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Use of Tomato Juice Supplemented With Glucose as a Medium for Growing Fungi

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Abstract
In this study, the possibility of using tomato juice as an alternative medium for growing fungi was tested. Four isolates; *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp. and *Candida* sp. were inoculated on Tomato Juice Agar (TJA), Tomato Juice Agar supplemented with 1g of glucose (TJA+1g Glc), Tomato Juice Agar supplemented with 2g of glucose (TJA + 2g Glc) and Potato Dextrose Agar (PDA) as control medium. The diameters of the fungal colonies were measured at 24 hours intervals for four days. The fungal growths on the test media were compared with those on PDA. The results for *Fusarium* sp., *Penicillium* sp. and *Asprgillus* sp. were significantly higher ($P < 0.05$) on TJA than PDA. This study shows that tomato juice agar (TJA) is suitable for the growth of all the tested fungi and can be used as an alternative medium for growing fungi.
Key words: tomato juice, fungi, PDA, glucose.

INTRODUCTION

In nature, many species of fungi and other microorganisms are found growing together in oceans, lakes and soil and on living and dead organic matter. These materials might be thought of as natural media. Growing fungi in laboratory requires knowledge of their nutritional needs and ability to provide the needed substances in a medium (Black, 2004).

In developing countries like Nigeria microbiological researches are hindered by high cost and scarcity of culture media (Adesemoye and Adedire, 2005). Therefore in recent years some researchers concentrated on screening alternative culture media from locally available cheap materials. Ogundana and Udoh (1984) prepared fungal media with infusion of waste including among others, peels, from banana, plantain and pineapple fruits. These workers showed that media prepared from infusion of waste supported comparable or better level of growth and sporulation than Potato Dextrose Agar and Potato Dextrose Broth.

Cassava whey (Ikenebomen and Chikwendu, 1997; Adesina and Akinyosoye, 2006); Avocado pear (Famurewa and David, 2008), Sago, raw dried palmyrah tuber flour, tuber of sweet potato and cassava (Jeyaseelam *et al.*, 2011) among other agricultural products have been used as both mycological and bacteriological media.

Tomato juice agar for the cultivation of fungi as described by Samson *et al.* (1996) composed of tomato juice: 200ml, $CaCO_3$: 3g, dextrose 5g, and agar 20g per litre of distilled water.

Proper diagnosis and treatment of mycotic diseases rest upon isolation and accurate identification of the etiological agents. Correct identification of moulds causing human and animal mycoses is incumbent on visualization of characteristic morphological features by the clinical microbiologist (Rinaldi, 1982). The isolation and accurate identification of fungi involves the use of mycological media.

Based on the market value and scarcity of culture media, screening of alternative media for the growth of fungi is found to be an important task for microbiologist in developing countries (Jeyaseelan *et al.*, 2011).

MATERIALS AND METHODS

Fungal test isolates

Four fungal isolates; *Aspergillus* sp., *Penicillium* sp., *Candida* sp. and *Fusarium* sp were used for the study. The moulds (*Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp) were obtained from the Department of Microbiology, Ahmadu Bello University (ABU), Zaria while the yeast (*Candida* sp.) was obtained from Microbiology laboratory, Sick Bay of ABU, Main campus, Samaru, Zaria. The fungi were sub cultured on fresh PDA slants. Slide culture technique was used for the moulds while wet preparation was used for the yeasts.

All the fungal cultures were identified and characterized both macroscopically and microscopically (Samson *et al.*, 1996). The fungi were then stored on PDA slant in the refrigerator.

Preparation of Tomato Juice

Fresh tomatoes were obtained from Samaru Market, Zaria. The tomatoes were rinsed with water and then placed in a polythene bag. The tomatoes were aseptically crushed mechanically with the hands and the juice was transferred into a conical flask and plugged with cotton wool. The flask was labeled appropriately (Jeyaseelan *et al.*, 2011).

Preparation of Tomato Juice Media and PDA

Solid media of the tomato juice agar (TJA) was prepared as described by Ramalingam *et al.* (2007) with modification in the volume of tomato juice and the quantity of agar, 250ml of tomato juice and 15g of agar per liter of distilled water modified to 200ml of tomato juice and 30g of agar to prepare 10 plates of tomato juice agar. Two hundred (200ml) of distilled water was heated to about 90^0C and then 6g of agar (MERCK) was dissolved in it. The solution was heated to boiling to dissolve the agar and then 40ml of tomato juice was added and the solution was heated to boiling to give a completely homogenous solution. The medium in the conical flask was then plugged with cotton and sterilized in an autoclave at 121^0C for 15 minutes at 15 lbs/inch^2.

Two other media, tomato juice agar + 1g of glucose (TJA + 1g Glc) [200ml of distilled water, 6g of agar, 40ml of tomato juice and 1g of glucose] and tomato juice agar + 2g of glucose (TJA + 2g Glc) [200ml of distilled water, 6g of agar, 40ml of tomato juice and 2g of glucose] were prepared and sterilized in an autoclave. PDA medium (MERCK) was prepared according to manufacturer's instruction. About 20ml of sterilized media were poured into sterile Petri dishes, allowed to solidify on the bench and labeled appropriately (Jeyaseelan *et al.*, 2011).

Assay for fungal growth

TJA, TJA + 1g Glc, TJA + 2g Glc and PDA were inoculated with the test fungi each using standard microbiological techniques and incubated at room temperature. The mean radial growth of mycelia (or diameter of the colonies in the case of yeasts) was measured using a transparent meter rule at 24 hours interval. The spore formation and colony morphology were also observed and recorded (Famurewa and David, 2008; Jeyaseelan *et al.*, 2011).

Statistical Data Analysis

The results obtained from the measurement of colonial diameter on the test media were subjected to analysis using ANOVA.

RESULTS

Table 1 shows the mean radial growth of *Aspergillus* sp. on the test and control media. The mean radial growth of *Aspergillus* sp. after 24 hours of incubation was 8.0mm on PDA, 11.5mm on TJA+2g Glc, 9.5mm on TJA+1g Glc and 16.5mm on TJA. However, after 96 hours of incubation the mean radial growth was 42.0mm on PDA, 45.5mm on TJA+2g Glc, 45.0mm on TJA+1g Glc and 43.0mm on TJA.

Table 1: Mean radial growth (mm) of *Aspergillus* sp.

	Mean radial growth (mm)			
Media/Time (Hr)	24	48	72	96
PDA	8.0	21.0	30.0	42.0
TJA+2g Glc	11.5	25.0	34.5	45.5
TJA+1g Glc	9.5	22.5	32.0	45.0
TJA	16.5	20.5	29.5	43.0

ANOVA = 0.1601, ($P < 0.05$).
PDA = potato dextrose agar
TJA = tomato juice agar
Glc = glucose

Table 2 shows the mean radial growth of *Penicillium* sp. on the test and control media. The mean radial growth of *Penicillium* sp. after 24 hours of incubation was 19.0mm on PDA, 34.0mm on TJA+2g Glc, 33.5mm on TJA+1g Glc and 30.5mm on TJA. However, the mean radial growth after 96 hours of incubation was 90.0mm on PDA, 92.0mm on TJA+2g Glc, 91.0mm on TJA+1g Glc and 89.0mm on TJA.

Table 2: Mean radial growth (mm) of *Penicillium* sp.

	Mean radial growth (mm)			
Media/Time (Hr)	24	48	72	96
PDA	19.0	61.0	80.0	90.0
TJA+2g Glc	34.0	90.5	92.0	92.0
TJA+1g Glc	33.5	78.5	84.5	91.0
TJA	30.5	77.0	83.0	89.0

ANOVA = 0.1831, ($P < 0.05$)
PDA = potato dextrose agar
TJA = tomato juice agar
Glc = glucose

Table 3 shows the mean radial growth of *Candida* sp. on the test and control media. The mean radial growth after 24 hours of incubation was 5.0mm on PDA, 4.0mm on TJA+2g Glc, 2.0mm on TJA+1g Glc and 2.5mm on TJA. However, after 96 hours of incubation the mean radial growth was 7.0mm on PDA, 7.5mm on TJA+2g Glc, 5.5mm on TJA+1g Glc and 5.0mm on TJA.

Table 3: Mean radial growth of *Candida* sp.

	Mean radial growth (mm)			
Media/Time (Hr)	24	48	72	96
PDA	5.0	6.0	7.0	7.0
TJA+2g Glc	4.0	5.0	6.0	7.5
TJA+1g Glc	2.0	2.0	3.5	5.5
TJA	2.5	3.0	3.5	5.0

ANOVA = 5.11, ($P < 0.05$)
PDA = potato dextrose agar.
TJA = tomato juice agar.
Glc = glucose.

Table 4 shows the mean radial growth of *Fusarium* sp. on the test and control media. The mean radial growth after 24 hours of incubation was 0mm on PDA, 10.5mm on TJA+2g Glc, 9.5mm on TJA+1g Glc and 14.5mm on TJA. However, after 96 hours of incubation the mean radial growth was 12.0mm on TJA, 45.0mm on TJA+2g Glc , 46.0mm on TJA+1g Glc and 53.0mm on TJA.

Table 4: Mean radial growth (mm) of *Fusarium* sp.

	Mean radial growth (mm)			
Media/Time (Hr)	24	48	72	96
PDA	0.0	4.0	10.0	12.0
TJA+Glc (2g)	10.5	22.5	34.0	45.0
TJA+Glc(1g)	9.5	20.5	35.0	46.0
TJA	14.5	26.5	40.0	53.0

ANOVA = 2.89, ($P < 0.05$)
PDA = potato dextrose agar
TJA = tomato juice agar
Glc= glucose

Discussion

This study revealed that all the four test fungi were able to grow on all the test media and as expected, in all the cases the mean radial growth increases with time, even though the rate of increase varies with the type of fungi.

Aspergillus sp. grew well on TJA and had the highest mean radial growth on it after 24 hours of incubation but on subsequent days, it showed higher mean radial growth on TJA + 2g Glc, suggesting that supplementation with Glc enhanced the performance of the medium. This is not surprising as Glc serves as energy source for many fungi (Black, 2004).

The *Penicillium* sp. showed better growth on TJA + 2g Glc after 24 hours incubation up to the 96th hour. Spores were observed on the 2nd day. The test fungus *Candida* sp. showed better growth on PDA compared to the test media.

This could be attributed to the different compositions of the media. This finding however contradicts that of Alves *et al*. (2006) who reported better performance of TJA in the isolation of *Candida* sp. compared with other mycological madia. The most interesting result was obtained for *Fusarium* sp. on TJA, where the mean radial growth was higher compared to PDA which shows no growth after 24 hours. Statistical analysis of the results using analysis of variance showed that the mean radial growths of the moulds differ significantly ($p < 0.05$) while that of yeast did not differ significantly ($p < 0.05$).

This also shows that the mean radial growth of *Aspergillus* sp., *Penicillium* sp. and *Candida* sp. increases with increase in glucose concentration. The mean mycelial spread of *Fusarium* sp. on TJA was better compared to its spread on TJA+ glucose. *Fusarium* sp. may have the ability to utilize other substrates than glucose for energy generation.

The results of this study reveal that tomato juice can be used as a medium for *in vitro* cultivation of different fungal species. The colony morphology, spore formation and growth of mycelia of the test fungi on the test media were found to be similar to that on PDA.

Previous studies by Jeyaseelan *et al*. (2011) and Famurewa and David (2008) have also proven the possibility of replacing PDA with alternative local material. Powdery substances in pod of *Parkia biglobosa* and cereals were used in different studies and all these studies revealed the possibility of replacing PDA with these local materials (Adesemoye and Adedire, 2005; Rajasab, 2007).

In a study conducted in Jaffna, Sri Lanka by Jeyaseelan *et al*. (2011), the use of sajo and palmyrah tuber as alternative media for the growth of fungi has demonstrated a good performance.

This present study has shown that tomato juice can be as a medium for growing fungi. The medium is cheap and easy to prepare; thus it can be used as an alternative to the commercially prepared potato dextrose agar (PDA).

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