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Growth temperatures of isolates of *Sporothrix schenckii* from disseminated and fixed cutaneous lesions of sporotrichosis

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Abstract

In 1979 Kwon Chung described two varieties of *Sporothrix schenckii* based on the thermotolerance of isolates from fixed cutaneous (35 °C) and that of disseminated cutaneous forms (37 °C) of sporotrichosis. Since we had not noted such a difference previously in a study of 100 cases of this disease (55% localized and 45% disseminated) wherein all the isolates grew at 37 °C, we decided to repeat this work.

Our results differ from those reported by Kwon Chung, since the isolates of both the fixed and disseminated forms of sporotrichosis grew at 37 and 38 °C, even when we used inocula of 30 conidia (20–50) which according to Kwon Chung were needed to observe this difference.

Resumen

En 1979 Kwon Chung describe dos variedades de *S. schenckii* dependiendo de la termotolerancia de los aislados de formas cutáneas fijas (35 °C) y formas cutáneas diseminadas (37 °C). Como nosotros no habíamos observado esta diferencia al estudiar 100 casos de esta micosis, decidimos repetir este trabajo.

Nuestros resultados difieren de los de Kwon Chung al obtener crecimiento de los aislados de la forma cutánea fija y diseminada a 37 °C y 38 °C aun sembrando solo 30 conidias requisito que según la autora es indispensable para observar esta diferencia.

Introduction

Sporothrix schenckii is the only known causative agent of sporotrichosis. The different clinical forms described for this cutaneous mycosis (fixed, lymphocutaneous and extracuteneous) are produced by immunological alterations of the host (6). Up to now there has been no explanation of what determines the localization or dissemination of the cutaneous forms of sporotrichosis, since patients do not show any immunological differences (1, 5).

We have been interested in the results obtained by Kwon Chung (2), who differentiated two types of isolates on the basis of their temperature requirements. Those that grow at 35 °C but not at 37 °C only gave rise to fixed cutaneous forms. In contrast those that grow at 37 °C produced lymphangitic, cutaneous and disseminated extracutaneous forms. In 100 cases of cutaneous sporotrichosis studied in our laboratory 55% were of the fixed type and 45% were of the disseminated form. The isolates from both types of sporotrichosis grew at 37 °C.

Nevertheless, since it seemed necessary to confirm our finding by using inocula of 20 to 50 conidia, we decided to repeat our temperature studies (3).

Material and methods

Isolates studied

For this study we used 20 isolates of *S. schenckii* that had been isolated from skin lesions of sporotrichosis.

Isolation of conidia

The isolates were cultured on a modified Sabouraud dextrose medium (MSD) (2) at room temperature (24–28 °C) for 2 weeks. The cultures were resuspended in saline and mixed in a test tube mixer (Vortrex Genier)[RT]. In order to separate the conidia from the mycelium the suspensions were filtered either through layers of sterile cotton (4) or through Whatman No 1 filter paper. This latter method proved to be more efficient for removing the hyphae. We counted the concentration of conidia in the filtrates with a Neubauer chamber. The suspensions were diluted to obtain 20 to 50 conidia per inoculum. They were spread on petri dishes of MSD and incubated at 35 °C, and 38 °C for 2 weeks.

Results and discussion

Of the 20 isolates studied, 10 came from fixed cutaneous cases and 10 from disseminated cases. Three days after inoculation, growth started appearing in almost all the dishes and at all the temperatures used.

At 2 weeks all the isolates from fixed forms had growth at 35 °C and 37 °C and 7 of them at 38 °C. In general, colonies were larger at 35 °C than at 37 °C and 38 °C. Growth of the isolates from the disseminated forms was quite similar to that of the fixed forms except that in two cases we did not obtain growth at 37 °C. Only 3 grew at 38 °C (Table 1).

Growth was fillamentous in all cases. Slide cultures of these isolates showed hyaline or light tan hyphae and conidia with the various forms that

Table 1. Growth characteristics of *Sporothrix schenckii* isolates from fixed and disseminated cutaneous lesions incubated at different temperatures for 15 days on modified Sabouraud dextrose agar.

Isolate	Fixed clinical form	Characteristic of growth					
		35 °C	colony size	37 °C	colony size	38 °C	colony size
0261	infiltrated face	+	2 mm	+	1 mm	+	0.5 mm
9017	infiltrated elbow	+	3 mm	+	3 mm	0	-
7533	infiltrated face	+	1 mm	+	1 mm	0	-
8287	ulcer face	+	6 mm	+	4 mm	+	1 mm
4610	ulcer face	+	5 mm	+	4 mm	+	1 mm
7603	verrucous face	+	1 mm	+	1 mm	+	0.5 mm
0073	verrucous arm	+	3 mm	+	2 mm	+	1 mm
9300	granuloma face	+	3 mm	+	5 mm	+	0.5 mm
7684	granuloma face	+	7 mm	+	5 mm	+	0.5 mm
9129	ezcematoid arm	+	2 mm	+	2 mm	0	-
	Disseminated clinical form						
0254	lymphangitic arm	+	4 mm	+	4 mm	0	-
0200	lymphangitic leg	+	4 mm	+	1 mm	+	1 mm
9977	lymphangitic arm	+	5 mm	+	4 mm	+	4 mm
8784	lymphangitic arm	+	1 mm	+	1 mm	0	-
9976	ulcers arm	+	1 mm	0	-	0	-
9286	ulcers arm	+	3 mm	0	-	0	-
9256	ulcers arm	+	1 mm	+	2 mm	0	-
9537	ulcers nodule arm	+	1 mm	+	1 mm	0	-
8770	infil. noduls arm	+	1 mm	+	3 mm	0	-
9522	noduls face	+	2 mm	+	2 mm	+	1 mm

have been described for this fungus: oval, triangular, etc. (4).

The results obtained differed from those of Kwon Chung (2). We not only obtained growth at 37 °C by all the isolates from patients with the fixed forms of sporotrichosis but more of them even grew at 38 °C in contrast to those that we had obtained from disseminated cases.

Up to now it has been accepted that the cell-mediated immunologic capacity of the host determines the degree of invasion by pathogenic fungi. This is also valid for sporotrichosis in relation to its cutaneous and extracutaneous forms (5, 6), but does not seem to explain the different clinical forms – that are seen in cutaneous sporotrichosis, where cell-mediated immunity, measured by skin hypersensitivity to *S. schenckii* and *in vitro* lymphocytes stimulation, seems to be unaltered (6). This was the reason we were so interested in repeating Kwon Chung's study because it would have explained the basis for the various cutaneous clinical forms that have been described for this mycosis. On the basis of our study we conclude that the differentiation of the number of conidia in the inoculum is not a supportable hypothesis.

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