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Development of Watermelon agar medium and Muskmelon agar medium.

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ABSTRACT

Research about the use of watermelon pulp or musk melon pulp as a medium for the microbial growth is very minimal if not un noticed. The nutrients present in the Watermelon pulp and Muskmelon pulp provides a good ground for the growth of microbes. We developed the Watermelon agar medium and the Muskmelon agar medium and preliminarily evaluated the suitability of these developed media for the growth of bacteria namely *bacillus subtilis*, *bacillus megaterium* and *Escherichia coli* in comparison with Nutrient agar medium. All the three bacterial cultures used for the evaluation of suitability in developed medias showed a significant growth on comparison with Nutrient agar medium. Considering the low cost, world-wide presence and abundance in availability of watermelon and muskmelon, the watermelon agar medium and muskmelon agar medium can be considered as an alternative for the present day's conventional medias for the growth of microbes. However further studies are required by testing with other microbes or with any modifications or nutrient supplementation for much effective growth of microbes for a wider acceptance of these developed media.

Keywords: Watermelon agar medium, Musk melon agar medium, Nutrient agar medium, *bacillus subtilis*, *bacillus megaterium, Escherichia coli.*

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INTRODUCTION

Microorganisms can be grown in any medium that which has nutrients to support their growth. In addition to chemical requirements, microorganisms need specific conditions like temperature, pH etc. for optimal growth [1] [2]. Watermelon pulp is enriched with nutrients [3] mostly consisting of water, carbohydrates, several vitamins, minerals like calcium, magnesium, phosphorus, potassium, iron, zinc and also sodium. Muskmelon is also enriched with nutrients [4] like water, carbohydrates, vitamins, minerals like calcium, magnesium, phosphorous, Sulphur, potassium, zinc and iron. Both the watermelon and muskmelon are also rich in their amino acid content [5] that facilitates better growth of most microbes.

We all know that most microorganisms like bacteria and yeasts do best when the presence of glucose as primary source of energy. Both watermelon and muskmelons processes very high amounts of glucose with 1292.1mg and 2230.2 mg per 100grams of watermelon and muskmelon respectively [5]. All together the nutrients present in both of the watermelon pulp and muskmelon pulp can solely act as an effective medium and make each of them a ground for the growth of the microbes without any additional supplementation. Though several alternatives for conventional medias were developed in the past [6] only a few withstand the potentials of conventional media like Tender coconut water agar medium [7]. which is economical even for the production of recombinant proteins. While some of the alternative media are limited to grow specific microorganisms. In this case the Watermelon pulp and Muskmelon pulp have the rich nutrient profile to support the growth of varied microorganisms.

MATERIALS AND METHODS

Preparation of Watermelon agar medium:

Water melons were obtained from different places in Guntur, India. The Pure mesocarp pulp was obtained from the water melon by removing exocarp and seeds in the mesocarp and then the mesocarp pulp is made into juice using a juicer and is collected in a sterile duran bottle. Then the watermelon juice was made free from large pulp masses by filtering them using a traditional stainless steel sieve aseptically. 2 grams of agar agar powder was dissolved in 20ml sterile distilled water by taking it in a sterile conical flask. Then 80ml of water melon juice was added to the conical flask and it was kept on a rotary shaker at 30rpm for 2 minute in order to obtain uniform mixing. Later the conical flask is placed in waterbath at 50^0C for 12 minutes.

Preparation of Muskmelonagar media:

Musk melons (Cantaloupe) were obtained from different places in Guntur, India. The exocarp was removed from the fleshy mesocarp pulp of muskmelon. Later seeds were removed from the mesocarp pulp, and the pulp was made into juice using a juicer and collected in a sterile duran bottle. Then an autoclaved traditional stainless steel sieve is used to filter any large particles of muskmelon pulp if present in the muskmelon juice. 2grams of agar agar powder was dissolved in 20ml of sterile distilled water by taking it in a conical flask. Then 80ml of the filtered muskmelon juice was added to the conical flask aseptically and kept it on rotary shaker for 3 minutes at 30 rpm for uniform mixing. Then the conical flask is placed in water bath at 50^0C for 12 minutes.

After preparing the watermelon agar medium and muskmelon agar medium as mentioned above the conical flasks were allowed to cool for some time by keeping them in laminar air flow chamber. Nutrient agar medium (HIMEDIA) is prepared and autoclaved at 121^0C for 15 minutes and is also allowed to cool for some time in sterile conditions and then all the three medias were poured in to their respective sterile labelled Petri dishes as per the requirement and are left undisturbed for solidification. The pH of all the media were adjusted to 7.0.

Inoculating the Bacterial Cultures:

Standard broth cultures using nutrient broth (HIMEDIA) were prepared by matching the 0.5 McFarland standard turbidity. A loop full of inoculum (HIMEDIA inoculum loop with capacity of 0.005ml) from the broth cultures was streaked on respective media as per the labelling in sterile environment and then the

petri dishes of *Escherichia coli*, *bacillus subtilis* were incubated at 36^{0}C, and *bacillus megaterium* at 30^{0}C for a period of 24 hrs. The growth of cultures in the Watermelon agar medium and Muskmelon agar medium was compared with growth on Nutrient agar medium(Table-1).

Table 1.Visual Observation of the level of growth in Watermelon agar medium, Muskmelon agar medium and Nutrient agar medium.

Culture	Watermelon agar Medium	Muskmelon agar Medium	Nutrient agar Medium
Bacillus subtilis	**Luxuriant growth**	**Luxuriant growth**	**Luxuriant growth**
Bacillus megaterium	**Luxuriant growth**	**Luxuriant growth**	**Luxuriant growth**
Escherichia coli	**Considerable growth**	**Considerable growth**	**Luxuriant growth**

Antibiotic Sensitivity testing:

Standard broth cultures prepared by matching the 0.5 McFarland standard turbidity were used for testing of antibiotic sensitivity. The testing was done simultaneously with streak plate testing for growth. The antibiotic testing was done by following the modified Kirby-Bauer technique [8] for the four antibiotics Penicillin, Tetracycline, Gentamicin and Chloramphenicol (HIMEDIA disks). The plates were incubated along with the streak plate testing petri dishes in the temperatures as mentioned above. The results were tabulated in Table 2a,2b, and 2c.

Discs used with their potency:

Penicillin-G	(P)	2Units
Tetracycline	(TE)	10mcg
Gentamicin	(GEN)	10mcg
Chloramphenicol	(C)	30mcg

Table 2a.Antibiotic sensitivity testing in Watermelon agar medium, Muskmelon agar medium and Nutrient agar medium with *Bacillus subtilis*

Culture	P-2	TE-10	GEN-10	C-30
Nutrient agar	**04**	**22**	**14**	**20**
Watermelon agar	**04**	**21**	**06**	**21**
Muskmelon agar	**04**	**20**	**06**	**21**

Values in Millimeters (mm)

Table 2b.Antibiotic sensitivity testing in Watermelon agar medium, Muskmelon agar medium and Nutrient agar medium with *Bacillus megaterium*

Culture	P-2	TE-10	GEN-10	C-30
Nutrient agar	**04**	**20**	**17**	**19**
Watermelon agar	**04**	**21**	**06**	**21**
Muskmelon agar	**04**	**23**	**06**	**22**

Values in Millimeters (mm)

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Table 2c.Antibiotic sensitivity testing in Watermelon agar medium, Muskmelon agar medium and Nutrient agar medium with *Escherichia coli*

Culture	P-2	TE-10	GEN-10	C-30
Nutrient agar	06	20	10	17
Watermelon agar	04	22	06	19
Muskmelon agar	04	23	06	21

Values in Millimeters (mm)

RESULTS AND DISCUSSION

Growth is observed in the newly developed Watermelon agar medium and Muskmelon agar medium. *bacillus subtilis* (Figure-1) and *bacillus megaterium* growth is very good and showed a similar growth with the Nutrient agar medium upon visual observation. But *Escherichia coli* comparatively showed low growth in Watermelon agar medium and Muskmelon agar medium when compared with Nutrient agar medium. On comparing the zone of Inhibitions cultures of *bacillus subtilis* (Figure -2), *bacillus megaterium* and *Escherichia coli* in the newly developed media showed similar if not a bigger zone of inhibition responding to tetracycline and chloramphenicol discs but a very small zone of inhibition was observed for gentamicin and penicillin discs when compared with cultures in nutrient agar medium. The use of 1.5% or 2% of agar agar in watermelon or muskmelon medias is causing tearing of media while streaking due to lack of desired hardness. So use of 2.5% agar agar in the media is essential.

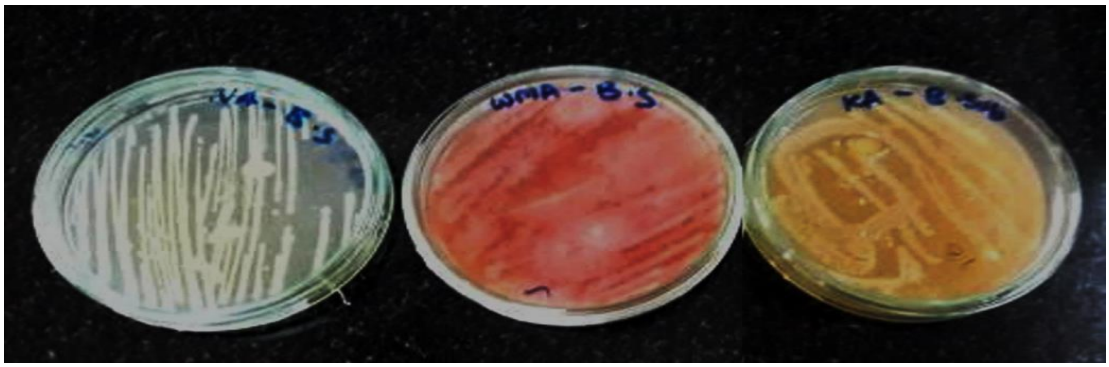

Figure 1:Growth of *Bacillus subtilis* observed in Nutrient agar medium, Watermelon agar medium and Muskmelon agar medium.

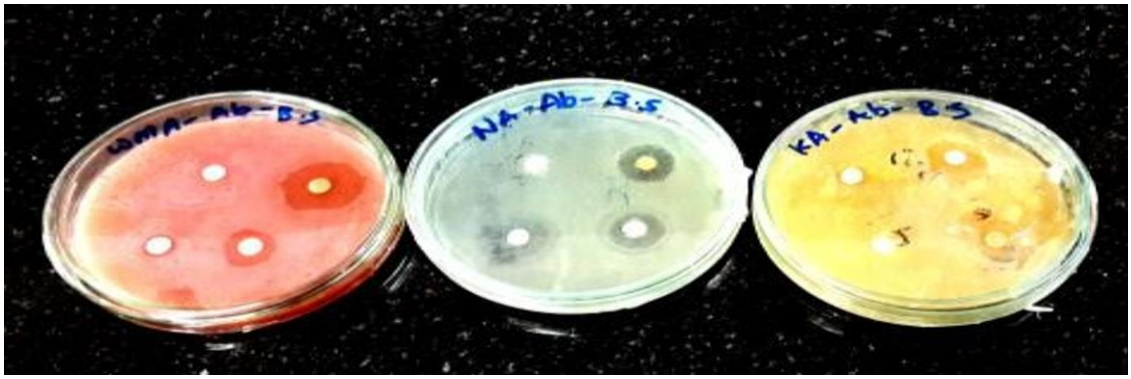

Figure 2:Zone of Inhibitions observed in Watermelon agar medium, Nutrient agar medium and Muskmelon agar medium with *Bacillus subtilis* culture.

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CONCLUSION

The watermelon juice and muskmelon juice made from their mesocarp pulp used for the preliminary study of developing the new media showed a competitive growth for the organisms tested similar to Nutrient agar medium. Hence they can be used as a potential alternative to several conventional medias. Keeping in mind the easy availability of these fruits worldwide and their low costs the Watermelon agar and Muskmelon agar media developed can be an economical alternative to many conventional media. However, more research was required to know the compatibility of these developed media with several other microorganisms. Research is also required to increase the efficiency (if required) for these developed media with any additional supplementation of specific nutrients. Due to the considerable growth of microbes in the Watermelon agar medium and Muskmelon agar medium they are needed to tested for their efficiency of producing valuable products like recombinant proteins, antibiotics etc. for a better acceptance.

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