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A MEDIUM FOR INDUCING CONVERSION OF *HISTOPLASMA CAPSULATUM VAR. CAPSULATUM* INTO ITS YEAST-LIKE FORM

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Histoplasma capsulatum var. *capsulatum is a dimorphic fungus that, under special conditions, converts from its more commom mycelial form to a yeast-like form. Achieving this conversion, however, has been problematical for researchers. The present study tested conversion rates in ten* Histoplasma capsulatum var. capsulatum *strains using seven culture media, four of which were conventional and three novel. One of our novel media, MLGema, induced complete conversion of two strains within five days of incubation at 35 °C, and of all strains that eventually converted by the time of the second subculturing transfer, under defined experimental conditions. MLGema is also inexpensive and easy to produce.*

Key words: *Histoplasma capsulatum var. capsulatum* – growth media – yeast-like form conversion

Histoplasma capsulatum var. capsulatum, the causative agent of Histoplasmosis, is a dimorphic fungus which grows in nature or at 25 °C in laboratory conditions in a mycelial or filamentous form. However, in infected hosts or under defined growth conditions at 37 °C, it can develop a yeast-like morphology (Lacaz et al., 1984; Rippon, 1988).

Histoplasmosis is a systemic mycosis that begins in the lungs and subsequently evolves into a generalized condition involving lymph nodes, spleen, bone marrow, liver and other organs, at which point it is normally found inside histiocytes. Since difficulties arise in the assessment of these biological materials, in the isolation and identification of the fungus, and in the lack of specific clinical manifestations, serological tests may offer a good alternative mean of diagnosing Histoplasmosis.

After several unsuccessful attempts to convert *H. capsulatum var. capsulatum* strains from their mycelial to their yeast-like form, we discovered in the literature that this is a common problem, as is clear, for example in the work of Zancopé-Oliveira & Wanke (1986). Their efforts to convert *H. capsulatum* strains gave rise to the statement: *"The reversibility of mycelial to yeast-like phase was difficult and laborious, corroboring with the international literature and indicating that more studies about the factors participating in this reversion are needed"*.

Lacaz et al. (1984) and Rippon (1988) reported the conversion of *H. capsulatim* strains from mycelial to yeast-like morphology *in vivo*, but these experiments are complex and time consuming.

In relation to the production of antigens from *H. capsulatum var. capsulatum* yeast-like cells, the development of media with a capacity to facilitate conversion would provide a useful contribution to the diagnosis of Histoplasmosis.

Previous studies have used a variety of media to induce conversion in *H. capsulatum* strains. Some routine media are not efficient, while the more elaborate ones are too complex and require components that are costly and difficult to obtain.

In the light of information in the literature regarding the nutritional requirements of various *H. capsulatum* strains, we developed three media that, allied with four conventional media, served as the basis for the present work.

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MATERIALS AND METHODS

Ten strains of *H. capsulatum var. capsulatum* were used: Hc0187, Hc2586, Hc6514, Hc6623, Hc6624, HcMMHC3, HcEP:04, HcLDLE, Hc348 and HcRP. All these strains are preserved in a culture collection (Setor de Microbiologia Clínica – Laboratório de Ensino e Pesquisa em Análises Clínicas-DAC/UEM), with monthly transfers in Sabouraud Dextrose Agar (SDA) and maintenance at 25 °C in an incubator.

Growth media (conventional) – SABHI agar (Lacaz et al., 1984); (BHI) Brain Heart Infusion (Difco) with 1% and 2% agar and 10% sheep blood (Howell, 1948); (PFE) Potato Flour-Egg Medium (Kurung & Yegian, 1954).

Growth media (novel) – ML – BHI, 3.7g; Pepton Proteose n° 3 (Difco). 0.5g; Magnesium sulphate heptahydrate, 0.025g; agar (Merck), 1.0g; Yeast Extract (Difco), 2.0g; Dextrose, 2.0g; L-Cysteine hydrochloride (Sigma), 0.2g; distilled water q.s.p. to 100 ml.

MLMelaço (MLM) – BHI, 3.7g; agar, 1.0g; 10 ml molasses (commercial sugar cane molasses) 10%; L-cysteine hydrochloride, 0.2g; distilled water q.s.p. to 100 ml.

MLGema – (MHB) Mueller Hinton Broth (Difco), 2.1g; agar, 1.0g; Dextrose, 2.0g; L-cysteine hydrochloride, 0.2g; Hen egg yolk, 15 ml; distilled water q.s.p. to 100 ml.

The base medium was adjusted to pH 7.0 and autoclaved for 20 min at 121 °C. After cooling (to 50 °C), thermo-labile constituents (yolk, molasses, dextrose and L-cysteine) were aseptically added. Solutions of molasses, dextrose and L-cysteine were pre-sterilized through Millipore or Seitz filters. The egg yolk was aseptically obtained and measured. The media were prepared in accordance with the usual aseptic procedures, and then each medium was distributed in 7.0 ml batches into 16 x 150 mm pre-sterilized screw-capped tubes. Following sterility checks, all tubes were stored at 4 °C until use.

Growth conditions – Small Sabouraud dextrose agar fragments (5 mm in diameter) from the ten cultivated strains under study, were transferred inside a cabinet (provided with a sterile laminar air flow) into tubes (two per strain) of each media. All strains were initially incubated for 10 days at 35 °C. Subculturing was performed weekly, and the procedure maintained until the tenth transfer.

Valuation of cells conversion – At various stages during the cycle of ten transfers, smears of cultured material were stained with Giemsa and examined under a microscope for evidence of morphology changes. Total conversion (100%) was assumed to have occurred when all cells examined in the glass slide showed a yeast-like form. Unstained cells were also examined under a microscope in 1% formaldehyde saline solution.

TABLE I

Behavior of ten *Histoplasma capsulatum var. capsulatum* strains in seven studied media

	Media						
	MLGema	ML	MLM	BHIS1%	BHIS2%	SABHI	PFE
Hc0187	–	–	–	–	–	–	–
Hc2586	1 (70%)	2 (80%)	–	1 (80%)	–	2 (95%)	–
Hc6514	1 (100%)	1 (95%)	–	2 (100%)	7 (80%)	2 (90%)	2 (20%)
Hc6623	1 (100%)	1 (95%)	2 (95%)	2 (90%)	7 (70%)	3 (80%)	3 (90%)
Hc6624	1 (95%)	2 (80%)	–	–	–	3 (50%)	4 (50%)
HcMMHC3	1 (40%)	–	–	2 (95%)	–	–	4 (100%)
HcEp:04	–	–	–	–	–	–	–
HcLDLE	–	–	–	–	–	–	–
Hc348	1 (30%)	–	–	–	–	–	–
HcRP	1 (95%)	1 (20%)	1 (60%)	1 (50%)	–	2 (70%)	–

The number before the parentheses indicates the transfer at which conversion from mycelial to yeast-like morphology was observed to have taken place, with transfer being numbered from 1 (the first) to 10 (the last). The number in parentheses stands for the percentage of yeast-like cells found. (–) Signifies that no conversion occurred.

TABLE II

Highest percentage of yeast-like cells of *Histoplasma capsulatum var. capsulatum* obtained in the media under study

	Media						
	MLGema	ML	MLM	BHIS1%	BHIS2%	SABHI	PFE
Hc2586	100%	80%	–	100%	–	100%	–
Hc6514	100%	100%	–	100%	80%	100%	90%
Hc6623	100%	100%	90%	100%	70%	80%	80%
Hc6624	100%	100%	–	–	–	50%	50%
HcMMHC3	100%	–	–	100%	–	–	100%
Hc348	100%	–	–	–	–	–	–
HcRP	100%	100%	80%	70%	–	80%	–

RESULTS

The results obtained with ten strains of *H. capsulatum var. capsulatum* are shown in Tables I and II. Table I presents data indicating how the strains behaved in the various media: it shows the stage at which, in each cycle of transfers, the conversion of mycelial to yeast-like forms was observed to have taken place, and also gives the percentage of yeast-like cells found at the time of the relevant transfer. Table II shows the highest percentage of yeast-like cells observed in each medium. Growth characteristics of *H. capsulatum var. capsulatum* strains that converted after the first and second transfer with MLGema are shown in Figs 1 and 2.

DISCUSSION

A change in incubation temperature from 25 °C to 37 °C is not sufficient to stimulate the conversion of *H. capsulatum var. capsulatum* from a mycelial to a yeast-like morphology. Additional nutrients, as well as increase in temperature, are necessary (Garraway & Evans, 1984).

Pine (1954), Littman (1955), McVeigh & Houston (1972) and Maresca et al. (1978) added components that were capable of maintain a low oxidation-reduction potential in the media, in order to induce conversion of mycelial to yeast-like forms. Among the compounds used, L-cysteine, L-cystine and DL-lanthionine provide good yields of yeast-like cells under conditions of aerobic growth. According to Salvin (1949a), Pine (1954), Scherr (1957), and more recently Maresca et al. (1977), L-cysteine is preferable because *H. capsulatum* normally requires free SH groups, owing to its limited capacity to reduce molecules with sulphur.

According to Garraway & Evans (1984), Biotin is important in cellular processes, functioning as a cofactor to acetyl CoA carboxylase, an important enzyme in fatty acid synthesis. Al-Doory (1960) reported that yeast-like cells have higher lipid contents than mycelial cells, indicating that the inclusion of lipids, or factors which induce their synthesis, in growth media may improve conversion results. In this regard, the whole egg would be a good source of appropriate nutrients, as suggest by Kurung & Yegian (1954). In our MLGema medium, however, only egg yolk was used, since it was thought that avidin might produce a bioinactive complex with biotin.

It has been reported (McVickar, 1951) that, during the conversion process, albumin absorbs toxic substances present or produced in the media as result of fungal metabolism. Albumin may also function as a fatty acid carrier, thus helping to regularize these molecular constituents. Zarafonetis (1952) reported that albumin stimulated growth rates and, in addition, allowed development of yeast-like cells from small inocula. This observation is important because, as discussed by Scherr (1957), a commonly held view has been that, in order to initiate or maintain conversion to yeast-like morphology, considerable numbers of cells are required. Previous studies (Pine, 1954) have demonstrated that starch can be used instead of albumin. In our MLGema medium, albumin was substituted with starch (present in the MHB medium).

Based on the medium proposed by Salvin (1949b), in which casamino acids were in-

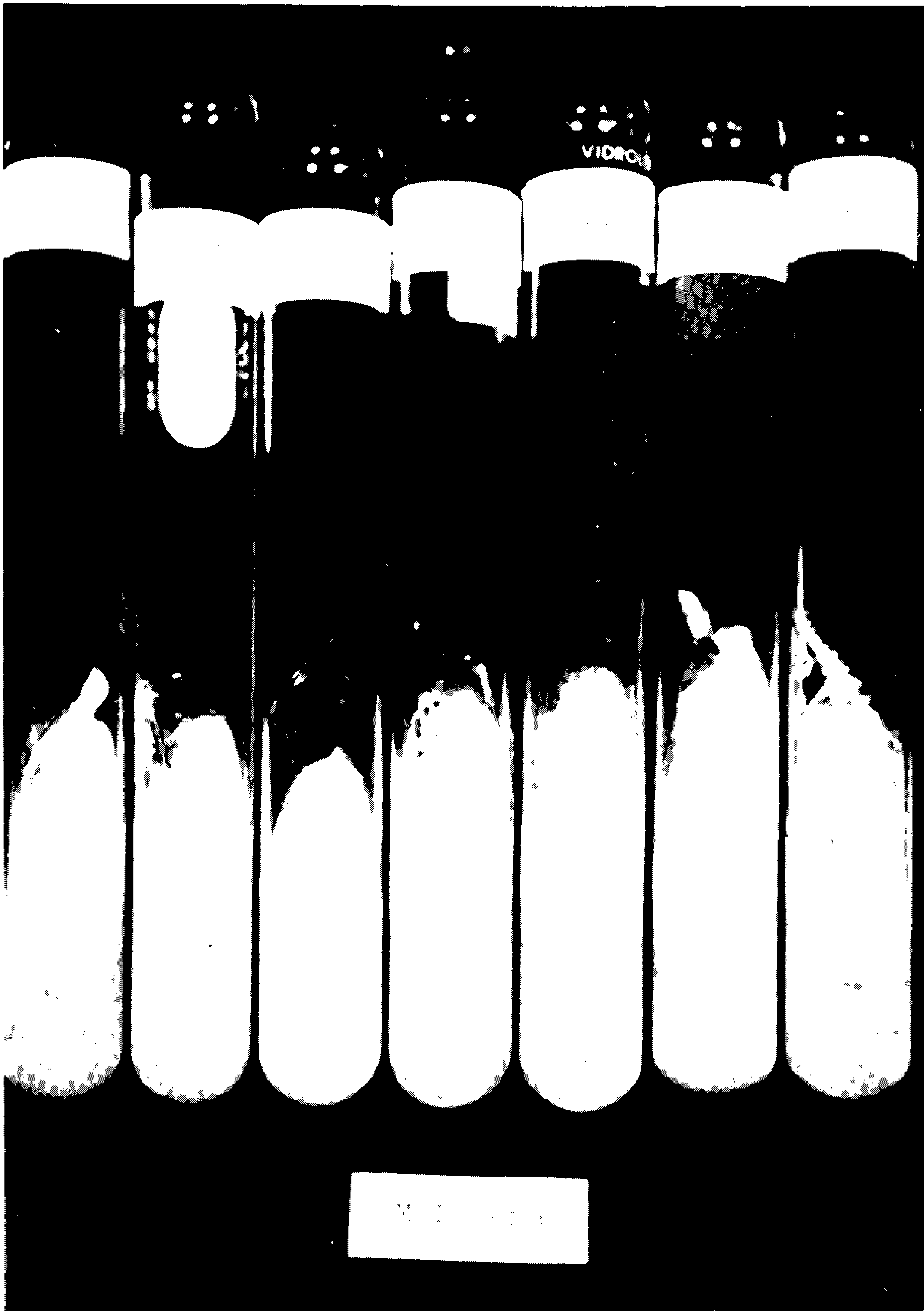

Fig. 1: *Histoplasma capsulatum var. capsulatum* strains converted to yeast-like morphology (100%) after the second transfer in MLGema. Good yields of cell mass were obtained with this medium. Strains from left to right are: Hc2586, Hc348, HcMMHC3, HcRP, Hc6624, Hc6623 and Hc6514.

cluded, MHB with 1% agar was selected as the base medium in our study. Agar (1%) was used because, as Northey & Brooks (1962) observed, humidity is important for development of yeast-like forms. To prevent water evaporation during the long incubation periods screw-capped tubes were used.

Comparing the conventional and the novel media used in our study, MLGema was found to be the most efficient with regard to: the rate of cell development (good development was observed five days after conversion); the percentage of cells converted; and the number of strains converted (Table I). It should be noted

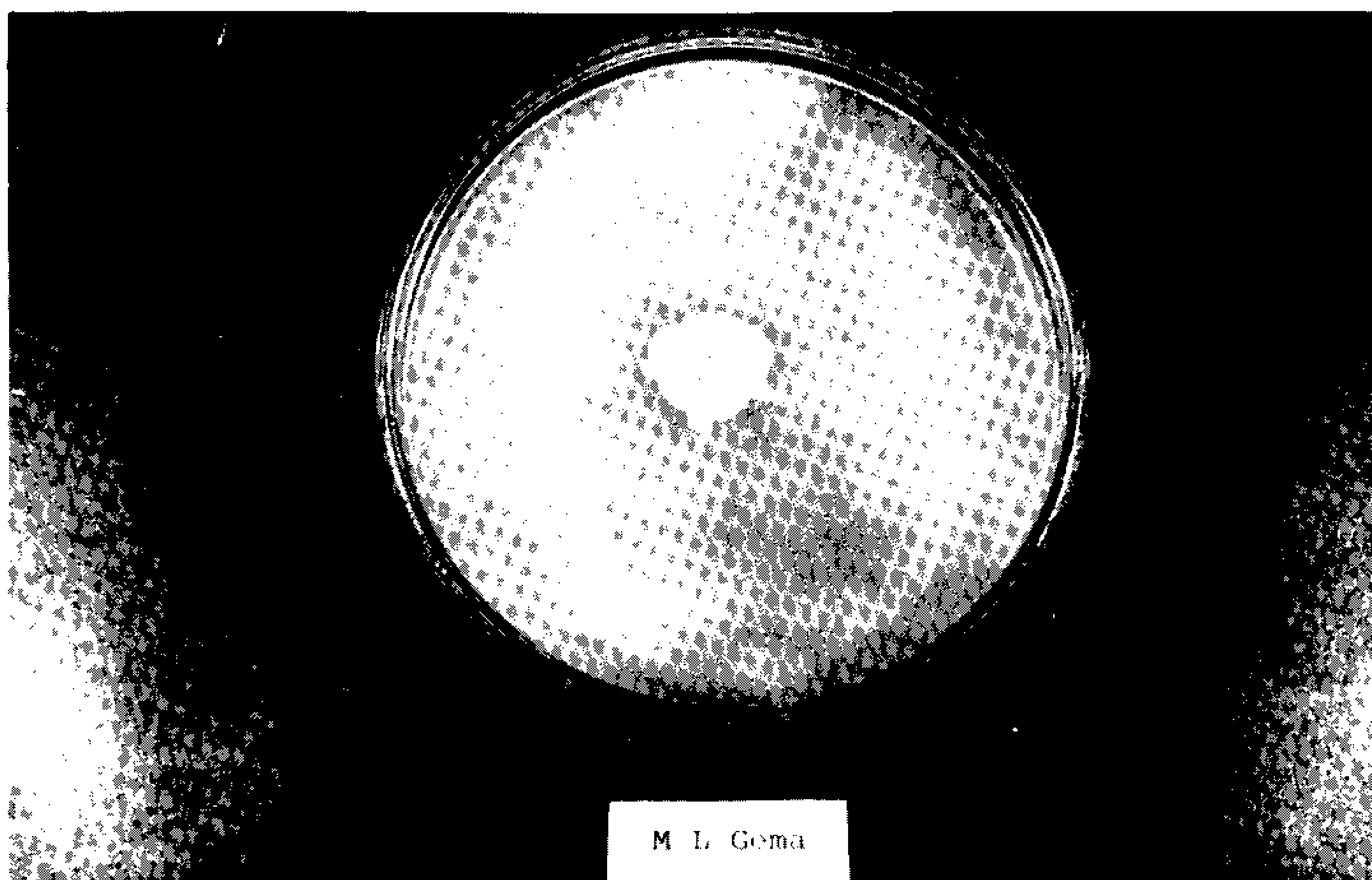

Fig. 2: growth characteristics of *Histoplasma capsulatum var. capsulatum* strain 6623, after the first transfer in MLGema medium. All cells showed the yeast-like morphology.

that the three strains that did not convert with MLGema (Hc0187, HcEP:04 and HcLDLE) also failed with all the other media used. This may be due to an eventual loss of conversion capacity in some strains when subcultured for long periods in the laboratory.

With our MLGema medium, the same percentage of yeast-like cells was maintained over several subcultures, and good yields in terms of cellular mass were obtained in all strains that converted (Figs 1, 2). After the tenth transfer in MLGema, all seven converted strains – having already attained 100% yeast-like morphology – were seeded in SDA (1 and 2%) and incubated at 25 °C and 35 °C. All the yeast-like strains were observed to revert to mycelial morphology, even when alterations were made in agar content and incubation temperature.

Given MLGema's effectiveness in inducing conversion, its facility of preparation and its low cost, we conclude that it should be the medium of choice when production of yeast-like cells of *H. capsulatum var. capsulatum* is required.

Further testing with other strains of *H. capsulatum var. capsulatum* would provide useful additional information on the effectiveness of MLGema in promoting conversion. It would also be interesting to explore the effect of this medium on other dimorphic fungi.

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