The proposition of an inexpensive chemically defined culture medium (MycoDef) for yeast studies

A proposta de um meio de cultura barato e quimicamente definido (MycoDef) para estudos de leveduras

DOI:10.34117/bjdv9n2-113

Recebimento dos originais: 17/01/2023
Aceitação para publicação: 19/02/2023

Antoniella de Aguiar
Mestranda em Farmácia
Instituição: Pontifícia Universidade Católica do Paraná
Endereço: Rua Imaculada Conceição, 1155, CEP: 80215-901
E-mail: antoniella.aguiar@gmail.com

Nicoly Subtil de Oliveira
Mestre em Ciência Animal
Instituição: Pontifícia Universidade Católica do Paraná
Endereço: Rua Imaculada Conceição, 1155, CEP: 80215-901
E-mail: nicolysubtil@gmail.com

Rosimeire Takaki Rosa
Mestre em Biologia Buco-Dental
Instituição: Pontifícia Universidade Católica do Paraná
Endereço: Rua Imaculada Conceição, 1155, CEP: 80215-901
E-mail: rosimeire.rosa@pucpr.br

Angela Bonjiorno Arantes
Doutora em Odontologia
Instituição: Pontifícia Universidade Católica do Paraná
Endereço: Rua Imaculada Conceição, 1155, CEP: 80215-901
E-mail: angela.arantes@pucpr.br

Romeu Cassiano Pucci da Silva Ramos
Doutor em Odontologia
Instituição: UniSociesc
Endereço: Avenida Getúlio Vargas 268, Jaraguá do Sul, Santa Catarina, CEP: 89251-970
E-mail: romeucs@gmail.com

Luiz Fernando Bianchini
Doutor em Odontologia
Instituição: Pontifícia Universidade Católica do Paraná
Endereço: Rua Imaculada Conceição, 1155, CEP: 80215-901
E-mail: fernando.bianchini@pucpr.br

Patrícia Maria Stuelp-Campelo
Doutora em Bioquímica
Instituição: Pontifícia Universidade Católica do Paraná
Endereço: Rua Imaculada Conceição, 1155, CEP: 80215-901
E-mail: patricia.campelo@pucpr.br

Edvaldo Antonio Ribeiro Rosa
Doutor em Biologia e Patologia Buco Dental
Instituição: Universidade Católica do Paraná
Endereço: Rua Imaculada Conceição, 1155, CEP: 80215-901
E-mail: edvaldo.rosa@pucpr.br

ABSTRACT
Chemically defined culture media (CDCMs) have their compositions qualitatively and quantitatively known. They are constituted of components able to meet the nutritional requirements of microorganisms. This study evaluated the employability of a multivitamin as the basis for the elaboration of a CDCM for experiments with yeasts. *Candida albicans* reference strains ATCC®90028™ and SC5314 were used. YNB® without amino acid (Difco Co.) was used as the standard in evaluations. For the preparation of the experimental culture medium (MycoDef), commercial multivitamin tablets had their coatings removed and were crushed until obtaining a fine powder; missing constituents were added. Comparisons were carried out evaluating aerobic and anaerobic planktonic growth rate, biofilm formation rate, whole-cell protein patterns by SDS-PAGE, minimum inhibitory concentrations and minimal fungicidal concentrations for clotrimazole, fluconazole, nystatin, and griseofulvin. Growth in MycoDef and YNB® did not differ between them ($p>0.05$) in both normoxic and anoxic conditions. Regarding employability for sensitivity testing, MycoDef showed performance like YNB®. The protein profiles of cells grown on both media did not differ in the number and positioning of bands. The results obtained allowed us to infer that MycoDef is a reliable low-cost culture medium useful for trials involving *C. albicans*.

Keywords: *Candida*, chemically defined culture medium, multivitamin, resistogram, yeasts.

RESUMO
Os meios de cultura quimicamente definidos (CDCMs) têm suas composições qualitativa e quantitativamente conhecidas. Eles são constituídos de componentes capazes de atender às exigências nutricionais dos microorganismos. Este estudo avaliou a empregabilidade de um multivitamínico como base para a elaboração de um CDCM para experimentos com leveduras. Foram utilizadas as cepas de referência Candida albicans ATCC®90028™ e SC5314. O YNB® sem aminoácidos (Difco Co.) foi usado como padrão nas avaliações. Para a preparação do meio de cultura experimental (MycoDef), os comprimidos comerciais multivitamínicos tiveram seus revestimentos removidos e foram triturados até obter um pó fino; os constituintes em falta foram adicionados. Foram realizadas comparações avaliando a taxa de crescimento planctônico aeróbio e anaeróbio, taxa de formação de biofilme, padrões de proteína de célula inteira por SDS-PAGE, concentrações inibitórias mínimas e concentrações fungicidas mínimas para coagrimazole, fluconazol, nistatina e griseofulvina. O crescimento em MycoDef e YNB® não diferiu entre eles ($p>0,05$) tanto em condições normoxicais como anóxicas. Quanto à empregabilidade para testes de sensibilidade, o MycoDef mostrou desempenho como o

YNB®. Os perfis proteicos das células cultivadas em ambos os meios não diferiram quanto ao número e posicionamento das bandas. Os resultados obtidos nos permitiram inferir que o MycoDef é um meio de cultura confiável e de baixo custo, útil para testes envolvendo C. albicans.

Palavras-chave: *Candida*, meio de cultura quimicamente definido, multivitamínico, resistograma, leveduras.

1 INTRODUCTION

Chemically defined culture media (CDCMs) for microbiological purposes are those whose composition is known both qualitatively and quantitatively. Their components serve to meet the microbial nutritional requirements when specific nutritional needs are known.

For various purposes, it is desirable to have a CDCM that (i) provides reproducibility of chemical composition; (ii) avoids unnecessary excess of nutrients, facilitating the adjustment of their levels; (iii) meets the experimentally determined nutrient requirements of various strains; and (iv) guarantees growth at a reasonably high rate (FOUCAUD et al., 1997). CDCMs may be useful for biotechnological purposes, allowing consistency between batches and enhancement of formulations (FALZONE et al., 2013, KHAN et al., 2013, KONGO et al., 2003).

CDCMs for yeasts are often relatively expensive, which restricts their use for many biotech or clinical research and educational labs with budget limitations. On the other hand, the composition of multivitamins is very close to those of the CDCMs. This study was conceived on the idea that it is possible to use multivitamins (with formulation corrections) as the basis for the formulation of complex culture media with defined composition.

2 MATERIALS AND METHODS

2.1 STRAINS

Reference strains *Candida albicans* ATCC®90028™ and SC5314 were employed. Throughout the study, the strains were maintained in Sabouraud Dextrose Agar (SDA) and sub-cultured in Sabouraud Dextrose Broth (SDB).

2.2 EXPERIMENTAL CULTURE MEDIUM (MYCODEF)

CDCM, hereinafter called MycoDef, was prepared from the dissolution of uncoated tablets of a multivitamin found in the Brazilian market (Centrum®, Pfizer Inc). Corrections were made with the addition of missing constituents [p-aminobenzoic acid (B10), inositol, boric acid, sodium chloride and monopotassium phosphate], based on YNB® w/o amino acids (BD Difco Co.). As both media had no nitrogen and carbon sources, ammonium sulfate and glucose were added (Table 1).

Table 1. Composition of YNB®, Centrum® multivitamin and experimental MycoDef

Component	**YNB® (L^{-1})**	**Centrum® (1.495 g $tablet^{-1}$)**	**MycoDef (L^{-1})**
Vitamin A	-	400 µg	133.33 µg
Vitamin C	-	45 mg	15 mg
Vitamin D	-	5 µg	1.66 µg
Vitamin E	-	6.7 mg	2.23 mg
Vitamin K	-	65 µg	21.66 µg
Thiamin HCl (B1)	400 µg	1.2 mg	400 µg
Riboflavin (B2)	200 µg	1.3 mg	4.33 mg
Niacin (B3)	400 µg	16 mg	5.33 mg
Calcium pantothenate (B5)	400 µg	5 mg	1.66 mg
Pyridoxine HCl (B6)	400 µg	1.3 mg	430 µg
Biotin (B7)	2 µg	30 µg	10 µg
Folic acid (B9)	2 µg	240 µg	80 µg
p-aminobenzoic acid (B10)	200 µg	-	200 µg
Cobalamin (B12)	-	2.4 µg	0.80 µg
Inositol	2 mg	-	2 mg
Boric acid	500 µg	-	500 µg
Calcium chloride	100 mg	250 mg	83.33 mg
Sodium chloride	100 mg	-	100 mg
Ferric chloride	200 µg	8.1 mg	2.70 mg
Monopotassium phosphate	1 g	-	1 g
Potassium iodide	100 µg	33 µg	11 µg
Sodium molybdate	200 µg	23 µg	7.66 µg
Copper sulfate	40 µg	450 µg	150 µg
Chromium sulfate	-	18 µg	6 µg
Magnesium sulfate	500 mg	100 mg	33.33 mg
Manganese sulfate	400 µg	1.2 mg	400 µg
Selenium sulfate	-	20 µg	6.66 µg
Zinc sulfate	400 µg	7 mg	2.33 mg
Ammonium sulfate	-	-	5 g
Glucose	-	-	20 g

Tablets had their coating removed by abrasion with 400-grit sandpaper and were ground with added components until obtaining uniform powder of #200 mesh. The obtained powder was stored in a black plastic flask in a desiccator under vacuum and with silica gel.

2.3 PREPARATION OF MYCODEF

A mass of 1.5 g of MycoDef powder was dissolved in 300 mL of type II reagent water at 37 °C on a magnetic stirrer (500 rpm) for 30 min. The soluble portion was separated by centrifugation (5000 ×*g*, 5 min). The supernatant was filter sterilized by 0.22

μm membrane (Millipore Co.) into three sterile flasks. When diluted, the content of each flask allows the production of 1 L of MycoDef. To prepare the MycoDef, the prepared volume (100 mL) was aseptically combined with 900 mL of sterile water.

2.4 AEROBIC PLANKTONIC GROWTH

Cells were grown in tubes with 5 mL of SDB at 120 rpm, ~20% pO_2 and 35 °C, for 12 h. Aliquots of 100 μL of cell suspensions were transferred to 5 mL tubes of MycoDef or YNB® (according to the manufacturer), for cell adaptation. After 24 h, cells were obtained by centrifugation (5000 ×*g*, 5 min) and washed twice with sterile 145 mM NaCl. Cell suspensions had their concentrations corrected to *ca*. 10^8 cells mL^{-1}, with control done in a modified Neubauer hemocytometer.

Sterilized prescription vials of 30 mL received 10 mL of MycoDef or YNB®. Sterile butyl rubber caps have been adapted and the vials will receive an aluminium seal by recapping.

Volumes of 10 μL suspensions (*ca*. 10^6 cells) were transferred to these bottles, which were incubated at 6 rpm, ~20% pO_2 and 35 °C in an incubator adapted with a blood homogenizer. At the time intervals 2 h, 4 h, 8 h, 10 h, 12 h, 24 h and 48 h, 10 μL aliquots were taken and immediately transferred to microplates. Absorbances were determined on an EPOCH-2™ microplate reader (BioTek Instruments Inc.) at 540 nm.

The tests were carried out in six replicates in three different situations, performing 18 replicates per strain.

2.5 ANAEROBIC PLANKTONIC GROWTH

Yeast strains were grown in YEPD and adapted to MycoDef or YNB®, as previously described.

Sterilized prescription vials received MycoDef or YNB® and were processed as above. The bottles were placed in an ultrasonic bath at maximum power for 10 min to remove dissolved oxygen. An atmosphere of O_2-free CO_2 replaced the flasks' headspaces.

Further steps were carried out as above with an atmosphere of 0% pO_2 inside the vials.

2.6 BIOFILM FORMATION

Cells were grown in SDB and transferred to 5 mL tubes of MycoDef or YNB®, for adaptation as described above.

U-bottomed polystyrene plate wells were treated with 200 µL of sterile 1% arginine (120 rpm, ~20% pO_2, 35 °C) for 2 h to form an adhesive pellicle. The supernatant was removed by aspiration and 100 µL volumes of suspensions (*ca*. 10^7 cells) were transferred to these wells and incubated at 120 rpm, ~20% pO_2 and 35 °C, for 2 h (adhesion phase). After adhesion, wells were depleted and washed with sterile 145 mM NaCl to reduce the number of non-adhered cells. Wells received 200 µL of MycoDef or YNB® and plates were incubated for 72 h at 120 rpm, ~20% pO_2 and 35 °C.

Biofilm biomasses were estimated by retention of crystal violet (CV). Supernatants were removed by aspiration and the biomasses were treated with 99% methanol for 5 min. Methanol was removed, wells were dried with forced air and biofilm was stained with 0.5% CV (200 µL, 10 min). Retained CV was eluted in 200 µL of isopropanol. Aliquots of 100 µL were transferred to flat-bottom microtitration plates and the absorbances were obtained at 540 nm. The absorbance values of blanks were used to eliminate spurious results derived from background interference.

Tests were conducted with 30 individual replicates in three different situations, performing 90 replicates for each strain.

2.7 POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE) OF WHOLE CELL PROTEINS

Cells were grown in MycoDef or YNB® exactly as described in "Evaluation of aerobic planktonic growth", for 12 h. After that, they were harvested by centrifugation at 2000 ×*g* for 3 min and the pellets were washed four times with cold sterile water to remove either culture media traces or extra-cellular metabolites. The last washed pellets were transferred to 2 mL microcentrifuge tubes and acid-washed glass beads (v/v) plus 200 mL of cold sterile water were added. Cells were lysed using a Mini-Bead Beater® cell disrupter (Biospec Inc.) at 4600 rpm, repeating four times for 30 sec at 5-min intervals, and placed in an ice bath. After cell disruption, tubes were centrifuged at 10,000 ×*g* for 2 min, and the supernatant's protein concentrations were determined by Bradford's method and adjusted to 80 µg mL^{-1} with sterile 100 mM PBS pH7.0. Lysates were centrifuged (12000 ×*g*, 3 min) and aliquots of 100 µL of the supernatants were transferred to 1.5 mL microtubes. Equivalent volumes of denaturing buffer [5 mM Tris-HCl (pH 6.8), 2.5% β-mercaptoethanol, 20% glycerol, 1.5% SDS and 0.025% bromophenol blue] were added (ROSA et al., 2000). Tubes were heated in a boiling water bath for 10 min. After cooling in an ice bath for 5 min, samples were immediately processed in SDS-PAGE.

Protean® II mini-plates (Bio-Rad Labs Inc) were prepared with 4.5% stacking gel and 12% running gel (LAEMMLI, 1970) in a batch buffer system (pH 8.9) containing 25 mM Tris, 192 mM glycine and 0.1% SDS. The resulting gels were 1 mm thick.

Aliquots of 50 µL of protein extracts were applied to upper wells of stacking gels. Upper and lower tanks were filled with the discontinuous buffer system (pH 8.9) described above. Runs were conducted under a constant voltage of 75 V (15 min) followed by 125 V, which was maintained until the end of the runs. SigmaMarker™ wide-range mol wt 6,500-200,000 Da reference standard (Sigma-Aldrich Co) was applied in wells #1 and #5.

At the end of the run, gels were transferred to tanks containing 0.025% Coomassie G-250 blue (in methanol), where they remained for 12 h. Gels were decolourized with methanol: acetic acid:water (25:10:75).

After discolouring, gels were scanned and transferred to the Sigma-Gel™ for Windows® software interface (Sigma-Aldrich Co.) where densitograms were obtained. Densitograms were compared according to the bands' Rf values and molar masses. The linear positioning of bands allowed the comparison of banding patterns.

2.8 MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM FUNGICIDAL CONCENTRATION (MFC)

Antifungal agents applied in the sensitivity tests were fluconazole, nystatin, clotrimazole and griseofulvin. Stock solutions with 2 mg mL^{-1} were prepared diluting Tween 80® in MycoDef and YNB® to 0.5% followed by the dissolution of 10 mg of each antifungal. Stock solutions were membrane-filtered for sterile tubes, stored at -20 °C and protected from light. To prepare working antifungal solutions, 25 µL aliquots of stock solutions were diluted in 975 µL of MycoDef or YNB® to a concentration of 50 µg mL^{-1}.

Yeast cells were grown overnight in 5 mL of SDB at 120 rpm, ~20% pO_2 and 35 °C. Suspension aliquots of 100 µL were transferred to 5 mL of MycoDef or YNB®, for adaptation. After 24 h, cells were harvested by centrifugation (5000 ×*g*, 5 min) and washed twice with sterile water. Suspensions were corrected with sterile saline solution to *ca*. #0.5 tube from McFarland's scale. Obtained suspensions were diluted (1:100) in MycoDef or YNB® and then diluted (1:20) again in both broths, thereby obtaining the working inocula.

Volumes of 50 µL of working inocula were transferred to all wells of microtitration plates. Aliquots of 50 µL of working antifungal solutions were dispensed into the first in-

line wells of the plates. Finally, 50 µL volumes of the first wells were transferred to the second wells, the second wells to the third wells and so on up to the tenth-second wells. Serial dilutions were carried out with 12 antifungal concentrations, being 25.0 µg mL^{-1}, 12.5 µg mL^{-1}, 6.25 µg mL^{-1}, 3.12 µg mL^{-1}, 1.06 µg mL^{-1}, 0.53 µg mL^{-1}, 0.26 µg mL^{-1}, 0.13 µg mL^{-1}, 0.06 µg mL^{-1}, 0.03 µg mL^{-1}, 0.015 µg mL^{-1}, and 0.007 µg mL^{-1} (CLSI, 2008). Broths were buffered to pH 7.0 with MOPS.

Plates were incubated at 35 °C and ~20% pO_2, for 48 h. For each strain, three replicates of the tests were performed. When MIC was achieved, no visible growth was observed in subsequent wells. For determination of MFC, the contents of wells with no growth were reinoculated in SDA and incubated at 35 °C and ~20% pO_2, for 48 h. If no growth was noticed, MFC was achieved in the first well with no growth.

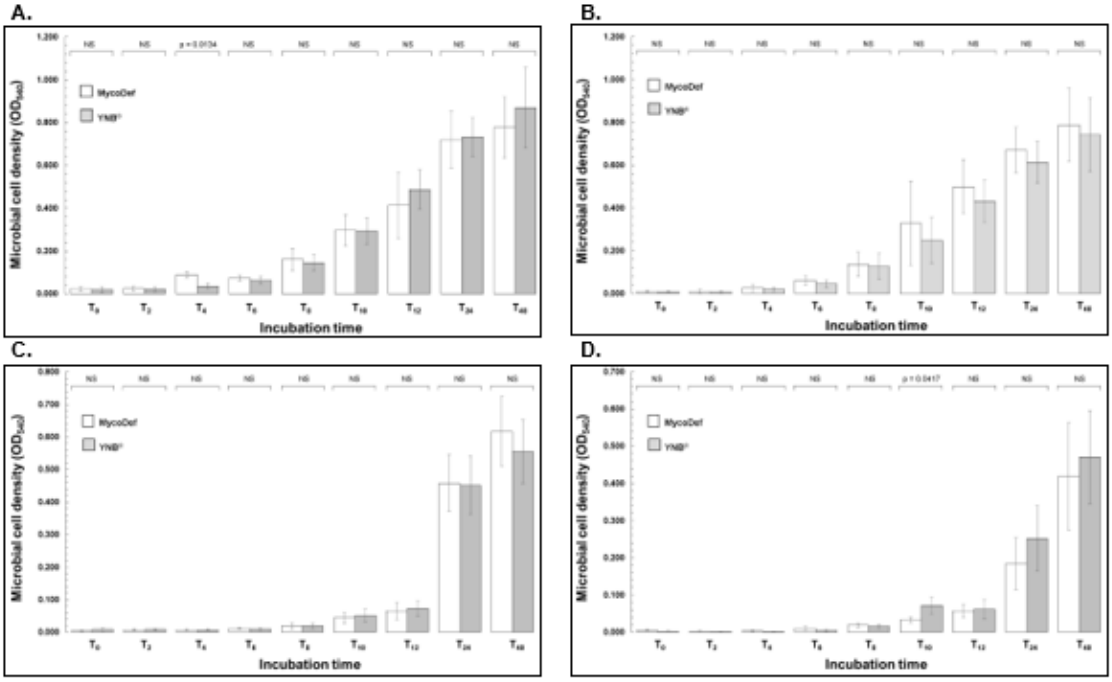
3 RESULTS AND DISCUSSION

Culture media such as YNB® and RPMI1640 are MCQDs universally employed for assays involving yeasts (UPPULURI et al., 2009; KUCHARÍKOVÁ et al., 2011). Each one has advantages and rather directed indications. In this study, it was proposed the use of a multivitamin (Centrum®) as a precursor of nutrients for the preparation of a culture medium, once corrected the concentrations of the components, as well as the addition of others absent. RPMI1640 was not included in the study, since that medium has a very different composition than that obtained with multivitamins and because it contains several amino acids (WEERASEKERA et al., 2016), which can serve as a source of nitrogen. Both media tested here are free from nitrogen sources, which negatively compromises yeast growth. As this study evaluated the full cell growth and not specific auxotrophic demands, we chose ammoniacal nitrogen to be used as a precursor of the chemical element (LORENZ et al., 2004).

3.1 AEROBIC AND ANAEROBIC PLANKTONIC GROWTH

Regarding aerobic planktonic growth of C. albicans ATCC®90028™, a higher growth was verified in MycoDef ($p = 0.0134$) after 4 h of incubation. At other time intervals, no significant differences were obtained (figure 1A). Candida albicans SC5314 presented continuous growth, without marked differences between both media (figure 1B).

Figure 1. Planktonic growth of *C. albicans* in experimental broth MycoDef and YNB®. A) Strain ATCC®90028™ in normoxia; B) Strain SC5314 in normoxia; C) Strain ATCC®90028™ in anoxia; D) Strain SC5314 in anoxia.



Anaerobic planktonic growth comparisons showed that *C. albicans* ATCC®90028™ continuously grew equally in MycoDef and YNB® (Figure 1C). For *C. albicans* SC5314, growth in YNB® was higher at 10-h post-inoculation ($p = 0.0417$). In other time intervals, no relevant differences were detected (Figure 1D).

Many yeasts are facultative aerobes and may grow in the presence or absence of molecular oxygen (ROSA et al., 2008; RYMOVICZ et al., 2011). Studies involving the metabolism of yeasts under different atmospheric conditions need a culture medium that provides nutrients essential for fungal development. The data obtained here suggest that the MycoDef provides necessary nutrients at concentrations suitable for yeast development like YNB® at different oxygen concentrations.

The log phase corresponds to a time interval in which mother cells continuously generate buds and daughter cells until nutrient reduction, accumulation of toxic substances or establishment of critical population size (LE MARC et al., 2009). The performance in this phase depends on the genetic potential of the yeast, the incubation temperature and, fundamentally, the constitution of the medium. Our results showed that the log phases were reached as a function of the strain and the atmospheric condition, but not of the culture medium. Such fact is important to propose that both culture media allow very close growth patterns.

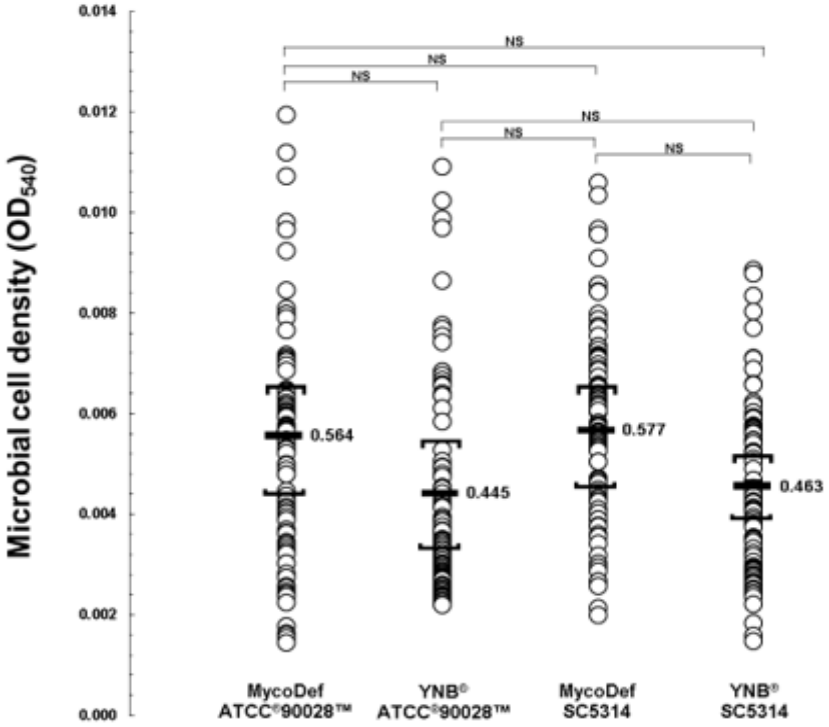
Punctual growth differences, as observed at T4 of the aerobic culture of ATCC®90028™ and T10 of the anaerobic culture of SC5314, cannot be singled out as determinants of overall behavioural differences since growth invariability was readily restored at subsequent measured times.

3.2 BIOFILM FORMATION

Retention of CV in biofilms grown in both broths did not diverge amongst experimental groups ($p \geq 0.325$). Although it seems that MycoDef slightly favoured biomasses obtaining, they were not different (Figure 2).

The results presented here are preliminary but strongly indicative of the good performance of MycoDef. Subsequent studies involving other yeast genera, species or strains, as well as clinical and industrial strains, will probably state that multivitamin formulation can serve as a basis for the formulation of a new CDCM suitable for biofilm studies.

Figure 2. Biofilm growth of *C. albicans* ATCC®90028™ and SC5314 in experimental broth MycoDef and YNB®, after 72 h of incubation in normoxia.



3.3 SDS-PAGE OF WHOLE CELL PROTEINS

The whole cell protein profile accessed by SDS-PAGE showed that both culture media allowed fungal growth without discrepancies in terms of protein synthesis. No differences in banding patterns were observed for ATCC®90028™ or SC5314 (Figure 3).

It is known that the same strain, when grown in different culture media, shows varied total protein banding patterns (HÖFLING et al., 1998, 2001). Since there were no variations in the banding patterns, it is plausible to assume that the constitutive similarity of the two media leads to a synthesis of the same proteins. This fact is very interesting because it shows that it is possible to use MycoDef in studies involving metabolic assays, in which YNB® is already widely used (PENDRAK et al., 2004; SILVA et al., 2008; LIMJINDAPORN et al., 2003; MORALES et al., 2013).

3.4 MIC AND MFC

Microbial sensitivity tests using the microdilution technique are considered quantitative (BALOUIRI et al., 2016) and allow determining, by approximation, the lower concentrations of a drug that can control cell growth (MIC) or eradicate fungi (MFC).

MycoDef allowed obtaining MIC and MFC values identical to those obtained in YNB® for the different antifungals and strains (Table 2). Variations of values were observed as a function of strains, but not about the media employed.

Table 2. Minimum Inhibitory Concentrations (MIC)* and Minimum Fungicidal Concentrations (MFC)* for antifungals determined in YNB® and MycoDef, following the adapted CLSI M27-A2** protocol

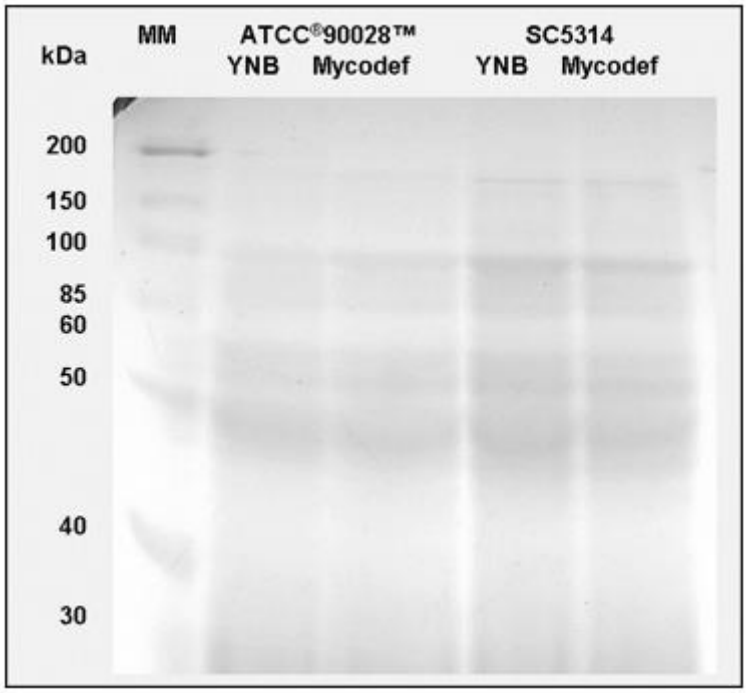
Strains	Culture media	Clotrimazole	Griseofulvin	Nystatin	Fluconazole
		MIC (μg mL^{-1})			
ATCC®90028™	YNB®	6.25	6.25	12.5	6.25
	MycoDef	6.25	6.25	12.5	6.25
SC5314	YNB®	1.25	12.5	12.5	12.5
	MycoDef	1.25	12.5	12.5	12.5
		MFC (μg mL^{-1})			
ATCC®90028™	YNB®	25.0	>25.0	12.5	12.5
	MycoDef	25.0	>25.0	12.5	12.5
SC5314	YNB®	3.12	>25.0	12.5	25.0
	MycoDef	3.12	>25.0	12.5	25.0

* Values determined by unanimity of consensus after three repetitions.
** CLSI. National Committee for Clinical Laboratory Standards. 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts. M27-A2. National Committee for Clinical Laboratory Standards, Wayne, PA.

The choice of the culture medium to be employed in resistance/susceptibility studies is very important, since it may interfere positively or negatively with the results

(MAZZOLA et al., 2009). YNB® has been used for this purpose (GHANNOUM et al., 1992; SHOKRI et al., 2011; MORES et al., 2011) and the similarity of results suggests that MycoDef may also be indicated for this purpose, with lower costs. However, we suggest future studies with an expanded number of strains to confirm this proposition.

Figure 3. SDS-PAGE whole cell protein patterns of 12 h-o aerobic planktonic *C. albicans* ATCC®90028™ and SC5314 grown in experimental broth MycoDef and YNB®.



3.5 FINANCIAL CONSIDERATIONS

In terms of profitability, MycoDef is more advantageous than YNB®, since 3 L of MycoDef can be produced with a single multivitamin tablet. Although more laborious, it is noteworthy to mention that the costs to prepare 1 L were inferior to US$ 0.15 (including added salts and sandpaper; excluding the 0.22 µm membranes, glucose and ammonium sulfate, common to both broths), while preparing the same volume of YNB® it required US$ 9.30 (invoices issued on September 10th, 2018). This is an important point of interest because an effective CDCM with reasonable costs becomes very attractive to research groups or academic labs with low budgets.

4 CONCLUDING REMARKS

The experimental culture broth MycoDef was considered satisfactory for the cultivation of *C. albicans* once it did not differ from YNB®, even in different atmosphere compositions. Furthermore, its application in the determination of MIC and MFC was considered similar to the control medium. The use of multivitamins as the basis for the preparation of CDCM may be an alternative to be considered for laboratories that handle yeasts and that have budgetary limitations.

ACKNOWLEDGEMENT

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES, PhD scholarship)and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brazil (CNPq, Graduate scholarship).

REFERENCES

1. Foucaud C, Francois A, Richard J. Development of a chemically defined medium for the growth of *Leuconostoc mesenteroides*. Appl Environ Microbiol. 1997;63(1):301-4

2. Falzone M, Martens E, Tynan H, Maggio C, Golden S, Nayda V, Crespo E, Inamine G, Gelber M, Lemence R, Chiappini N, Friedman E, Shen B, Gullo V, Demain AL. Development of a chemically defined medium for the production of the antibiotic platensimycin by Streptomyces platensis. Appl Microbiol Biotechnol. 2013;97(21):9535-9.

3. Khan H, Flint SH, Yu PL. Development of a chemically defined medium for the production of enterolysin A from Enterococcus faecalis B9510. J Appl Microbiol. 2013;114(4):1092-102.

4. Kongo JM, Gomes AMP, Malcata FX. Development of a chemically defined medium for growth of Bifidobacterium animalis. J Food Sci. 2003;68:2742–2746.

5. Rosa EA, Rosa RT, Pereira CV, Boriollo MF, Höfling JF. Analysis of parity between protein-based electrophoretic methods for the characterization of oral Candida species. Mem Inst Oswaldo Cruz. 2000;95(6):801-6.

6. Laemmli UK. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227(5259):680-685.

7. CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of yeasts; Approved Standard-Third Edition. CLSI document M27-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

8. Uppuluri P, Dinakaran H, Thomas DP, Chaturvedi AK, Lopez-Ribot JL. Characteristics of Candida albicans biofilms grown in a synthetic urine medium. J Clin Microbiol. 2009;47(12):4078-4083.

9. Kucharíková S, Tournu H, Lagrou K, Van Dijck P, Bujdáková H. Detailed comparison of Candida albicans and Candida glabrata biofilms under different conditions and their susceptibility to caspofungin and anidulafungin. J Med Microbiol. 2011;60(9):1261-9.

10. Weerasekera MM, Wijesinghe GK, Jayarathna TA, Gunasekara CP, Fernando N, Kottegoda N, Samaranayake LP. Culture media profoundly affect Candida albicans and Candida tropicalis growth, adhesion and biofilm development. Mem Inst Oswaldo Cruz. 2016;111(11):697-702.

11. Lorenz MC, Bender JA, Fink GR. Transcriptional response of Candida albicans upon internalization by macrophages. Eukaryot Cell. 2004;3(5):1076-87.

12. Rosa EA, Rached RN, Ignácio SA, Rosa RT, José da Silva W, Yau JY, Samaranayake LP. Phenotypic evaluation of the effect of anaerobiosis on some virulence attributes of Candida albicans. J Med Microbiol. 2008;57(10):1277-81.

13. Rymovicz AU, Souza RD, Gursky LC, Rosa RT, Trevilatto PC, Groppo FC, Rosa EA. Screening of reducing agents for anaerobic growth of Candida albicans SC5314. J Microbiol Methods. 2011;84(3):461-6.

14. Le Marc Y, Valik L, Medved'ova A. Modelling the effect of the starter culture on the growth of Staphylococcus aureus in milk. Intl J Food Microbiol. 2009;129:306-311.

15. Höfling JF, Rosa EAR, Rochelle SLA, Spolidório DMP, Campos AS. Comparative SDS-PAGE of soluble proteins of oral Candida (Fungi: Saccharomycetales) using different culture media for the cultivation of cells. J Comparat Biol. 1998;3:21-6.

16. Höfling JF, Rosa EA, Pereira CV, Boriollo MF, Rodrigues JA. Differentiation and numerical analysis of oral yeasts based on SDS-PAGE profiles. Influence of the culture media on the whole-cell protein extracts. Braz J Biol. 2001;61(3):507-16.

17. Pendrak ML, Chao MP, Yan SS, Roberts DD. Heme oxygenase in Candida albicans is regulated by hemoglobin and is necessary for metabolism of exogenous heme and hemoglobin to alpha-biliverdin. J Biol Chem. 2004;279(5):3426-33.

18. Silva WJ, Seneviratne J, Parahitiyawa N, Rosa EA, Samaranayake LP, Del Bel Cury AA. Improvement of XTT assay performance for studies involving Candida albicans biofilms. Braz Dent J. 2008;19(4):364-9.

19. Limjindaporn T, Khalaf RA, Fonzi WA. Nitrogen metabolism and virulence of Candida albicans require the GATA-type transcriptional activator encoded by GAT1. Mol Microbiol. 2003;50(3):993-1004.

20. Morales DK, Grahl N, Okegbe C, Dietrich LEP, Jacobs NJ, Hogan DA. Control of Candida albicans metabolism and biofilm formation by Pseudomonas aeruginosa phenazines. mBio. 2013;4(1):e00526-12.

21. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71-79.

22. Mazzola PG, Jozala AF, Novaes LCL, Moriel P, Penna TCV. Minimal inhibitory concentration (MIC) determination of disinfectant and/or sterilizing agents. Braz J Pharm Sci. 2009;45(2): 241-248.

23. Ghannoum MA, Ibrahim AS, Fu Y, Shafiq MC, Edwards JE Jr, Criddle RS. Susceptibility testing of Cryptococcus neoformans: a microdilution technique. J Clin Microbiol. 1992 Nov;30(11):2881-6.

24. Shokri H, Khosravi AR, Yalfani R. Antifungal efficacy of propolis against fluconazole-resistant Candida glabrata isolates obtained from women with recurrent vulvovaginal candidiasis. Int J Gynaecol Obstet. 2011;114(2):158-9.

25. Mores AU, Souza RD, Cavalca L, de Paula e Carvalho A, Gursky LC, Rosa RT, Samaranayake LP, Rosa EA. Enhancement of secretory aspartyl protease production in biofilms of Candida albicans exposed to sub-inhibitory concentrations of fluconazole. Mycoses. 2011;54(3):195-201.