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Mimicking Microaerophilic/Anaerobic Environment for Culture of Anaerobic Bacteria Using Oxygen Scavengers in Agar Well Technique

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ABSTRACT *A cheap and simple method for culture of anaerobic bacteria using oxygen scavengers in agar well technique is described. A recognized bacterial strain was taken which was known to be anaerobic by nature. In order to replicate the natural microaerophilic growth environment, the bacteria were cultured in a modified media with oxygen scavengers in agar well technique. It was found that this method successfully simulated the natural microaerophilic growth of the bacteria which is difficult to achieve in the laboratory. This technique will be very useful for microbiological studies on growth and culture of anaerobic bacteria and to ensure that organisms are well protected from oxygen.*

INTRODUCTION

Bacteria can often be characterized on the basis of amount of oxygen they need. Bacteria that absolutely require oxygen are called obligate aerobes, whereas those that react negatively or may even die in presence of oxygen are called as obligate anaerobes. Those bacteria which can switch between aerobic and anaerobic lifestyles are categorized into facultative anaerobes. Microaerophilic bacteria are those which require a very low concentration of oxygen. Often these bacteria can also grow in complete absence of oxygen as stated by Forbes, Betty A., Daniel F. Sahm, and Alice S. Weissfeld., (1998) [1].

Anaerobic bacteria cultures are performed to identify bacteria that grow only in the absence of oxygen and which may cause human infection. Because anaerobes are the predominant components of the skin's and mucous membranes normal flora, they are the common cause of infections of endogenous origin. Because of their fastidious nature, anaerobes are hard to isolate and are often not recovered from infected sites. This makes their study very essential. The growth of anaerobic bacteria is rarely straightforward. Many methods have pre existed in view of this. The simplest is though the use of autoclave. When the medium is sterilized by autoclaving, the dissolved oxygen is 'driven out' of the liquid. Upon cooling, the headspace can be flushed or the liquid can be purged with an oxygen-free gas (N_2, Ar, He). Oxygen is not very soluble in water and it diffuses only slowly into the medium; that is, only the first few mm near the top of a tube will contain oxygen as long as it is not mixed with air as suggested by V. I. Baranenko, L. N. Fal'kovskii, (1990) [2]. J. G. Collee, B. Watt, Elizabeth B. Fowler and R. Brown (2008) [3] demonstrated the use of gas-pack which contains an oxygen indicator and oxygen removal gas packets. One of the more successful methods of anaerobic culturing is by using glove box, which serves the purpose best. However, the availability of glove box set-up is low due to its high cost. By far the best method of anaerobic culture is by the use of thioglycollate broth, (Michael T. Madigan, Brock biology of microorganisms, Thirteenth edition, Microbial growth, Chapter 5th). [4]. Sodium thioglycolate in the medium consumes oxygen and permits the growth of anaerobes. But the price and sensitivity towards certain classes of bacteria makes it less viable. This makes it necessary for a cheaper and convenient method for culture of anaerobic bacteria, which is more viable than all the above mentioned methods.

ISOLATION AND SEPARATION

The bacterial strain was procured from Lonar Lake, located in the Buldhana District of Maharashtra, India (lat.19°58′ N, long. 76°34′E). The Lonar Lake, situated in a hypervelocity meteorite impact crater, is the only crater in the world to be formed in basaltic rock and the lake water is saline and alkaline (pH 9.5–10.0, CaCO3 alkalinity – 3.6 g/l, NaCl (as chloride) – 3.0 g/l) reported by Rajasekhar, R. P. and Mishra, D. C., (2005) [5]. Lonar Lake provides an optimum environment for the growth of anaerobes such as Magnetotactic bacteria, which are usually found in an oxic-anoxic transition zone (OATZ), the transition zone between oxygen-rich and oxygen-starved water or sediment as reported by Thakker, C. D. and Ranade, D. R., (2002) [6].

The soil samples were collected from various sites of the lake and taken to laboratory in tightly sealed glass jars. These were covered from the outside to protect from over-exposure to sunlight. The soil samples were carefully preserved in refrigerated condition until the first step of isolation was carried out. For the preliminary isolation, 2 gm of soil sample was suspended in a solution of 8.5-9 pH, obtained using NaOH in 100ml distilled water as demonstrated by Blakemore, R. P., D. Maratea and R.S. Wolfe., (1979) [7] . A strong magnet was attached to one side of the beaker and the suspension was left undisturbed for about a week. The solution in proximity to the magnet was used for the culture. This was designated as IS-1(isolated sample-1)

MATERIALS AND METHODS

The culture media was modified so as to contain Ferrous and Ferric ions which are essential for the formation of magnetosomes within the bacteria. The paper by Kundu, S. and G. R. Kulkarni., (2010) [8] also modifies the original MSGM culture media. The media was prepared by mixing 5 g of Sodium chloride, 3 g of Beef extract and 5 g of tryptone in 1.0 liter of distilled water and then modified with 20 ml of 0.1M ferric quinate solution. The pH of the medium was adjusted to 6.7 using 0.1 N NaOH. For the preparation of solid media, 15 g/l agar was added to the above prepared culture media. In addition to this 0.2g of sodium sulphide (Na_2S) per plate is required which serves as oxygen-scavenger. This culture media was similar to that prepared by Sharma, Gyan Prakash and Balomajumder, Chandrajit, (2011) [9] but with the addition of an oxygen Scavenger, namely Na_2S. All the chemicals were sterilized by autoclaving. This media is designated as MMSC SELM Q.

In order to achieve micro-aerophilic conditions required for the culture of these anaerobic bacteria, a small well was created in solidified agar plates. 40µl of IS-1 was used in culture of one plate. After spreading, a measured amount of sodium sulphide (Na_2S) was added to the well. The petri-plate was then sealed and incubated. It was regularly monitored at 12 hours, 24 hours and 48 hours. The bacteria were also grown

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in aerobic conditions without the use of sodium sulphide, in order to compare the viability of agar-well technique.

RESULTS

Initially the plate turned greenish in colour, fig [a]. This is due to the fact that Na_2S reduces the ferric quinate present in the media.. The Na_2S thus removes the oxygen by getting oxidized. Fig. [b] shows the condition of plate as monitored after 12 hour. As seen from the images it is very well clear that the Na_2S reacts with ferric quinate, giving Fe^{+2} and hence the green color.

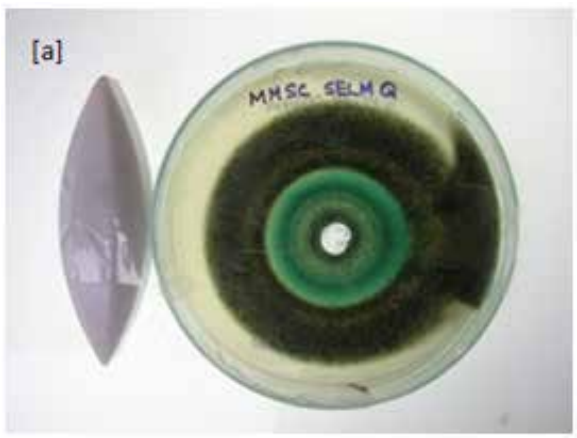

Fig.[a] Condition of plate after plating.

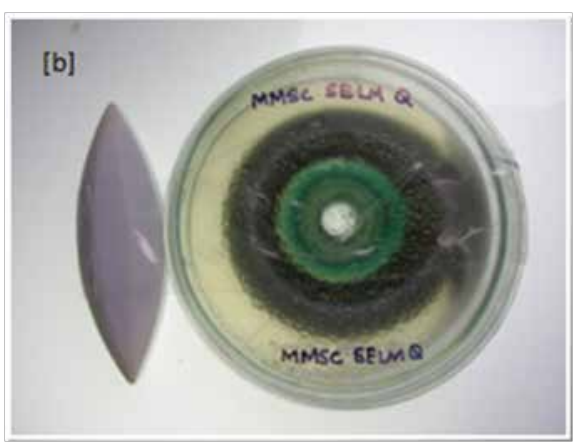

Fig.[b] Condition of plate after 12 hours of incubation

Fig.[c] Condition of plate after 24 hours of incubation

Fig. [d] Control plate vs Incubated plate.

However, it is only temporary as the plate 'cures' itself i.e. it eventually loses its green tinge with time after a period of 24 hours, fig [c].

A culture was also prepared without the use of Na_2S and this was used as control against our standard media. The results show no growth of bacteria, as seen in fig. [d].

DISSCUSION

It is evident from the data that the Agar well technique with the use of an oxygen scavenger is a success. It shows consistency and the presence of Na_2S does not affect the bacterial growth in any way. The curing is a natural phenomenon which again does not affect the culturing process.

CONCLUSION

Using this way, we are successfully able to culture anaerobic bacteria, such as MTB with quite ease. The use of superfluous additives and tools is avoided and hence the culture is less expensive and unhindered.

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REFERENCE

[1] Forbes, Betty A., Daniel F. Sahm, & Alice S. Weissfeld., 1998, "Anaerobic Bacteriology," Bailey & Scott's Diagnostic Microbiology.10th ed. St. Louis Mosby. | [2] V. I. Baranenko, L. N. Fal'kovskii, "Solubility of oxygen and carbon dioxide in water" UDC 621.039.57 | [3] J. G Collee, B. Watt, Elizabeth B Fowler & R. Brown, J. awl. B. 35 (l), 71-82. | [4] Michael Madigan (2011), Brock biology of microorganisms, Thirteenth edition. Pearson Publication, Chapter 5th, Microbial growth. | [5] Rajasekhar, R. P. & Mishra, D. C., 2005, "Analysis of gravity and magnetic anomalies over Lonar lake, India: An impact crater in a basalt province," Curr. Sci., 2005, 88, 1836–1840. | [6] Thakker, C. D. & Ranade, D. R., 2002, "An alkalophilic Methanosarcina isolated from Lonar crater," Curr. Sci., 2002, 82, 455–458. | [7] Blakemore, R. P., D. Maratea & R.S. Wolfe., 1979, "Isolation and pure culture of a freshwater magnetic spirillum in chemically defined medium," J. Bacterial. 140(2): 720-729. | [8] Kundu, S. & G.R. Kulkarni., 2010, "Enhancement of magnetotactic bacterial yield in a modified MSGM medium without alteration of magnetosomes properties," Indian J. Exp. Biol. 48: 518-523 | [9] Sharma, Gyan Prakash & Balomajumder, Chandrajit., 2011, "Preliminary isolation report of aerobic magnetotactic bacteria in a modified nutrient Medium," Recent Research in Science and Technology 2011, 3(11): 71-75.