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TEXTURIZED SOY PROTEIN AS AN ALTERNATIVE LOW-COST MEDIA FOR BACTERIA CULTIVATION

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ABSTRACT

Most of the culture media used in bacterial growth is composed of complex ingredients, increasing the value of the product. This makes its acquisition unavailable by educational institutions without sufficient funding, making even more difficult the practical teaching of microbiology. Therefore, the development of an alternative medium of simple composition and low-cost becomes necessary. This work aimed to use texturized soy protein (TSP) as a low cost culture medium that allows the bacterial growth. For the composition of the broths, concentrations between 0.5% and 10% were prepared. Thirty-eight bacteria, including important pathogens associated with food, were inoculated and the concentration of 7.5% TSP allowed the growth of 100% of the tested bacteria, with a production cost of approximately 86% and 68% lower than tryptic soy broth and agar, respectively. This work demonstrates that the use of a culture medium of easy acquisition and low cost is feasible and has good results.

Keywords: low-cost culture medium, textured soy protein, bacterial growth, practical teaching of Microbiology

INTRODUCTION

Soybeans are important source of low cost protein and have high nutritional value, besides their health benefits (**Vagadia *et al*., 2017**). In Brazil, soybean is the main crops both in area and in production. Only at the end of 2019, Brazil exported a record 10.3 million tons of soybeans (**Cattelan and Dall'Agnol, 2018**; **USDA/FAS, 2019**).
Different products can be obtained from soybeans, as miso, soymilk, soy cheese, soy yogurt, soy sauce, tofu, textured vegetable protein and textured soy protein (**Jayachandran and Xu, 2019**). Textured vegetable proteins are obtained from edible protein sources and are characterized by structural integrity and texture that resist the procedures used in preparing food for consumption. The textured soy protein (TSP) is generally obtained by high temperature extrusion of defatted soy flour (**Wu *et al*., 2018**) and its main application, without a doubt, is in human food, in substitution to ground meat, but it can also be used in animal feed (**Stein *et al*., 2008; Jovanovic *et al*., 2019**).
Many culture media used for the growth of microorganisms also have some soy derivatives in their composition, but in general, they are made up of complex ingredients, which increase the value of the product.
Taking into account the high nutritional content of TSP and its relatively low cost for the domestic market, this work aimed to develop a non-selective culture medium based on TSP in order to enable its use for the growth of various types of bacteria in educational and research institutions with limited resources, validating their effectiveness in the growth of several species of bacteria.

MATERIAL AND METHODS

Formulation of TSP medium

Four TSP brands have been acquired in local markets. For the composition of the broths, the samples were ground in an electric blender and different concentrations (0.5%, 1.0%, 2.5%, 5%, 7.5% and 10% [w/v]) were prepared in distilled water, filtered on filter paper to remove debris and autoclaved. For the composition of the solid medium, 1.5% [w/v] agar-agar was added.

Evaluation of bacterial growth on TSP agar

For the initial evaluation of the most promising formulation, fifteen species of Gram-negative and Gram-positive bacteria isolated from food, previously identified by mass spectrometry (MALDI-TOF) and with different levels of nutritional requirements, were subjected to growth in the six formulations of TSP. Important food pathogens such as *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus* were included. The bacteria were grown on tryptic soy agar (Casoy agar, Himedia, São Paulo, Brazil) and diluted in 0.85% [w/v] saline solution, to a concentration of approximately 1.5 x 10^8 CFU.ml^{-1}. Twenty microliters of each suspension were inoculated on the surface of the solid media and the plates were incubated at 37 °C for 18 h.

Quantification of bacterial growth in TSP broth

With the formulation that provides the best results (the lowest concentration used that allowed the growth of the largest number of bacteria) in the previous step, a total of 38 bacteria belonging to different species were tested for growth. Tryptic soy broth (Casoy broth) was used as a positive control. The bacteria were grown on Casoy agar at 37 °C for 18 h and inoculated in 0.85% [w/v] saline solution, to a concentration of approximately 1.5 x 10^8 CFU.ml^{-1}. Aliquots of this dilution (corresponding to 10^6 CFU.ml^{-1}) were inoculated in tubes with TSP broth and Casoy broth, which were then incubated at 37 °C for 18 h. After this period, bacterial quantification was performed on Casoy agar, to compare the bacterial growth in the two culture media.

Statistical analysis

Statistical analysis of the mean values of CFU.ml^{-1} obtained from bacterial growth in TSP and Casoy media was performed by unpaired two-tailed t-test (GraphPad QuickCalcs Web software), considering $p \leq 0.05$ as statistically significant.

Economic evaluation

A price research of TSP medium, Casoy medium and agar-agar (necessary for making TSP agar) of at least five different brands and marketed by several companies in Brazil was carried out in order to calculate the average production value of the TSP medium.

RESULTS AND DISCUSSION

Some works described in the literature have also tried to develop low-cost culture media, using different substrates. Adesemoye & Adedire (2005), for example, used different cereals extracts (corn, millet and sorghum) as basal medium for the growth of fungi. In a study conducted in India, an alternative culture medium for bacterial growth from fruit and vegetable remains was developed (Jadhav *et al*., 2018). Recently, Gabunia *et al*. (2019) verify that corn husk extract can be used for the growth of *Staphylococcus aureus* and *Escherichia coli*. In another study, the main food pathogens such *as S. aureus, E. coli, Bacillus cereus, Pseudomonas aeruginosa* and some types of molds showed satisfactory growth in culture media developed from vegetables such as lentils, chickpeas and peas. (Sharref, 2019). Uthayasooriyan et al. (2016), found that *Klebsiella* sp. showed more abundant growth in a culture medium developed from chickpeas than in commercial nutrient agar. The authors also used soy flour to prepare a culture medium, but the results were more promissory with the fungi then the bacteria tested.
However, the present work appears to be the first to use textured soy protein as the only source of nutrient for the composition of a low-cost culture medium. The TSP agar formulation containing concentrations of 7.5% and 10% TSP proved to be more promising, with the growth of all of the tested bacteria, as shown in

Table 1. Some species also showed a proteolysis halo in the 10% TSP medium, a characteristic of the culture medium to be studied in further works. It is worth mentioning that there was no difference in bacterial growth in the 4 brands of TSP tested. The appearance of TSP is shown in Figure 1.

Table 1 Initial assessment of the bacterial growth at different concentrations of TSP agar

Bacteria tested	Concentration of textured soy protein on TSP agar						Control
	0.5%	1.0%	2.5%	5.0%	7.5%	10%	(Casoy agar)
Gram-positive							
Bacillus cereus	++	++	+	+	++	++*	++
Micrococcus luteus	++	+	+	++	+	++*	++
Staphylococcus aureus	++	+	+	++	++	++	++
Gram-negative							
Enterobacter absuriae	-	+	++	+	++	++	++
Escherichia coli	++	+	+	+	++	++*	++
Hafnia alvei	-	+	+	+	+	++	++
Klebsiella ornythinolytica	++	++	++	++	++	++	++
Klebsiella pneumoniae	-	++	++	++	++	++	++
Leclercia adecarboxylata	+	+	-	-	+	+	+
Pantoea agglomerans	++	+	++	++	++	++	++
Serratia liquefaciens	++	++	++	++	+	++	++
Serratia marcescens	++	+	++	++	+	++*	++
Stenotrophomonas maltophilia	+	-	+	+	++	++	+

Legend: -: no growth; +: sparse growth (isolated colonies); ++: abundant growth; *: presence of a proteolysis halo.

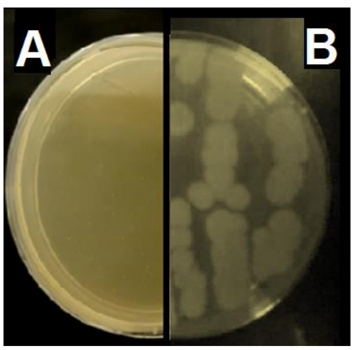

Figure 1: Appearance of 7.5% TSP agar. A: non-inoculated; B: inoculated with *Bacillus* sp.

Once the 7.5% concentration was selected as ideal, 38 isolates from different food matrices were subjected to growth in 7.5% TSP broth for 24h. All showed good to excellent growth, similar to the control in Casoy broth, with counts between 1.1 x 10^9 and 5.1 x 10^{12} CFU.ml^{-1} after the incubation period, as shown in Table 2. Statistical analysis of the mean values of CFU.ml^{-1} obtained from bacterial growth in TSP and Casoy media showed that the two-tailed P value was 0.9031. By conventional criteria, this difference was considered to be not statistically significant, showing the potential of using the TSP broth to replace the Casoy broth.

Table 2 Quantification of different bacteria isolated from food in 7.5% TSP broth and in Casoy broth after incubation for 24 h

Genera	Species	Counts* (CFU.mL^{-1}) after 18h incubation	
		TSP Broth	Control (Casoy broth)
Acinetobacter [1]	*A. bereziniae*	1,1 x 10^{10}	5,0 x 10^{12}
	A.guillouiae	5,0 x 10^{12}	9,5 x 10^{10}
	A.ursingii	3,4 x 10^{10}	1,6 x 10^{11}
Enterobacter [2]	*E. asburiae*	1,0 x 10^{10}	5,0 x 10^{10}
	E. cloacae	5,0 x 10^{10}	5,0 x 10^{10}
	E. hormaechei	1,5 x 10^{10}	6,0 x 10^{9}
	E. kobei	8,6 x 10^{11}	5,0 x 10^{12}
Escherichia [3]	*E. coli*	5,0 x 10^{12}	5,0 x 10^{10}
Klebsiella [2]	*K. oxytoca*	4,8 x 10^{11}	2,9 x 10^{10}
	K. pneumoniae	5,0 x 10^{12}	1,6 x 10^{10}
	K. varicola	9,6 x 10^{10}	5,0 x 10^{12}
Leclercia [2]	*L . adecarboxylata*	3,6 x 10^{11}	8,9 x 10^{10}
Pantoea [2]	*P. agglomerans*	2,2 x 10^{11}	9, 0 x 10^{9}
Pseudomonas [4]	*P. chloraphis*	9,6 x 10^{11}	5,0 x 10^{12}
	P. extremorientalis	9,5 x 10^{11}	5,0 x 10^{12}
	P. koreensis	8,3 x 10^{10}	1,4 x 10^{11}
	P. libanensis	5,0 x 10^{12}	9,0 x 10^{11}
	P. mosseli	5,0 x 10^{12}	8,6 x 10^{10}
	P. nitroreducens	8,7 x 10^{10}	5,0 x 10^{12}
	P. plecoglossicida	2,2 x 10^{11}	2,6x 10^{10}
	P. synxantha	6,5 x 10^{11}	1,0 x 10^{10}
	P. tolaasii	1,5 x 10^{10}	6,7 x 10^{11}
	P. vancouverensis	5,4 x 10^{10}	5,1 x 10^{12}
Raoutella [2]	*R. ornithinolytica*	1,0 x 10^{11}	1,0 x 10^{9}
Serratia [2]	*S. liquefaciens*	5,0 x 10^{10}	1,1 x 10^{9}
Stenotrophomonas [2]	*S. maltophilia*	5,0 x 10^{12}	8,3 x 10^{11}
Bacillus [3,5]	*Bacillus* sp. A	1,3 x 10^{10}	2,0 x 10^{10}
	Bacillus sp. B	1,0 x 10^{10}	1,3 x 10^{11}
	Bacillus sp. C	3,5 x 10^{10}	4,0 x 10^{11}
	Bacillus sp. D	3,2 x 10^{10}	4,5 x 10^{10}
	B. cereus	3,7 x 10^{11}	5,0 x 10^{10}
	B. megaterium	5,0 x 10^{10}	5,0 x 10^{10}
	B. stearothermophilus	4,2 x 10^{11}	5,0 x 10^{10}
Staphylococcus [5]	*Staphylococcus* sp. A	4,2 x 10^{11}	2,5 x 10^{10}
	Staphylococcus sp. B	5,8 x 10^{11}	6,5 x 10^{10}
	Staphylococcus sp. C	5,0 x 10^{10}	5,0 x 10^{10}
	Staphylococcus sp. D	4,2 x 10^{10}	1,3 x 10^{11}
	Staphylococcus sp. E	5,0 x 10^{10}	3,0 x 10^{10}

Legend: *The values represent the average of two independent experiments. Sources of isolates: [1]Ramos & Nascimento, 2019; [2]Ramos, 2019; [3]Bank of bacteria from the Microbiology Laboratory of IFRJ; [4]Ramos & Nascimento, 2020; [5]Oliveira et al., 2012.

In developing countries, research and classes involving microorganisms are often not carried out due to the scarcity of resources for the acquisition of the necessary inputs (**Uthayasooriyan *et al*., 2016**). The value of conventional culture media, both in agar and in casoy broth, is extremely high because it has complex ingredients. For this reason, a price survey was carried out to verify whether the TSP would be a good substitute for conventional culture media with a more accessible value. It was found that the production cost of TSP broth in Brazil is, on average, about 88% less than Casoy broth and 69% less than Casoy agar, respectively, as shown in Table 3.

Table 3 Comparison of production costs between Casoy and TSP media

Value per gram (USD[1])		**Approximate average value needed for prepare 1 liter of culture medium (USD)**		**Cost difference**
Casoy broth	0.114 ± 0.019	Casoy broth (30g/L)	3.42	87.7%
Textured soy peptone	0.006 ± 0.002	7.5% TSP broth (75g/L)	0.42	
Casoy agar	0.183 ± 0.067	Casoy agar (45g/L)	8.23	68.2%
agar-agar	0.147 ± 0.024	7.5% TSP agar[2]	2.62	

Legend: [1]The values in dollars were quoted in March 2020, from the values in reais (Brazilian currency). [2] TSP broth 7.5% plus 1.5% (w/v) agar.

CONCLUSION

The TSP culture medium, at a concentration of 7.5%, presents efficiency comparable to the growth patterns of commercial media, at a lower cost, being more accessible mainly to educational institutions that do not have large resources for Microbiology and small research laboratories, in activities that aim only at bacterial growth. The use of more bacteria isolated from food, especially Gram-positive ones, and statistical analysis of the results are being carried out in order to expand and improve the data on the potential use of the TSP medium.

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