Test Some New Media for Cultivation of Gram Positive and Gram Negative Bacteria

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Abstract

Possibility of using the wastes of used paper to prepare a culture medium for bacterial growth was carried out in this study, five experimental culture media (paper waste extract agar, glucose paper waste extract agar, yeast extract paper waste extract agar, NaCl paper waste extract agar and GYN paper waste extract agar) that made from the extract of used papers were prepared to cultivation two species of Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus* spp.) and two species of Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) in comparison with readymade media (nutrient agar and brain-heart infusion agar). Heavy growth of bacteria was appeared on yeast extract paper waste extract agar and GYN paper waste extract agar media, while no growth was happen on the other three experimental culture media. The results of statistical analysis found that there is no significant different at $P \leq 0.05$, using LSD test among yeast extract paper waste extract agar , GYN paper waste extract agar and nutrient agar or brain-heart infusion agar media .These results indicate likelihood of using two experimental culture media instead of nutrient agar or brain-heart infusion agar to cultivation of bacteria.

Keywords: *Culture medium, used paper, bacterial growth, paper wastes*

Introduction

Culture medium is the medium containing all the essential nutrients for growth of microorganisms [(1)],including sources of carbon, nitrogen, , phosphorus, energy and different minerals [(2)] .The main purpose of it is either to grow a specific microorganism or to study the biochemical characteristics of bacteria particularly the pathogenic one [(3)].Different culture media are used in the laboratory [(4)], whilst media are used to support the growth of many types of microorganisms such as nutrient agar, others are particularly designed for the isolation and identification of specific kinds [(5)]. Generally, there are three commonly kinds of media; differential, enriched and selective media [(3)]. Differential media are used to cultivation more than one bacteria of interest but distinguish between them whose growth they support, usually by means of a coloured indicator such as MacConkey agar [(5)]. Enriched media are used to support the growth of fastidious bacteria such as blood agar [(3)] and the selective media are preferentially support the growth of specific bacteria, such as mannitol salt agar [(6)].

Many researchers mentioned using of plant materials as components to prepare culture media for microorganisms, Osman *et al*. [(2)] used many fresh vegetables, legumes and seeds as promoters to growth of some fungi like *Curvularia lunata, Aspergillus niger*, and *Fusarium oxyaporum*. Arulanantham *et al*. [(4)] used several protein sources; namely legume seeds, cowpea, soya meat (processed soya bean), green gram, and black gram for cultivation of some microorganisms. Kadhim and Ali [(7)] reported using the leaves and stems of *Portulaca oleracea oleracea* L. for growing

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of some pathogenic bacteria such as *Escherichia coli, Pseudomonas flouresence, Staphylococcus aureus , Klebsiella pneumoniae , Proteus mirabilis* and *Proteus vulgaris*. Tzintzun-Camacho *et al*. [8] designed a culture medium from hydrolysed avocado seeds waste for growth of *E. coli*.

Paper is known as a mixture of organic materials (cellulose, hemi-cellulose, lignin, and different compounds of lignin) and inorganic or non-fibrous filling and loading materials like clay, calcium carbonate and titanium oxide depending on the type of paper. The previously neglected products of paper or paperboard is known as waste paper [9] .To our knowledge, there is no study about using the paper wastes as an alternative medium for growing of bacteria. So, the aim of the present study is to evaluate the bacterial growth on the wastes of used paper as a culture medium in comparison with nutrient agar and brain-heart infusion agar media.

Materials and Methods

Bacterial isolates:

Staphylococcus aureus, *Streptococcus* spp., *Escherichia coli*, and *Pseudomonas aeruginosa* were obtained from department of Biology, College of Science, Al-Mustansiriya University, Baghdad, Iraq.

Paper waste extract:

It was prepared by mixing 5 kg of paper waste pieces with 10 liters of distilled water; the mixture was left for 2 to 4 hours and then filtered, the extract was used to prepare culture media used in this study.

Preparation of culture media:

a- Paper waste extract agar medium: it was made by adding 500 ml of paper waste extract to 20 g of agar-agar (Hi-Media, India) and then the volume was completed to 1 liter with distilled water.

b- Glucose paper waste extract agar medium: it was made by adding 500 ml of paper waste extract to 10 g of glucose (BDH, England) and 20 g of agar-agar and then the volume was completed to 1 liter with distilled water.

c- Yeast extract paper waste extract agar medium: it was made by adding 500 ml of paper waste extract to 5 g of yeast extract (Hi-Media, India) and 20 g of agar-agar and then the volume was completed to 1 liter with distilled water.

d- NaCl paper waste extract agar medium: it was made by adding 500 ml of paper waste extract to 1 g of sodium chloride (BDH, England) and 20 g of agar-agar and then the volume was completed to 1 liter with distilled water.

e- GYN paper waste extract agar medium: it was prepared by mixing 500 ml of paper waste extract, 10 g of glucose, 5 g of yeast extract, 1 g of sodium chloride and 20 g of agar-agar and then the volume was completed to 1 liter with distilled water.

f- Brain-heart infusion agar (BHIA) and nutrient agar (NA) (Hi-Media, India) were prepared according to the manufacturing company instructions.

The pH value for all the media was adjusted at 7.2 and autoclaved at 121°C for 15 minutes and then used for cultivation of *S.aureus , Streptococcus spp., E.coli , and P.aeruginosa*.

Growing of bacteria on culture media:

Serial decimal dilutions (10^{-1} – 10^{-6}) were made for each bacterial species namely; *S.aureus*, *Streptococcus spp., E.coli*, and *P.aeruginosa* by using normal physiological saline solution. Spread-plate method was used [3] , in brief ; 0.1 ml from each dilution was spread separately on the following media: paper waste extract agar, glucose paper waste extract agar, yeast extract paper waste extract agar, NaCl paper waste extract agar, GYN paper waste extract agar, BHIA medium and NA medium and then incubated at 37°C for 24 h. After incubation, the numbers of colonies was counted and the results were recorded.

Statistical Analysis

Different between means of culture media for each bacterial species have analyzed using least significant differences (LSD) at $P \leq 0.05$ and complete randomized design (CRD) to evaluate the bacterial growth on the culture media. Using SPSS program 2010 and excel application to find the results and charts [10, 11].

Results and Discussion

Culture medium is used to cultivate numerous kinds of microbes, it is formed of numerous nutrients which are necessary for microbial growth depending on the requirements of growth of each microbe, when culture media are formed from all nutrients that most bacteria consume for growth and aren't selective for specific type of bacteria, they called general media.

In the present study, in order to evaluate five experimental culture media prepared from the wastes of used paper in comparison with readymade media; nutrient agar (NA) and brain-heart infusion agar (BHIA), *S.aureus , Streptococcus spp., E.coli ,* and *P.aeruginosa* were cultivated on these media using spread-plate method. The results showed that yeast extract paper waste extract agar and GYN paper waste extract agar were excellent as culture media (Figures 1 and 2), since heavy growth of bacteria was appeared on them indicate that all the important and essential nutrients requirements for bacterial growth is available in these media. The presence of used paper extract with yeast extract only was enough to obtain copacetic growth for both Gram positive and Gram negative bacteria.

On the other hand, the presence of paper extract alone or with glucose or sodium chloride failed to stimulate bacterial growth and thus there is no growth was happen on the paper waste extract agar, glucose paper waste extract agar, and NaCl paper waste extract agar. It could be because the absence of the some essential nutrients for growth.

Statistical analysis showed no significant differences among yeast extract paper waste extract agar, GYN paper waste extract agar and NA or BHIA media at $P \leq 0.05$ (Tables 1 and 2). So, both of the experimental culture media can be used instead of NA or BHIA to cultivation of Gram positive and Gram negative bacteria.

NA is one of the general media used for cultivation of wide range of non-fastidious bacteria. It is formed of peptone as a source of organic nitrogen, beef or yeast extract, agar and NaCl [12]. BHIA is a rich medium used for cultivation of numerous fastidious microbes (e.g. *Streptococci*, *Pneumococci* and *Meningococci*). BHI is formed by gathering an infusion of boiled bovine or porcine brain and heart (as a source of amino acids), in addition to numerous other nutrients, salts, Na_2HPo_4 as a buffer, and glucose as a source of carbon [13].

The primary structural element of paper products is cellulose fibers [14]. Cellulose is a linear polysaccharide of glucose residues linked by β-1, 4-glycosidic linkages. Different accommodation products are made from cellulose, but unfortunately a lot of cellulosic wastes are used for burning especially in developing countries, which represent a global concern. Cellulolytic bacteria convert cellulose to glucose which is a multi- use material for many different cheaper and biological favorable processes [15]. As all know, glucose is the simplest carbon source for many bacteria due to its ability to consume simply and production of energy for a faster bacterial growth [16]. For nitrogen source ammonia is the preferred nitrogen source especially for *E. coli* [17]. Yeast extract is considered as stimulant for microbial growth as mentioned by [1].It is autolyzed formed of yeast cells, soluble in water due to strict control in order to maintain B complex vitamin which considered as bacterial growth stimulator [18].

So, depending on the results of this study, we can conclude that extract of used paper could be used as a culture medium for cultivation of bacteria, just like other general media like nutrient agar, especially after addition of the other important nutrients like yeast extract. By this way we could benefit from paper wastes without increasing environmental pollution and prepare new substitute media as the synthetic commercial media. Future studies are needed to create new media from different environmental wastes to culture numerous microbes.

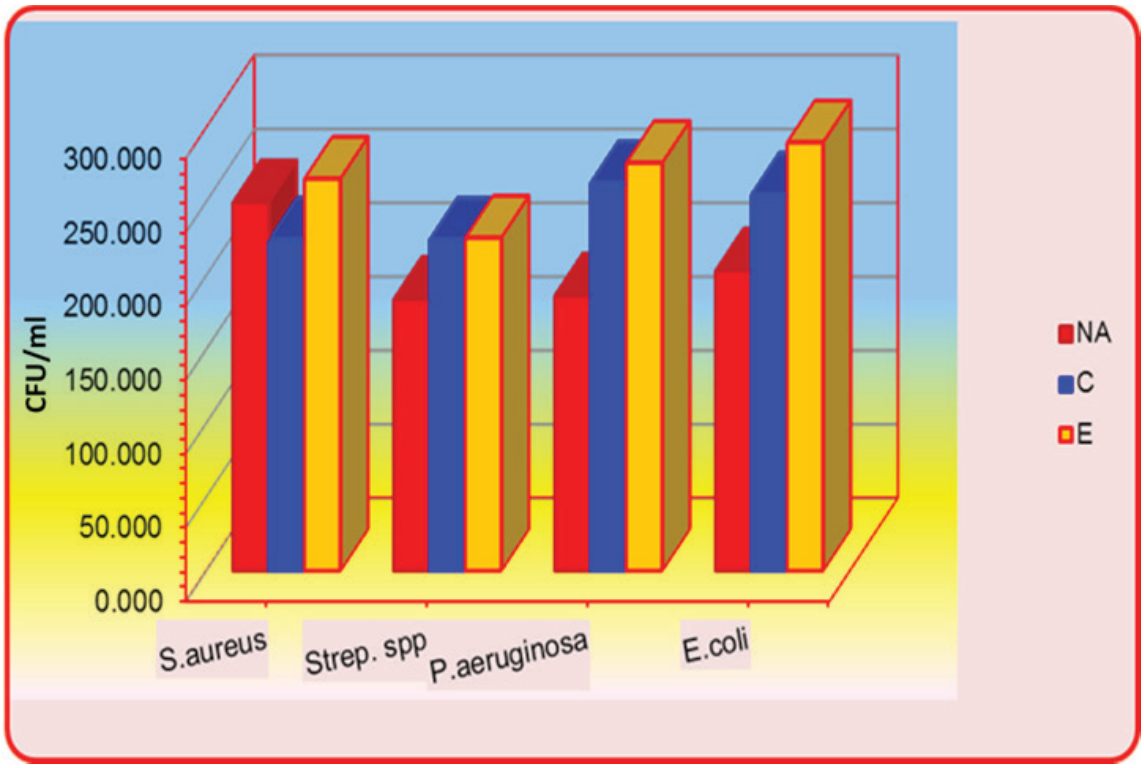

Figure 1: Growth of *Staphylococcus aureus* , *Streptococcus* spp., *Escherichia coli* , and *Pseudomonas aeruginosa* on the culture media, NA, Nutrient agar; C, yeast extract paper waste extract agar ; E, GYN paper waste extract agar

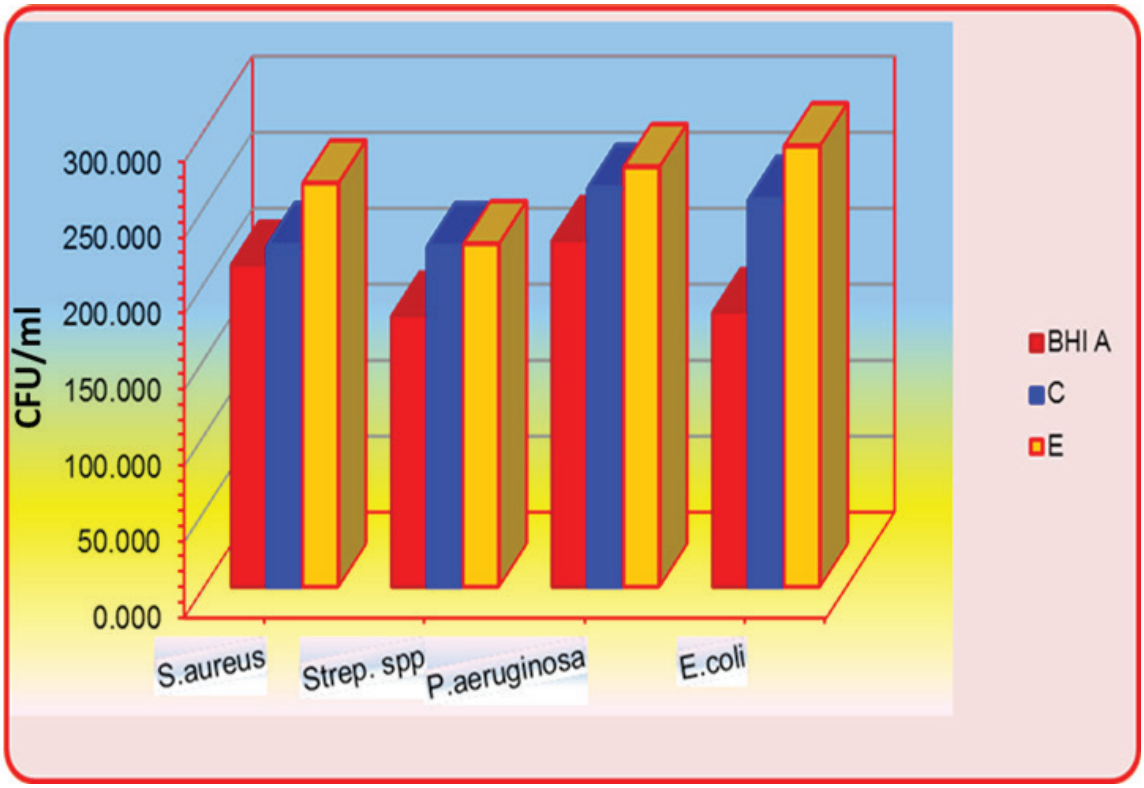

Figure 2: Growth of *Staphylococcus aureus , Streptococcus spp., Escherichia coli* , and *Pseudomonas aeruginosa* on the culture media, BHIA, Brain-heart infusion agar; C, yeast extract paper waste extract agar ; E, GYN paper waste extract agar

Table 1: Growth of bacteria on the culture media

Bacteria Culture media	S.aureus	Strep. spp	P.aeruginosa	E.coli
NA	249.50 a	184.00 a	186.33 a	203.17 a
	±	±	±	±
	36.15	40.59	48.74	50.91
C	224.17 a	224.00 a	262.33 a	254.50 a
	±	±	±	±
	31.16	41.47	33.06	35.59
E	265.50 a	225.50 a	276.17 a	290.00 a
	±	±	±	±
	33.25	48.03 a	18.55	18.05
LSD P ≤ 0.05	101.208	131.068146	107.4382	112.5516

NA= Nutrient agar; C,= yeast extract paper waste extract agar ; E= GYN paper waste extract agar.

The letters indicate to comparison between means in each column, and the similar letters are not significant different at (P≤0.05),using (LSD test).

Table 2: Growth of bacteria on the culture media

Bacteria Culture media	S.aureus	Strep. spp	P.aeruginosa	E.coli
BHI A	211.83 a	178.33 a	228.00 a	180.67 a
	±	±	±	±
	56.58	31.92	44.51	56.27
C	224.17 a	224.00 a	262.33 a	254.50 a
	±	±	±	±
	31.16	41.47	33.06	35.59
E	265.50 a	225.50 a	276.17 a	290.00 a
	±	±	±	±
	33.25	48.03	18.55	18.05
LSD P ≤ 0.05	126.408668	123.596707	101.7321	120.0244

BHIA= Brain-heart infusion agar; C= yeast extract paper waste extract agar ; E= GYN paper waste extract agar. The letters indicate to comparison between means in each column, and the similar letters are not significant different at (P≤0.05),using (LSD test).

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