Potential of Banana Fruit Peel Extract-Based Agar as Isolation Medium for Microorganisms

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Abstract— Microorganisms need nutrients and certain environmental conditions in order to grow and reproduce. Culture media used in the laboratory for the cultivation of microorganisms supply the nutrients required for growth and maintenance. As the readily available culture media are expensive, there is a need to develop cheaper and natural source of alternative media for laboratories with less facility.This study aimed to determine the viability of Musa paradisiaca fruit peel extract-based agar as primary isolation medium for selected microorganisms. Six microorganisms were tested in this study, namely; Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Candida albicans. Two agar preparations were made: one preparation from aqueous extract of the fruit peel of ripe Musa paradisiaca and the 95% alcohol extract of the oven-dried fruit peel of ripe Musa paradisiaca.These extracts were prepared in four concentrations namely; 25%, 50%, 75% and 100% and were incorporated in a pharmaceutical agar to form the Musa paradisiaca extract-based agar. The pH of the prepared media was adjusted to pH 6.0, 6.5, 7.0, and 7.5 before sterilization and inoculation. The growth of the microorganisms was determined through the colony count after 24 hours of incubation at 370C. Results of the experiment revealed that not all the microorganisms cultured on agar containing different concentrations of aqueous and ethanol Musa paradisiaca extract at different pH levels exhibited growth. However, growth is more pronounced in the culture medium with 75% extract of Musa paradisiaca.Moreover, it is concluded that ethanol extract based-agar at 75% concentration at pH 7.0 can be used as a cheap and cost-effective isolation medium for non-fastidious bacteria. Similar extract concentration of 75% at pH 6.0 can be used also in the culture of fungi, particularly Candida albicans.

Keyword: based-agar, culture medium, isolation medium, and non-fastidious

INTRODUCTION

Banana is a tropical fruit cultivated by most farmers in developing countries. According to the latest agricultural data, the Philippines ranks fourth among the major banana producing countries in the world. It is a top dollar earner for the country with an agricultural production of 9.45 million metric tons in 2015 valued at P59.48 billion (Smith, 1987).

The peel which shields the banana fruit is thrown away as rubbish after the inner flesh portion is consumed. Banana peels are major agricultural wastes. Wastes are materials that have not yet been fully utilized. They are leftovers from production and consumption. However, waste is an expensive and sometimes unavoidable result of human activity. The disposal of agricultural wastes on land and into water bodies is common and has been a serious ecological hazards (Ofuya, 1990).

In developing countries, there is a growing interest regarding the utilization of organic wastes generated by the food processing sector and through other human activities so that little or no residue is left to pose pollution problems (Famurewa, 2008).

In general, banana peels are highly perishable and are considered a problem to the processing industries and pollution monitoring agencies. This environmental problem has something to do with its high nitrogen and phosphorus content. But this problem can be recovered by utilizing its high value compounds, including the dietary fiber fraction that has a great potential in the preparation of locally-made culture media.

The soaring cost of culture media have motivated the search for new formulations that support microbial growth. The development of culture media from economical bio-products, which are able to support and carry out nutritional requirements for microbial growth, is currently receiving remarkable research attention. Different materials derived from plants, such as groundnut, sorghum extracts, cassava, whey, cereals, and local food waste have been used to create microbiological growth media (Molina, 2015).

The increased production of banana fruit peels in the Philippines and other countries requires the development of sustainable technological applications for this waste material. The presence of carbohydrates and other micronutrients in banana peel make it an attractive source for the preparation of a culture medium for microorganisms.

This study aimed at developing a culture medium from banana fruit peel that allowed the primary isolation of six non-fastidious microorganisms. Specifically, it sought to determine the: (a) colony growth characteristics of the microorganisms grown on the aqueous and ethanol extracts of the banana (Musa paradisiaca) fruit peel; (b) colony count of the microorganisms grown on the different concentrations of the aqueous and ethanol Musa paradisiaca fruit peel extract-based agar at pH 7.0; (c) significant variations in the colony count of microorganisms grown on the aqueous and ethanol Musa paradisiaca fruit peel extract-based agar at 75% concentrations and at different pH at 370C; and (d) formulation of the Musa paradisiaca fruit peel extract-based agar that can be used as primary isolation medium for microorganisms.

METHODOLOGY

This study utilized the experimental research design conducted in actual laboratory setting. The experimental set-up required the use of Musa paradisiaca fruit peel extracts – aqueous extract and alcohol extract. Four (4) concentrations (100%, 75%, 50%, and 25%) were prepared from each extract of the banana fruit peel. These different concentrations of the banana fruit peel extracts were integrated in the pharmaceutical agar. The final pH was adjusted to 6.0, 6.5, 7.0, and 7.5.

Pure cultures of clinical isolates of Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes were acquired from a private tertiary hospital while Pseudomonas aeruginosa (ATCC 27853), Salmonella typhi (ATCC 19430) and Candida albicans (ATCC 90028) were acquired from the Department of Medical Microbiology, College of Public Health, University of the Philippines, Manila.

Bacterial Suspension

Tube serial dilution using Normal Saline Solution (NSS) was performed on the six organisms used in the experiment.

Standardization of inoculum utilized the McFarland's Nephelometry method wherein a loopful of inoculum was mixed to homogeneity (by manual shaking) with NSS, serially diluted and the turbidity of the suspension was standardized using McFarland's Standard Tube 1.

Pour Plate Technique

One hundred microliters of each standardized suspension of the 6 organisms was transferred aseptically into 10 petridishes per concentration and per pH level. Fifteen millilitres each of the prepared extract-based agar was dispensed on each of the petridishes. After mixing, the preparation was incubated at 370C. The growth of the microorganisms was determined through colony count after 18-24 hours of incubation.

Data Processing

Descriptive statistics (mean and standard deviation) were computed and later used for making statistical inferences. One-way ANOVA was done to compute the significant F-Test, which indicated the presence of true difference between readings of the experimental and control groups. Post hoc tests were employed only when F-tests were significant.

RESULTS AND DISCUSSION

Bacterial Colony Characteristics

Table 1 presents the characteristics of the colonies of microorganisms grown on the Musa paradisiaca aqueous fruit peel extract-based agar.

Table 1
Characteristics of the Colonies of Microorganisms Grown on the Musa paradisiaca Aqueous Fruit Peel Extract-Based Agar

Microorganisms	Colony Characteristics				
	Size	Shape	Color	Margin	Texture
Staphylococcus aureus	Pinhead	Circular	Tan	Entire	Rough, shiny
Streptococcus pyogenes	Pinpoint	Circular	White	Entire	Smooth, shiny
Escherichia coli	Pinpoint	Circular	Colorless	Irregular	Smooth, shiny
Pseudomonas aeruginosa	Pinhead	Circular	Colorless	Entire	Smooth, shiny
Salmonella typhi	Pinpoint	Circular	Colorless	Entire	Smooth, shiny
Candida albicans	Pinpoint	Circular	White	Entire	Smooth, shiny

Four of the test organisms (Steptococcus pyogenes, Escherichia coli, Salmonella typhi and Candida albicans) produced pinpoint, circular, smooth and shiny colonies while Staphylococcus aureus and Pseudomonas aeruginosa exhibited pinhead and round colonies with entire margins on Musa paradisiaca fruit peel aqueous extract-based agar. Surprisingly, Staphylococcus aureus produced tan (yellow) pigment with rough texture. Most of the colonies were non-chromogenic. These results agreed well with the study of Molina (2015) when she used Smallanthus sonchifolius extract-based agar to isolate some of the organisms used in the experiment.

Similarly, all organisms exhibited round with entire margin colonies on Musa paradisiaca fruit peel alcohol extract-based agar. In terms of size, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa, produced medium-sized colonies described as pinhead. These results are in agreement with Delost (1997). Interestingly, Staphylococcus aureus and Pseudomonas aeruginosa exhibited yellow and light-blue colonies respectively. These results agreed well with the ones obtained in the Nutrient agar (control) preparations and in most literature. The ability of the organisms to produce certain pigment caused the coloration of the colonies and this can be an important tool in the preliminary identification of the organisms. Pseudomonas aeruginosa has the ability to produce two pigments, pyocyanin (blue) and pyoverdin (green), and this caused the production of light-blue colonies when inoculated on the banana extract-based agar.

Table 2 presents the characteristics of the colonies of microorganisms grown on the Musa paradisiaca ethanol fruit peel extract-based Agar.

Table 2
Characteristics of the Colonies of the Microorganisms Grown on the Musa paradisiaca Ethanol Fruit Peel Extract-Based Agar

Microorganisms	Colony Characteristics				
	Size	Shape	Color	Margin	Texture
Staphylococcus aureus	Pinhead	Circular	Yellow	Entire	Rough, shiny
Streptococcus pyogenes	Pinpoint	Circular	White	Entire	Smooth, shiny
Escherichia coli	Pinhead	Circular	Colorless	Irregular	Smooth, dull
Pseudomonas aeruginosa	Pinhead	Circular	Light blue	Entire	Smooth, shiny
Salmonella typhi	Pinpoint	Circular	Colorless	Entire	Smooth, shiny
Candida albicans	Pinpoint	Circular	White	Entire	Smooth, shiny

As further observed, organisms plated on the alcohol extract-based agar produced better growth characteristics than the organisms cultivated on aqueous extract-based agar of the Musa paradisiaca. This could be attributed to the better extracting power of alcohol than that of the distilled water. There were phytochemical components in the banana peel that are not water-soluble and as such, could not be extracted by merely using water and are best extracted using the 95% ethanol. This potent ability of the ethanol as extracting agent was confirmed by the growth proliferation of the different organisms used in the study. It can be inferred that in using ethanol, more sugars, proteins and microelements were extracted which are vital to the growth and development of microorganisms. Accordingly, with more nitrogen, carbohydrates and micronutrients extracted, various cellular organelles are readily developed and the bacterial cell rapidly divided.

Bacterial Colony Count

Table 3
Colony Count of Microorganisms Grown in Four Different Concentrations of the Aqueous Extract of Musa paradisiaca Fruit Peel-Based Agar at pH 7.0 and at 370C

Microorganisms	Concentrations (%)				Control (Nutrient agar)
	25	50	75	100	
Escherichia coli	3	7	16	7	36
Staphylococcus aureus	5	9	27	12	41
Streptococcus pyogenes	3	12	19	12	35
Pseudomonas aeruginosa	5	12	17	10	28
Salmonella typhi	5	13	18	10	31
Candida albicans	5	6	23	24	43

Colony count of the various test organisms in the aqueous-extract based agar of the Musa paradisiaca at pH 7.0 and at 370C is presented in Table 3 and Figure 3. Generally, as the concentration of the extract increases, the colony count also increases. However, the exponential increase in the colony count stops at 75% extract concentration. At 100%, the colony counts of all the organisms declined. All the test microorganisms (both the bacteria and the fungus) registered favorable growth, with Candida albicans and Staphylococcus aureus having the highest number of colonies at 75%. Surprisingly for Candida albicans, as the concentration of the extract increases, the colony count also increases even beyond 75% concentration. This result is contrary to the growth patterns of bacteria plated at different extract concentrations which declined at 100% concentration. The antibacterial action of the banana extract may have affected the growth of the test organisms.

Figure 3 below elaborates further what is contained in Table 3:

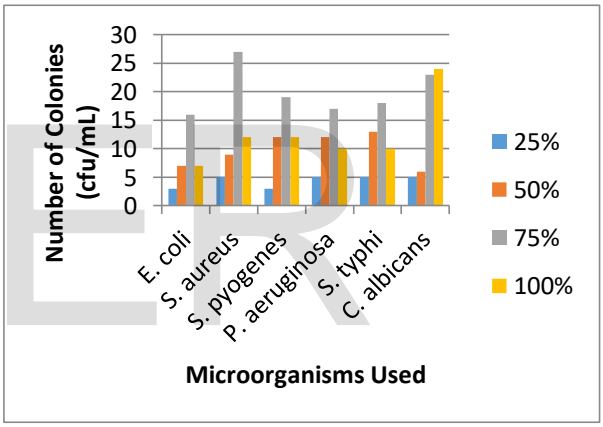

Figure 3. Colony count of microorganisms at different aqueous extract concentrations at pH 7.0 and at 370C.

Table 4 and Figure 4 below present the colony count of microorganisms at different ethanol extract concentrations at pH 7 and at 370C.

Table 4
Colony Count of Microorganisms Grown in Four Different Concentrations of the Alcohol Extract of Musa paradisiaca Fruit Peel-Based Agar at pH 7.0 and at 370C

Microorganisms	Concentrations (%)				Control
	25	50	75	100	
Escherichia coli	8	11	33	10	47
Staphylococcus aureus	15	18	36	16	53
Streptococcus pyogenes	8	15	29	13	32
Pseudomonas aeruginosa	10	19	33	11	38
Salmonella typhi	10	20	34	17	32

Candida albicans	10	15	31	33	43

As observed in Table 4, the 75% extract-based agar exhibited the highest number of colonies. The 100% extract is surprisingly less effective than the 75% as manifested by fewer colony counts. This phenomenon can be explained by the presence of bacterial activity in the banana peel. Obviously, the 100% extract has greater antibacterial effect than the other concentrations causing greater growth inhibition among the test organisms. When the 100% extract was diluted to form the 75% and 50%, the antibacterial effect was lessened due to the dilution effect. The antibacterial actions of the banana peel extract is attributed to the presence of phytochemicals such as flavonoids, phenols, and tannins. The mechanism of action of flavonoid and tannin on the inhibition of bacterial cultures could be credited in the alteration of the cellular membrane resulting in the escape of cellular components which could possibly be in the form of structural degeneration which is beyond repair due to cell leakage as observed by irregular, wrinkle shape and loss in rigidity.

Likewise, drying the banana peel prior to extraction concentrated the chemical components present through the process of dehydration. Drying removed the water content of the plant material that comprised about 90 percent of the total weight of the fresh peel. During the extraction process, phytochemicals could be readily extracted in large amounts resulting to the enrichment of the culture medium.

The colony count of the microorganisms grown in the 4 concentrations of the alcohol extract of the Musa paradisiaca fruit peel-based extract at pH 7.0 and at 370C

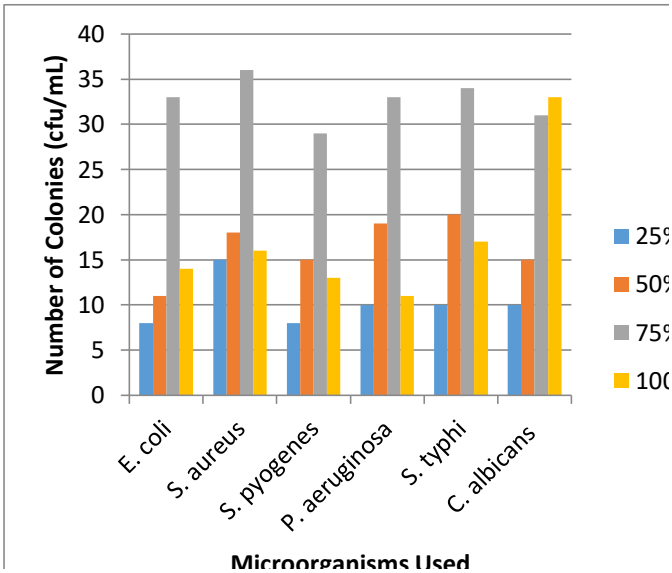

Figure 4. Colony count of microorganisms at different ethanol extract concentrations at pH 7 and at 370C.

Figure 4 shows that colony counts increased as the concentrations increased from 25% to 75%; but growth rate declined at 100% concentration. The 75% extract concentration exhibited the highest colony count among the test bacteria. However, the colony count of C. albicans remained high even at 100% extract concentration. Candida albicans and Salmonella typhi registered the greatest number of colonies among the test organisms at 100% concentration. Both organisms require complex nutrients, like amino acids and vitamins especially thiamine and niacin for their development (Gretler, et al., 2010). Aside from the carbohydrates content, the banana peel extract also contains high thiamine and niacin which enabled the proliferation of these organisms. Candida albican has the ability to convert organic materials in the extract to organic acid thereby creating an acidic environment which favored its optimum growth and development.

Variations in the Colony Count of the Microorganisms

Table 5
Variation in the Colony Count of Microorganisms Grown in 75% Aqueous Extract of Musa paradisiaca Fruit Peel-Based Agar at Different pH and at 37 0C

Variables	Df	Critical Value (at α 0.05)	Statistics *F*	Analysis	Decision
Colony Count at 75 % Aqueous Extract pH 6.0	5/54	2.39	87.19	*Significant*	*Reject H_0*
Colony Count at 75 % Aqueous Extract pH 6.5	5/54	2.39	52.58	*Significant*	*Reject H_0*
Colony Count at 75 % Aqueous Extract pH 7.0	5/54	2.39	42.55	*Significant*	*Reject H_0*
Colony Count at 75 % Aqueous Extract pH 7.5	5/54	2.39	139.80	*Significant*	*Reject H_0*

Table 6
Variation in the Colony Count of Microorganisms Grown in 75% Ethanol Extract of Musa paradisiaca Fruit Peel-Based Agar at Different pH and at 37 0C

Variables	Df	Critical Value (at α 0.05)	Statistics *F*	Analysis	Decision
Colony Count at 75% at pH 6.0	5/54	2.39	3489.30	*Significant*	*Reject H_0*
Colony Count at 75% at pH 6.5	5/54	2.39	11.44	*Significant*	*Reject H_0*
Colony Count at 75% at pH 7.0	5/54	2.39	18.96	*Significant*	*Reject H_0*
Colony Count at 75% at pH 7.5	5/54	2.39	367.14	*Significant*	*Reject H_0*

Tables 5 and 6 present the variation in the colony counts of the test organisms grown in the 75% aqueous and ethanol extracts of the Musa paradisiaca fruit peel-based agar at different pH and at 370C.

Both tables show that significant variations exist in the number of colonies of the microorganisms as documented by their computed F-values as compared to the critical F-value of 2.39 (all pH conditions) at 0.05 level of significance. This indicates that the microorganisms cultivated at pH 7.0 and pH 6.5 culture media produced the most number of colonies as compared to pH 6.0 and pH 7.5. This finding is not surprising since microorganisms grow optimally at a neutral or nearly neutral pH levels.

Formulation of the Musa paradisiacal-Based Agar:

Based on the result of the study, the researcher proposes the following formulation in the preparation of the Musa paradisiaca-based agar:

75% ethanol extract of the banana pee.....750 mL
NaCl..... 5 grams
Agar.....15 grams
Distilled water.....1.0 liter
pH.....7.0/6.0
(pH 7.0 for bacteria and pH 6 for fungi)

This formulation is prepared from ethanol extraction of the dried banana fruit peel. The pulverized raw material is treated with 95% analytical grade ethanol in 1:2 solute to solvent ratio and allowed to stand for 72 hours. The mixture is filtered using a Whatman filter paper and concentrated in a rotary evaporator. Seventy-five percent (75%) concentration of the extract is obtained through dilution and incorporation in a pharmaceutical agar. To sustain maximum growth, the microorganisms are incubated overnight at 370C.

CONCLUSION

Based on the findings of the study, the researcher concluded the following:

Ethanol Musa paradisiaca fruit peel extract-based agar can be used as a cheap and cost-effective primary isolation medium for microorganism. Seventy five percent (75%) of the ethanol Musa paradisiaca fruit peel extract may be incorporated in a pharmaceutical agar to make a primary isolation medium for the growth of Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi.

One hundred percent (100%) of the ethanol Musa paradisiaca fruit peel extract may be incorporated in a pharmaceutical agar to make a primary isolation medium for the growth of Candida albicans.

RECOMMENDATIONS

Based on the findings of the study, the researcher strongly recommends that further investigation on the use of other parts and varieties of banana plant to formulate a bacterial or fungal culture media be conducted; that utilization of other non-fastidious bacteria and fungi to be grown in the Musa paradisiaca fruit peel extract-based agar be considered; and the use of Musa paradisiaca fruit peel extract-based agar as a selective or inhibitory culture media be further explored.

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