The beetroot juice as a bacterial growth and maintenance medium for many pathogenic bacteria.

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Abstract

In a pioneer study in Iraq, a water extract (juice) from the root of *Beta vulgaris* (beetroot) was prepared under sterile conditions, then used for the first time as experimental bacterial growth medium for the growth of the bacteria: *Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae*; the juice was used as alternative growth medium for the nutrient agar and nutrient broth for the growth of these genera in the laboratory.

All the genera showed active growth after (24) hours when the juice used directly as a liquid growth medium, the medium preserve these bacteria viable for (7) days. The juice was used also to enrich the agar-agar and cultured with the same bacteria; it showed a noticeable growth lasted for (10) days. The results suggest that this juice is a suitable growth medium for these bacteria, and it could be used instead of the growth media that mentioned above which used in the cultivation of these bacteria in the laboratory. It also represents important, rich nutritional environment not less important than those that is found in the other media.

عصير نبات الشوندر كوسط لنمو وديمومة عدة أجناس من البكتريا المرضية

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الخلاصة

تم تحضير مستخلص مائي (عصير) من جذور نبات الـشوندر (أو الـشمندر) *Beta vulgaris* تحت ظروف معقمة، ثم استخدم لأول مرة في هـذا البحـث كوسـط زرعي تجريبي لتنمية البكتريا:

Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia.

استُخدِم هذا العصير كوسط تنمية بديل عن وسط آلاگار المغـذي Nutrient agar ووسط المرق المغذي Nutrient broth لتنمية هذه الاجناس في المختبر.

أظهرت جميع أنواع هذه البكتريا نمواً فعالاً بعد 24 ساعة من الحـضن عنـد استعمال العصير كما هو بشكله السائل وأمكن المحافظة عليها حية فيه لمدة 7 أيام، كما استعمل العصير في اغناء الاكار agar-agar وزرعت عليـه نفـس انـواع البكتريـا وأظهرت نمواً ملفتاً للانتباه واستمرت حيويتها فيه لمدة 10 أيام، تقترح النتائج ان هذا العصير يوفر وسطاً مناسباً لتنمية هذه البكتريا وممكن الاستعاضة بـه عـن الأوسـاط الزرعية المذكورة أعلاه التي تستعمل لتنمية هذه البكتريا في المختبر. كما يـوفر هـذا العصير بيئة مغذية غنية و مهمة لا تقل أهمية عن البيئات المغذية التي توفرها الأوساط الأخرى.

Introduction

For any bacterium to be propagated for any purpose it is necessary to provide the appropriate biochemical and biophysical environment. The food base that supports the growth of an organism is called culture medium; the biochemical (nutritional) environment is made available in this culture medium (5; 6; 17; 18).

A growth medium is a mixture of nutrients, moisture and other chemicals that bacteria need for growth in a laboratory environment. The food base depending upon the special needs of particular bacteria (as well as particular investigators), so that a large variety and types of culture media have been developed with different purposes and uses. These include sources of organic carbon, nitrogen, phosphorus, sulfur and metal ions including iron (5; 6; 8; 15; 17; 18). Culture media are employed in the isolation and maintenance of pure cultures of bacteria and are also used for identification of bacteria according to their biochemical and physiological properties (6; 17; 18).

The manner in which bacteria are cultivated, and the purpose of culture media, varies widely. Media can be solid, such as Jell-O-like agar that is poured into the bottom half of a Petri dish, or media can be liquid to allow for bacterial growth in test tubes (5; 6; 15; 17; 18). Liquid media are used for growth of pure batch cultures, it include media may be made from animal tissue and fluids or from vegetable tissue(6; 18). Solidified media are used widely for the isolation of pure cultures, for estimating viable bacterial populations, and a variety of other purposes; the usual gelling agent for solid or semisolid medium is agar, a hydrocolloid derived from red algae. Agar is used because of its unique physical properties (it melts at 100^{o}C and remains liquid until cooled to 40^{o}C, the temperature at which it gels) and because it cannot be metabolized by most bacteria(6; 17; 18).

Basically, the culture media are of three types: natural, synthetic and a complex (undefined) media. Natural medium is that which contains the natural products such as, for example diluted blood, urine, milk, vegetable juices, peptone or animal cells/tissues/organs. In such medium the exact chemical composition is not known. A synthetic medium is one chemically-defined in which the exact chemical composition and concentration is known. A complex (undefined) medium is one in which the exact chemical constitution of the medium is not known(5; 6; 11; 15; 17; 18). Other

concepts employed in the construction of culture media are the principles of selection and enrichment. An enrichment medium contains some component that permits the growth of specific types or species of bacteria, usually because they alone can utilize the component from their environment(2; 6; 7; 14; 15; 16; 17; 18).

In a pioneer study in Iraq, a water extract (juice) from the root of *Beta vulgaris* was prepared under sterile conditions, then used as an experimental bacterial growth medium to examine its ability to produce a suitable source of nutritional requirements for many types of (Gram +ve) and (Gram -ve) bacteria as alternative growth and maintenance medium for the nutrient agar and nutrient broth that used for the growth of these types in the laboratories; the types of bacteria were: ***Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae***. The juice used into two ways, directly as a liquid growth medium and used to enrich the (agar-agar) as a solidified growth medium. The aim of this study is to find an alternative medium characterized by cheap cost and simple preparation could be used instead of the routinely prepared media that is used in the laboratories.

Beetroot was used in this research because of its nutritional components; it is a rich source of carbohydrates, a good source of protein, has high levels of important vitamins and micronutrients and good source of minerals. All beetroot preparations contain only trace amounts of saturated and mono-unsaturated fatty acids, and 0.1gm of polyunsaturated fatty acid per 100gm. Boiling beetroot increases its carbohydrate and protein content compared to raw beetroot, with a corresponding increase in energy value[12,13]. The root of ***Beta vulgaris*** contains about a tenth portion of pure sugar, which is one of the glucoses or fruit sugars; as well as, it has as much as a third of its weight in starch and gum(9). Chlorine, manganese, traces of selenium occurs with all preparation methods, trace levels of vit. E and biotin occur in all cases and betaine (nitrogenous compound found in beetroot which in its structure like an amino acid). The exact components of the beetroot juice which prepared by the cooking (or boiling) of the root appear in (table, 1) below(10; 13):

Table (1): The components of the cooked (boiled) beetroot per (100gm) of the root.

Components	The value in 100gm	Components	The value in 100gm
Energy	180 kJ (43 kcal)	Vitamin B_6	0.067 mg (5%)
Carbohydrates	9.96 gm	Folate (vit. B_9)	80 µg (20%)
Sugars	7.96 gm	Vitamin C	3.6 mg (6%)
Dietary fiber	2 gm	Calcium	16 mg (2%)
Fat	0.18 gm	Iron	0.79 mg (6%)
Protein	1.68 gm	Magnesium	23 mg (6%)
Vitamin A	2 µg (0%)	Phosphorus	38 mg (5%)
Thiamine (vit.B_1)	0.031 mg (2%)	Potassium	305 mg (6%)
Riboflavin (vit. B_2)	0.027 mg (2%)	Sodium	77 mg (3%)
Niacin (vit. B_3)	0.331 mg (2%)	Zinc	0.35 mg (3%)
Pantothenic acid (B_5)	0.145 mg (3%)		

Materials and Methods

The preparation of the beetroot juice:

The preparation of the juice done according to Al-Azzauy(1). The red root of *Beta vulgaris* were collected and washed carefully with tap water to remove the dust and other materials; these root were peeled and segmented to many small quadrangular segments (or pieces), then 250gm of these segments were putted in 1.5L beaker and 1L of distilled water were poured in the beaker, the mixture were heated for 45minutes in 100˚C. The resulting solution (the juice) refined by the refinery then cooled to room temperature, and then it is filtered by Whatman filter paper; the pH adjusted at 7.4. After then the juice were sterilized by millipore filter 0.22µm pores, putted in sterile bottles and preserved in 4˚C to be ready for use then. It was clear and reddish-brown in color.

The use of the juice in the bacterial growth and maintenance:

1. The juice as liquid growth medium:

The juice were dispensed into many sterile plain tubes (2 ml in each one) and used as a liquid growth medium. The bacteria were selected from 24 hrs. incubated bacterial suspensions, turbidity was visually adjusted to that of (0.5) McFarland turbidity standard 1.5×10^8

Colony Forming Unit (CFU)/ml. The plain tubes of the juice divided into three groups in each group 4 tubes were devoted for each type of the used bacteria; the first group inoculated with 0.1ml of the bacterial suspensions of the standard strains of (***P. aeruginosa*** ATCC 27853***, S. aureus*** ATCC 25923 **and** ***E. coli*** ATCC 25922) which obtained from the central public health laboratory, the second group inoculated with 0.1ml of the bacterial suspensions of **(*P. aeruginosa, P. vulgaris, S. aureus, E. coli, and K. pneumoniae*)** which collected from patients, it was obtained from the laboratory of Al-Yarmuk hospital and were identified by cell morphology, colony morphology, relevant biochemical tests and also Epi system (Oxoid) was used according to the procedure described by Brok *et al.*(3) and Collee *et al.*(4); the third group consists of 4 tubes left without inoculation as a control. The same design was used with nutrient broth for the comparison.

After 24 hrs of incubation at 37˚C the bacterial growth, the color of the medium and the pH of the medium were investigated. The intensity of the growth measured by the turbidity, color and pH change. The viability of the bacteria investigated every day after the preliminary period of incubation (the 24 hrs.) in the experimental medium (the beetroot juice) by subculturing; the purpose of this step were to estimate the ability of this medium in the maintenance of these bacteria.

2. The solidified growth medium (the juice as enrichment substance to the agar-agar):

The preparation of the agar-agar (Biolife company) done by dissolving 10gm of agar-agar powder in 250ml of distilled water, it sterilized by autoclave at 121˚C for 15min. then left to cool for 42˚C and enriched with 250ml of the prepared beetroot juice. The medium then poured into sterile Petri dishes which divided into three groups, in each group 4 dishes were devoted for each type of bacteria; the first group cultured by spreading 0.1ml from the bacterial suspensions by loop, these suspensions prepared from selected colonies of 24-48hrs. nutrient agar incubated cultures for the standard strains of (***P. aeruginosa*** ATCC 27853***, S. aureus*** ATCC 25923 **and** ***E. coli*** ATCC 25922), the inoculums picked up by an inoculating loop to prepare bacterial suspensions its turbidity equal to 0.5 McFarland turbidity standard 1.5×10^8 CFU/ml. The second group cultured by spreading of (0.1)ml of bacterial suspensions 0.5 McFarland turbidity standard 1.5×10^8 CFU/ml by loop for the bacteria **(*P. aeruginosa, P. vulgaris,***

***S. aureus, E. coli, and K. pneumoniae*)** which collected from patients; the third group consists of 4 dishes left with out cultivation as a control. For the comparison, the same design was used with the nutrient agar.

After the incubation at 37˚C for 24hrs. the bacterial growth, the color of the medium and the colonies were investigated. The viability of the bacteria investigated by culturing every day after the preliminary period of incubation (the 24 hrs.).

3. The examination of the growth cells (in the liquid medium) and the colonies (in the solidified medium) using Gram stain and biochemical tests:

After the incubation period and the appearance of the growth in the both experimental media (liquid medium and enriched solidified medium), the necessary biochemical tests and Gram stain were done for the growing bacteria in the two types of the experimental growth medium to ensure that the growth in the experimental media was from the certain selected isolates and not because of any contamination.

Results and discussion

After the incubation period, each type of media (the liquid and enriched solidified media) showed noticeable bacterial growth. The color and pH were changed in the liquid medium; they are synchronized with the average bacterial growth which is detected by the turbidity of the medium. All the used types of bacteria still viable in the experimental liquid medium for 7 days at 37˚C, where they lasting for 7-9 days in the nutrient broth.

The color of the experimental liquid medium converted from mahogany (reddish-brown) to light brown in the tubes inoculated with the bacteria, this is conspicuous in the figures (1,2,3 and 4). The pH of the liquid medium was decreased from 7.4 to 6 after the growth of ***S. aureus, E. coli and K. pneumoniae*** in the medium (become acidic); while decreased to 5.5 in the medium inoculated with ***P. aeruginosa, P. vulgaris***. (Table, 2) summarized these results.

The results suggest that the liquid medium (the juice) is a suitable alternative medium for the nutrient broth in the growth and the maintenance of ***P. aeruginosa, P. vulgaris, S. aureus, E. coli*** and ***K. pneumoniae***; it represents a suitable medium for all the used types of bacteria although it is from plant origin, this fittings very clear from

the intensity of the growth that obtained from the inoculation of the medium with the above types of bacteria which measured by the turbidity, the color and the pH change as it showed in the figures (1, 2, 3 and 4) and (table, 2). Moreover, the medium maintained all the used types of bacteria with full viability for a period approximately similar to the period in the nutrient broth (7 days in the experimental medium at 37˚C compared with 7-9 days in the nutrient broth). This efficiency of the experimental medium interpreted as that this medium provides these types of bacteria with all the essential requirements for their growth because the juice rich with many essential substances for the energy production (such as the amino acids, sugars, carbohydrates) and cell wall construction (such as the fatty acids, carbohydrates) and other metabolic activities of these bacteria, (table, 1)(10; 13).

The experimental medium is clear and has a reddish-brown color; this color resulting from the natural stain that present in the beetroot cells (Anthocyanin stain) which become free in the juice solution after the destruction of the cells by the heat action during the boiling of the beetroot to prepare the juice. The red stain of the red beetroot juice converted to this color because of the stain oxidation. The color of the medium changed slightly after the bacterial growth in the medium as a result of the pH change which result from the bacterial metabolism in the medium, the pH become acidic because of the end metabolic products resulting from the fermentation of the sugars and the carbohydrates which is present in the extract (the juice) components; and because the Anthocyanin color changing with pH, then the stain's color changed since the pH of the medium changed. This change in the color gave a good indicator for the presence of the growth, as well as to the turbidity, from one hand and used as an evidence for the pH change (which then approved by the litmus paper) from the other hand; this character reduced the need for adding any dye to the prepared medium.

The solidified enriched medium showed considerable bacterial growth, the color of the medium changed with the growth; it was converted from yellow to brown or light brown. The color of the bacterial colonies was transparent in all the types of the cultured bacteria, (table, 3). The success of this medium in its job approved by the bacterial growth that obtained after the culturing of the bacteria as it appears in the (figures 5, 6, 7, 8, 9, 10 and 11). All the used types of the bacteria still viable in this medium for 10 days at 37˚C in comparison with 10-12 days in the nutrient agar, that means it was produced a suitable environment for the bacterial growth and met the

nutritional requirements for the growth of these types of bacteria, so it could consider this medium suitable for the maintenance of these types too. The progressive growth of the used bacteria in the enriched medium was because the juice very rich with the nutritional substances as mentioned above which supplies these bacteria with its growth requirements, and indicates that these components meet all the essential requirements for the growth of these types of bacteria. As a conclusion, this experimental medium considers a suitable alternative medium for the nutrient agar which used for the growth of these types especially because the culturing of these bacteria lasted for 10 days and it is very approximate from the period in the nutrient agar.

Although the growth of ***S. aureus*** needs a medium meet their special nutritional and environmental requirements and provide it with suitable growth components similar to those that presents in their infected foci, which may absent in our enriched solidified medium, it showed normal growth and normal survivance in our medium as in the nutrient agar because the beetroot juice has a rich source of protein, high levels of important vitamins, minerals, micronutrients, saturated, mono-unsaturated and polyunsaturated fatty acids, as well as to the other useful components such as carbohydrates, pure sugar, starch, gum, Betaine (the nitrogenous compound in its structure like an amino acid)(10, 13).

The color of the enriched solidified medium before the cultivation with any bacteria was yellow (figure, 5); it is changed slightly after the growth of each of the bacterial types and become light brown to yellowish. The colonies' color of the certain type of the bacteria was not different in all the cultured types in the enriched medium, it was transparent, (table, 2).

The biochemical tests confirmed that the diagnosed samples are the same types of the selected bacteria, this step improve that the obtained growth was not because of any contamination in the medium or during the cultivation of the bacteria.

After the staining of the slides which prepared from the growing isolates in both the liquid and solidified media with Gram stain, the microscopic examination showed (Gram -ve) rods present as single bacteria or in pairs or in short chains in the slides prepared from the cultures of ***P. aeruginosa***; in the slides prepared from the cultures of ***P. vulgaris*** there were (Gram -ve) rods, while in the slides prepared from ***S. aureus*** cultures there were (Gram +ve) cocci single, pairs, tetrads and chains arranged in irregular clusters. The microscopic

examination of ***E. coli*** cultures showed short (Gram -ve) rods; the slides of ***K. pneumoniae*** showed (Gram -ve) rods with large capsule.

As a conclusion, it could use the liquid form of this medium as a liquid culture medium directly instead of the nutrient broth and get very acceptable results. If the juice used as an enriched substance for the agar-agar it produces very suitable source for nutrients and promote the growth of the certain type of the bacteria. It could not consider this medium as a selective medium because it provides a rich nutritional environment for many types of the microbes since it supports the growth of (Gram +ve) and (Gram -ve) bacteria. It also could not consider as a differential medium because it isn't providing colonies with different color according to the type of the cultured bacteria.

Generally, this medium represents a cheap, simple prepared natural medium used for the growth and the maintenance of different type of bacteria and it could use it as alternative medium instead of the routine medium in the growing and the maintenance of many types of bacteria such the types used in this research.

Table (2): The color and pH changes in the liquid medium (the juice) before and after the inoculation with different types of pathogenic bacteria.

Type of Bacteria	The color of the liquid medium (the juice)		The pH of the liquid medium (the juice)	
	Before growth	After growth	Before growth	After growth
Pseudomonas aeruginosa	Reddish-brown	Light brown	7.4	5.5
Proteus vulgaris	=	=	=	=
Staphylococcus aureus	=	=	=	6
Escherichia coli	=	=	=	=
Klebsiella pneumoniae	=	=	=	=
Pseudomonas aeruginosa ATCC 27853	=	=	=	5.5
Staphylococcus aureus ATCC 25923	=	=	=	6
Escherichia coli ATCC 25922	=	=	=	6

Table (3): The color of the solidified medium (the juice) and the colonies of the bacteria.

Type of Bacteria	The color of the solidified medium		The color of the colonies
	Before growth	After growth	
Pseudomonas aeruginosa	Yellow	Light brown to yellowish	Transparent
Proteus vulgaris	=	=	=
Staphylococcus aureus	=	=	=
Escherichia coli	=	=	=
Klebsiella pneumoniae	=	=	=
Pseudomonas aeruginosa ATCC 27853	=	=	=
Staphylococcus aureus ATCC 25923	=	=	=
Escherichia coli ATCC 25922	=	=	=

Figure (1): experimental liquid medium, control without growth.

Figure (2): experimental medium, from right: ***E.coli*** (L), ***E.coli*** (S), control with out growth.

Figure(3):from right: nutrient broth control 1,nutrient broth *Staphylococcus aureus*(S), nutrient broth *Staphylococcus aureus*(L), experimental medium *Staphylococcus aureus* (L), experimental medium *Staphylococcus aureus* (S), experimental medium control 2.

Figure(4):experimental medium, from right: *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa Escherichia coli, Proteus vulgaris*, control

Figure (5): Solidified enriched medium without cultivation.

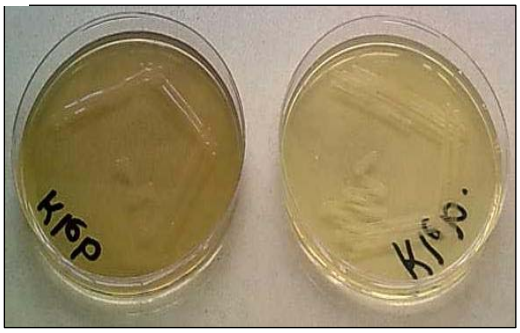

Figure (6): ***Klebsiella pneumonia*** right: nutrient agar, left: solidified medium.

Figure (7): *E.coli* (L.), experimental medium.

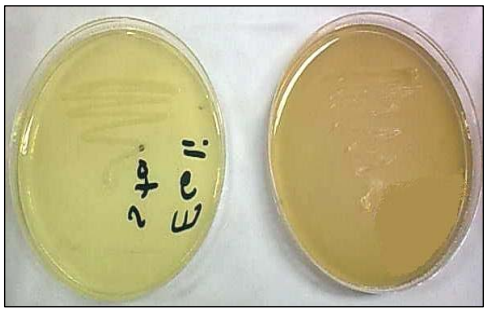

Figure (8): ***E. coli*** (S), right: experimental medium, left: nutrient agar.

Figure (9): *Proteus vulgaris*, experimental medium.

Figure (10): *Pseudomonas aeruginosa*, right: nutrient agar, left: experimental medium

Figure (11): *Staphylococcus aureus*, right: experimental medium, left: nutrient agar

S: Standard strain, *L: Local isolates.

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