

CULTURE MEDIUM ALKALINIZATION BY DERMATOPHYTES (Influence of time and temperature of incubation)

Maria Helena PAVEIA

Laboratory of Mycology*, University Department of Dermatology, Santa Maria Hospital, Lisbon

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Abstract

95 dermatophyte strains (12 of *Trichophyton mentagrophytes*, 12 of *T. tonsurans*, 11 of *T. rubrum*, 12 of *T. megninii*, 12 of *T. violaceum*, 2 of *T. schoenleinii*, 1 of *T. soudanense*, 12 of *M. canis*, 8 of *Microsporium gypseum*, 1 of *M. ferrugineum* and 12 of *Epidermophyton floccosum*), 1 of *Aspergillus niger*, 1 of *A. ochraceus*, 1 of *Paecilomyces* sp., 1 of *Penicillium* sp. and 1 of *Candida albicans* were grown in Sabouraud liquid medium for the study of pH variation over 6 weeks at room temperature and after 4 weeks at 37°C.

All the dermatophyte strains alkalinized the medium. The highest pH values after 2–3 weeks' development at room temperature were produced by *T. mentagrophytes*, *M. gypseum* and *M. canis*, and the lowest by *T. rubrum* and *T. violaceum*. After the 4th week the alkalinizing activity became more marked for *T. mentagrophytes* and remained stationary for *T. violaceum*. In the 5th week the values produced by *T. tonsurans* were higher than those for *M. gypseum*, and those for *E. floccosum* and *T. megninii* were higher than those for *M. canis*. A similar behaviour was observed for *T. rubrum* and *T. megninii* and for *M. canis*, *M. gypseum* and *M. ferrugineum*. There seems to be a relation between the alkalinizing capacity and the rapidity and amount of the growth. At 37°C both alkalinization and range of variation of the pH values of the medium became more noticeable for the strains of each species.

The fact that dermatophytes quickly alkalinize the culture medium – contrary to what happens with the saprophytic fungi, which, normally, produce acidification – has been used by some authors (Goldfarb et al., 1956; Wiegand et al., 1968; Taplin et al., 1969; Kurup et al., 1969) for the

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early detection of pathogenic and contaminating species.

Goldfarb & Herrmann (1956) report, without indicating values, pH variations in the culture medium even before visible growth of the fungi and throughout the entire period of development of the colonies. The studies we found in the literature regarding these alterations were made at room temperature.

In the present work the evolution of pH changes of the culture medium produced by dermatophytes over 6 weeks at room temperature and after 4 weeks at 37°C is studied.

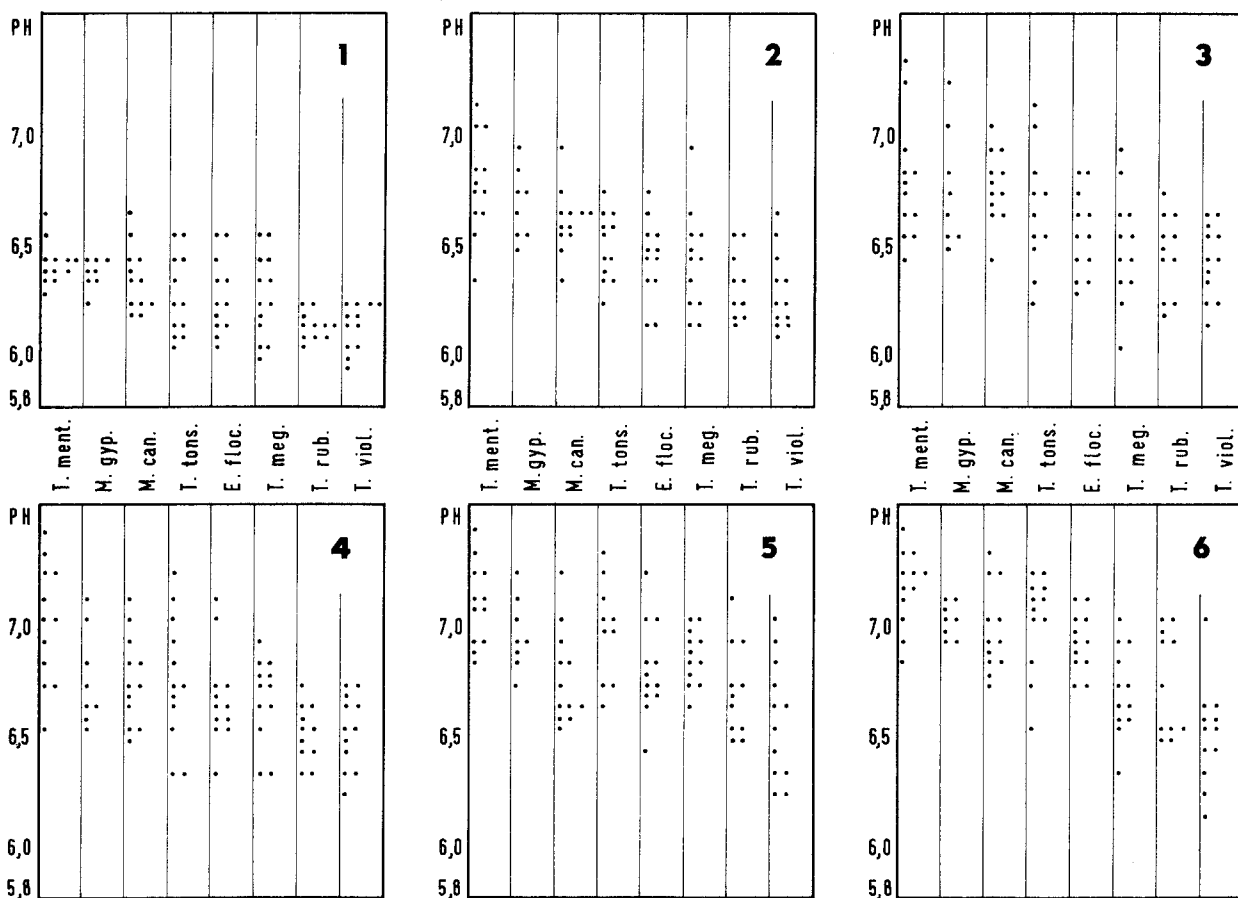
Materials and methods

Dermatophytes, other filamentous fungi and yeasts were inoculated in test tubes (170×25) containing approximately 40 c.c. of Sabouraud liquid medium (2% glucose + 1% peptone), with pH 5.8±0.1.

The dermatophytes studied were *Trichophyton mentagrophytes* (12 strains), *T. tonsurans* (12), *T. rubrum* (11), *T. violaceum* (12), *T. megninii* (12), *T. schoenleinii* (2), *T. soudanense* (1), *Microsporium canis* (12), *M. gypseum* (8), *M. ferrugineum* (1) and *Epidermophyton floccosum* (12); the other filamentous fungi: *Aspergillus niger* (1), *A. ochraceus* (1), *Paecilomyces* sp. (1) and *Penicillium* sp. (1); the yeasts: *Candida albicans* (1).

All the dermatophyte strains were of human origin, with the exception of two of *T. mentagrophytes* isolated from mice.

6 Cultures were made from each strain and kept at room temperature for 1 to 6 weeks. 68 Dermatophyte strains were also incubated at 37°C for 4 weeks. After the lapse of the above-mentioned incubation periods, the colony was taken from the test tube and the culture medium was poured into a glass for pH measurement.



Figs. 1-6. Scattergram showing pH values of the culture medium after 1 to 6 weeks' development of dermatophytes at room temperature. Fig. 1. after 1 week. Fig. 2. after 2 weeks. Fig. 3. after 3 weeks. Fig. 4. after 4 weeks. Fig. 5. after 5 weeks. Fig. 6. after 6 weeks.

The pH measurements were made with two electrode 'Model 5' pH meter (Corning).

Results

All the dermatophyte strains alkalinized the culture medium. In figures 1 to 6 (pH values of the medium per week of incubation at room temperature) it will be noted that, for identical incubation times, the pH values of the medium varied for the strains of each species; this variation increased for the various species, though to a certain extent those values coincide for strains of different species.

Comparing figures 7 and 4 (pH changes of the medium after 4 weeks, respectively at 37 and 24°C.) it will be noted that, in general, the alkalinization of the medium and the range of variation of pH values for the strains of

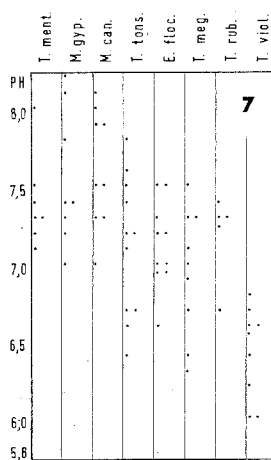
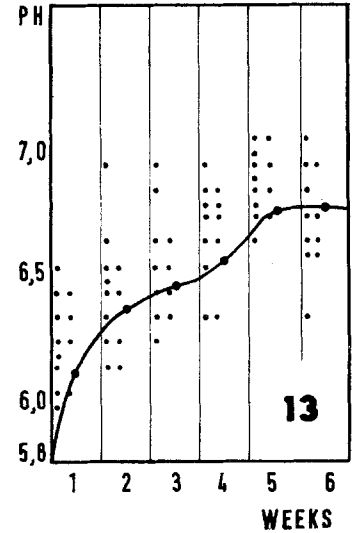
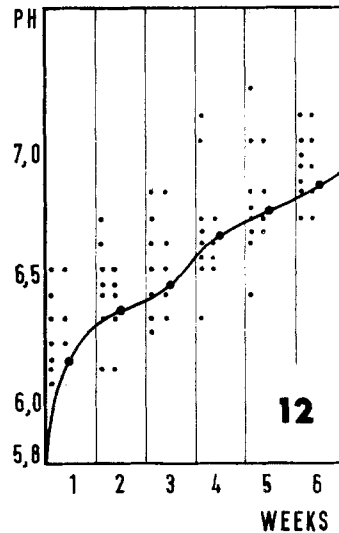
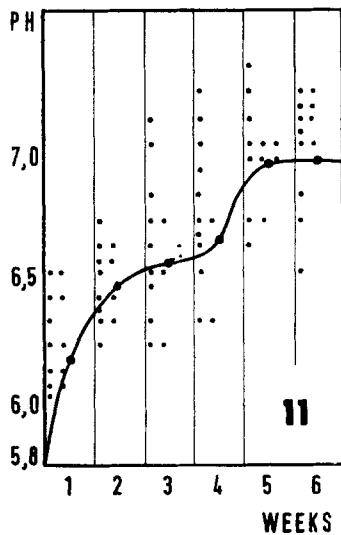
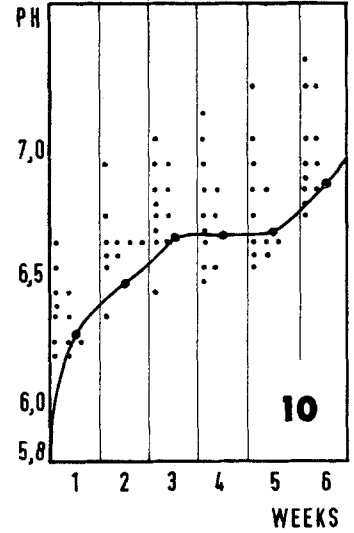
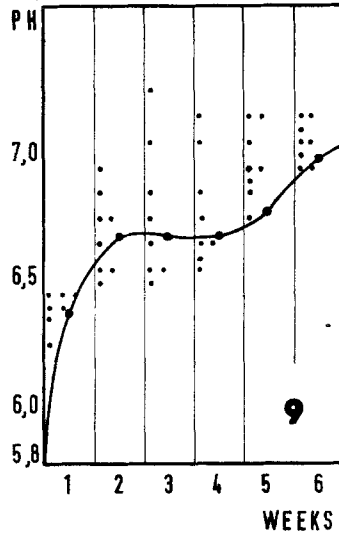
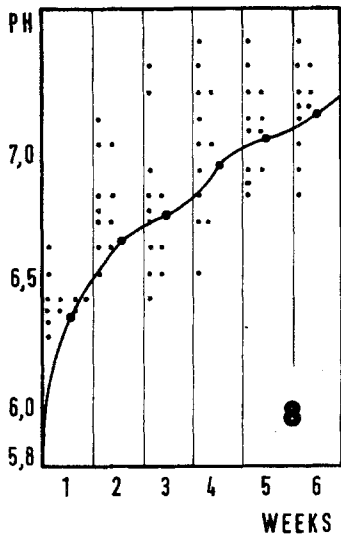


Fig. 7. Scattergram showing pH values of the culture medium after 4 weeks' development of dermatophytes at 37°C.



Figs. 8-15. Scattergram showing pH values of the culture medium produced by each dermatophyte during the 6 weeks' development at room temperature and curves obtained from the mean values for the strains of each species per week of incubation.

Fig. 8. *T. mentagrophytes*. Fig. 9. *M. gypseum*. Fig. 10. *M. canis*. Fig. 11. *T. tonsurans*. Fig. 12. *E. floccosum*. Fig. 13. *T. megninii*.

each species became more noticeable at 37°C; the relative position of the various species seems to be maintained with the exception of *T. rubrum*, for which considerably higher values were obtained.

Figures 8 to 15 show the pH values of the medium for the strains of each dermatophyte during the 6 weeks of incubation at room temperature and also the curves obtained from the mean value for each week. The tracing of these curves indicates irregularity in the alkalization for the various dermatophyte species, this being also noted for the various strains of each one: whereas some gradu-

ally alkalized the medium, in others alkalization occurred more quickly at first, diminishing or even becoming stationary after a certain point. Despite the dispersion of points observed in figures 8 to 15, the same curves given together in figure 16, make it possible to compare the pH evolution of the medium in the various species and throughout the indicated incubation times. It is found that alkalization was quicker in the first two weeks for all dermatophytes, the peak values being for *T. mentagrophytes* and *M. gypseum* and the lowest ones for *T. rubrum* and *T. violaceum*. At the end of 4 weeks, the

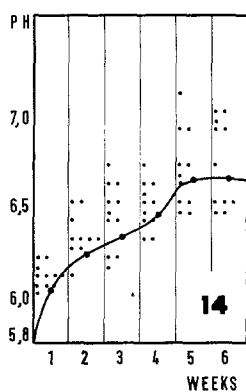


Fig. 14. *T. rubrum*.

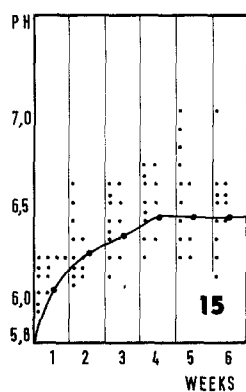


Fig. 15. *T. violaceum*.

pH values of the medium ranged only between 6.5 and 6.7, with the exception of *T. mentagrophytes*, which reached 7.0. After 6 weeks, the highest values were for *T. mentagrophytes* and the lowest for *T. violaceum*.

The figures relating to *T. soudanense*, *T. schoenleinii* and *M. ferrugineum*, respectively 17, 18 and 19, were not placed along with those of the other dermatophytes as the number of utilized strains was not comparable.

The results obtained for the remaining studied fungi are given in figure 20.

Commentary

The highest pH values of the medium after two to three weeks' development at room temperature were produced by *T. mentagrophytes*, *M. gypseum* and *M. canis*, and the lowest by *T. rubrum* and *T. violaceum*. This is, in a general way, in accordance with the results obtained by Cardoso (1969), who made readings after 18 days of incubation of the cultures at 24°C. In this study very few pleomorphized strains were used, the variation with regard to the recently isolated ones being slight, which is in accordance with the findings of the same author.

No term of comparison for the values recorded after the 4th week was found in the literature. Our experience enabled us to observe that the order of mean values, which had remained stable until then, changed in the last 2 weeks except for *T. mentagrophytes*, whose alkalizing activity became gradually more noticeable, and for *T. violaceum*, whose results became stationary after that date. In the 5th week the values for *T. tonsurans* rose and exceeded those for *M. gypseum*, and those for

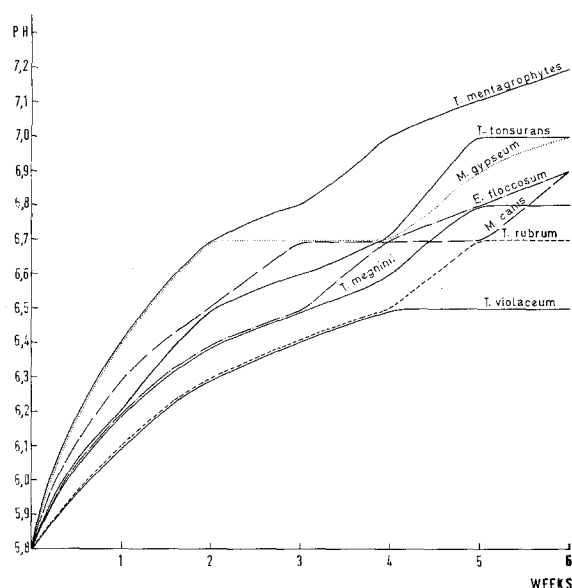


Fig. 16. Ensemble of the curves represented in figs. 8–15.

E. floccosum and *T. megninii* became higher than those for *M. canis*.

The curves relating to *T. rubrum* and *T. megninii* ran parallel after the first week. For the 3 species of the genus *Microsporum* similar curves were found, with stationary periods of alkalization during 2 weeks.

The strains of *T. mentagrophytes* for which the highest pH values of the medium were obtained were isolated from mice and possessed abundant microconidia and spiral hyphae; however, these values were very close to those recorded for the other species of human origin mostly belonging to the cottony variety.

For *T. schoenleinii* high pH values of the medium were obtained; it should be noted that, though similar to *T. violaceum* microscopically and in its slow and scanty development in solid medium, in liquid medium the development of *T. schoenleinii* is quicker and more abundant than that of *T. violaceum*.

A correlation is to be admitted between the morphological complexity and the capacity of alkalizing the culture medium, as was suggested by Cardoso (1969). It seems to us also that there is a relation between the alkalizing capacity and the rapidity and amount of growth, since the species which develop faster produced higher pH values of the medium and the same applies to the various strains of each species; it should be noted that, in general, at 37°C the development of the colonies

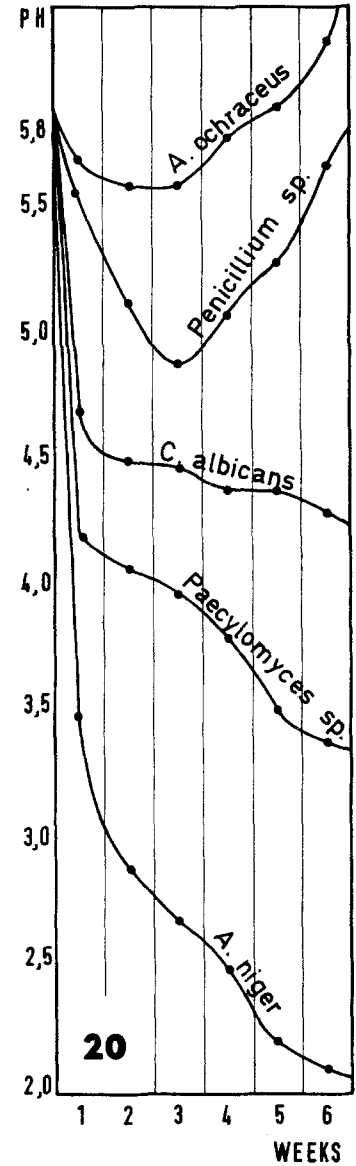
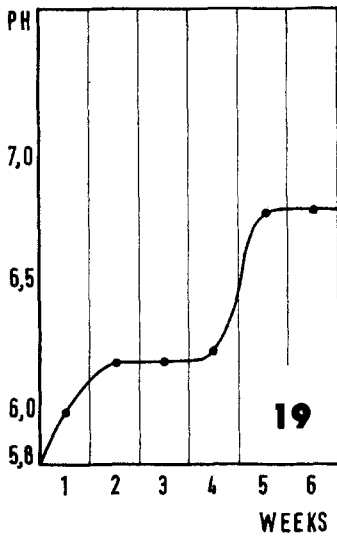
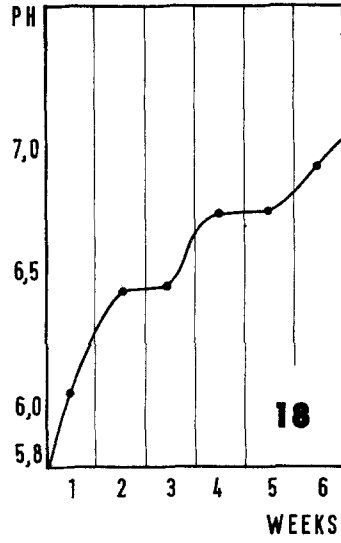
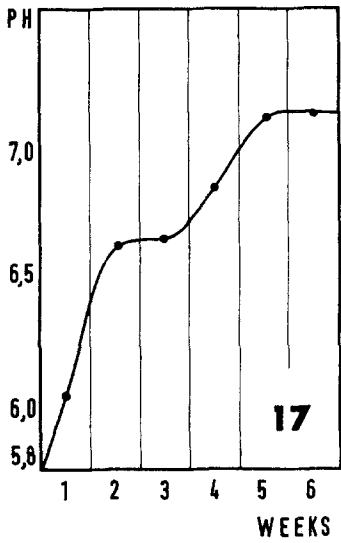


Fig. 17. pH changes of the culture medium produced by a strain of *T. soudanense* during the 6 weeks' incubation at room temperature.
 Fig. 18. pH changes of the culture medium produced by *T. schoenleinii* during the 6 weeks' incubation at room temperature (curve obtained from the mean values of two strains).
 Fig. 19. pH changes of the culture medium produced by a strain of *M. ferrugineum* during the 6 weeks' incubation at room temperature.
 Fig. 20. pH changes of the culture medium produced by 4 filamentous fungi (1 strain of each one) and by *C. albicans* (1 strain).

was much quicker and the pH values of the medium remarkably higher.

The fact that there is no noteworthy difference between the pH values of the medium for recent and for old pleomorphic strains suggests preservation of physiological properties in strains whose morphological char-

acteristics were lost.

As regards the other fungi studied we observed two distinct types of behaviour: *C. albicans*, *Paecilomyces sp.* and *A. niger* gradually acidified the medium; *A. ochraceus* and *Penicillium sp.*, after 3 weeks of poor acidification, slightly alkalized the medium.

Résumé

95 souches de dermatophytes (12 de *T. mentagrophytes*, 12 de *T. tonsurans*, 11 de *T. rubrum*, 12 de *T. violaceum*, 12 de *T. megninii*, 2 de *T. schoenleinii*, 1 de *T. soudanense*, 12 de *M. canis*, 8 de *M. gypseum*, 1 de *M. ferrugineum* et 12 de *E. floccosum*), 1 de *A. niger*, 1 de *A. ochraceus*, 1 de *Paecilomyces* sp., 1 de *Penicillium* sp. et 1 de *C. albicans* ont été cultivées en milieu de Sabouraud liquide pour étudier la variation de pH pendant 6 semaines à la température du laboratoire et après 4 semaines à 37°C.

Toutes les souches de dermatophytes ont alcalinisé le milieu. Après 2-3 semaines de développement à la température du laboratoire les valeurs les plus élevées de pH ont été produites par *T. mentagrophytes*, *M. gypseum* et *M. canis* et les moins élevées par *T. rubrum* et *T. violaceum*. Après la 4^{ème} semaine l'action alcalinisante de *T. mentagrophytes* s'est accrue et celle de *T. violaceum* est restée stationnaire. A la 5^{ème} semaine, les valeurs produites par *T. tonsurans* sont devenues supérieures à celles de *M. gypseum* et celles de *E. floccosum* et *T. megninii* supérieures à celles de *M. canis*.

Un comportement pareil a été observé, d'un côté concernant *T. rubrum* et *T. megninii* et de l'autre *M. canis*, *M. gypseum* et *M. ferrugineum*.

Une relation semble exister entre le pouvoir alcalinisant et la vitesse et l'abondance de développement. A 37°C l'alcalinisation et l'amplitude de variation des valeurs de pH du milieu ont augmenté pour toutes les souches de chaque espèce.

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