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Research Article

New Enriched Culture Media for Culturing Streptococcus pyogenes by using Spirulina Powder

Ahmed Mohammed Abdelazim [1]*, Abdelrahman Eldaw Mohammed [2], Omer Hassan Arabi [2]

[1] *Department of Medical Microbiology, Faculty of Medical Laboratory Science, University of Dongola, Al Dabbah, Sudan*

[2] *Department of Medical Microbiology, Faculty of Medical Laboratory Science, University of Gezira, Madani, Sudan*

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*Address for Correspondence:

Ahmed Mohammed Abdelazim, Department of Medical Microbiology, Faculty of Medical Laboratory Science, University of Dongola, Al Dabbah, Sudan.

Abstract

Background and Aim: Fastidious bacteria require special nutrients and growth factors to grow on enriched culture media. Many enriched culture media were developed for culturing and isolating fastidious bacteria in pure cultures. The present study investigates the ability of spirulina powder to support the fastidious bacteria growth in pure culture compared to ordinary enriched media, e.g., Blood agar and Chocolate agar.

Methods: Spirulina powder was used as a nutrient source with some additives to prepare different types of Spirulina media. Reference strain ATCC (19615) and three clinical isolates of *Streptococcus pyogenes* were examined for their growth on the developed candidate medium. Three formulations of spirulina powder were used: Medium 1, Medium 2, and Medium 3.

Results: All spirulina media supported the growth of *streptococcus pyogenes* and there was no significant difference in the morphology and cultural characteristics on the blood agar and chocolate agar media. Colonies size of *streptococcus pyogenes* were slightly smaller size on spirulina media.

Conclusion: Spirulina media is a possible candidate that can be used as enriched culture media for culturing and isolating fastidious bacteria such as *Streptococcus pyogenes*.

Keyword: Spirulina powder, enriched culture media, *Streptococcus pyogenes*.

1. INTRODUCTION

Cultivating and maintaining microorganisms in suitable culture media are crucial for conducting microbiological investigations and studies [1]. Furthermore, it is vital to obtain pure bacterial colonies from cultures to study their morphology, virulence, antibiotic sensitivity patterns, and genome sequence to understand better and treat bacterial diseases [2]. There are many lines for the classification of bacterial growth; based on the consistency, media are classified into solid, semi-solid, and liquid preparations. Furthermore, according to the function, there are basic, enriched, selective, differential, and transport media [3] (Monica, 1984). Specialized media are essential for the isolation and identification of some microorganisms. These include media for antibiotic sensitivity testing, water, and food analysis, industrial microbiology, and other activities. In such cases, the function of the medium also will determine its composition. Generally, bacteria grow naturally in almost all habitats and utilize the essential, necessary sources of nutrients to maintain their growth and reproduction. Knowledge of the typical bacterial habitat is often valuable for selecting an appropriate culture medium because its nutrient requirements reflect it s natural surroundings [4]. Different microorganisms grow in different environments and have various growth requirements, like nutrients, pH, osmotic conditions, and temperature. The current limitations of microbial cultivation in the laboratory need to be addressed by formulating newer media. Microorganisms require various macro elements, namely C, H, O, N, S, P, K, Ca, Mg, and Fe, to synthesize carbohydrates, lipids, proteins, and nucleic acids. Moreover, all microorganisms require several microelements like Mn, Zn, Co, Mo, Ni, and Cu, which are generally part of the enzymes and cofactors. Microorganisms also require organic compounds as growth factors [5]. To obtain energy and construct their cellular components, they must have a supply of raw materials or nutrients [6]. Failure of an organism to grow on a specific medium is probably due to the absence of one or more of the essential nutrients requirements. The high cost of the culture medium is one of the thoughtful challenges in developing countries; therefore, it is crucial to consider introducing alternative media and agar to reduce the cost involved and consider the nutritional value of the investigated alternative [7].

Ordinary culture media has many disadvantages, Blood agar contains inhibitors for certain bacteria, such as members of the *Neisseria* and *Haemophilus* genera, and the blood must be heated to inactivate these inhibitors [8]. There was also the problem of collecting sufficient blood from the smaller animals. Although cow blood is easily available, the variability of the hemolytic reactions by some of the organisms and the fact that it did not support adequate growth of *klebsiella pneumoniae*, the most commonly used

blood for the isolation of Microorganisms from human tissue and fluids. However, expired blood from blood banks is still used despite the risk of exposure to HIV and other blood–borne infection, human blood may contain antibodies and anti–microbial agents which may also inhibit growth or cause false haemolysis [9]. When half-blood agar plates are used for culturing specimens, a narrow central strip of medium can be cut out (ditching) to prevent the spread of Proteus from one specimen to the other [3]. When making blood or chocolate agar plates, the formation of bubbles during mixing and pouring can be removed by applying the flame directly to the surface of the agar plate, but this increases the possibility of contamination. It is believed that bacteria floating in the air enter the agar medium [10].

2. MATERIALS AND METHODS

Organisms used:

Reference *Streptococcus pyogenes* ATCC(19615) strain and three clinical isolates collected from the Ear, Nose and Treachal (ENT) Khartoum hospital were used in this study.

Formulation and inoculation on solid media:

Spirulina powder was placed in warm water, mixed, and boiled at 100°C. Filtered and 12gms agar were added to 250 ml distilled water. NaCl, peptone water, and pericarp powder were used as additives to formulate different culture media. In all experiments pH of the media was adjusted to 6.5 – 7.0. The dissolved media were sterilized in a water bath at 100°C for 30 minutes, then were poured into sterile Petri dishes separately (Table 1).

Ingredients	Medium 1	Medium 2*	Medium 3**
Spirulina powder	05 gm	10 gm	15 gm
Nacl	05 gm	05 gm	05 gm
Agar	12 gm	12 gm	12 gm
Pericarp powder		02 gram	02 gram

*The mixture was dissolved in250 ml distilled water.

** The mixture was dissolved in 250 ml distilled water and 10ml of peptone water.

Streptococcus pyogenes reference strain ATCC (19615), and three clinical isolates were inoculated on to the spirulina media 1, 2 and 3 and incubated under an anaerobic atmosphere with CO_2 (10%CO_2) at 37 °C. Significant bacterial growth was checked every 12 hours for three days. Colonies size, shape, and color were reported and compared with those produced on blood agar (B.A.) and Chocolate agar (C.A.) media.

Growth and bacteria count on liquid media:

Serial suspensions of *Streptococcus pyogenes* were made as follow to calculate the bacterial count:

1. 9 ml of sterile distilled water, mixed with1 ml bacterial suspension in tube 1, then transferred 1 ml from tubes3 serially.
2. Dilution factor in tube 1 = 1/10, in tube 2 =1/100 and in tube 3= 1/1000.
3. Different suspensions of bacteria from different tubes were used for inculcation on spirulina broth media (10 gm), blood and chocolate broth media.
4. Bacterial growth turbidity was counted after 24hours using a spectrophotometer, and the bacterial cells number was calculated according to the following formula:

   Optical density of test / Optical density of STD * $_x$ Concentration of STD= CFU 106/ ml** $_x$ D.F***.

   STD*= McFarland STD (known concentration).

   CFU 106/ ml **= Colony-forming unit compared to McFarland STD

   D.F***= Serial bacterial dilution [11].

3. RESULTS

3.1 Growth on solid media: Spirulina media (1, 2 and 3) supported *Streptococcus pyogenes* on growth in all the formulated culture media. There was no significant variation in the colony morphology. Colony size was slightly smaller than that produced on ordinary enriched media (B.A., CA) (Table 2).

Table 3.1: Growth of *Streptococcus pyogenes* on different spirulina media (1, 2, and 3), B.A. and C.A. media.

Type of culture media	Beginning of growth	Complete growing	Colony Size	Colony Shape & Color	Condition of The growth
Medium1	At 24 hours	After 48 hours	Smaller than that produced on Chocolate and Blood agar (4-4.5mm)	Same as that produced on Blood agar white in color	37 °C aerobic and anaerobic with a CO_2 atmosphere
Medium 2	At 24 hours	After 48 hours	Smaller than that produced on Chocolate and Blood agar (4-4.5mm)	Same as that produced on Blood agar white in color	37 °C aerobic and anaerobic with a CO_2 atmosphere
Medium 3	At 24 hours	At 48 hours	Smaller size colonies than that produce on Blood agar(4-4.5mm)	Moist, convex, white color	37 °C aerobic and anaerobic with a CO_2 atmosphere
B.A.medium	At 24 hours	After 48 hours	Small size colonies (5mm)	Moist, convex, white color with beta hemolysis	37 °C aerobic and anaerobic with a CO_2 atmosphere
C.A.medium	At 24 hours	After 48 hours	Small size colonies (5mm)	Moist, convex, greenish-white in color	37 °C aerobic and anaerobic with a CO_2 atmosphere

3.2 Bacterial count: Spirulina powder was used to prepare Spirulina Broth media; the nutrient broth was used to prepare blood and chocolate broth media. The bacterial count for *Streptococcus pyogenes* was then cheeked using serial dilution methods. The bacterial turbidity was adjusted using a spectrophotometer and applied the formula mentioned above.

Table 3.2: Counts of *Streptococcus pyogenes* suspensions on Spirulina Broth, blood broth, and Chocolate broth media (CFU/ml*) using Spectrophotometer.

D.F*	Spirulina (CFU*/ml)	Blood Broth (CFU/ml)	Chocolate broth (CFU/ml)
0.1	2.100X106 CFU/ml	1.350X106 CFU/ml	1.893X106 CFU/ml
0.01	102X106 CFU/ml	71.1X106 CFU/ml	60X106 CFU/ml
0.001	4.2X106 CFU/ml	1.7X106 CFU/ml	2.5X106 CFU/ml

D.F*= Dilution factor

CFU*= Colony-forming unit

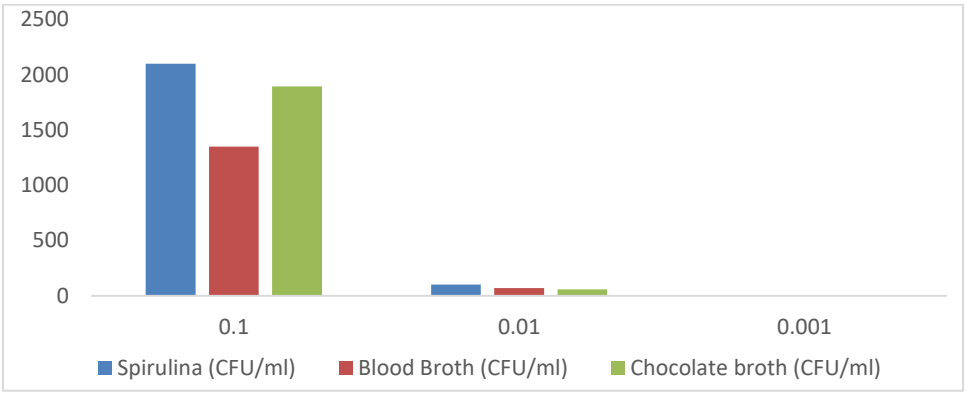

Figure 1: Bacterial Cell count of *Streptoccus pyogenes* on Spirulina, blood, and Chocolate Broth using Spectrophotometer.

4. DISCUSSION

This study was constructed to investigate and assess the capability of spirulina powder to provide nutrients and growth factors required to support fastidious bacteria's growth. Three formulations of spirulina media were examined for their ability to support *streptococcus pyogenes* growth as an example of a fastidious organism. The growth of *Streptococcus pyogenes* on spirulina media indicates that this candidate medium provides the essential elements and growth factors to support *S.pyogenes* recovery. Commonly used enriched culture media like blood agar and chocolate agar media contain blood as enriched nutrients to support the growth of fastidious bacteria. However, these media are prone to contamination during the pouring process of blood. Dried spirulina is a good nutritional source with high protein content and significant lipid content. Spirulina is high in unsaturated and polyunsaturated fatty acids in particular (25% - 60% of the total fatty acids), such as oleic acid, linoleic acid, gamma-linolenic acid and docosahexaenoic (DHA). It also contains amino acid with a high nutrient digestibility. In addition, spirulina contains substances such as pigments (for example carotenoids such as β-carotene and zeaxanthin), phycobiliproteins (for example phycocyanin, which is unique in the cyanobacteria), vitamins, polysaccharides, macro and micro mineral elements and antioxidants [12-14].

Spirulina media prepared in this study contained a small amount of spirulina powder, NaCl, agar for solidification, and other additives (peptone water and pericarp powder). This study showed that: Spirulina media supported *Streptococcus pyogenes* growth on all formulated culture media, with no significant variation in the colony morphology, but the colony size was slightly smaller than that produced on ordinary enriched media (BA., CA). Regarding the bacterial cell counts, spirulina media showed high bacterial counts compared with blood broth and chocolate broth media. This result indicated that spirulina powder could be used as an enriched medium to support *Streptococcus pyogenes* culturing.

In conclusion, the present study showed that spirulina medium supported *Streptococcus pyogenes'* growth compared to ordinary BA and CA media. Based on these findings, the proposed spirulina medium formulation will be a possible medium candidate to support the growth of fastidious bacteria.

5. CONCLUSION:

The study concluded that the Spirulina media is a possible candidate that can be used as enriched culture media for culturing and isolating fastidious bacteria such as *Streptococcus pyogenes*.

Competing interests:

Authors have declared that no competing interests exist.

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