Ahmed A Mohammed et al /J. Pharm. Sci. & Res. Vol. 10(8), 2018, 1881-1884

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# Beetroot juice as an alternative growth and maintenance medium for six isolates of *Mycobacterium* spp.

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**Abstract**

In a pioneering study, a water extract from ***Beta vulgaris*** root (beetroot juice) was prepared in sterile conditions and used for the first time as an experimental medium for the growth of six isolates of ***Mycobacterium* spp.** All the used isolates displayed active growth after 3 days. Moreover, the medium maintained the viability of the growing bacteria for 21 days. The prepared juice was also used to enrich the agar-agar and used to culture the same isolates, which displayed noticeable growth after 7 days.

The obtained results recommend that the beetroot juice providing a suitable growth medium for this genus of bacteria as either a liquid or enriched and solidified agar medium, with the latter able to substitute for Lowenstein-Jensen (LJ) medium. The liquid medium represents an important, rich nutritional environment not less significant than other liquid media used to grow and maintain this genus in the laboratory.

**Keywords**—beetroot juice; culture medium; experimental medium; ***Mycobacterium* spp.**

## 1. Introduction

Bacterial growth in laboratory environment requires a suitable growth medium consists of a mixture of nutrients, moisture and other chemical substances which can be used by bacteria for growth. Using a certain composition and food base for bacterial growth depends upon the specific requirements of the particular bacteria, as well as the particular investigation. Accordingly, a wide range of culture media has been developed for a variety of purposes and utilizes. These media contain various sources of organic carbon, nitrogen, phosphorus, sulfur and metal ions, together with iron [1-6]. Based on the biochemical and physiological properties of bacteria, culture media had been developed to use them in the identification of bacteria, and also employed for the isolation and maintenance of pure cultures. [1-3].

Different techniques are used for bacterial cultivation, depending on the purpose in which the particular species are cultivated. and alsothe used culture medium. Accordingly, media can be solid (such as gelatin agar in a Petri dish), or liquid, which allows for bacterial growth in test tubes [1-5].

Liquid media have different compositions and intended for the growth of pure culture. It can be composed of animal tissues and, or fluids e.g., nutrient broth, serum broth, carbohydrate broth and milk, or it can be derived from vegetable tissues, such as malt extract (germinated barley), beer wort, yeast extract, hay infusion, natural fruit juices, and wines (fermented fruit juices) [2,3].

Solidified media on other hand are extensively utilized to isolate pure cultures, in estimating the viability of bacterial populations and many other objectives. Typically, a solidifying agent called agar is used in the preparation of solid and semisolid media, which is a hydrocolloid substance derived from red algae [1-3].

Generally, there are three types of culture media: natural, synthetic and complex (undefined) media. The exact chemical composition of natural media is unknown. However, it contains natural products like milk, vegetable juices, peptone diluted blood, animal cells, urine, tissues and organs. In contrast, a synthetic media is chemically defined, which means that its exact chemical component and concentration is identified, while undefined medium does not possess a known chemical constitution [1-5, 7]. In addition, an enrichment medium is slightly different compared to the former three types. The advantage of using such this type of media is that they allow the growth of particular species of bacteria because only these bacteria can consume of their components, consequently, an enrichment medium may have selective features [1,3, 5, 9, 10].

Although there are many reviews on using medicinal plants activity such as *Neem, Ginger, Tulsi, Garlic* as anti-bacterial agent against tuberculosis [11], and in a recent study, it has been proven the enormous significance of plant mediated creation of silver nanoparticles as an antimicrobial [12]. Yet, other plants have been used in the activation and modification of bacterial flora like the juice of ***Beta vulgaris*** (beetroot juice) [13, 14].

In a pioneering study, the beetroot juice of ***Beta vulgaris*** had prepared under sterile conditions and it has been used as an experimental bacterial growth medium for six isolates of ***Mycobacterium* spp.** as an alternative growth and maintenance medium for Lowenstein-Jensen (LJ) medium, the medium commonly employed for the growth of this genus in the laboratories, and the ability of the beetroot juice to meet the nutritional requirements of ***Mycobacterium* spp.** were examined. The juice was utilized into two manners: firstly, it has been used directly as a liquid growth and maintenance medium, and secondly, it has been added to the agar-agar to prepare an enriched solidified growth medium, as previously described by [14].

The beetroot ***Beta vulgaris*** has superior nutritional components such as carbohydrates, protein, elevated levels of many important vitamins, micronutrients, minerals, and trace amounts of fatty acids. The different preparations of beetroot have no effect on its nutritional value. For instance, when beetroot is boiled, the carbohydrate and protein content become more than their amount in the raw beetroot, and the energy value would correspondingly increase [15,16]. In addition, around one-tenth of ***Beta vulgaris*** root components are glucoses or fruit sugars which are found as pure sugars, as well as gum and starch consist approximately one-third of its weight [17]. The beetroot juice has also an important nutritional value which is derived from its components represented by the energy, carbohydrates, sugars, dietary fiber, fat, thiamine (vitamin $B_1$), riboflavin (vitamin $B_2$), niacin (vitamin $B_3$), pantothenic acid ($B_5$), vitamin $B_6$, folate (vitamin $B_9$), vitamin C, calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, chlorine, manganese, trace amount of selenium, which can be found in all the prepared forms, in addition to trace amounts of biotin and vitamin E, which found in whole preparations, and betaine (a nitrogenous compound establish in beetroot, structurally similar to an amino acid) [16,18]. The exact beetroot juice components that are present when the root is prepared by cooking (or boiling) are listed in Table 1.

For all these properties, the beetroot was used in this research as a growth and maintenance medium for *Mycobacterium* spp. This study was aimed to find an alternative low-cost medium for *Mycobacterium* spp., which can easily prepare for the laboratory use instead of the routine media.

## 2. Materials and Methods

*2.1. Preparation of the beetroot juice*

The juice preparation was performed as previously described by [19]. ***Beta vulgaris*** red roots were washed carefully with tap water, dirt and other materials are removed, then peeled and chopped into small pieces. A 250 gm of chopped roots were placed into a 1.5 L beaker and immersed in 1 L of distilled water, the mixture was heated for 45 minutes at 100°C. The resultant solution (the juice) was refined and cooled to room hotness and filtered by Whatman filter paper. The pH of the prepared juice was adjusted to 7.4, then sterilized using a 0.22 µm Millipore filter. The juice then stored in sterile bottles at 4°C until use and appeared clear with a reddish-brown color.

The availability of suitable nutrients and the applicability of both liquid and solidified media (which mainly composed of the juice) for the growth and maintenance of ***Mycobacterium* spp.** isolates were investigated via the observation of bacterial growth.

*2.2. The use of beetroot juice for bacterial growth and maintenance*

*2.2.1. Samples*

The six isolates of ***Mycobacterium* spp.** were collected from the National Reference Laboratory of TB in Baghdad during October 2010. Four of these isolates were ***M. tuberculosis*** while the other two isolates were ***M. bovis***. The identification of these isolates was based on biochemical tests (catalase, niacin, nitrate reduction).

*2.2.2. The beetroot juice as a liquid growth medium*

In this experiment, the beetroot juice was dispensed into sterilized screw-capped tubes and directly employed as a liquid growth medium. 6 ml of beetroot juice was placed into each tube, and the tubes were then distributed into seven groups, with each group consisting of 3 tubes. Each group of the first six groups of tubes were devoted to one of the six isolates while the seventh group of tubes was left none inoculated as a control. Bacterial suspensions for all six isolates were prepared for subculturing after being adjusted using a McFarland turbidity standard No.1, which is $3\times10^{8}$ colony forming units (CFU)/ml. Each bacterial suspension (200 µl) was transferred to the prepared screw-capped tubes and then incubated at 37°C. The color of the medium and the rate of bacterial growth was continuously investigated. When bacterial growth had observed in the experimental medium (the juice), 100 µl from each tube with growth was cultured in LJ medium every three days for one month to determine how long the growing isolates would survive in the experimental medium and to provide an estimation to the ability of this medium to maintain this genus.

*2.2.3. The solidified beetroot juice with agar-agar as a growth medium*

This medium was prepared by dissolving 15 gm of agar-agar (Biolife company) in one liter of distilled water, which was followed by the addition of 10 ml of glycerol per 1 liter of the liquid experimental medium. The mixture then sterilized in an autoclave at 121°C for 15 minutes, then left until the temperature dropped to 42°C and enriched by an equal volume of sterilized beetroot juice. The agar was then poured into sterile screw-capped tubes (6 ml per tube) and placed at a sloped position until the agar solidified. The tubes were divided into seven groups with each group consisting of 3 tubes. Each of the first six groups was employed for one of the six isolates. In addition, the fourth tube of LJ medium was added to each of the seven groups to serve as positive controls for growth. The tubes in the seventh group were not inoculated and served as negative controls. The culturing was performed by adjusting each suspension using a McFarland standard No. 1 and then transferring 100 µl from each bacterial suspension. The tubes were incubated in a sloped position for 72 hr. to ensure that the inoculums contacted the medium. The tubes were then maintained in a vertical position for 2 weeks. The growth of bacteria, medium color and any visible colonies were continuously investigated. Bacterial viability was investigated after the preliminary period of incubation (7 days).

*2.2.4. Examining bacterial growth in the liquid medium and on the solid medium using acid-fast staining technique*

Following the appearance of acid fast bacilli growth in experimental media (liquid and enriched solid media) after an incubation period, the acid-fast staining technique was performed on the bacteria growing in the liquid experimental medium to ensure that the growing bacteria were ***Mycobacterium* spp.** and it is not contaminants and to investigate the colonies growing on the solidified medium.

## 3. Results

After the primary incubation period (3 days in the liquid medium and 7 days on the solidified medium), each type of medium demonstrated noticeable bacterial growth. The color of liquid medium was altered corresponded with the average rate of bacilli growth, which was detected based on the turbidity of the medium (Figs. 1, 2). All the isolates remained viable in the liquid medium for 21 days (Table 2).

The color of the liquid new medium changed from red - brown to little brown or yellowish in the tubes inoculated by the acid-fast bacilli, which may have been the result of a pH change in the medium (Figs. 1 and 2) [14].

The solidified enrichment medium demonstrated considerable bacterial growth, and its color changed after growth appeared from yellowish to milky (Fig. 3). The color of the growth colonies was creamy for the cultured bacterial isolates (Fig. 4). The appearance of the ***M. tuberculosis*** colonies was rough while the ***M. bovis*** colonies were smooth. Bacterial growth appeared after approximately 7 days on this medium.

The microscopic examination using slides prepared from bacteria from both liquid and solidified media, which were prepared by acid-fast staining have verified our results.

Figure (1): the color of the liquid experimental medium after growth.

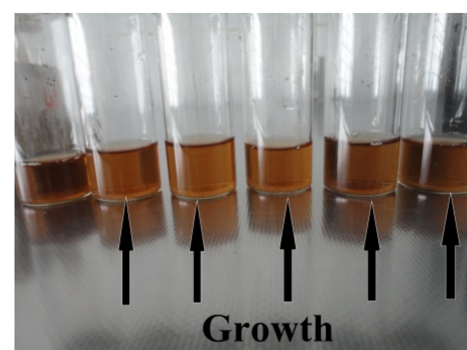


Figure (2): the growth and turbidity in the liquid experimental medium.

Figure (3): the color of the solidified and enriched medium.

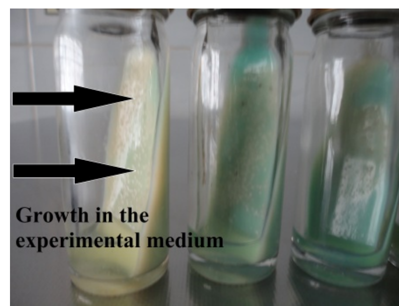


Figure (4): the colors of the colonies on the solidified and enriched medium (left) and LJ medium (right).

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

| Components | The value per 100 gm | Components | The value per 100 gm |
|---|---|---|---|
| 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%) | | |

Table 2 The viability period after the appearance of growth in the liquid medium (the juice).

| Isolates | Viability period in the liquid medium (the juice) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 days | 6 days | 9 days | 12 days | 15 days | 18 days | 21 days | 24 days |
| $Isolate_1$* | + | + | + | + | + | + | + | - |
| $Isolate_2$* | + | + | + | + | + | + | + | - |
| $Isolate_3$* | + | + | + | + | + | + | + | - |
| $Isolate_4$* | + | + | + | + | + | + | + | - |
| $Isolate_5$** | + | + | + | + | + | + | + | - |
| $Isolate_6$** | + | + | + | + | + | + | + | - |

* ***Mycobacterium tuberculosis***
** ***Mycobacterium bovis***
\+ growth is present
\- growth is absent

## 4. Discussion

The observed results propose that the prepared liquid medium (the juice) represents a highly qualified alternative medium to LJ medium when used to culture ***Mycobacterium* spp.**, and this medium is capable of maintaining and preserving this genus. The liquid medium has proved that it considers an appropriate growth medium for all the isolates in this study. Although the juice is of plant origin, it was very efficient as a growth medium, which can be clearly detected from the high intensity of bacterial growth which was determined using turbidity and color changes, as demonstrated in the figures above. This effectiveness of the new medium can be explained as this medium is providing these types of bacteria with all of their necessary supplies of growth. The juice is rich with many necessary substances, as mentioned in Table 1, and many other undefined components required for the growth of these bacteria, which are used for the production of energy (like sugars, carbohydrates and amino acids), cell wall and capsule construction (such as fatty acids, carbohydrates) and the additional metabolic activities of these organism. In addition, it is important to note that the growing bacteria can directly use these components to build their structural units and may sometimes indirectly use them because some of these components enter into other complex structures. Such components are present in cell walls and capsules which contain a polypeptide layer, a peptidoglycan layer, free lipids, sulfolipids, a complex structure of fatty acids such as mycolic acids, which appear glossy, in addition to the layers of the arabinogalactan and peptidoglycan which is found above the plasma membrane [20,21]. In a recent study on rats, a positive modulation of gut metabolic activity and microflora were demonstrated in the groups of rats fed on a diet supplemented with fermented beetroot juice. It has been found that fermented beetroot juice had encouraged *Bacteroides-Prevotella* bacteria to adhere to the gut epithelium which leads to increase bacterial counts in the groups treated with the juice compared to the control groups, and fermented juice serve in reducing the oxidation processes in the organism and also useful in the modulation of intestinal flora and fauna and its enzymatic actions [13].

Interestingly, all the used isolates were maintained with full viability in the medium for a considerable period, which indicates that this medium is also useful in the preservation of these bacteria and provides the medium with more value as a culture medium. Therefore, our experimental medium could be a very good alternative medium for other liquid media, such as Middlebrook 7H9 medium.

The color of this experimental medium is reddish-brown, which results from the natural stain (anthocyanin stain) which is found in the beetroot cells. The stain will liberate from the cells to the juice solution after they destroyed during the boiling process which has used in the preparation of the juice. The red dye of the

red juice had altered to reddish-brown color for the reason that using heat during juice preparation resulted in oxidizing of the stain. This color was slightly changed after the appearance of bacterial growth in the medium, which altered the pH of the medium. This modification in the pH of the medium was a result of the metabolic products which was resulted from sugars and complex carbohydrates fermentation which are found in the juice components. Because the color of anthocyanin is pH sensitive, the stain's color is also changed. The color change of the medium serves as a good indicator for bacterial growth, as does the turbidity. This unique characteristic negates any need to add any indicating color to the prepared medium.

The solidified growth medium also produced a suitable environment for bacterial growth. Success in using this medium was indicated by the bacterial growth obtained after culturing (Figs. 3 and 4). Therefore, this medium is also suitable and a good alternative medium for LJ medium, which is typically employed on these types of microorganisms growth.

Before cultivation, the color of the enriched solid medium was yellowish, which slightly changed after bacterial growth to a milky color. The colors of the bacterial colonies did not differ significantly in any of the isolates on the enriched medium, meaning the bacteria did not produce pigments during their growth on this medium.

## 5. Conclusion

In conclusion, beetroot juice can be directly employed as a liquid growth medium for *Mycobacterium* spp. and provides satisfactory results. If the *Beta vulgaris* root juice would be used as an enriched material for agar-agar, it produces a good source of nutrients and enhances the *Mycobacterium* spp. for growth. In addition, this medium represents an inexpensive and simple to prepare natural medium. The liquid medium can be employed for the growth and maintenance of *Mycobacterium* spp. while the solidified medium can be used for growth studies. These media could be employed as an alternative media for routine media which are used for the culturing and maintenance of this genus in the laboratories, which would depend on if either the liquid or enriched solid medium was employed.

## References

[1] Todar, K., 2004. Todar's Online Textbook of Bacteriology. http://textbookofbacteriology.net/nutgro.html

[2] Winn, W. C. and Koneman, E. W., 2006. Koneman's Color Atlas and Textbook of diagnostic microbiology, 6th ed., Lippincott Williams and Wilkins, Philadelphia, USA.

[3] Engelkirk, P. G. and Duben-Engelkirk, J., 2007. Laboratory Diagnosis of Infectious Diseases: Essentials of Diagnostic Microbiology, Lippincott Williams and Wilkins, USA, pp. 133-134.

[4] Dubey, R. C. and Maheshwari, D. K., 2009. Practical Microbiology, 6th ed., S. Chand & Company LTD, India, pp. 24-25.

[5] Parija, S. C., 2009. Textbook of Microbiology & Immunology, Elsevier, India.

[6] Fox, A., 2010. Microbiology and Immunology on-line,http://pathmicro.med.sc.edu/book/bact-sta.htm.

[7] Madigan, M. T., Martinko, J. M. and Brock, T. D., 2006. Brock Biology of Microorganisms, 11th ed., Pearson Prentice Hall, USA.

[8] Baron, S., 1996. Baron's Medical Microbiology, 4th ed., Univ. of Texas Medical Branch, USA.

[9] Forbes, B. A., Sahm, D. F. and Weissfeld, A. S., 2007. Bailey and Scott's Diagnostic Microbiology, Mosby Inc, China.

[10] Ochei J. and Kolhatkar, A., 2008. Medical Laboratory Science: Theory and Practice, 10th ed., Tata McGraw-Hill publishing company LTD, New Delhi, India.

[11] Dubey, N. and Goyal, A. (2017). The role of nutritional value in causing tuberculosis and growth of TB microbes in the presence of some herbal plant extract. World Journal of Pharmacy and Pharmaceutical Sciences, 6(6): 796-804. doi: 10.20959/wjpps20176-9092.

[12] Ahmed, S., Ahmad, M., Swami, B. L and Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. Journal of advanced research. 7(1): 17–28. doi.org/10.1016/j.jare.2015.02.007.

[13] Klewicka, E., Zduńczyk, Z., Juśkiewicz, J., and Klewicki, R. (2015). Effects of Lactofermented Beetroot Juice Alone or with N-nitroso-N-methylurea on Selected Metabolic Parameters, Composition of the Microbiota Adhering to the Gut Epithelium and Antioxidant Status of Rats. Nutrients, 7(7): 5905–5915. doi: 10.3390/nu7075260.

[14] Al-Azzauy, A. A. M. and Salman, A. M. H., 2011. The beetroot juice as a bacterial growth and maintenance medium for many pathogenic bacteria, Iraqi J. of Market Res. and Consumer Protection, Vol. 3, No. 5, pp. 147-161.

[15] McCance, R. A. and Widdowson, E. M., 1995. The Composition of Foods, the Royal Society of Chemistry, 5th ed., Cambridge, UK.

[16] Nottingham, S., 2004. Stephen Nottingham's meticulously researched online book, Beetroot. http://www.stephennottingham.co.uk/beetroot6.htm

[17] Grieve, M., 2010. Botanical.com A modern herbal. http://botanical.com/botanical/mgmh/b/beetro28.html.

[18] Grubben, G. J. H. and Denton, O. A., 2004. Plant Resources of Tropical Africa 2, Vegetables. Backhuys, Leiden CTA, PROTA Foundation, Wageningen.

[19] Al-Azzauy, A. A. M., 2002. The extraction of (RA) and (BA) plant dyes and use them in the viability test of *Echinococcus granulosus* protoscolices, patent, registered by the central organization for standardization and quality control, patent no. 3031, 2002, Iraq-Baghdad.

[20] Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Eiglmeier, K., Gas, S. and Barry, C. E., 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence, Nature, Vol. 393(6685), pp. 537-544.

[21] Riley, L. W., 2006. Of mice, men and elephants: *Mycobacterium tuberculosis* cell envelope lipids and pathogenesis, American Society for Clinical Investigation, Vol. 116, pp. 1475-1478.