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Full Length Research Paper

Industrialization of a microbial medium coined from industrial food waste

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A microbial medium, designated DYSP medium, was produced from food wastes that included defatted soya, clear beer spent yeast, potato solid waste and opaque beer spent grains. Various combinations of the food wastes were used for the media formulations and evaluated for extent of supporting microbial growth of pure cultures. A 3.0 M sodium hydroxide hydrolysis followed by a neutralization process using concentrated hydrochloric acid has been established and standardized for the preparation of the medium. A dry pulverized medium was produced that could be reconstituted in distilled water (dH_2O) without settleable solids.The formulated DYSP medium supported the growth of *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Aspergillus niger* and *Saccharomyces cerevisiae*. The composition of the medium was 35.45% protein, 5.7% nitrogen, 56.6% ash, 8.6% moisture and 0.012 mg/ml of total reducing sugar. The pH of the DYSP broth was 6.6 when reconstituted in distilled water. Traditional classical microbiological studies demonstrated that the test cultures could grow and retain normal phenotypic and morphological properties when cultured on the formulated medium.The DYSP medium containing ampicillin, isopropyl ß-D-1-thiogalactopyranoside and 5-bromo-4-chloro-3-indolyl-ß-D-galactoside proved to be an equally alternative medium in molecular biology for selection and screening *E. coli* TG1 cells transformed with pUC18 plasmid. Other preliminary biotechnological results showed that the formulated medium could form a base for studying and optimizing the production of penicillin by *P. chrysogenum*.

Key words: Food wastes, chemical hydrolysis culturing medium, fermentation and molecular biology.

INTRODUCTION

The food industries produce a significant amount of processing wastes and residues that can accumulate in the environment, causing pollution, eutrophication, high biological oxygen demand (BOD) and chemical oxygen demand (COD) (Moyo, 1997).

In developed countries, they employ landfills and on-site burning, which, in turn, can contribute adversely to water, soil, air pollution and ecosystems (Scragg, 1999). The changing of economic, social, political and cultural values of the world has challenged many countries to effectively utilize industrial biotechnology for a friendly way of disposing wastes (Torres et al., 2004). Raw materials in the form of food industrial wastes have been channelled towards production of industrial, commercial and pharmaceutical products that include energy, and fine biochemical products (Turker, 2004; Scragg, 1999). The industrial food waste contains vast amounts of nutrients, which can be harnessed for growth of microorganisms and subsequent production of useful primary and secondary products like enzymes (Ellouz et al., 2001; Vásquez Álvarez et al., 2004; Chipeta et al., 2002), energy production (Oliveira, 2001; Sun and Cheng, 2002), biomass production (Duru and Uma, 2003; Tripodo et al., 2004) and organic acids like lactic acids, butanol, methanol and ethanol. The high costs of synthetic media have negatively impacted on the development of studies in the fields of microbiology, fermentation, and molecular biolo-gy in developing countries. The introduction of microbio-logy practicals at high schools, even at up-coming new university institutions is severely influenced or handicapped by high costs incurred in purchasing reagents.

It is against this background that this project focuses on formulation and standardization of a microbial medium that can be commercialized. This medium is anticipated to pave the way for development of research in microbial

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Table 1. Quantities of different mixture formulations (g/l) of DYSP medium before alkaline hydrolysis

Substrate	DYS1	DYS2	DYS3	DYS4	DYS5
Defatted soya	10	5	10	10	10
Clear beer spent yeasts	10	5	5	10	5
Potato solid waste	7.50	3.75	7.50	7.50	7.50
Opaque beer spent grains	7.5	3.75	3.75	3.75	1.875

biotechnology. The application of the formulated medium was demonstrated in areas that encompass microbiological culturing, fermentation, and molecular biology. The study looked at standardizing the microbial medium formulation and preparation, determination of the microbial spectrum that can be supported by the medium, and ascertaining different applications of the formulated medium.

MATERIALS AND METHODS

Preparation of the DYSP medium

DYSP medium raw materials and test microbes

A medium was formulated from food waste obtained from Zimbabwean local food industries. The food wastes were defatted soya, clear beer spent yeast, potato solid waste and opaque beer spent grains. The medium was designated DYSP (defatted soya-yeast extract- sorghum-potato) medium. Defatted soya was obtained from an oil manufacturing company, potato solid waste was collected from a potato processing company, opaque spent grains were obtained from the opaque brewery plant and dried spent yeasts were obtained from a clear beer brewery plant. The following microorganisms were incubated under suitable conditions: *E. coli*, *B. subtilis* CHZ1, *B. cereus*, *S. aureus*, *A. niger*, *S. cerevisiae* and *P. chrysogenum*. Our basis for choice was that *E. coli* is the most widely used cloning host system and is used widely in other biotechnological research. *A. niger* and *S. cerevisiae* are industrially valuable microbes for the production of lactic acid, acetic acid, baking, brewing and wine making (Pintado et al., 1998; Korbanoglu and Korbanoglu, 2004).

Pretreatment and pulverization of food waste material

The opaque beer spent grains and the potato solid waste were dried in an oven at 80^{o}C for 72 h. The spent yeasts and defatted soya were obtained dry; however, all samples were ground to a powder using a grinding machine from the Institute of Mining Research (IMR), University of Zimbabwe. The opaque beer spent grains outer coats and coarse materials were fractionated by sieving through a 110 µm mesh sieve and then followed by a 100-µm sized sieve to remove the husks. The ground materials were once more sieved through the 75 µm sieve mesh to obtain very fine powders. The pulverized materials of each raw material were kept sealed in media plastic bottles and stored at room temperature.

Food waste media formulations

The compositions of different medium formulations are summarized in Table 1. The formulations were based on the CWW medium designed by Zvauya and Zvidzai (1996). The nutrient contribution by each component of the raw materials had been previously studied. The formulations were hydrolyzed using 3 M sodium hydroxide at 80^{o}C for 24 h, using a rotary shaker at 150 rpm, and then boiled at 100^{o}C for 1 h. The media formulation mixtures were cooled to ambient temperatures and then brought to a pH of 7.0 ± 0.2 with concentrated hydrochloric acid. This was followed by centrifuging at 3 000 rpm for 6 min and drying in an oven at 55^{o}C for 72 h. The final clear broth was used to prepare solid media by adding 1.2% (w/v) bacteriological agar (Oxoid). For the purpose of comparison during microbial cultivations, Nutrient Agar medium was also used concurrently as reference medium. All media types were sterilized by autoclaving at 121^{o}C at 15 lb/inch^{2} for 15 min.

Hydrolysis of composite waste media formulations

The formulation DYSP4, which constituted 10 g of defatted soya, 10 g of clear beer spent yeasts, 7.5 g of potato solid waste and 3.75 g of opaque beer spent grains in 1 L of distilled water, was found to be the most suitable medium in terms of supporting the growth of the spectrum of microbes tested. Therefore, the mixture composition of DYSP4 was selected to improve on its qualities. There was a need to improve on its clarity. The medium had opaque characteristics and a high amount of solids, which could be cleared by treating with sodium hydroxide. Sodium hydroxide hydrolysis of the DYSP4 medium was performed by modifying the method of Kurbanoglu and Kurbanoglu (2004). The procedure involved hydrolyzing with 3.0 M sodium hydroxide in a rotary shaking water bath set at 200 rpm and 80^{o}C for 24 h. The hydrolyzed mixture was then subjected to boiling at 100^{o}C for 1 h. The resultant hydrolysates were allowed to cool to room temperature and then brought to a pH of 7.0 with concentrated hydrochloric acid. After neutralization, the solution was centrifuged at 3 000 rpm for 6 min and the filtrate was dried in an oven at 55^{o}C for 72 h. The dried material was ground to a fine powder and the medium was termed DYSP medium.

Nutrient evaluation of DYSP medium

DYSP medium was analysed for ash content (AOAC, 1990), sodium chloride (AOAC, 1990), PO_4 by the Vanadomolybdophosphoric acid colorimetric method (APHA, 1974), moisture content (AOAC, 1990), pH, protein content by the Biuret method, reducing sugar content by the DNS method (Ghose, 1978), nitrogen content by the Kjeldahl method, and total sugar content by the modified phenol-sulphuric acid method (Fox and Robyt, 1987) to ascertain the shelf-life of the medium during the 6 months course of the project.

Test cultivation of microbes using DYSP medium formulations

Pure cultures were maintained on agar plates that were kept at 4^{o}C and sub-cultured fortnightly on nutrient agar, except for *A. niger* which was sub-cultured on Sabouraud's dextrose agar. Discrete colonies were picked and then inoculated into previously sterilised 10 ml of nutrient broth in a 50 ml Erlenmeyer flask and Sabouraud's dextrose broth, respectively, for bacteria and fungi. Bacterial cultu-

res were incubated at 37^{o}C and fungal cultures at room temperature with shaking at 150 rpm. The cell density for bacterial cultres was monitored until an absorbance at 600 nm ($A_{600\ nm}$) of 0.7 was reached, while the fungal cultures were serially diluted in physiological normal saline after 5 days and spore counts adjusted to 1 x 10^8 spore units/ml (Korbanoglu and Korbanoglu, 2004). A standardized inoculum size of 0.1 ml of the bacteria and fungal cultures were spread plated on agar plates of the 5 DYSP media formulations. The growth-promoting capabilities of each medium were compared by determining colony forming units (log cfu/ml) on the DYSP plates. Another set of experiments were carried out to determine biomass levels, using a batch cultivation system, in DYSP broth by monitoring $A_{600\ nm}$ values of the cultures in a rotary shaker set at 150 rpm. Triplicate preparations were made for all inoculated plates and batch cultivations were then incubated at 37^{o}C for bacterial and at room temperature for fungal cultures. Colonies were counted with the aid of a colony counter.

Various reconstituted concentrations (0.2, 0.4, 0.6, 0.8, 1.0 and 1.5 % [w/v]) of the DYSP medium were tested for supporting growth of microorganisms. A nutrient broth flask was included as a reference medium for each microbial strain. The best medium formulation, whose reconstitution percentage was determined from culturing with the same microbes, was then used for application purposes.

The maximum cell densities ($maxA_{600nm}$) were determined as the growth curves assume stationary phase. Specific growth rate (μ_{max}), for each microbe was determined in the exponential phase, as follows:

$$\mu_{max} = \frac{\log A_{600nm}}{t}$$

where t = time in minutes (Zwietering et al., 1990)

Cumulative results allowed comparisons between different concentrations of DYSP media and nutrient broth which was used as a reference medium.

Phenotypic characterization and biochemical tests of test microbes on DYSP medium

The capability of supporting microbial growth of pure bacterial and fungal cultures were analyzed by wet-mount microscopy to check for retention of cell morphology properties, catalase reaction, Gram staining and other classical biochemical reactions as described by Gerhardt et al. (1994).

Applications of DYSP medium

Use of DYSP medium in microbial evaluation of samples

The pulverized medium was reconstituted and tested for its applicability in several microbial techniques that include spread, streak, screening and selection of transformed *E. coli* TG1 cells for molecular biology research experiments. The reconstituted 1% (w/v) of DYSP medium was selected as the best medium and was then compared to commercial Nutrient Agar (Difco), Trypticase Soy Agar (Difco), Plate Count Agar (Oxoid) and Sabouraud's dextrose agar (Oxoid) for microbial analysis of environmental samples (air, soil filtrate and water samples). Plates prepared from the different media were then subjected to inoculation with air, soil and water samples. The inoculated plates were incubated at 37^{o}C for 48 h. An inoculum volume of 0.5 ml water sample was used, as stipulated in the methods for microbiological examination foods (APHA, 1994). An amount of 1.0 g of soil was serially mixed in 10 ml of sterile physiological saline and then filtered. A volume of 0.5 ml of the soil-filtrate sample was then spread plated onto the agar media plates. Colonies were counted after 48 h and expressed as log cfu/ml. All inoculations were done in triplicate and the averages calculated.

Use of DYSP medium in penicillin production

Spores of *P. chrysogenum* were initially obtained on medium described by Bhuyan and Johnson (1957), and then a 10% (v/v) inoculum prepared over 3 days was subsequently inoculated into a 250 ml Erlenmeyer flask containing 50 ml DYSP broth supplemented with mineral salts. A control was included in parallel and contained the penicillin germination medium (El -Marsaf et al., 2001). The culturing temperature was maintained at 26^{o} C. Agitation speed was maintained at 200 rpm in a rotary shaker for 6 days. After 6 days of cultivation, cultures were centrifuged at 3 000 rpm for 6 min to remove fungal cells and then the supernatant was used for determination of inhibition zones on a plate inoculated with *S. aureus* as a test microorganism (Gerhardt et al., 1994). A reference sample of penicillin G (Sigma) at a concentration of 100 IU/ml was used for comparison.

Use of DYSP preparation, transformation and screening of competent E. coli (TG1 strain) cells

A sterile inoculation loop was used to pick a colony of *E. coli* cells (strain TG1) growing on a 2YT agar plate and used to prepare competent cells with $CaCl_2$ treatment (Sambrook et al., 1990). After transformation of *E coli* host cells with 10 µl of pUC18 plasmid DNA, the cells were cultured on sterile 2YT and DYSP broth, respectively, in 1.5 ml-Eppendorf tubes. Then, 50 µl of the cell suspensions were plated on 2YT and DYSP agar plates, respectively, containing 50 µg/ml ampicillin, 60 µl of isopropyl ß-D-1-thiogalactopyranoside (IPTG) and 50 µl of X-gal (5-bromo-4- chloro- 3-indolyl-ß-D- galactoside). After spread plating, transformed *E. coli* cell colo-nies were observed after incubating at 37^{o}C for 12 h.

RESULTS

Formulation of DYSP medium

Several combinations of the raw material amounts were prepared in an attempt to optimize the medium formulation. Initially, 5 types of DYSP media formulations were screened for their ability to support the growth of pure cultures of bacteria. However, the formulations contained high amounts of solids, which interfered with medium preparations, particularly in broth optical density determination. The designed medium using industrial food wastes was then subjected to chemical hydrolysis using 3.0 M sodium hydroxide to improve on nutrient extraction and reduction of dissolved solids that interfered with the clarity of DYSP broth.

Evaluating different DYSP agar medium formulations for microbial growth

When microbial growth was determined from plates, the DYSP4 formulation, containing: 10 g defatted soya, 10 g clear beer spent yeasts, 3.75 g potato solid waste and 1.875 g opaque beer spent grains in 1 L of distilled water,

Table 2. Comparison of growth of bacterial cultures (log cfu/ml) grown for 24 h on different formulations of DYSP medium on plates compared with nutrient agar

Microorganism	DYSP1	DYSP2	DYSP3	DYSP4	DYSP5	NA/SDA
A. niger	6.7 ± 0.0	6.5 ± 0.05	7.7 ± 0.01	7.9 ± 0.01	6.8 ± 0.01	7.5 ± 0.05
B. cereus	6.5 ± 0	6.3 ± 0.05	8.2 ± 0.01	8.6 ± 0.01	6.4 ± 0.1	8.6 ± 0.1
B. subtilis	6.5 ± 0.03	6.4 ± 0.1	8.4 ± 0.01	8.6 ±0	6.4 ± 0	8.6 ± 0.01
E. coli	6.5 ± 0.01	6.4 ± 0.03	8.4 ± 0.03	8.6 ± 0.03	6.5 ± 0.03	8.7 ± 0.03
S. aureus	6.5 ± 0.1	0	8.2 ± 0.1	8.5 ± 0.1	0	8.6 ± 0.1
S. cerevisiae	6.9 ± 0.1	6.8 ± 0.01	8.0 ± 0.3	8.3 ± 0.3	6.2 ± 0.1	8.4 ± 0.01

Table 3. A600 nm comparison of pure cultures of bacteria and yeasts grown on different broth media formulations cultured for 8 h

Microorganism	DYSP1	DYSP2	DYSP3	DYSP4	DYSP5	NB
B. subtilis	0.432	0.346	0.242	0.598	0.500	0.620
B. cereus	0.542	0.375	0.517	0.711	0.681	0.730
E. coli	0.598	0.475	0.661	0.769	0.573	0.912
S. aureus	0.465	0.265	0.234	0.693	0.548	0.680
S. cerevisiae	0.864	0.320	0.700	0.561	0.480	0.680

Table 4. Specific growth rates ($.h^{-1}$) of test microbes converted to percentage index value of maximal value of 0.4 h-1 obtained with a commercial Nutrient broth.

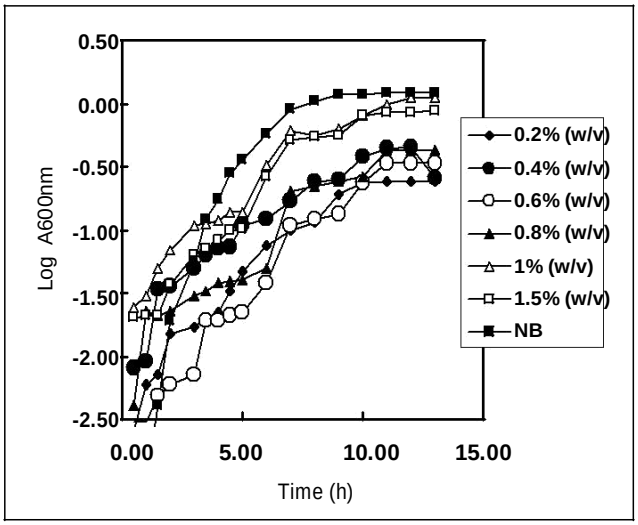
DYSP	*E. coli*	*S. aureus*	*B. subtilis*	*B. cereus*	total	rank	% best medium
0.2% (w/v)	0.2	0.1	0.1	0.1			
%/max	50	37.0	28.6	33.3	149	7	39
0.4% (w/v)	0.24	0.15	0.13	0.16			
%/max	60	55.6	37.1	45.7	198	6	52
0.6% (w/v)	0.26	0.2	0.15	0.17			
%/max	65	74.1	42.9	56.7	239	5	63
0.8% (w/v)	0.3	0.23	0.20	0.21			
%/max	75	85.2	69.0	70	299	4	78
1% (w/v)	0.35	0.25	0.21	0.28			
%/max	87.3	92.6	60	93.3	333	2	87
1.5% (w/v)	0.26	0.20	0.35	0.23			
%/max	65	74	100	76.7	316	3	82
NB	0.4	0.27	0.29	0.30			
%/max	100	100	82.9	100	383	1	100

produced the highest colony counts (8.6 ± 0.03 log cfu/ml) as compared to other food waste medium formulations (Table 2). All formulations of DYSP gave the lowest colony counts of 6.3 - 6.5 ± 0.01 log cfu/ml with all microbes, except for *S. aureus* that did not grow at all in formulations DYSP2 and DYSP5. The lowest growth for all microbial cultures were obtained in media formulations DYSP1, DYSP2 and DYSP5 with the highest growth DYSP1, DYSP2 and DYSP5 with the highest growth achieved in the DYSP4 formulation. In all the media formulations tested, *E. coli* showed high biomass production and *S. aureus* had the least biomass production.

When DYSP broth media formulations were tested for batch cultivations of the microbes, highest growth was achieved with *E. coli* reaching 0.769 A_{600nm} values in DYSP4 after 8 h, whereas Nutrient broth achieved 0.91 (Table 3). The amount of biomass obtained for all strains used were quite comparable to those of Nutrient broth. Generally, the least growth was obtained in formulation DYSP2 were the A_{600nm} values of *E. coli* and *B. cereus* were 0.475 and 0.375, respectively.

The formulation DYSP4 was observed to the best formulation in supporting growth against the tested microbes. DYSP4 mixture was then used for chemical hydrolysis to improve on the clarity and nutrition quality. The various concentrations of DYSP4, which was termed

B. cereus



E. coli

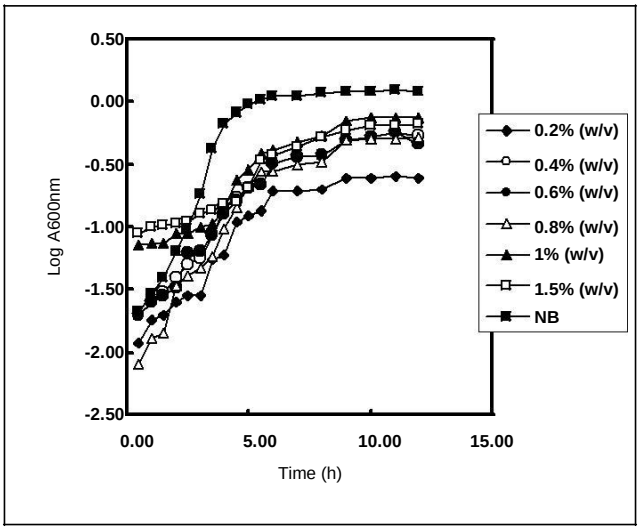
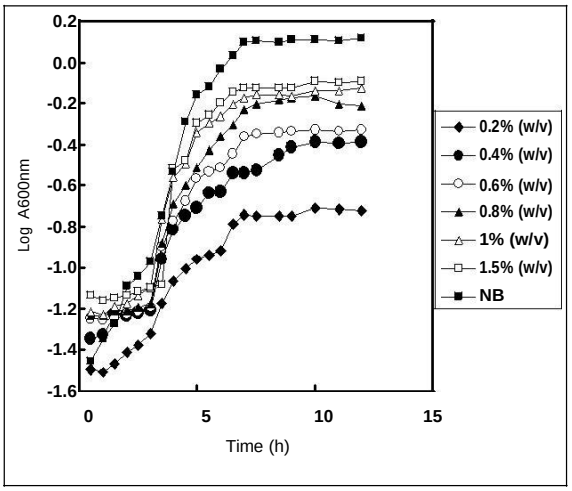


Figure 1. Growth profiles of *Bacillus cereus* and *Escherichia coli* under various concentrations of DYSP medium.

B. subtilis



Staphylococcus aureus

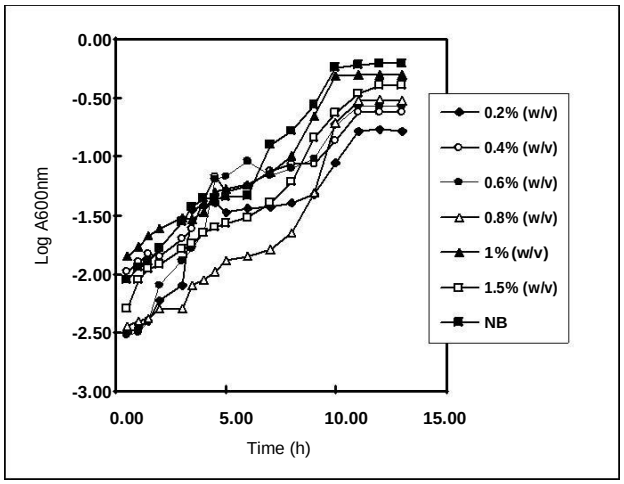


Figure 2. Growth profiles of *Bacillus subtilis* and *Staphylococcus aureus* under various concentrations of DYSP medium.

DYSP medium, were tested against Nutrient broth to determine the appropriate % (w/v) reconstitution. The growth profiles for the tested microbes are shown in Figures 1 and 2.

Table 4 shows data analysis for differently reconstituted % (w/v) of DYSP broth medium used during shake flask cultivations. The maximum specific growth rates and cell densities for *E. coli*, *B. subtilis* CHZ1, *B. cereus* and *S. aureus* on DYSP broth were calculated. The 1.0 % (w/v) DYSP broth gave the highest specific growth rates with all the microbial strains and achieved maximal cell densities, except with *B. subtilis* which attained its highest values at 1.5% (w/v). Generally, it should be noted that all four tested microbes yielded highest cell densities and specific growth rates in Nutrient broth. However, their growth profiles were quite similar when DYSP was reconstituted at various concentrations. DYSP medium at 0.2 to 0.8% (w/v) yielded low biomass and growth rates compared to the Nutrient broth with maximum cell densities ranging between 0.225 and 0.511 for *E. coli* and 0.245 to 0.424 for *B. cereus* (Table 4).

Nutrient composition of DYSP4 medium

The chemical analysis of DYSP medium produced a total nitrogen content of 5.7 and 35.45% crude protein, 11.3% dry matter, 8.6% moisture content, 56.6% ash, 0.03 mg/ml sodium chloride, 0.012 mg/ml total reducing sugar,

Table 5. A comparison of colony developments from water, soil and air samples on DYSP agar, nutrient agar, plate count agar and trypticase soy agar after 48 h of incubation of the plates

Medium	Air (colony numbers)	Water (log cfu/ml)	Filtered Soil sample (log cfu/ml)	Gram stain
Nutrient agar	7 ± 4.7	1.3 ± 0.3	3.1 ± 0.1	+ve rods & cocc
Plate count agar	6 ± 1.1	0	2.95 ± 0.03	fungi, +ve cocci
Trypticase soy agar	10 ± 0.9	1 ± 0.03	4.0 ± 0.01	+ve cocci; -ve rods
DYSP agar	6 ±1.8	0.6 ± 0.9	3.0±0.03	+ve rods & cocc

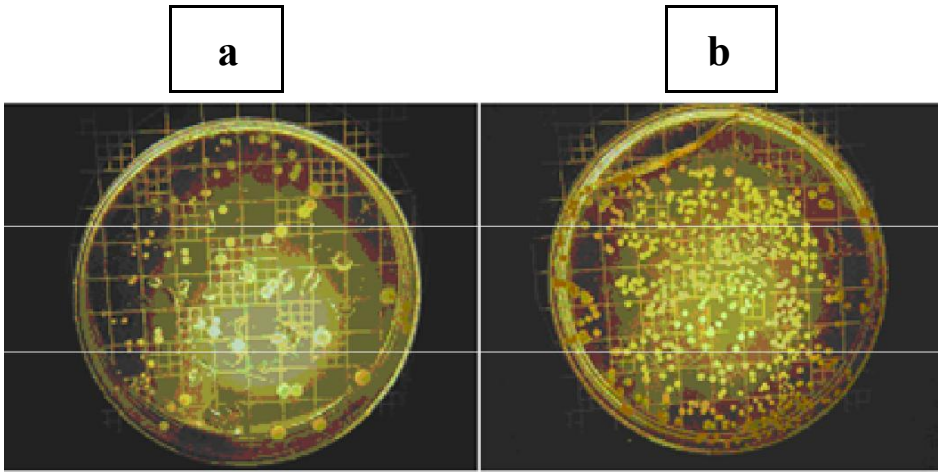


Figure 3. Comparison of growth obtained with S. aureus on DYSP agar medium (a) and on Nutrient Agar (b).

0.45 mg/ml total sugar and the non-reducing sugar content was 0.44 mg/ml. When reconstituted with distilled water the pH value was 6.6 ± 0.4. Notably, DYSP4 medium contains the basic essential substances required in microbial media such as nitrogen, carbon sources (reducing and non-reducing sugars), proteins and other unanalyzed nutrients for microbial growth. The pulverized DYSP4 medium contained a low moisture content and low ash content. The medium contains nutritional components for microbial growth that are quite comparable to the synthetic nutrient medium. A follow- up of the shelf-life of the medium was done by analyzing for moisture con-tent, ash content and pH of medium after reconstituting it in distilled water at 2-month intervals for 6 months. The medium showed stability of moisture content, 9.0 ± 0.61% (w/w); pH, 6.21 ± 0.56 and ash content, 6.35 ± 0.31% (w/w).

Application of DYSP medium

Microbiological analysis of natural samples: The DYSP medium was compared with Trypticase soya agar, Plate Count Agar and Nutrient Agar regarding the growth of cultures from air, soil, and tap water samples. Table 5 shows the number of colonies obtained after exposure of open agar plates to the respective environmental samples. Trypticase soy agar developed the highest number of colonies under all sample inoculations. Plate Count Agar gave lowest and only fungal colony counts of 6 ± 1.1 cfu. DYSP agar medium had microbial counts that were comparably high to all the media types.

Evaluation of DYSP medium on microbial phenotypic characterization

A spread plate comparison of *S. aureus* and *A. niger* on DYSP agar and nutrient agar (NA) plates is shown in Figures 3 and 4, respectively. The colonies of *S. aureus* showed a golden yellow pigment on both the DYSP agar medium and the nutrient agar. However, fewer colonies were obtained on DYSPA medium as compared to nutrient agar. The *A. niger* managed to produce mycelia and its normal morphological characteristics on both the DYSP agar and on the recommended Sabouraud's dextrose agar medium. All the strains tested on DYSP medium showed retention of their normal phenotypic characteristics as observed on wet-mount microscopy, gram reaction, catalase reaction and other biochemical tests performed.

Use of DYSP medium in selecting and screening transformed competent cells

Figure 5 shows the growth of recombinant *E. coli* TG1 host cells transformed with pUC18 plasmid DNA on DYSP and 2YT media containing ampicillin, IPTG and X-

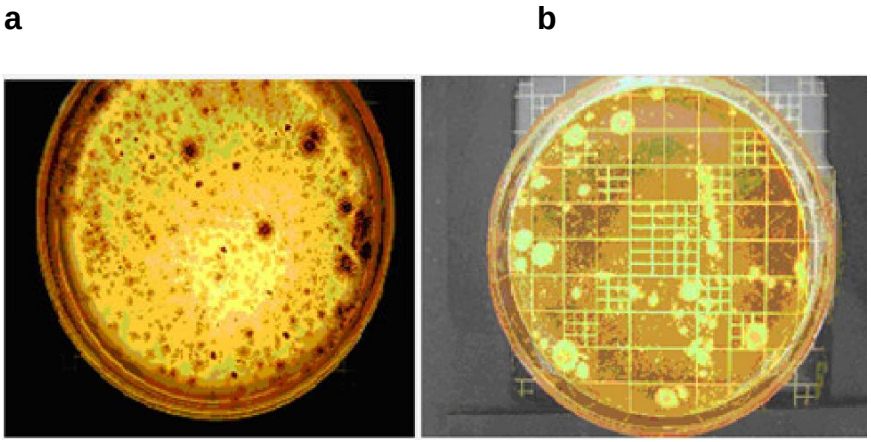


Figure 4. Comparison of growth obtained with *A. niger* on DYSPA medium (a) and Sabouraud's dextrose agar medium (b).

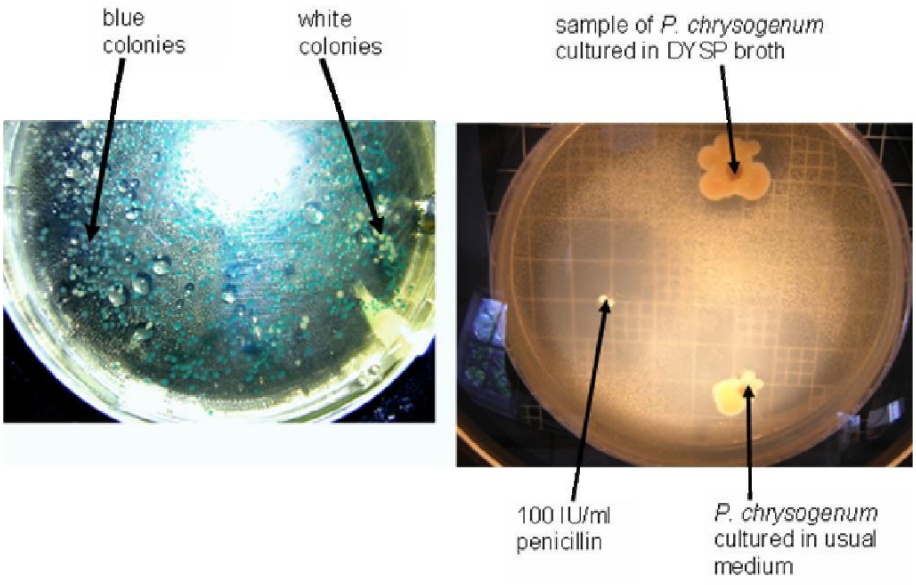


Figure 5. Screening blue and white colonies of transformed *E. coli* TG1 cells with pUC18 on DYSP agar medium using X-gal and IPTG (a), and inhibition zones produced by *S. aureus* in determining bacterium sensitivity to a crude sample of *P. chrysogenum* cultivation broth in DYSP medium.

gal, which are usually used for selecting and screening transformants, respectively. White colonies are *E. coli* cells that took up foreign pUC18 plasmid with a modified *lacZ* gene, while blue colonies had intact pUC18 plasmid. This was a normal result of colony development on DYSP and 2YT media types.

Use of DYSP broth in penicillin production

The DYSP medium was used as a fermentation medium for the production of penicillin using a penicillin producer, *P. chrysogenum.* The fermentation broth was assayed using *S. aureus* as an indictor microorganism. The bio-assay agar diffusion method (Figure 5) showed zones of inhibition on the indicator microorganism *S. aureus*.

DISCUSSION AND CONCLUSION

The main objective of the study was to standardize the microbial medium formulated from food wastes and compare it with a commercial medium like nutrient agar commonly used in microbiological cultivation.

The formulated medium could support growth of the tested microbes, indicating that DYSP medium has com-

ponents for supporting the growth of fungi and bacteria. Chemical treatment of the DYSP medium with 3.0 M sodium hydroxide improved the nutritional availability from the raw materials. Similar treatments of other organic materials have realised bioavailability of such raw materials for fermentation processes (Korbanoglu and Algur, 2002; Sun and Cheng, 2002).

The content of DYSP medium indicated 0.49% (w/w) NaCl and an ash content of 56.6% (w/w). The use of 3.0 M sodium hydroxide for hydrolysis contributed to the final high levels of sodium. The resultant high ash content of DYSP medium cannot be reduced due to the neutralization by concentrated hydrochloric acid (Kristinsson and Rasaw, 1990) . During the alkaline hydrolysis some amino acids such as arginine, asparagine, glutamine, and serine can be destroyed, while others are racemized although this can be avoided if enzymatic hydrolysis is carried out (Daven, 1990). The DYSP4 medium contains a high percentage of proteins and nitrogen. This is due to the incorporation of defatted soya and spent yeast, which is rich in nitrogenous substances and other growth factors (vitamins and co- enzymes). Spent yeast has been established to be rich in proteins, minerals and vitamins (e.g. pantothenic acid and niacin) (Miller and Churchill, 1986). The complete nutrient evaluation of the DYSP medium will serve as a guide to the development and production of a commercial medium able to sustain and maintain a wide range of microorganisms.

The bacterial counts for the formulation DYSP4 differ significantly ($p<0.05$) from the formulations DYSP2, DYSP3 and DYSP5, and notably from DYSP1 with respect to growth achieved with *E. coli*, *B. subtilis*, *S. cerevisiae* and *A. niger*. The total colony yield (log cfu/ml) for DYSP4 medium was 8.6 ± 0.01 for *B. cereus* and 7.9 ± 0.01 for *A. niger*, respectively. This compared favorably well with nutrient agar and Sabouraud's dextrose agar, which yielded 8.6 ± 0.1 log cfu/ml and a spore forming unit of 7.5 ± 0.01 log cfu/ml. When the DYSP4, nutrient agar and Sabouraud's dextrose agar were compared against each other, it showed that the yield on nutrient agar is not statistically significant ($p<0.05$). All microbes, here studied, managed to grow on DYSP medium. The lowest growth observed for the formulation DYSP2 may arise from the low concentrations of defatted soya and spent yeasts used in this formulation (Zvauya and Zvidzai, 1996; Zvidzai, 2003; Uzeh et al., 2006). However, it should be realised that Figure 3 showed that the DYSP4 medium formulation may be nutritionally deficient of certain amino acids that could have realised low colony counts of *S. aureus* compared to nutrient agar. The difference between the results of nutrient agar, DYSP and other media types were not significant ($p < 0.05$).

Microscopic examinations of microbes after gram staining show that cellular morphology is similar when grown on DYSP agar and nutrient agar. Similar research work, using different food industrial wastes, has shown retention of phenotypic characteristics (Knochel, 1989; Korbanoglu and Algur, 2002). It has similarly been reported that low concentrations of the hydrolytic enzymes (proteases and amylases) and biomass were produced with *B. subtilis* CZH1 when low defatted soy and spent yeast were used in medium formulation. However, low amounts of the highly hygroscopic spent yeast extract helps to reduce moisture levels of the medium preparations. The chibuku spent grains and potato solid waste contribute to the sugar requirements, even though chemical hydrolysis degrade complex polysaccharides like xylose, pullulan and arabinose (Sziponar et al., 2003).

Food and industrial wastes like saw dust, sugar cane pulp, barley, spent grains, fish wastes and ram horns (Green and Kramer, 1979; Kristinsson and Rasco, 2000; Poenomo and Buckle, 2002; Kurbanoglu and Kurbanoglu, 2002; Chinedu-Nwodo et al., 2003; Sziponar et al., 2003) have also been reported to support the growth of microorganisms favourably well compared to nutrient broth. In the fermentation industry, various objectives can be pursued either a high product attainment or a high biomass level.

Screening tests for competent *E. coli* cells produced normal blue/white transformants on the DYSP and 2YT media, showing the possibility of working with either medium and achieving the same objectives. The growth of transformed cells on DYSP and 2YT media, containing ampicillin, shows that the blue colonies are due to the transformed *E. coli* cells transformed with pUC18 plasmids containing genes for ampicillin resistance and an intact *lacZ* encoding the enzyme - galactosidase. The IPTG induces the production of the functional galactosidase that cleaves X- gal and results in the blue colored metabolite. There was no statistical significance between the number of white and blue colonies on DYSP medium and the conventional 2YT medium considering the transformation efficiency obtained. It follows that the DYSP medium can be used in gene manipulations since transformed *E. coli* cells grew well on DYSP and was quite comparable to that on 2YT.

Fermentation tests with *P. chrysogenum* yielded positive results in the production of penicillin, as reported elsewhere with industrial food wastes (El-Marsaf et al., 2001). The DYSP medium supports the growth of *P. chrysogenum* with concomitant production of penicillin in a growth-dependent manner. DYSP medium has the potential to be a valuable supplement for use in penicillin production and other microbiology laboratory investigations. The DYSP medium is recommended for preliminary routine rudimentary experiments before finally using commercial types for such purposes. It can be noted that the DYSP medium can be used in secondary education for basic microbiology experiments without incurring huge costs on purchasing media reagents.

Finally, the cost of DYSP production was not considered in this study. It remains to be optimized and determined whether supplementation of DYSP medium would be economic under commercial conditions.

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