

Original Research Article

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Formulated Sorghum Media as Alternative to Nutrient broth in Cultivation of *Staphylococcus aureus* (NCTC 6571) and *Bacillus subtilis* (NCTC 8241)

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ABSTRACT

Microbiological culture media are expensive been imported from developed countries, and there is need to formulate our local media from vast raw materials available to improve our foreign reserves. This study was aimed at formulation of culture media from sorghum grains to cultivate test bacteria commonly used in research purposes. The sorghum grains free from debris were soaked in distilled water for 24 hr. and ground into paste. The fibers were removed by filtration followed by centrifuged at 9000 rpm for 10 minutes to obtained gluten and starch deposits which were separated and dried at 25°C. The dried gluten and starch were digested with pepsin and amylase enzymes to obtained protein and sugar digests which was combined proportionally and pH.7.2 adjusted. A pure culture each of *Staphylococcus aureus* (NCTC 6571) and *Bacillus subtilis* (NCTC 8241) were inoculated into sterile formulated sorghum and nutrient media. A visualized turbidity was observed. The OD (0.66 and 0.65) of *S. aureus* and (0.57 and 0.47) for *B. subtilis* in sorghum and nutrient media respectively. The mean viable count of $6.0 \times 10^{21} \pm 0.004$ and $5.7 \times 10^{21} \pm 0.003$ CFU/mL of *Staphylococcus aureus* in sorghum and nutrient media followed by $4.3 \times 10^{20} \pm 0.003$ and $4.7 \times 10^{21} \pm 0.005$ CFU/mL of *Bacillus subtilis* in nutrient and sorghum media respectively. There is no significant difference in viable counts of bacteria ($p < 0.05$) in these media. The growth curves exhibited by *Staphylococcus aureus* and *Bacillus subtilis* in sorghum and nutrient media showed similar pattern of no lag phase and lengthen log phase. The formulated sorghum media promotes and support the bacteria. The study recommends that sorghum media can be an alternative to nutrient broth as routine culture media.

Keywords

Formulated Sorghum media, Nutrient Broth, Viable count, *Staphylococcus aureus*, *Bacillus subtilis*, Culture media, Sorghum grains, Enzymes

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Introduction

Cultivation of bacteria and fungi required media composed of nutrients including meat or yeast digest, proteins, carbohydrate and

mineral salts for these microorganisms to grow. Microbiological culture media with exact defined compositions are defined media when compared with complex media with compositions made up of peptones, yeast or

meat digest, which promote the growth of microorganisms. Therefore, many sources could be used in the cultivation of microorganisms based on their diversified mode of degradation of materials in sourcing for energy. Bacteria and fungi also require macronutrient and microelement to grow (Basu *et al.*, (2015). The use of Nutrient broth as a routine media is composed of digested peptones, yeast digests and sodium chloride in the cultivation of *Staphylococcus aureus* and *Bacillus subtilis* both Gram positive bacteria used for research purposes, therefore, a general-purpose media for cultivation of non-fastidious microorganisms. The use of dehydrated media has gained a global acceptance in both mycological and bacteriological laboratories (Franhauser, 2005). The Nutrient broth is obtained as dehydrated media and mostly imported from the developed countries and are expensive (Adesemoye and Adedire, 2005). There is need to explore vast resources in the development of media to substitute the commonly used media. Plant materials have been used to recover both fungi and bacteria from different samples sources. Local food stuff waste (Tortora *et al.*, 2018), maize and beans (Oloke and Famurewa, 1991), sorghum extracts (Akinola *et al.*, 1997), Groundnut (Akinola *et al.*, 2004), cassava whey (Adesina and Akinyosoye, 2006), three-leaf yam (Eleke *et al.*, 2006), African oil bean (Egenu and Njoku, 2006), pigeon pea (Laleye *et al.*, 2007) among other agricultural products have been used as both mycological and bacteriological media. Other researchers had sourced for cheap raw materials to formulate media to grow bacteria and fungi, which resulted in supporting and promoting the growth of microorganisms used (Laleye *et al.*, 2007; Deivanayaki and Iruthayaraj, 2012; Ravathie *et al.*, 2012; Vasantha *et al.*, 2013; Basu *et al.*, 2015; Pratibha *et al.*, 2018 and Izebe *et al.*, 2020). Famurewa and David (2008) in their study used *Pseudomonas aeruginosa*, *Bacillus*

subtilis and other fungi species in the formulation of culture media sourced from local materials. Similarly, Shareef (2019) also in his study used *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Pseudomonas aeruginosa* as microorganisms in the formulation of microbiological culture media from local sources. Culture media formulated from chickpea, rice, corn soy flour have been used by Mekala *et al.*, (2016) in culturing *E. coli*, *Pseudomonas sp.*, *Bacillus sp.*, *Staphylococcus sp.*, and *Klebsiella sp.*

Sorghum (*Family* Andropogaceae) is the fourth most important cereal crop in the world surpassed only by rice, wheat and maize respectively. It belongs to the family Andropogaceae. In Nigeria, Sorghum is a staple food for most of the households. Sorghum is grown in most of the States of Nigeria and large quantity harvested annually. Sorghum production exceeds 6 million tons annually (Etuk *et al.*, 2012). Sorghum had been modified to have improved yield and high carbohydrate, protein and minerals content. The sorghum grain is rich in amino acids, fatty acids and micro and macronutrients that can be explored in the formulation of microbiological culture media for the cultivation of bacteria and fungi as alternative to the dehydrated media obtained commercially. This study is aimed at developing microbiological culture media from sorghum grains in the cultivation of *Staphylococcus aureus* and *Bacillus subtilis*.

Materials and Methods

Collection of materials

Sorghum grain of short kaura variety (SK 5912) was used in this study. It was obtained and identified by Institute of Agricultural Research, Ahmadu Bello University Zaria, Nigeria. The choice of sorghum was based on high nutritious value (Tables 1 and 2) and

improved varieties developed (Etuk *et al.*, 2012)

Treatment of Materials

Approximately, two thousand grams (2000 g) of sorghum grains were cleaned up by removing debris and stones and soaked in distilled water for 24 hours. After 24 hours soaking, the grains were ground with miller machine (Honda, Japan) and centrifuged at 9000 rpm for 10 minutes where the gluten (protein) was separated from the starch. Both the gluten and starch (whitish) were separated with a dryer at 25°C for three days and stored at 4°C in refrigerator until requested for use.

Starch Digestion

Twenty grams (20 g) of the dried starch was dissolved in 100 mL distilled water in 1 Litre capacity flask and placed in water-bath at 70°C. About 5.0 mL of 0.1% amylase (*Hitempase*) was added and cooled to 60°C after 1 hour. This was followed by the addition of 1.0 mL *Glycoamylase* for 30 minutes (Cruickshank *et al.*, 1983). The mixture was allowed to cool and centrifuged at 9000 rpm for 5 minutes. The supernatant was used for the estimation of sugar content. Ten millilitre of the supernatant was estimated using oxidase test (Bask, 2007).

Gluten digestion

Twenty grams of the dried gluten was dispensed into 100 mL distilled water in 1.0 L capacity flask and placed in water-bath shaker at 52°C. Pepsin solution was added and mixed for 4 hours as explained by Cruickshank *et al.*, (1983) and Zangne *et al.*, (2011). The mixture was centrifuged at 9000 rpm for 5 minutes in Beckman centrifuge. The supernatant was used to estimate the total protein content using Biuret method adopted from Fenk *et al.*, (2007).

Sorghum medium composition

The medium composition was based on addition of sorghum starch digest to sorghum protein digest in different ratios of 1:19, 2:18, 3:17, 4:16, 5:15, 6:14 and 7:17 as shown in the Table 3 below. For each mixture, the pH was adjusted to 7.4 and filtered in Whatman filter paper. The filtrates were dispensed into flasks and bottles and sterilized at 121°C for 15 minutes.

Bacteria inoculation

Strains of *Staphylococcus aureus* (NCTC 6571) and *Bacillus subtilis* (NCTC 841) were obtained from the National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria. The 24 hours' pure culture of each bacterium was diluted serially in sterile peptone water in 1:10 where 0.2 mL diluents were separately inoculated into labelled tubes containing 4.8 mL of the formulated sorghum medium in triplicates. Similar inoculation process was carried out on the prepared industrially made nutrient broth. All the inoculated tubes were incubated at 37°C for 24 hours. Negative control tubes containing 5.0 mL sorghum and nutrient without test bacteria were also incubated at 37°C for 24 hours.

Monitoring the Bacterial Growth in liquid media

After the end of 24 hours incubation, the labelled culture tubes of *S. aureus* and *B. subtilis* were monitored for the turbidity appearance.

The spectronic 20 (spectrophotometer) was switched on and the absorbance was adjusted to 540 nm. The blank cultures without the test organisms were used to calibrate the reading followed by treatments testing against the blanks.

Monitoring bacterial growth in solid medium

Nineteen folds serial dilutions (1:10) of the 24 hours-culture from the incubated sorghum medium with *S. aureus* were prepared. Similar dilutions were prepared from the nutrient broth cultures of *S. aureus* and *B. subtilis* and 1.0 mL from different dilutions were aseptically dispensed into sterile tryptic soya agar plates in triplicates. The plates were incubated at 37°C for 24 hours. The colony forming unit (CFU) was estimated using colony coulter. The colony in the tryptic soya agar plates each for *S. aureus* and *B. subtilis* were counted and expressed as colony forming units (CFU/mL).

Determination of growth curves

The Bio-screen C (Bio-screen C (200 wells), Biolink Laboratories, Finland) was put on to boot to maintain stability of the equipment. The sterile micro-wells were each dispensed with 0.3 mL of the formulated Sorghum broth in the honey wells. Similarly, 0.3 mL of the sterile nutrient broth was dispensed into the labeled honey wells. This was followed by addition of 0.05 mL of 3 hours' culture each of the *S. aureus* and *B. subtilis* into the labeled wells of sorghum and dehydrated nutrient broth. The inoculated wells were transferred into chamber and the absorbance

was taken at 540nm with an adjusted time interval of 0, 1, 2, 3, 4, 5 and 6 hours.

Data analysis

The data was analysed using SPSS (20.0 Version Inc., Chicago, USA). The mean and standard error of mean (\pm SEM) of the sorghum media and nutrient media values were calculated and presented in graphical and tabular forms. The p-value were considered significant at $p < 0.05$.

Results and Discussion

The compositions of sugar and protein digest in 15.0 mL capped tubes containing the 24 hours cultures of *S. aureus* and *B. subtilis* in formulated sorghum media revealed that 6:15 ratio of sugar to protein had higher turbidity while the higher sugar content and low protein gave moderate to low turbid culture (Table 4).

The growth profile of the test bacteria (*S. aureus* and *B. subtilis*) on both sorghum and commercially processed nutrient broth revealed varied from zero to six hours-growth time (Figure 1 and 2). At zero hour, the absorbance for test bacteria was relatively on the low value. But six hours' growth, the absorbance had increased to 0.60 except for *B. subtilis*

Table.1 Composition of nutrients in sorghum grains

Kernel fraction	% Kernel weight	Protein (%)	Ash (%)	Oil (%)	Starch (%)	Niacin (mg/100g)	Riboflavin (mg/100g)	Pyridoxine (mg/100g)
Whole kernel	100.0	12.30	1.67	3.60	73.80	4.50	0.13	0.47
Endosperm	82.30	12.30	0.37	0.60	82.50	4.40	0.09	0.04
Germ	9.80	18.90	10.40	28.10	13.40	8.10	0.39	0.72
Bran	7.90	6.90	2.00	4.90	34.60	4.40	0.40	0.44

(Etuk *et al.*, 2012)

Table.2 Amino acid composition in sorghum grains

Parameters	Dry	Nit	Arg	Cys	Gly	His	Leu	Lys	Met	Phe	Ser	Thr	Try	Tyr	Val
Compositions	870	14.1	3.4	1.6	3.5	4.2	11.4	2.1	1.6	4.2	3.9	2.9	1.0	3.8	5.3

Key: Dry = Dry matter, Nit = Nitrogen, Arg = Arginine, Cys = Cysteine, Gly = Glycine, His = Histidine, Leu= Leucine, Lys = Lysine, Met= Methionine, Phe= Phenylalanine, Ser = Serine, Thr = Threonine, Try = Tryptophan, Tyr = Tyrosine, Val = Valine. (Etuket *et al.*, 2012)

Table.3 Combinations of starch and protein digest in the formulated sorghum media

Starch digest (mL)	50	100	150	200	250	300	350
Protein digest (mL)	950	900	850	800	750	700	650

Table.4 The turbidity profile of *S. aureus* and *B. subtilis* in the sugar to protein ratio composition after 24 hours incubation

Starch digest (mL)	50	100	150	200	250	300	350
Protein digest (mL)	950	900	850	800	750	700	650
<i>S. aureus</i>	++	++	++	+++	++	++	+
<i>B. subtilis</i>	++	++	++	+++	++	+	+

Key: (+) turbid, (++) moderate turbid, (+++) highly turbid

Table.5 OD at 540 nm and viable count of 24hours cultures of *S. aureus* and *B. subtilis*

Growth profile	Sorghum medium		Nutrient broth	
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis.</i>
Absorbance (540nm)	0.660	0.571	0.650	0.478
Viable count (CFU/mL)	$6.0 \times 10^{21} \pm 0.004$	$4.7 \times 10^{20} \pm 0.005$	$5.7 \times 10^{21} \pm 0.003$	$4.3 \times 10^{20} \pm 0.006$

Fig.1 Growth profile *Staphylococcus aureus* in Nutrient broth (A) and formulated Sorghum broth (B)

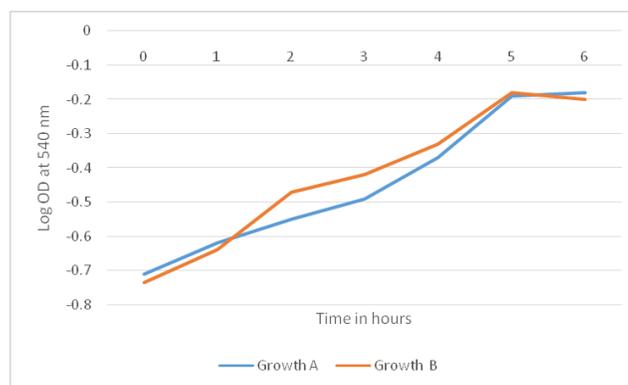
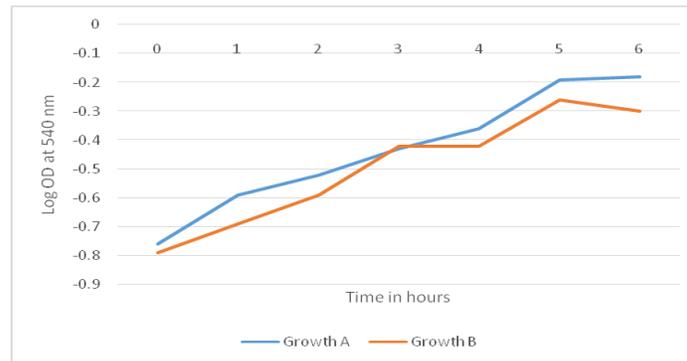


Fig.2 Growth profile of *Bacillus subtilis* in Nutrient broth (A) and Sorghum broth (B)

The bacteria growth after 24 hours-time in both media showed some turbid response with different values (Table 5). The absorbance values were ranged from 0.600 to 0.478 for both bacteria in nutrient broth while 0.600 to 0.571 were obtained from the culture in the formulated sorghum medium. The viable counts for the formulated sorghum medium and nutrient broth gave mean viable count of $6.0 \times 10^{21} \pm 0.004$ CFU/mL and $4.7 \times 10^{21} \pm 0.005$ CFU/mL for *S. aureus* and *B. subtilis* respectively (Table 5) in formulated sorghum medium respectively. The mean viable counts of $5.7 \times 10^{21} \pm 0.003$ and $4.3 \times 10^{20} \pm 0.003$ CFU/mL estimated for *S. aureus* and *B. subtilis* in nutrient broth respectively.

The sorghum grains of short variety used in this study were found to be rich in proteins, sugars, and amino acids that are necessary in the formation of microbiological culture media (Ekut *et al.*, 2012; Izebe *et al.*, 2020). The findings of this study showed that the sorghum media promotes the growth of *S. aureus* and *B. subtilis*. The OD values at 540 nm after 24 hours culture gave 0.660 and 0.571 for the *S. aureus* and *B. subtilis* in sorghum medium while 0.650 and 0.478 were obtained in nutrient broth culture respectively. The estimated mean CFU of *S. aureus* and *B. subtilis* in 24 hours. Sorghum medium 24-hour culture when sub-cultured in tryptic soya agar gave CFU of 6.0×10^{20} for *S. aureus* while *B. subtilis* gave CFU of $5.0 \times$

10^{20} . Similarly, in nutrient broth, the counts included 4.6×10^{21} and 5.1×10^{20} CFU respectively. These results agreed with similar work carried out by Izebe *et al.*, (2020) using sorghum medium to growth *Pseudomonas aeruginosa* and *Escherichia coli*. The growth curves of *S. aureus* and *B. subtilis* in sorghum and nutrient broth showed similar pattern of minimum lag phase, which was followed by log phase and briefed stationary phase. The growth curves of *S. aureus* and *B. subtilis* in sorghum and nutrient broth also agreed with the growth curves of *P. aeruginosa* and *E. coli* in sorghum and nutrient broth as reported by Izebe *et al.*, (2020). The need for preparations of microbiological culture media from local resources has led researchers to into materials readily available to us. This is similar to study carried out by Pratibha *et al.*, (2018) that reported formulations from seeds and peels extracts to grow *E. coli*, *Serratiasp.*, and *P. aeruginosa*. Similarly, Deivanayaki and Iruththayaraji (2012) who reported the use of formulations from Gooseberry and use of carrot, tomato, cabbage and pumpkin to cultivate bacteria and fungi (Wasas *et al.*, 1999; Uthayasooriyan *et al.*, 2016). In Nigeria, other researchers have formulated microbiological culture media from local materials (Akinola *et al.*, 1997; Ikenebomen and Chikwendu, 1997; Oloke and Famurewa, 1991; Akinola *et al.*, 2004; Eleke *et al.*, 2006; Adesina and Akinyosoye, 2006; Laleye *et al.*, 2007). In this is study, the formulated

Sorghum medium was found to contain 0.103g sugars and 2.4g proteins per litre when compared with Nutrient broth composition of 6.0g of peptone, 3.0g of yeast extract and 1.5g beef extract to give 28g per liter.

In conclusion formulated Sorghum broth is a complex media containing 0.103 g of sugar and 2.4 g of protein that promotes the cultivation of *Staphylococcus aureus* and *Bacillus subtilis*, alternative to Nutrient broth as basal media for routine use in the microbiological laboratory. The Sorghum media is formulated from locally sourced and cheap material for the development of the sorghum medium. The study recommended further work to define sugar and protein digest.

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