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 NCPF352 and one Microsporum distortum were included in this study. Statistical analysis of the results indicated that despite limited variations within the pattern of carbohydrate utilization, no correlation was observed between strain and rate of carbohydrate assimilation. The results also revealed that erythritol and trehalose were best utilized for sporulation by the typical and atypical strains of Microsporum canis. Production of abundant macroconidia, microconidia and chlamydoconidia by use of erythritol and trehalose suggested that these two carbohydrates were effective in production of fluffy appearance in colonies examined. The Microsporum canis NCPF352 strongly utilized glucose, mannitol and melibiose in addition to the two above-mentioned carbohydrates. Weak erythritol assimilation was observed by Microsporum distortum. Carbohydrate utilization pattern is unable to differentiate typical and atypical strains of Microsporum canis, but it could be regarded as a valuable aid for identification of Microsporum distortum as well as a marker in epidemiological investigations. Acta Medica Iranica 38 (3): 132-137; 2000

Key Words: Microsporum canis, Microsporum distortum, carbohydrate assimilation

INTRODUCTION

Biochemical techniques such as enzymatic activities, SDS polyacrylamid gel electrophoresis (SDS-PAGE), carbohydrate assimilation, urea hydrolysis, assimilation of nitrogen compounds as sodium nitrate and also ammonium sulphate, casein, tyrosin and gelatin hydrolysis 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 carbohydratic environments. However the results showed that the ability of isolates to assimilate some compounds was more related to the pathogenesity rather than their morphological characteristics, but in the where routine morphological features fail to identify the unknown isolates, carbohydrate assimilation test can be used as an aid in identification of such organisms (3). Mayroudea et al showed that combination of 5gl-1 glucose and 0.6 gl-1 thiamin 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 1952 to 41.1% 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 Tucker (5).

Basal Medium

Potassium dihydrogen phosphate (1.0g), magnesium sulphate 7H2O(0.5g), ammonium sulphate(5.0g), and purified agar (Difco) (15.0g), were mixed in distilled water and made up to 800 ml. The medium was autoclaved at 10 lb/20 min.

Carbohydrate Compounds

The carbohydrate sources were prepared as 5% solution in sterile distilled water and sterilized by filtration through 0.45 μ 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 aseptically in 3 ml amounts as slopes, in bijoux 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 Merk except mannitol, inositol and xylose (Difco).

Procedure

Strains were grown on 4% sabouraud dextrose agar including 0.5grl^{-1} cyclohexamid and 0.05 grl^{-1} chloramphenicol (SCC) (Fig. 1), and for the carbohydrate test were subcultured twice on transfer medium containing dipotassium hydrogen phosphate (0.3gr), pepton (bacto- pepton, Difco) (2.0gr), and purified agar (Difco)(15 gr) in distilled water (1 lit). 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. 7,14,21 days and graded in relation to control, by means of production of fluffy colonies with aerial mycelium as follows: nil to ± = negative (-). + to .+/++ = weakly positive (w),++ to +++ = strongly positive(+). Microscopic features of colonies also were 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 $p<0.9$ 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 only weak utilization of erythritol has been found in Iranian Microsporum distortum. Floccose mycelia were only obtained on media containing erythritol, trehalose, glucose, levulose and mannitol. Erythhtol 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 tables 1,2.

Table 1. Carbohydrate assimilation patterns of typical and atypical strains of Microsporum canis, NCPF352 strain and Iranian strain of Microsporum distortum

Isolate	origin	Ad	Ar	Er	Ga	Gl	In	Le	Mlt	Man	Melib	Mel	Suc	Tr	Xy
Typical	Tehran	-	-	+	-	-	-	-	+	-	-	-	+	+	-
Atypical	Tehran	-	-	+	-	-	-	-	+	-	-	-	+	+	-
NCPF352	UK	-	-	W	W	+	-	-	+	+	+	W	W	+	-
M.dist.	Tehran	-	-	W	-	-	-	-	-	-	-	-	-	-	-

Table 2. The sporulation in media with or without determinant carbohydrates in respect to the type of strains and production of fluffy colony

Carbohydrate / Fluffy colony	Strain	Er	Tr	Suc	Mel	Control
+	T	mic	mic.	mycel	mycel	mycel
	At	chl-mac	chl	mycel	mycel	mycel
-	T	chl	chl	mycel	mycel	mycel
	At	mac	mic	mycel	mycel	mycel

Ad: Adonitol, Ar: Arabinose, Er: Erythritol, Ga: Galactose, Gl: Glucose, In: Inositol, Le: Levulose, Mlt: Maltose, Man: Mannitol, Melib: Melibiose, Mel: Melitose, Suc: Sucrose, Tr: Trehalose, Xy : Xylose, T: Typical, AT: Atypical, mic: microconidia, mac: macroconidia, chl: chlamydoconidia, mycel: mycelium

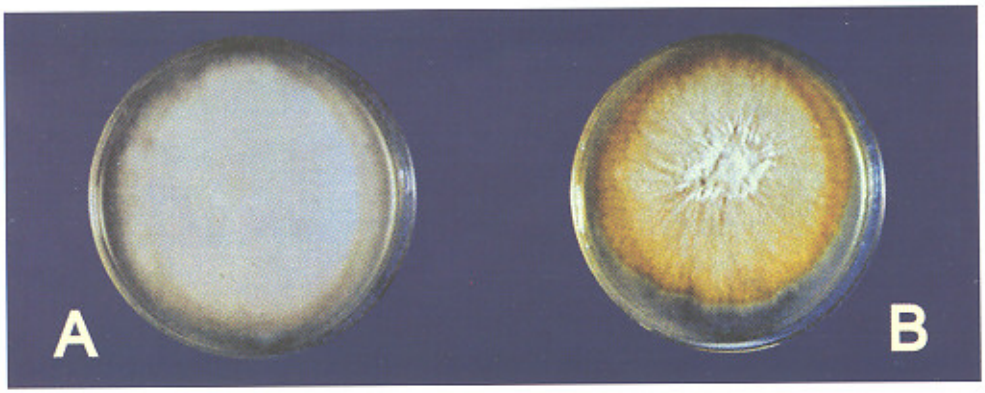

Fig. 1. Colony of Microsporum canis in Soc medum after fourteen days at 30 ℃.
A: atypical strain, B: typical strain

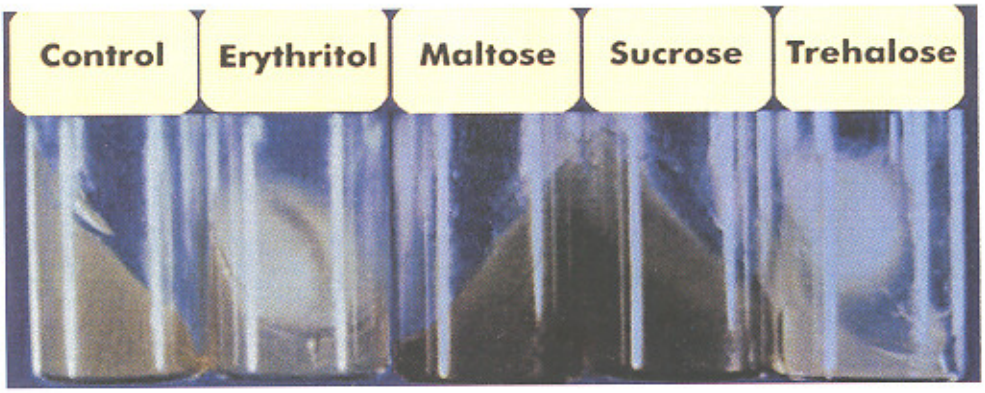

Fig. 2. Typical strain of Microsporum canis with fluffy mycelium in determinant carbohydrate media.

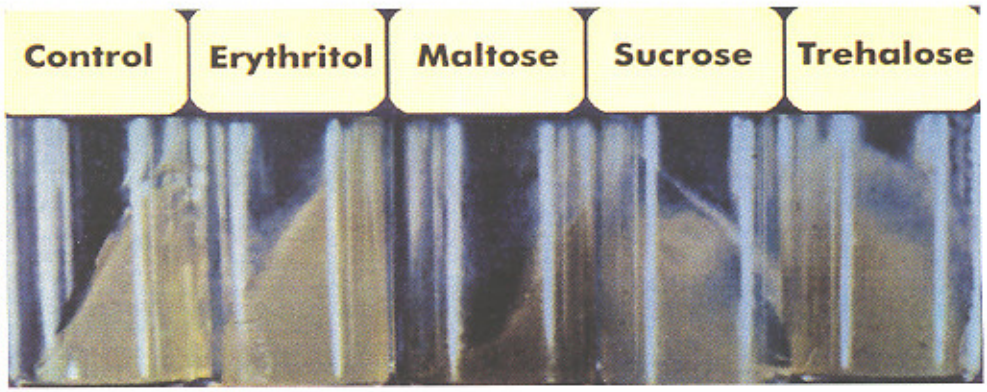

Fig. 3. Typical strain of Microsporum canis without fluffy mycelium in determinant carbohydrate media.

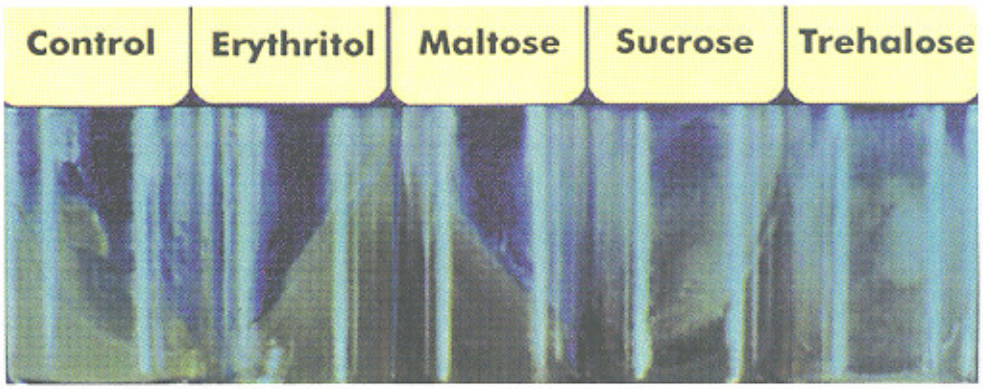

Fig. 4. Atypical strain of Microsporum canis with fluffy mycelium in determinant carbohydrate media.

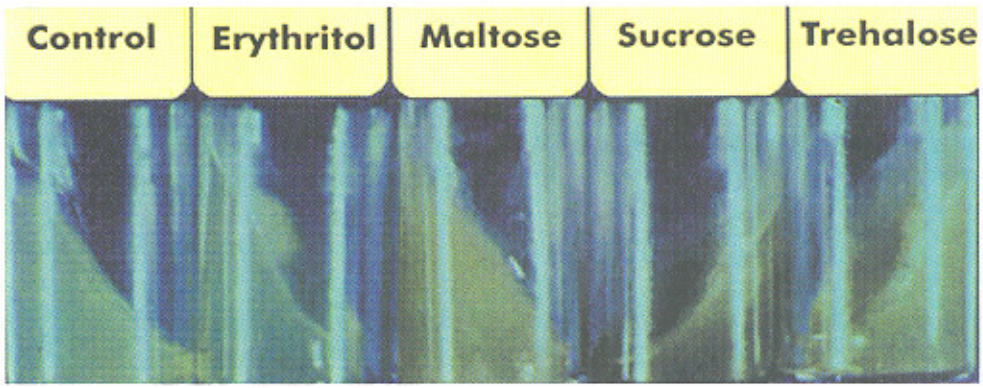

Fig. 5. Atypical strain of Microsporum canis without fluffy mycelium in determinant carbohydrate media

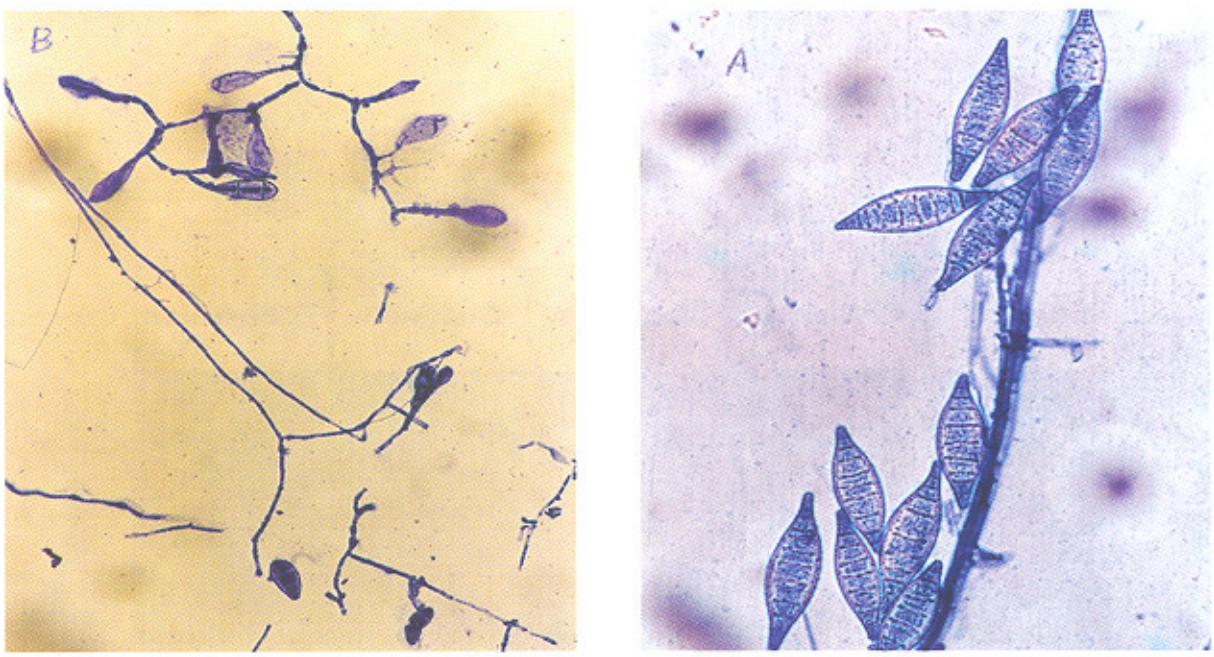

Fig. 6. Macroconidia of Microsporum canis in erythritol medium. A: Typical strain, B: Atypical strain

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 floccose 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 Mavroudea's investigations (4). macroconidia production was enhanced by erythritol in atypical forms with or without floccose myeelium. In all strains of Microsporum canis sporulation enhancement occurred by erythritol and trehalose assimilation (Fig. 6). Although on media containing sucrose and melitose only myeelium 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 intersting that in contrast to strong assimilation of glucose, mannitol, melibiose and weak assimilation of galactose and melitose by strain NCPF352, all Iranian typical and atypical strains of Microsporum canis failed to utilze those carbohydrates. Therefore reaction with these compounds was sufficient to differentiate Iranian and British NCPF352 strains of Microsporum canis and also Microsporum distortum. On erythritol, trehalose and levulose media, NCPF352 was able to produce floccose mycelia. Iranian isolates produced fluffy mycelia on glucose, galactose and mannitol, as well as the three above-mentioned carbohydrates. Microsporum distortum only weakly assimilated erythritol. This organism produces fluffy mycelia 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 technique is particularly useful in identification of Microsporum distortum and also NCPF-352. Even though only one strain from UK was examined, but the correlation of assimalation 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

1. Zaini F, Zarchi M. Enzymatic activity of pigmented and unpigmented strains of Trichophyton violaceum. Iranian Journal of Public Health. 17:11-22; 1988.

2. Zaini F. Enzymatic activity of Trichophyton rubrum, Trichophyton mentagrophytes and Trichophyton verrucosum. Iranian journal of Public Health.20: 33-40; 1991.

3. Philpot C. The use of nutritional test for the differentiation of dermatophytes. Sabouraudia. 151:141-150; 1977.

4. Mavroudea D, Velegraki A, Leonardopoulos J, Marcelou U. Effect of glucose and thiamine concentrations on the formation of macroconidia in dermatophytes. Occurrence of dysgonic Microsporum canis strains in Athens, Greece. Mycoses. 39(1) 2): 61-66; 1996.

5. Tucker W. Biotypes of the dermatophyte, Microsporum distortum. Mycoses 35:147-151: 1992.

6. Shokouhi T. Epidemiologic investigation and etiological agents of cutaneous and superficial infections in patients referred to medical mycology laboratory of School of Public Health. PhD. thesis. Tehran University of Medical Sciences. (1981-85).

7. Rezai AH. A survey for isolation of atypical Microsporum canis from the clinical materials in Iran. M.S.P.H. thesis. School of Public Health, Tehran University of Medical Sciences. (1993-94).

8. Essayag S. An atypical Microsporum canis isolate. Mycoses. 34:505-511; 1991.

9. Brasch J. Microsporum canis with polymorphous macroconidia. Mycoses 32 (1):33-38; 1989.

10. English M., Tucker W. Atypical strains of Microsporum canis. Mycopathologia. 63: (2) 113-120; 1978.

11. Kwon-chung K, Bonder R, Fethiere A, Blank F. Enigmatic relationship of two Microsporum species. Sabouraudia 15: 325-332; 1977.

12. Artis W.M, Odle B.M, Jones H.E. Griseofulvin resistant dermatophytosis correlates with in vitro resistance. Arch Derm. 117: 16-19; 1981.

13. Hantschke D, Gotz H. Resistance to griseofuvin. Z. Hautker. 56: 1326-32; 1981.

14. Zaini F, Madani M, Elmi Akhounie E. Polyacrylamid gel electrophoresis patterns of some Iranian Microsporum and Trichophyton species, Acta Medica Iranica 36: (1)9-13; 1998.