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Efficacy of seed-based media for the mould-yeast conversion of *Blastomyces dermatitidis*

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Abstract

The efficacy of 20 seed-based media is reported for the *in vitro* mould-yeast conversion of *Blastomyces dermatitidis*, employing pharmamedia agar, peptone glucose agar, glucose agar and water agar as controls. The mould-yeast conversion varied significantly according to the culture medium, fungal strains and incubation period ($p < 0.01$). Garden-pea, chick-pea, cow-pea, soyabean, peanut, green gram, French bean, lentil, okra and cottonseed converted all of the 7 *B. dermatitidis* test strains after 5 days of incubation at 37 °C. Although the efficacy of many of these seed media was found to be at par with pharmamedia agar – a commercial cottonseed embryo-derived protein, garden-pea seed agar is adopted because of the wider availability and low fat content of this seed. The recommended composition of the medium comprises 2% aqueous seed extract, 2% glucose and pH 6–7. Only nigerseed and sunflower seeds failed to support the conversion of *B. dermatitidis*. Of the control media, peptone glucose agar, glucose agar and water agar did not support the conversion of 2 of the *B. dermatitidis* test strains. The mechanism underlying variable mould-yeast conversion of *B. dermatitidis* on seed-based media is not clearly understood. However, most of the seeds supporting excellent mould-yeast conversion are known for their high protein content. The conversion was apparently not affected by the fat content of the seeds or by incorporation of glucose in the medium.

Introduction

The specific identification of *Blastomyces dermatitidis*, the etiologic agent of blastomycosis, requires verification of its dimorphic character by *in vitro* conversion of the mould form to the yeast form which is characterized by thick-walled and broad-based budding cells. Weeks [1] reported that pharmamedia (a commercial cottonseed embryo-derived protein) was more efficacious than brain heart infusion agar (with or without 5% blood) and Kelly's medium for the mould-yeast conversion of *B. dermatitidis*. Subsequently, pharmamedia was widely recommended for the mould-yeast conversion of *B. dermatitidis* [2–4]. In an earlier communication, we have reported that a simple cottonseed medium based on 1% aqueous extract of any of the eight indigenously available varieties of cotton representing *Gossipium hirsutum* and *Gossipium arboreum* was as efficacious as pharmamedia for the mould-yeast conversion of 19 *B. dermatitidis* test strains [5].

This paper reports a wide variety of additional seed-based media for the mould-yeast conversion of *B. dermatitidis*.

Materials and methods

Fungal strains. Seven *B. dermatitidis* strains were used for evaluating the efficacy of various seed-based media. They were selected from a pool of 19 strains representing diverse geographic origin and variable mould-yeast conversion on pharmamedia [5]. They were divisible into 3 groups. Group A comprised fast converting strains, S-3922, B-1145 and Kc-14 of American origin (full conversion in 5 days), Group B comprised intermediate converting strains, VPCI-S70 and VPCI-BR42 of Indian origin (full conversion in 7 days) and Group C comprised slow converting strains, IP-973 and IP-1000-70 of African origin (full conversion in 14 days).

Seeds. A wide variety of seeds were evaluated for the mould-yeast conversion of *B. dermatitidis*. This included black gram (*Phaseolus mungo*), chick-pea (*Cicer arietinum*), cow-pea (*Vigna sinensis*), French bean (*Phaseolus vulgaris*), garden pea (*Pisum sativum*), green gram (*Phaseolus aureus*), lentil (*Lens esculenta*), mat bean (*Phaseolus aconitifolius*), peanut (*Arachis hypogaea*), pigeon-pea (*Cajanus cajan*), soyabean (*Glycin max*), niger seed (*Guizotia abyssinica*), sunflower seed (*Helianthus annus*), cucumber (*Cucumis sativus*), red gourd (*Cucurbita maxima*), corn (*Zea mays*), rice (*Oryza sativa*), wheat (*Triticum vulgaris*), cottonseed (*Gossypium arboreum*) and okra (*Hibiscus esculenta*).

Culture media. Pharmamedia was received through the courtesy of the Buckeye Cellulose Corporation (Memphis, TN) and was prepared as described by Weeks [1]. The composition of the rest of the media was the same as given by Weeks [1] except that pharmamedia was substituted by 2% extracts of the test seeds. The extracts were prepared by pulverization of the seeds in an electric blender, followed by boiling for 30 min in distilled water and filtration through muslin cloth. Peptone glucose agar (peptone 1%, glucose 2% and agar 1.5%), glucose agar (glucose 2%, agar 1.5%) and water agar (agar 1.5%) were also included as controls.

Inoculum. The inoculum was prepared from the *B. dermatitidis* test strains cultured on modified Sabouraud glucose agar slants for 3 weeks at 28 °C. The growth was scraped and ground manually in a glass tissue grinder with 5 ml of sterilized physiological saline. Two drops of the mycelial culture suspension, the density of which had been adjusted to MacFarland standard No. 3, were inoculated with a Pasteur pipette onto the various seed media slants and incubated at 37 °C for up to 7 days.

Assessment of conversion. After 3, 5 and 7 days of incubation, fungal growth from the top end of slope cultures were removed with a loop needle (diameter 1 mm), transferred to 0.05 ml of lactophenol cotton blue on a glass slide and examined microscopically. The number of yeast cells seen was counted in 5 microscopic fields, employing the 40x objective and 10x ocular and their mean count were recorded. The hyphal fragments and the forms intermediate between the yeast cells and hyphae were not counted. The mould-yeast conversion was graded as very poor (1+) = < 5 yeast cells; poor (2+) = 6 – 15 yeast cells; good (3+) = 16–25 yeast cells; excellent (4+) = 26 – 35 yeast cells, and full (5+) = > 35 yeast cells and without any intermediate or hyphal forms.

Statistical analysis. Yeast cell counts were considered as poisson variates and transformed to achieve variance, stability and normality [6]. The data were subjected to analysis of variance considering the entire experiment as a tri-factorial design, i.e. 23 media (including the controls) × 7

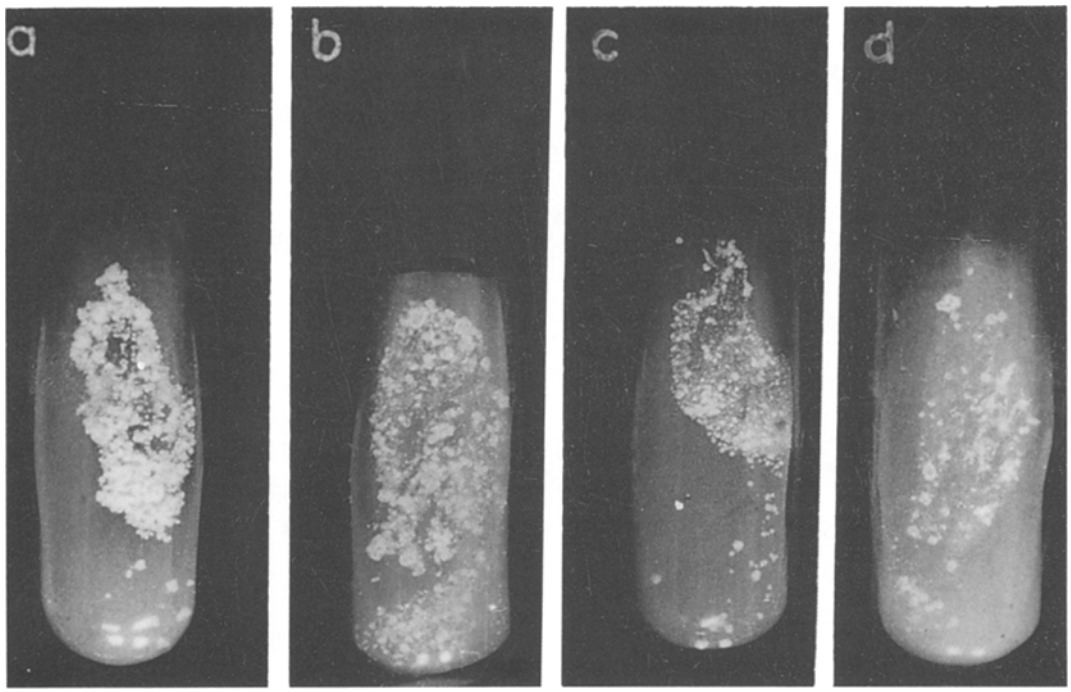

Fig. 1. Comparative efficacy of some seed-based media for mould-yeast conversion of *B. dermatitidis* VPCI-S70 after 7 days of incubation at 37 °C. Note, the full conversion to yeast form on (a) garden-pea agar, (b) pharmamedia agar, (c) excellent conversion on lentil agar, and (d) poor conversion on pigeon-pea agar.

strains × 3 incubation period, with 5 replicates of each observation after ascertaining homogeneity of variance (taking cognizance of those with conversion) and normality.

Results

The data on efficacy of the 20 seed-based culture media for *in vitro* mould-yeast conversion of the *B. dermatitidis* test strains are presented in Tables 1–2. Figures 1–2 illustrate the macroscopic and microscopic morphology of the yeast form of one of the *B. dermatitidis* test strains, VPCI-S70, on various seed media. The statistical analysis of the data revealed that the culture medium, fungal strain and incubation period were highly significant factors in the mould-yeast conversion of *B. dermatitidis* ($p < 0.01$). Of the 20 seed-based media investigated, 10 yielded conversion of all the 7 *B. dermatitidis* strains after 5 days of incubation (Table 1). This included the garden-pea, chick-pea, cow-pea, soyabean, peanut, green gram, French bean, lentil (Family Leguminosae), cottonseed and okra (Family Malvaceae). Eight of the remaining seed-based media, namely, pigeon-pea, black gram, mat bean (Family Leguminosae), corn, wheat and rice (Family Gramineae), cucumber and red gourd (Family Cucurbitaceae) yielded the conversion of 5 *B. dermatitidis* strains after 5 days of incubation (Table 2). Nigerseed and sunflower seeds (Family Compositae) did not support conversion of any of the test strains. Of the control media, only pharmamedia was found to be at par with the excellent efficacy of garden-pea, chick-pea, cow-pea, soyabean, peanut and cottonseed media for the conversion. Notwithstanding its high efficacy for conversion, peanut was unsuitable in that oil globules in this medium interfered with microscopic observations of yeast

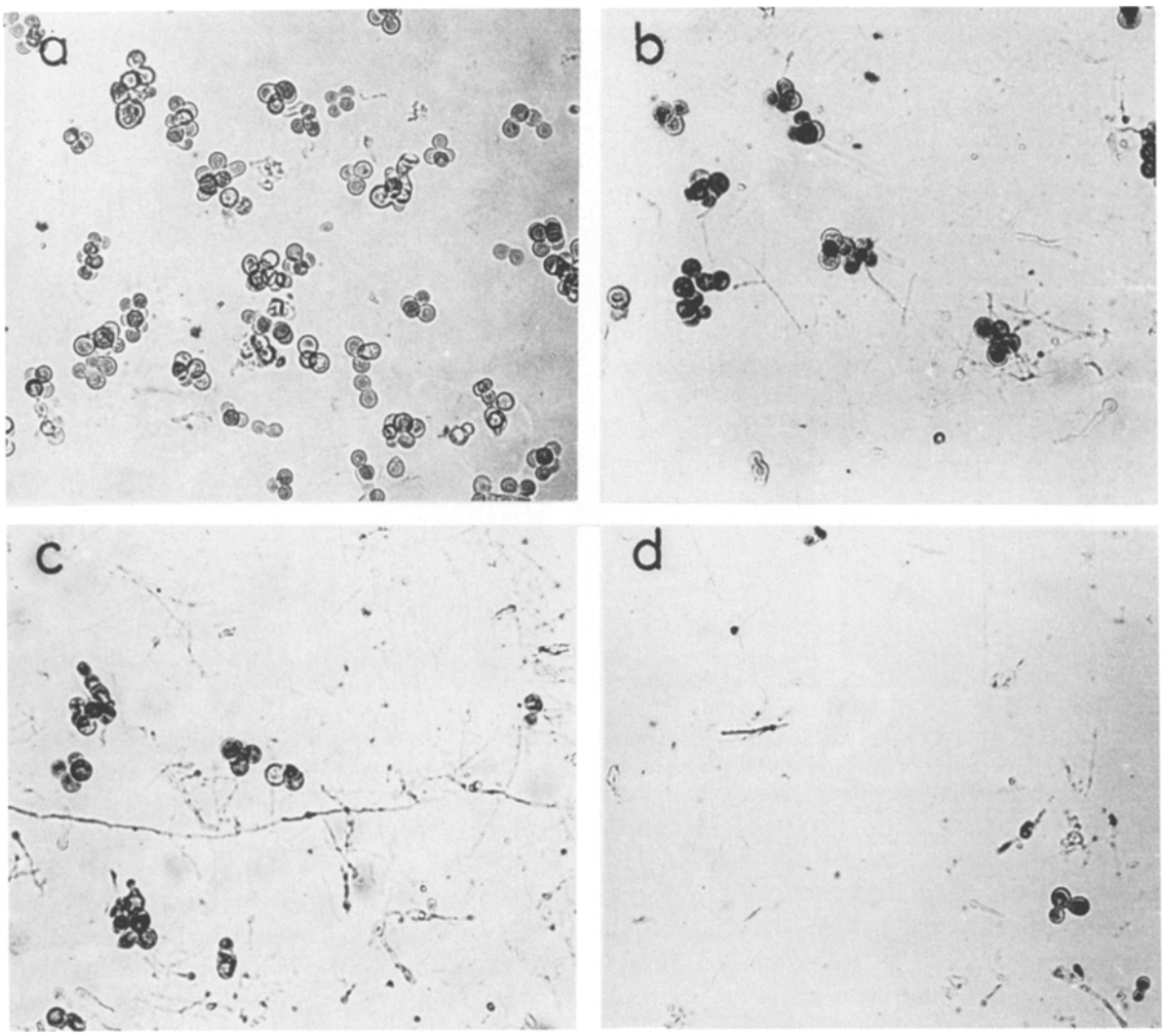

Fig. 2. A microscopic view of the mould-yeast conversion of *B. dermatitidis* VPCI-S70 on some seed-based media depicting degree of mould-yeast conversion. Lactophenol-cotton blue mounts showing (a) the full conversion denoted by >35 yeast cells per microscopic field on garden-pea agar, (b) excellent conversion with 26–35 yeast cells on lentil agar, (c) good conversion with 16–25 yeast cells on pigeon-pea agar, and (d) very poor conversion with 5 yeast cells on glucose agar.

morphology. The remaining control media, i.e. peptone glucose agar, glucose agar and water agar failed to convert 2 of the *B. dermatitidis* strains whereas the results were variable in the remaining 5 strains.

The mould-yeast conversion behaviour of *B. dermatitidis* test strains varied significantly ($p < 0.01$) with the test medium and the incubation period (Fig. 3). For instance, after 3 days of incubation, *B. dermatitidis* strains S-3922 and B-1145 showed excellent conversion on garden-pea, chick-pea, cow-pea, green grm, French bean, lentil, soyabean, peanut, okra, cottonseed, wheat and corn agar media whereas the conversion was poor on pigeon-pea, black gram, mat bean, red gourd, cucumber and rice. Furthermore, both of these strains showed negligible or no conversion on peptone glucose agar, glucose

Table 1. Comparison of 10 highly efficacious seed-based media for mould-yeast conversion of 7 representative *B. dermatitidis* strains after 5 days of incubation at 37 °C.

Glucose agar with seed extract of	Number strains showing conversion grade* of				Number of strains showing no conversion
	+ + + +	+ + +	+ +	+	
Garden-pea	3	2	1	1	0
Chick-pea	3	2	1	1	0
Cow-pea	3	2	1	1	0
Soyabean	3	2	1	1	0
Peanut	3	2	1	1	0
Cottonseed	3	2	1	1	0
Green gram	3	0	2	2	0
French bean	3	0	2	2	0
Lentil	3	0	2	2	0
Okra	3	0	2	2	0
Control media					
Pharmamedia glucose agar	3	2	1	1	0
Peptone glucose agar	0	0	0	5	2
Glucose agar	0	0	2	3	2
Water agar	0	0	2	3	2

*For details, please refer to 'Materials and methods'.

agar and water agar. The remaining 5 *B. dermatitidis* strains Kc-14, VPCI-BR42, VPCI-S70, IP-973 and IP-1000-70 were slow to convert as they showed negligible conversion on all of the seed-based media and the controls. After 5 days of incubation, *B. dermatitidis* S-3922 and B-1145 showed good to full conversion on all of the seed-based media. Strain B-1145 showed good conver-

Table 2. Comparison of 10 less efficacious seed-based media for mould-yeast conversion of 7 representative *B. dermatitidis* strains after 5 days of incubation at 37 °C.

Glucose agar with seed extract of	Number strains showing conversion grade* of				Number of strains showing no conversion
	+ + + +	+ + +	+ +	+	
Pigeon-pea	0	3	0	2	2
Black gram	0	3	0	2	2
Mat bean	0	3	0	2	2
Corn	0	3	0	2	2
Wheat	0	3	0	2	2
Rice	0	1	2	2	2
Cucumber	0	1	2	2	2
Red gourd	0	1	2	2	2
Sunflower seed	0	0	0	0	7
Niger seed	0	0	0	0	7
Control media					
Pharmamedia glucose agar	3	2	1	1	0
Peptone glucose agar	0	0	0	5	2
Glucose agar	0	0	2	3	2
Water agar	0	0	2	3	2

*For details, please refer to 'Materials and methods'.

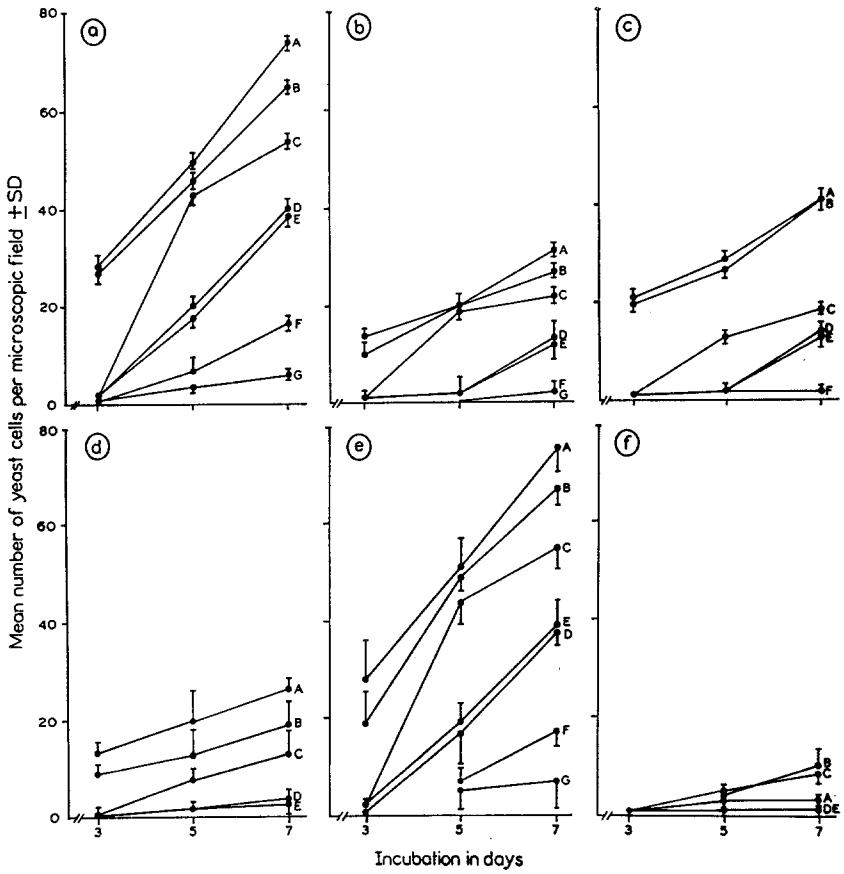

Fig. 3. Efficacy of various seed-based media for mould-yeast conversion of 7 representative *B. dermatitidis* strains at 37 °C: (a) Garden-pea representing group I seed-based media, (b) pigeon-pea representing group II seed-based media, (c) wheat representing group III seed-based media, (d) cucumber representing group IV seed-based media, (e) pharmamedia agar, (f) peptone glucose agar. Bars represent standard deviation. Abbreviations: *B. dermatitidis* strains, A = S-3922; B = B-1145; C = Kc14; D = VPCI-S70; E = VPCI-BR42; F = IP-973; G = IP-1000–70.

sion even on glucose agar and water agar media. Strain Kc-14 had fully converted after 5 days on garden-pea, chick-pea, cow-pea, soyabean, peanut and cottonseed media although it had shown no sign of conversion after 3 days of incubation.

The seed-based media were divisible into 4 groups depending upon the time required for mould-yeast conversion of all the *B. dermatitidis* test strains. Group I comprised those seed media which converted all of the 7 *B. dermatitidis* strains after 5 days of incubation. This included garden-pea, chick-pea, cow-pea, green gram, French bean, lentil, soyabean, peanut, cottonseed and okra media. Group II comprised pigeon-pea and black gram media which yielded conversion of all the 7 strains after 7 days of incubation. Group III included mat bean, corn and wheat media which converted 6 of 7 strains after 7 days of incubation. Group IV included rice, cucumber and red gourd media which converted only 5 of the strains after 7 days of incubation (Fig. 3).

Garden-pea agar as a preferential medium. Garden-pea agar was selected for more comprehensive evaluation because of the wide availability and low fat content of this seed. To determine the optimal composition of the medium, the effects of graded concentrations of seed extract (0.5 to

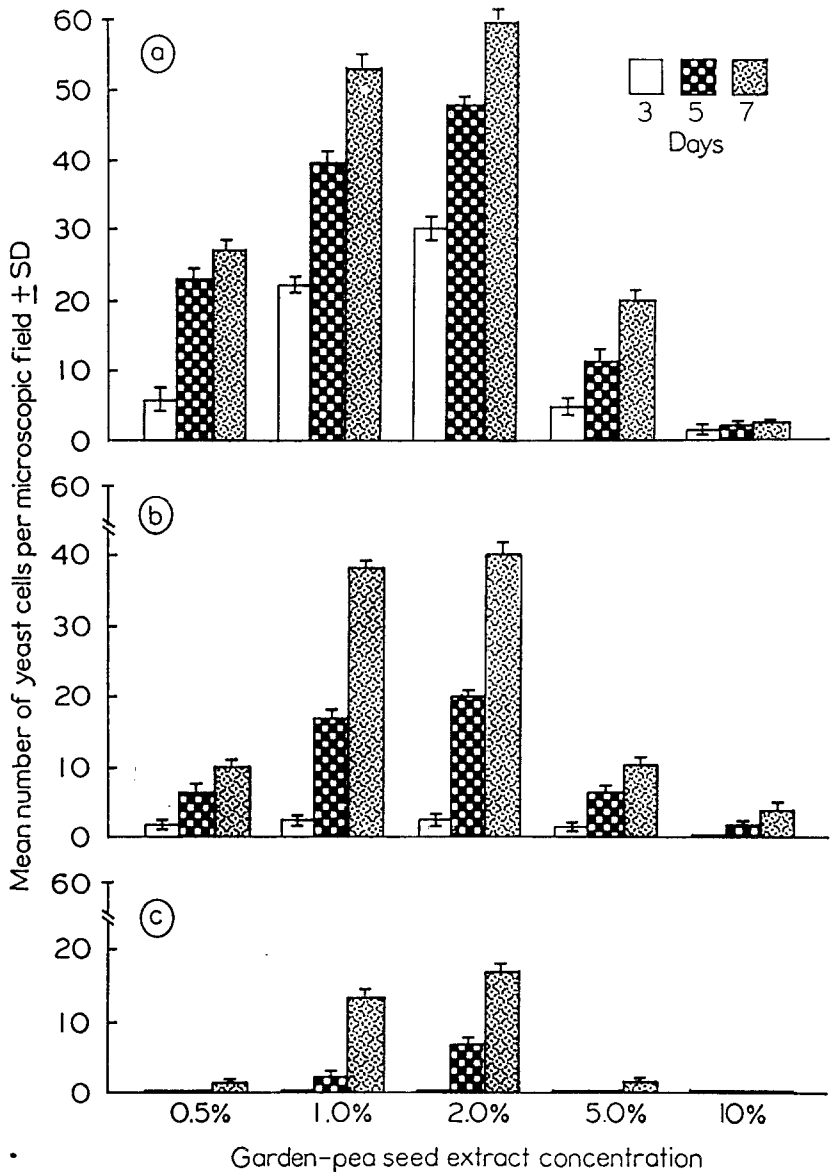

Fig. 4. Effect of variable garden-pea seed extract concentrations on mould-yeast conversion of *B. dermatitidis* strains: (a) B-1145, (b) VPCI-S70, (c) IP-973. Bars represent standard deviation.

10%), glucose (0.5 to 2%) and of variable pH (pH 4 to 10) upon mould-yeast conversion of 7 *B. dermatitidis* strains were investigated. It was found that the optimal seed extract concentration was 2% (Fig. 4). The conversion was adversely affected at 5% or higher concentrations including total inhibition in strains IP-973, and IP-1000–70. No difference in conversion was initially discernible on garden-pea seed agar with or without the supply of glucose. However, upon prolonged incubation the growth of the yeast form was enhanced by the supply of 0.5% to 2% glucose. It was shown that the optimum pH for the conversion ranged from 6 to 7 (Fig. 5). The conversion gradually declined at pH levels below 6 and above 7, with a complete inhibition at pH 4 and pH

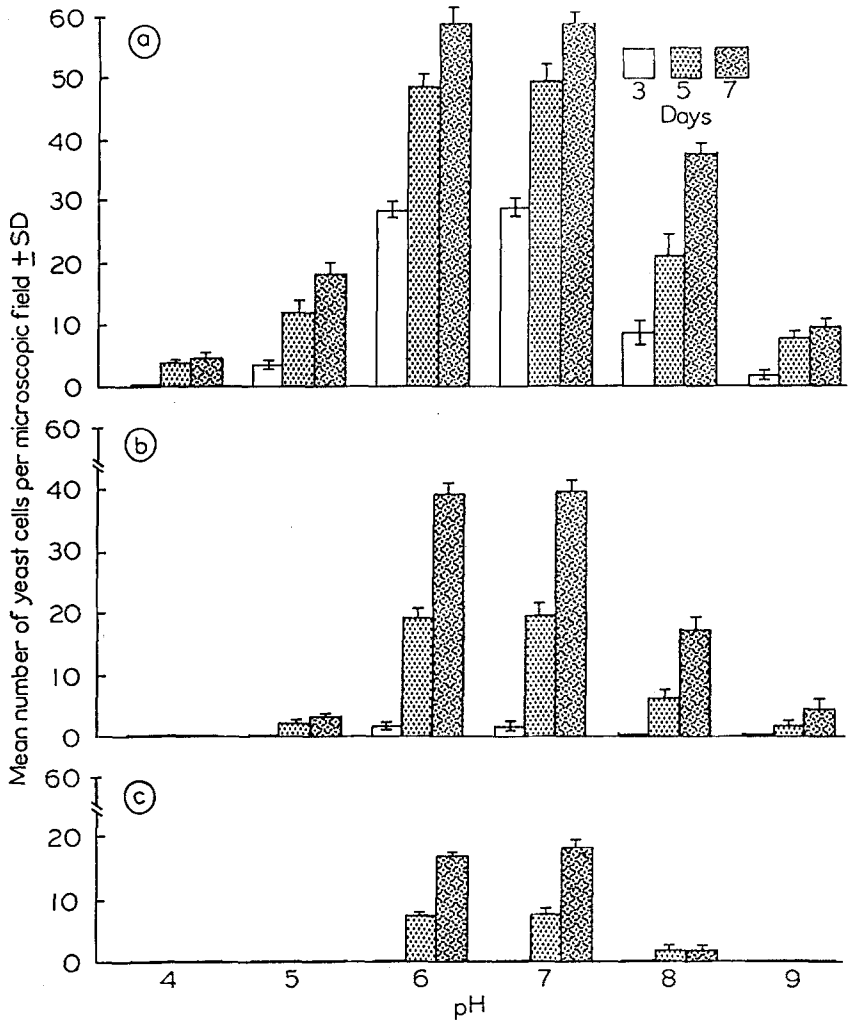

Fig. 5. Effect of variable pH of garden-pea seed agar on mould-yeast conversion of *B. dermatitidis* strains: (a) B-1145, (b) VPCI-S70, (c) IP-973. Bars represent standard deviation.

10. However, *B. dermatitidis* strain B-1145 was exceptional in that it showed a slight conversion at pH 4. On the basis of the preceding observations, the following composition for garden-pea seed agar is recommended for adoption: seed extract 2%, glucose 2%, agar 1.5%, pH 6. This medium was further evaluated by verification of mould-yeast conversion in 12 additional *B. dermatitidis* test strains (Table 3). Seven of the 19 strains showed excellent conversion after 3 days, 6 after 5 days, and 3 each after 7 and 14 days of incubation, respectively.

Discussion

The results demonstrate that the mould-yeast conversion of *B. dermatitidis* can be readily done on media based upon a variety of seeds such as garden-pea, chick-pea, cow-pea, soyabean, peanut, French bean, lentil, green gram, okra and cottonseed. In fact, the efficacy of many of these seed media was found to be at par with that of pharmamedia, a commercial product used for the mould-yeast conversion of *B. dermatitidis*. It may be pointed out that the seeds under reference are

Table 3. Time required for excellent* *in vitro* mould-yeast conversion of 19 *B. dermatitidis* strains cultured on garden-pea seed agar at 37 °C for 14 days.

Time for conversion in days	Strains showing conversion	
	Number	Accession numbers
3	7	B-1145, B-3393, B-779, BL-L, S-3920, S-3921, S-3922
5	6	784, 788, Kc-6, Kc-14, Kc-22, Kc-28
7	3	VPCI-S70, VPCI-BR42, VPCI-B53
14	3	IP-973, IP-268, IP-1000-70

*26-35 yeast cells per microscopic field with 10× ocular and 40× objective lenses.

widely available, inexpensive and their aqueous extracts are free from a precipitate encountered in the preparation of pharmamedia agar. However, garden-pea seed agar is adopted because of the wider availability and low fat content of this seed.

The mechanism underlying the variable mould-yeast conversion of *B. dermatitidis* on seed-based media is not clearly understood. However, 9 of the 10 seeds yielding excellent mould-yeast conversion are known for their high protein content, ranging from 22–37% [7, 8]. Pigeon-pea, mat bean and black gram were exceptional in being less efficacious for the mould-yeast conversion in spite of their equally high protein content. This observation may be ascribed to certain specific attributes of the proteins characterizing these seeds. In this context, it is interesting to note that the incorporation of peptone in agar media also adversely affected the conversion. The fat content of the seeds had apparently no role in the mould-yeast conversion of *B. dermatitidis*. Thus, peanut and soyabean which have fat contents of 48% and 17%, respectively, supported as good a conversion as did garden-pea, chick-pea and cow-pea whose fat content does not exceed 2% [7, 8]. The lack of mould-yeast conversion on nigerseed and sunflower seeds (protein 15–17%, fat 31–32.5%) might have been possibly due to a toxic effect of certain constituents on the metabolism of *B. dermatitidis*. From the parity of conversion observed on glucose agar and water agar media, it appeared that glucose was not essential for the conversion process. This observation is in conformity with that of Weeks [1] who reported that higher concentrations of glucose enhanced the growth of the yeast form but did not promote conversion.

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