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FORMULATION OF MICROBIAL GROWTH USING MILLET AND SWEET POTATO

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ABSTRACT

Culture media supply the nutrients required for the growth and maintenance of microorganism in the laboratory. This study aims to investigate the possibility of using locally available materials (sweet potato and millet) as basal media and as a substitute for commercially available media formulation of microbial growth. Four different media were formulated, namely, Millet agar (MA), Sweet Potato agar (SPA), Millet glucose agar (MGA) and sweet potato dextrose agar (SPGA). The test organisms chosen were bacterial genera Staphylococcus aureus and Escherichia coli and fungal genera Aspergillus spp and Rhizopus sp. The suitability of the formulated media was estimated by culturing the isolated species through the pour plating method. The results reveal that the growth of Staphylococcus aureus and Escherichia coli is higher in the nutrient agar followed by the formulated millet agar and least growth was seen on the sweet potato agar. The higher growth level in millet compared to sweet potato could be as a result of higher protein and fat content in the millet as bacteria proliferate more on high protein foods. The fungal isolates Aspergillus sp. and Rhizopus sp is higher in the potato dextrose agar followed by sweet potato glucose agar and the least growth was seen on the millet agar. The higher level of fungal growth on the sweet potato glucose agar is due to the higher carbohydrate content in the sweet potato when compared to the millet. These alternative formulations should be used more often in the laboratory for growth and study of microorganisms. More studies should be done on the shelf life of these alternative formulations.

KEYWORDS: Media, Growth, Formulation, Sweet Potato, Millet.

INTRODUCTION

The study of microorganisms depend on the ability to grow and maintain these microorganisms under laboratory conditions by providing suitable culture media that provide favourable environmental conditions (Prescott *et al*., 2002). The conditions needed for growth include good carbon source, nitrogen source such as protein, availability of enzymes, vitamins, mineral elements such as phosphorus and sulphur. Others include suitable pH, suitable temperature, relative humidity, inorganic salt and water (Soetan, *et al.*, 2010). The knowledge of the conditions is useful in the control of the growth of microbes that cause diseases and food spoilage but also in the effort to encourage the growth of helpful microbes and those to be studied (Amadi, and Moneke, 2012).

Sweet potato is one of the major roots and tubers and it was reported to be the 7th most important food crop in the world (Kays, 2004). The importance of sweet potato as a food crop is growing rapidly in some parts of the world and in sub-Saharan Africa, it is outpacing the growth rate of other staples. Sweet potato is used for human consumption and as a healthy cheap source of animal feed. It is considered an excellent food security crop in sub-Saharan Africa. It requires less agro-chemical inputs and survives/grows in diverse conditions (CIP, 2013).

Sweet potato like the other roots and tubers is mainly made of carbohydrates. Total carbohydrates, which are mainly starch (FAO, 2002), make up about 19% to 28% of the root. While the 86% (on dry basis) of total soluble sugars are glucose, the remainder are fructose and sucrose (Zhang *et al*., 2002), besides the fact of having a significant fiber content. Protein content is 1% to 2% and it has high biological value due to its high lysine content, whereas lipid level is low (0.1 to 0.4%) (FAO, 2002). Sweet potato root is therefore a major source of energy due to its high carbohydrate content. In addition to these nutrients, sweet potato is well known for its β-carotene content and other minor components such as vitamins B, C, E and phenolic acid, as well as potassium, calcium, iron, zinc and phosphorus (Burri *et al*., 2011). Moreover, it has been considered as a highly functional, low

calories food, with anti-diabetic effects. Due to these, it is often recognized as a health food (Padmaja, 2009).

Millets are unique among the cereals because of their richness in calcium, dietary fibre, polyphenols and protein (Devi *et al*., 2011). Millets generally contain significant amounts of amino acids particularly the sulphur containing amino acids i.e. methionine and cysteine (Obilana and Manyasa, 2002). The amino acid profile of the different varieties of millet per 100 grams with respect to essential amino acids show that Pearl millet has high isoleucine content (5.1g) compared to Foxtail (4.59g), Finger (4.3g) and Proso (4.1g) millet varieties (Obilana, 2003). This study therefore aims to assess the possibility of using sweet potato and millet as basal media and as a stand-in for commercially available media for formulation of the growth of bacteria and fungi.

METHODS

Sample Collection

Samples of sweet potato and millet were collected from Modern market Lafia, Nasarawa State and taken to the laboratory for analysis.

Preparation of Control Media

The commercial agar used as control was Nutrient agar and Potato dextrose agar and they were prepared according to manufacturer's specification.

Formulation of Media

Four different media were formulated, namely, Millet agar (MA), Sweet Potato agar (SPA), Millet glucose agar (MGA) and sweet potato glucose agar (SPGA).

For the millet agar, approximately 6 g of the millet grain was measured into a beaker; 160 ml of clean water was added and was allowed to boil. It was filtered through a muslin cloth 5g of agar–agar were added to the filtrate. The volume of the mixture was made to 200 ml with distilled water and heated on a hot plate with steady stirring until the solution boiled. The stirring was meant to achieve homogeneity. The resultant suspension was sterilized in the autoclave for 15 min at 121^{o}C. The same procedure was repeated for sweet potato agar (SPA).

For the millet glucose agar (MGA), approximately 6 g of the millet grain was measured into a beaker; 160 ml of clean water was added and was allowed to boil. It was filtered through a muslin cloth, and then 5g of glucose and about 5g of agar–agar were added to the filtrate. The volume of the mixture was made to 200 ml with distilled water and heated on a hot plate with steady stirring until the solution boiled. The stirring was meant to achieve homogeneity. The resultant suspension was sterilized in the autoclave for 15 min at 121^{o}C, and an antibiotic (gentamycin) solution was added to prevent bacterial growth. The procedure was repeated for sweet potato glucose agar (SPGA).

Test Organisms

The test organisms chosen were bacterial genera *Staphylococcus aureus* and *Escherichia coli* and fungal genera *Aspergillus* spp and *Rhizopus* sp. Inocula were drawn from the pure cultures of the isolates just before 96th hr of growth and transferred into a sterile broth and incubated for 3 hours and later aseptically inoculated onto all the formulated media and the control (PDA and NA). The inocula were taken at that time to ensure that growth was still high at logarithmic phase when cells would have uniform physiological characteristics.

Inoculation of Media

The suitability of the formulated media was estimated by culturing the isolated species through the pour plating method. Each of the four organisms from the pure cultures was serially diluted and the 3rd and 5th dilution was inoculated on the plates of formulated media (MA, SPA, MGA and SPGA) in like manner. The plates were then incubated at 31^{o}C inside the incubator for 72 hours. The growth of the organisms was evaluated by counting the number of colonies after 18 hours of incubation using a colony counter.

RESULTS

Table 1.0: Table showing the physical properties of the formulated media and commercially available media.

Sample	*pH*	*Colour*
MA	7.2	Cream
SPA	7.3	Yellow
MGA	7.1	Cream
SPGA	6.9	Light yellow
NA	7.4	Golden yellow
PDA	5.8	Yellow

Key: MA: Millet agar , SPA: Sweet potato agar , MGA: Millet glucose agar, SPGA: Sweet Potato Glucose agar, NA: nutrient agar, PDA: Potato Dextrose Agar

Table 2.0: Table showing the growth of *Staphylococcus aureus* on the formulated Millet agar, Sweet potato agar and control Nutrient agar.

Sample	10^{-3}	10^{-5}	*AVERAGE*
MA	2.8×10^4	9.0×10^5	4.6×10^5
SPA	2.1×10^4	5.0×10^5	2.6×10^5
NA	5.4×10^4	2.5×10^6	1.3×10^6

Key : MA: Millet agar , SPA: Sweet potato agar ,NA: nutrient agar

Table 3.0: Table showing the growth of *Escherichia coli* on the formulated Millet agar, Sweet potato agar and control Nutrient agar.

Sample	10^{-3}	10^{-5}	*AVERAGE*
MA	1.8×10^4	4.0×10^5	2.1×10^5
SPA	1.2×10^4	3.0×10^5	1.5×10^5
NA	4.5×10^4	2.1×10^6	1.1×10^6

Key: MA: Millet agar, SPA: Sweet potato agar , NA: nutrient agar.

Table 4.0: Table showing the growth of *Aspergillus* spp on the formulated Millet glucose agar, Sweet potato glucose agar and control Potato Dextrose agar after 72 hours.

Sample	10^{-3}	10^{-5}	*AVERAGE*
MGA	2.5 X 10^4	8.0 X 10^5	4.1 X 10^5
SPGA	3.7 X 10^4	1.9 X 10^6	1.1 X 10^6
PDA	4.8 X 10^4	2.5 X 10^6	1.2 X 10^6

Key: MGA: Millet glucose agar, SPGA: Sweet Potato Glucose agar, PDA: Potato Dextrose Agar

Table 5.0: Table showing the growth of *Rhizopus* spp on the formulated Millet glucose agar, Sweet potato glucose agar and control potato dextrose agar after 72 hours.

Sample	10^{-3}	10^{-5}	*AVERAGE*
MGA	1.6 X 10^4	3.0 X 10^5	1.5 x 10^5
SPGA	2.5 X 10^4	5.0 X 10^5	2.6 x10^5
PDA	4.6 X 10^4	2.3 X 10^6	1.1 x10^6

Key: MGA: Millet glucose agar, SPGA: Sweet Potato Glucose agar, PDA: Potato Dextrose Agar

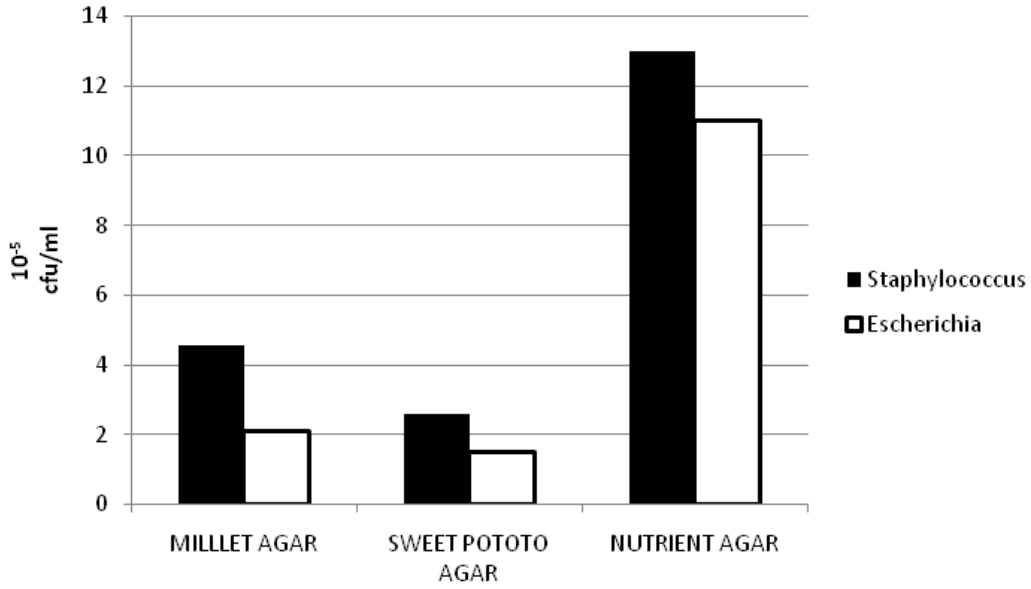

Figure 1: Chart showing the average growth of the test bacterial isolate on the formulated media and the commercially available media.

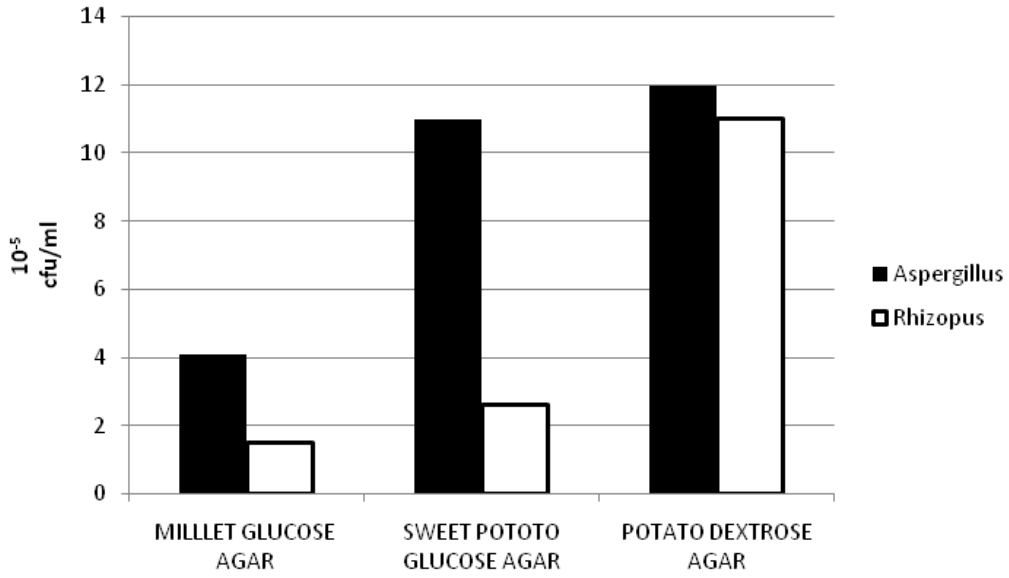

Figure 2: Chart showing the average growth of the test fungal isolate on the formulated media and the commercially available media.

DISCUSSION

The results obtained for this study are presented in table 1.0 to table 5.0 above. Table 1.0 shows the physical properties of the formulated media and commercially available media. Table 2.0 shows the growth of *Staphylococcus aureus* on the formulated Millet agar, Sweet potato agar and control Nutrient agar. Table 3.0 shows growth of *Escherichia coli* on the formulated Millet agar, Sweet potato agar and control Nutrient agar. Table 4.0 shows the growth of *Aspergillus* spp on the formulated Millet glucose agar, Sweet potato glucose agar and control Potato Dextrose agar after 72 hours. Table 5.0 shows the growth of *Rhizopus* spp on the formulated Millet glucose agar, Sweet potato glucose agar and control potato dextrose agar after 72 hours. While Figure 1 shows the average growth of the test bacterial isolate on the formulated media and the commercially available media. Figure 2 shows the average growth of the test fungal isolate on the formulated media and the commercially available media.

Microbial require nutrients (such as a carbon, nitrogen, vitamins, mineral elements, as well as the availability of enzymes) and certain environmental conditions (such as suitable pH value, suitable temperature, oxygen) in order to grow and reproduce. These nutrients and conditions are formulated into a media for their growth in the laboratory to ease the study of these microorganisms.

The growth of *Staphylococcus aureus* and *Escherichia coli* is higher in the nutrient agar followed by the formulated millet agar and least growth was seen on the sweet potato agar. The higher growth level in millet compared to sweet potato could be as a result of higher protein and fat content in the millet as bacteria proliferate more on high protein foods. This result is in conformity with that of Laryea, *et al.*, (2018) who reported higher content of protein and fat in millet flour than sweet potato flour.

The fungal isolates *Aspergillus* sp. and *Rhizopus sp* is higher in the potato dextrose agar followed by sweet potato glucose agar and the least growth was seen on the millet agar. The higher level of fungal growth on the sweet potato glucose agar is due to the higher carbohydrate content in the sweet potato when compared to the millet as reported by Laryea, *et al.*, (2018).

CONCLUSION

Nutrient agar and Potato dextrose agar are used as a general purpose culture medium to grow various bacteria and fungi respectively. The formulated medium though did not have high growth levels when compared to the nutrient agar and potato dextrose agar can be used as a substitute for the growth of microorganisms in the laboratory because they contain necessary nutrient for the growth of these organisms. Also cost of these commercially available medium is relatively higher whereas it costs less to prepare different alternative formulations. Thus the use of different alternative formulations as culture media in laboratories with basic facilities is very much feasible and cheaper when compared to commercially prepared medium.

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