original paper

Comparison of media for optimal recovery of *Candida albicans* and *Candida glabrata* from blood culture

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Abstract

Background *Candida* spp., mainly *Candida albicans*, are frequently responsible for complications in immunocompromised patients. There are limited data comparing recovery efficiency using simple non-selective basal broth media.

Aim To compare several commercially available basal growth media to determine the medium that gave highest yeast proliferation.

Method Eight commercially available non-selective basal broth culture media were evaluated for optimum recovery of clinical *C. albicans* and *C. glabrata*. They included nutrient broth (NB), nutrient broth no. 2 (NB2), Todd-Hewitt (TH) broth, tryptone soya broth (TSB), tryptone soya broth supplemented with yeast extract (0.5% w/v [TSBYE]), brain heart infusion broth supplemented with yeast extract (0.5% w/v [BHIYE]), salt meat broth (SMB) and 0.1% [w/v] peptone saline (PS). Differences in cell density were evaluated by spectrophotometrical analysis.

Results TSBYE>BHIYE>TSB>TH>NB2>NB>SMB>PS for the optimum proliferation of cells in vitro. Either TSBYE or BHIYE broth may be employed as suitable basal broth media for growth of *C. albicans* and *C. glabrata*. NB should be considered the least suitable medium for routine use when others are available.

Conclusion These data may be of value to laboratories setting up simple blood culture systems to detect *Candida* spp., particularly in developing and underdeveloped countries.

Introduction

Candida spp., mainly *Candida albicans*, are frequently responsible for infective complications in immunocompromised patients, which can lead to significant increases in morbidity and mortality.[1,2] Various automated detection systems are available for the routine detection of yeasts from blood culture and there are several reports comparing their efficacy.[3] However, there are very limited data in the literature comparing recovery efficiency using simple non-selective basal broth media.

Knowledge of which basal broth to employ to maximise cell proliferation is important for several reasons: for downstream propagation/harvest of cells following primary isolation on sophisticated automated detection systems; employment in biochemical diagnostic tests; use in routine diagnostic laboratories which have limited resources and/or media production facilities; and for employment in simple blood detection systems, particularly in developing and underdeveloped countries not able to afford expensive automated blood culture monitoring systems, such as BacTec or BacTAlert systems or the consumables to support such systems.

In the underdeveloped and developing world, candidaemia is often associated with several risk factors, including HIV/AIDS, and has been defined as a predictor of mortality.[4] In such areas where there is a high incidence of this infection, diagnostic problems associated with the detection of Candida have previously been reported.[5] Therefore, the aim of this study is to evaluate eight commercially available non-selective basal broth culture media to determine optimum recovery of clinical *Candida* spp, in particular *C. albicans* and *C. glabrata*.

Methods

Twenty-eight isolates of *Candida* spp. were examined in this study, as detailed in Table 1. All isolates were obtained from well-marked clinical cases of blood borne candidiasis presenting at Belfast City Hospital over the period 1994-2000, as well as a reference strain of *C. krusei* NCPF 3953. Initially, the identity of the isolates was confirmed by sequencing of the short internal transcribed spacer (ITS) region of rRNA (5.8S-ITS2 rRNA), as previously described.[6]

Eight commercially available non-selective basal broth media were examined in this study: nutrient broth ([NB] Oxoid CM0001, Oxoid Ltd., Basingstoke, UK), nutrient broth no. 2 ([NB2] Oxoid CM0067), Todd-Hewitt broth ([TH] Oxoid CM0189), tryptone soya broth ([TSB] Oxoid CM0129), tryptone soya broth supplemented with yeast extract (0.5% w/v [TSBYE] Oxoid CM0129+Oxoid LP0021), brain heart infusion broth supplemented with yeast extract (0.5% w/v [BHIYE] Oxoid CM+Oxoid LP0021), salt meat broth ([SMB] Oxoid CM0094) and 0.1% [w/v] peptone saline ([PS] Oxoid CM0733). All media were reconstituted in accordance with the manufacturers' instructions.

All strains were grown at 37°C (24 hour) on Sabouraud dextrose agar (Oxoid CM0041). Inocula were prepared of each isolate in PS to give approximately 10^5 cells/ml, of which 50µl aliquots were added to 250µl prepared media in a 96-well flat bottomed microtitre plate (Sarstedt GmbH, Germany). For each treatment series, an equal number of negative controls were established, containing uninoculated media, to check sterility and thus avoid false-positives. Plates were incubated statically at 37ºC for 60 hours with sealed lids to avoid evaporation of water. Plates were examined spectrophotometrically without lids at 0, 20, 40 and 60 hours and

Table 1. Comparison of growth (mean absorbance) of *Candida* spp. in eight basal broth media

Organism	No. isolates examined	NB	NB2	TH	TSB	TSBYE	BHIYE	SMB	PS
C. albicans	13	0.225	0.211	0.289	0.365	0.619	0.536	0.019	0.002
C. glabrata	7	0.094	0.080	0.144	0.321	0.873	0.773	0.020	0.006
C. parapsilosis	2	0.311	0.364	0.400	0.375	0.670	0.845	0.060	0.005
C. tropicalis	1	0.612	0.755	0.920	1.116	1.639	1.077	0.337	0.000
C. dubliniensis	1	0.407	0.518	0.553	0.604	1.313	1.113	0.000	0.001
C. krusei	4	0.119	0.143	0.177	0.325	0.857	0.783	0.035	0.001
Total	28	0.295	0.345	0.414	0.518	0.995	0.855	0.079	0.003

Mean absorbance (λ=405nm)

Table 2. Detailed listing of components of the eight basal media compared in this study Broth formulation

Media constituents (g/l)	NB	NB2	TH	TSB	TSBYE	BHIYE	SMB	PS
Lab-Lemco powder	1	10					10	
Infusion 450g fat-free beef			10					
Pancreatic digest of casein				17	17			
Brain heart infusion solids						3.5		
Papaic digest of soybean meal				3	3			
Natural heart muscle							30	
Yeast extract	2				5	5		
Peptone	5	10				25	10	1
Tryptone			20					
Glucose			2	2.5	2.5	2		
Sodium bicarbonate			2					
Sodium chloride	5	5	2	5	5	5	100	8.5
Disodium phosphate			0.4					
Disodium hydrogen phosphate						2.5		
Dibasic potassium phosphate				2.5	2.5			
PH	7.4±0.2	7.5±0.2	7.8±0.2	7.3±0.2	7.3±0.2		7.6±0.2	7.0±0.2

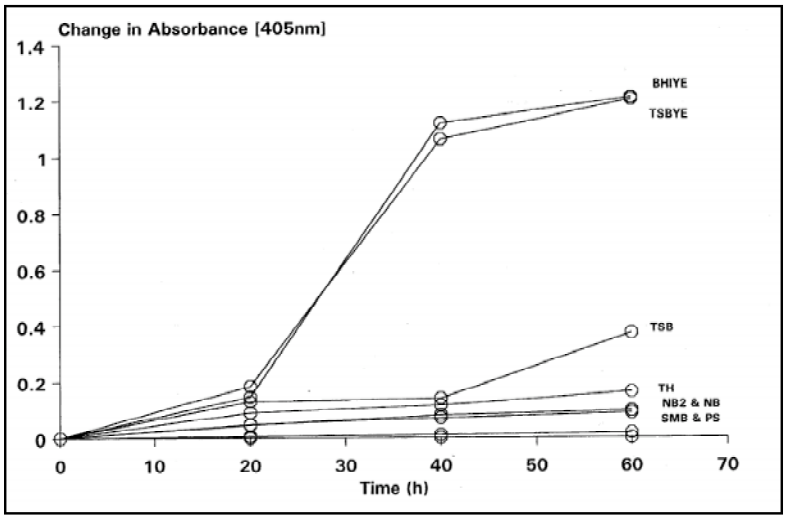

Figure 1. Time course (0-60 hours) showing changes in mean absorbance [λ=405nm] of *C. krusei* NCPF 3953 grown in eight basal broth media (see methods)

were shaken prior to triplicate readings being taken (λ=405nm) on an automatic microtitre plate reader (Emax, Molecular Devices Inc., CA, USA) and the mean absorbance values noted.

Results

Mean spectrophotometrical values of cell density were calculated for each isolate incorporating for the eight media screened (see Table 1), using the equation:

$$\lambda\,\text{Absorbance}_{[\lambda=405nm]} = \text{Absorbance}_{[t=60h]} - \text{Absorbance}_{[t=0h]}$$

Mean absorbance results of the broth media, comparison for each strain and statistical analysis of variance allowed the basal media to be ranked into the order (commencing with the media which gave greatest cell density) TSBYE>BHIYE>TSB>TH>NB2>NB>SMB>PS. An example of the growth dynamics for *C. krusei* NCPF 3953 over 60 hours in each broth medium is shown in Figure 1.

Overall, statistical analyses showed that there was no statistical difference between NB and NB2 (p=0.116), nor between TSBYE and BHIYE (p=0.106). However, there was a statistical difference between BHIYE and TSB (p=0.007). Supplementation of TSB with yeast extract demonstrated a significant difference between the supplemented formulation and the unsupplemented TSB media (p=0.001).

There were no significant differences between cell density achieved between *C. albicans* and *C. glabrata* (p=0.462), nor between *C. albicans* and *C. krusei* (p=0.341). Statistical analyses were not performed between the other species tested, due to the relatively small numbers of organisms examined. Additional observations noted the relative salt tolerance of the *C. tropicalis* isolate examined compared to the other species examined.

Discussion

This short report indicates that either TSBYE or BHIYE broth may be employed as suitable basal broth media and this may be attributed to these media containing higher concentrations of peptones, proteins and other nutritionally rich meat infusions (see Table 2). NB gave the lowest absorbance values of those tested and should be considered less suitable for routine use, when other nutritionally rich media are available. Such data may be of value to laboratories setting up simple blood culture systems to detect *Candida* bloodstream infections, particularly in developing and underdeveloped countries.

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