydrates based on the method used by Tekker (5).

**Basal Medium**

Potassium dihydrogen phosphate (1.0 g), magnesium sulphate 7H2O (0.5 g), ammonium sulphate (5.0 g), and purified agar (Difco) (15 g) were mixed in distilled water and made up to 800 ml. The medium was autoclaved at 100 °C for 20 min.

**Carbohydrate Compounds**

The carbohydrate sources were prepared as 5% solution in sterile distilled water and sterilized by filtration through 0.45 µm membrane. Twenty ml of each carbohydrate solution was incorporated separately into 80 ml melted and cooled basal medium giving a final concentration of 1%, and distributed isotropically in 3 ml amounts as slants in Piper bottles. Slopes of basal medium alone were prepared as a base-line control. The following carbohydrates were included in the study: adonitol, arabinose, erythritol, galactose, glucose, inositol, levulose, maltose, mannitol, melibiose, melitose, sucrose, trehalose, and xylose. All were obtained from Merck except mannitol, inositol, and xylose (Difco).

**Procedure**

Strains were grown on 4% Sabouraud dextrose agar including 0.5 g/l 1-ethylxanthine and 0.05 g/l 1-chloroanisole (SCC) (Fig. 1) and for the carbohydrate test were subcultured twice on transfer medium containing dipotassium hydrogen phosphate (0.3 g), pepton (tektan pepton, Difco) (2.0 g), and purified agar (Difco) (15 g) in distilled water (1 l). Slopes containing each specific carbohydrate were point-inoculated using sterile 1 µl loops. Tests were inoculated in duplicate and incubated at 30 °C for 21 days. Readings were recorded at 7, 14, and 21 days and graded in relation to control, by means of production of fluffy colonies with aerial mycelium as follows: 0 = negative (−), + to ++ = weakly positive (+), +++ = strongly positive (+++). Microscopic features of colonies were also studied by use of slide culture method. The Fisher and chi-square statistical tests were used to analyze the results. All results were considered significant at p<0.05 and \( \chi^2 < 1 \).

**RESULTS**

All strains assimilated erythritol, trehalose, sucrose, and maltose more strongly and rapidly than the other carbohydrates (Table 1) (Fig. 2-5). Therefore these sugars were considered as determinant carbohydrates and used throughout this study. As shown in Table 1, no weak utilization of erythritol has been found in Iranian Microsporum distortum. Fibrous mycelia were only obtained on media containing erythritol, trehalose, glucose, levulose, and mannitol. Erythritol stimulated production of macroconidia in only atypical strains. Erythritol and trehalose stimulated conidiation in both typical and atypical strains. All strains of Microsporum canis were unable to produce conidia in sucrose and melitose media (Table 2). The results are summarized in Table 1, 2.

**Table 1.** Carbohydrate assimilation patterns of typical and atypical strains of Microsporum canis, NCFS352 strain and Iranian strain of Microsporum distortum

<table>
<thead>
<tr>
<th>Isolate</th>
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<th>Fr</th>
<th>Ga</th>
<th>Gl</th>
<th>In</th>
<th>Te</th>
<th>Mt</th>
<th>Man</th>
<th>Mel</th>
<th>Mol</th>
<th>Suc</th>
<th>Tr</th>
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<td>Typical</td>
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<td>NCFS352</td>
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**Table 2.** The sporulation in media with or without determinant carbohydrates in respect to the type of strains and production of fluffy colony

<table>
<thead>
<tr>
<th>Carbohydrate</th>
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<th>Tr</th>
<th>Suc</th>
<th>Mol</th>
<th>Control</th>
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<tr>
<td>Fluffy colony</td>
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</tbody>
</table>

Ad: Adonitol; Ar: Arabinose; Er: Erythritol; Ga: Galactose; Gl: Glucose; In: Inositol; Te: Trehalose; Mt: Maltose; Man: Mannitol; Mel: Melibiose; Mol: Melitose; Suc: Sucrose; Tr: Trehalose; Xy: Xylose; T: Typical, AT: Atypical, nke: nonconidial; nke: nonconidial; nke: nonconidial; nke: nonconidial; echinon: echinon; echinon: echinon; echinon: echinon; echinon: echinon; chl: chlmydioscolia; chl: chlmydioscolia; chl: chlmydioscolia; chl: chlmydioscolia

www.SID.ir
Fig. 1. Colony of *Microsporum canis* in Soc medium after fourteen days at 30 °C. A: atypical strain, B: typical strain.

Fig. 2. Typical strain of *Microsporum canis* with fluffy mycelium in determinant carbohydrate media.

Fig. 3. Typical strain of *Microsporum canis* without fluffy mycelium in determinant carbohydrate media.
Fig. 4. Atypical strain of Microsporum canis with fluffy mycelium in determinant carbohydrate media.

Fig. 5. Atypical strain of Microsporum canis without fluffy mycelium in determinant carbohydrate media.

Fig. 6. Macroconidia of Microsporum canis in erythritol medium. A: Typical strain, B: Atypical strain.
DISCUSSION

Upon reviewing the data on fungal infections in medical mycology laboratory of School of Public Health, Tehran University of Medical Sciences, we find an increase in numbers of cases caused by Microsporum canis especially after the imposed Iraq-Iran war (6). Atypical Microsporum canis was first described by Sabouraud in 1910 (7). Since then there have been numerous reports from different parts of the world on the isolation of atypical strains from human and animal specimens (8,9,10,11). Rezaei's study in Tehran (1993) showed that of the 114 isolated Microsporum canis, 43 strains (37%) were atypical (7).

In spite of the fact that griseofulvin is the drug of choice for the treatment of dermatophytic infection, numerous atypical clinical isolates are resistant to this drug (12,13). Therefore definitive diagnosis of this type of Microsporum canis is quite necessary. Also due to the fact that atypical colonies on Sabouraud dextrose agar routinely used in medical mycology laboratories are mostly glabrous and lack typical characteristics, these require several media and a long period of time for identification. On the other hand, use of SDS-PAGE for this purpose is not possible in all laboratories, so finding a simple and rapid method seems unavoidable. For this reason the present study was undertaken to determine the assimilation patterns of typical and atypical form of Microsporum canis and also evaluate this method as a tool in rapid identification. However, statistical analysis indicated that there was no correlation between the type of strain and the assimilation pattern and also production of glucose mycelium, but there was a relevance to the carbohydrate assimilation pattern and country of origin. The microscopic examination of Microsporum canis strains on carbohydrates media showed that contrary to results obtained by Morten's investigations (14), melibiose production was enhanced by erythritol in atypical forms with or without glucose mycelium. In all strains of Microsporum canis sporulation enhancement occurred by erythritol and trehalose assimilation (Fig. 6). Although melibiose containing sucrose and melibiose only mycelium was produced without any fluffy colony, but on media with erythritol and trehalose a large number of macroconidia, microconidia and chlamydoconidia were produced and gave a fluffy appearance to the colonies (Table 2). It is interesting that in contrast to strong assimilation of glucose, mannitol, melibiose and weak assimilation of galactose and melibiose by strain NCPF352, all Iranian atypical and typical strains of Microsporum canis failed to utilize those carbohydrates. Therefore reaction with these compounds was sufficient to differentiate Iranian and British NCPF352 strains of Microsporum canis and also Microsporum distans. On erythritol, trehalose and levulose media, NCPF352 was able to produce fructose mycelium. Iranian isolates produced fluffy mycelium on glucose, galactose and mannitol, as well as the three above-mentioned carbohydrates. Microsporum distans only weakly assimilated erythritol. This organism produces fluffy mycelium on glucose, trehalose, and levulose, whereas typical and atypical strains of Microsporum canis also showed this capability on erythritol, mannitol and glucose. Finally it must be concluded that the carbohydrate assimilation technique, unlike the SDS-PAGE method (14), has a limited application in identification of Iranian typical and atypical forms of Microsporum canis, but this techniquem is particularly useful in identification of Microsporum distans and also NCPF-352. Even though only one strain from UK was examined, but the correlation of assimilation patterns with country of origin suggested that simple tests could provide a marker for global epidemiological investigations. Growth effects of some carbohydrates could possibly be considered in pharmaceutical sciences and also antigenic capabilities for further investigations in future.

REFERENCES


