PRO EXPERIMENTIS

A New Simple Method for Rapid Acquisition of Chlamydospores in *Candida albicans*

The high incidence of *Candida albicans* infections due to a large scale use of antibacterial and immunosuppressive drugs[1], requires new rapid and reliable tests for clinical diagnosis of this organism. Chlamydospores represent a morphological element of high specificity for the species *albicans*. The formation of chlamydospores, however, is inconstant, depends on many factors and requires 4–10 days long cultivation periods[2].

In this paper we describe a new and simple method for obtaining in a short time and in a reproducible way a large quantity of *C. albicans* chlamydospores.

In all experiments 30 strains of *C. albicans* and 20 strains belonging to other species of *Candida* were tested.

Suspensions of blastospores of *Candida* (O.D. $\frac{1cm}{560nm}$ = 1,0) grown during 24 h at 37 °C in a Sabouraud medium (BBL) were used throughout this study. 0.1 ml of a suspension were inseminated into 0.9 ml of the culture medium consisting of horse serum diluted with distilled water (1:2.5) containing 0.01% of soluble starch (Merck) and 0.25% of trypan blue (Fluka). The culture medium was incubated in a water bath maintained at 37 °C. The formation of chlamydospores was controlled by means of microscope after 6–8 h of incubation and eventually at 24 h after insemination. Chlamydospores formed in these conditions may give subsequently vegetative forms and therefore incubation periods longer than 24 h were not used.

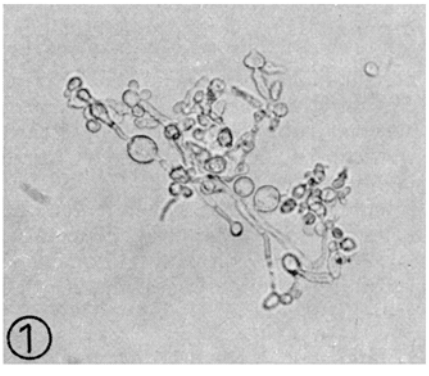

Fig. 1. Chlamydospores obtained with culture medium described in text incubated for 8 h at 37 °C. Leitz Ortholux – Orthomat microscope. × 320.

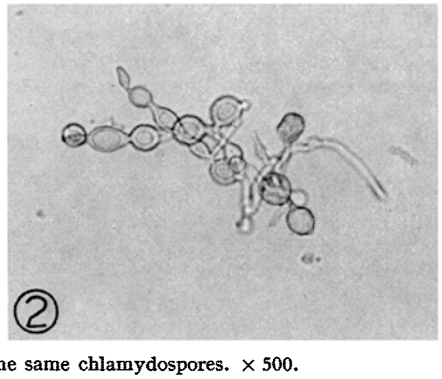

Fig. 2. The same chlamydospores. × 500.

Microscopical examination of morphological elements grown in a culture medium indicated above shows that chlamydospores were constantly formed only by strains of *C. albicans*. These spores were formed apically and eventually intercalary and had a typical round or irregular structure (Figures 1 and 2). Besides chlamydospores, blastospores and pseudomycelia were also found. It has to be stressed that the culture medium described above may be used for the pseudogermination test[3] because pseudogermination takes place in the above indicated conditions in less than 2 h of incubation.

The culture medium described in this paper is unique in its property to allow the rapid formation of numerous chlamydospores of *C. albicans* in an extremely short period of incubation so far not reported in the relevant literature. It has to be stressed that formation of chlamydospores takes place at 37 °C, the temperature considered by many authors inhibitory for this process[4]. Addition of trypan blue in a concentration 25 times higher than that used by NICKERSON[5] facilitates microscopical examination of chlamydospores, stimulates their formation and exerts bactericidal action. The possibility of using the culture medium described in this paper for a pseudogermination test which is considered the most reliable test for the diagnosis of *C. albicans* is another of the useful features of this medium.

Riassunto. Viene descritto un nuovo e semplice metodo per ottenere in un tempo molto breve (6–8 ore) e con grande riproducibilità le clamidospore dalla *C. albicans*.

N. SIMONETTI and V. STRIPPOLI[6]

Institutes of Microbiology, University of Camerino and Rome, I-00100 Roma (Italy), 25 January 1971.

[1] M. S. SEELIG, Bact. Rev. *30*, 442 (1966).
[2] D. W. R. MACKENZIE, in *Symposium on Candida infections* (Eds. H. I. WINNER and R. HURLEY; E. and S. Livingstone Ltd., Edinburgh and London 1966), p. 29.
[3] C. L. TASCHDJIAN, J. J. BURCHALL and P. J. KOZINN, Am. J. Dis. Child. *99*, 212 (1960).
[4] L. WICKERHAM and L. F. RETTGER, J. trop. Med. Hyg. *42*, 1 (1939).
[5] W. J. NICKERSON and Z. T. MANKOWSKI, J. infect. Dis. *92*, 20 (1953 x).
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Beobachtung von Furchungsteilungen mit Chromosomen-Elimination in lebenden Embryonen der Gallmücke *Heteropeza pygmaea*

Die Gallmücke *Heteropeza pygmaea* (syn. Oligarces paradoxus; Cecidomyiidae syn. Itonididae; Diptera) kann sich vivipar pädogenetisch fortpflanzen[1,2]. Dabei gelangen unbefruchtete Eier aus dem Ovar der Larve in ihre Leibeshöhle. Dort wachsen sie zu Larven heran und schlüpfen schliesslich aus der inzwischen abgestorbenen Mutterlarve. Bei bestimmten Nahrungsbedingungen sind alle schlüpfenden Tiere weiblich[1,3]. In diesem Fall be-