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ORIGINAL ARTICLE

# Isolation and Study of Cellular Components of *Aerobacillus Polymyxa* along with Its Comparison in Soil Layers

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## ABSTRACT

**Objectives:** The main objective of this research was to isolate and to study the cell morphology and biochemical reactions of *Aerobacillus polymyxa* and *Bacillus megaterium* along with its habitat either in deep soil or aerobic soil.
**Background:** *AeroBacillus* is from the family of *PaneBacillus* polymxa and *Bacillus megaterium* belongs from "*Bacillaceae*" family. These two organisms are gram positive, non-pathogenic bacteria found in soil that helps in nitrogen fixation. They both are equally important today but the main aim of this research to isolate them from the soil due to the characteristic importance of *A. polymyxa* to produce antibiotic and helps to remove biofilm formation where as B. *megaterium* is a good a source of producing industrial proteins due to its larger size than any other organisms.
**Methodology:** Total16 samples were collected from aerobic & anaerobic soil, water and milk. The soil samples were cultured on TGB media and NA for four days.
**Results:** Out of 16 samples 9 samples have shown positive results for the colonies of *A. polymyxa* and out of 16 samples 12 samples showed positive results for *B. megaterium* further confirmed by biochemical reactions.

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## Introduction

Microorganisms are the microscopic organisms which may be single cell or multi-cellular. The study of microorganisms became vast after the discovery made in 1674 by Antonie van Leeuwenhoek(1).They may be either prokaryotic or eukaryotic. Microorganisms are the diverse groups that are divided into bacteria, fungi, viruses, protozoa and algae. In every part of biosphere microorganisms are found anywhere. For example, we naturally contain microorganisms in every part of the body as the normal flora to protect us from other harmful microorganisms and their pathogenicity.These microbes are also present in soil that helps in the better growth of plants and soil expansion by nitrogen fixation (2).

*Aerobacillus polymyxa* and *Bacillus megaterium* both are equally the most important micro-organisms today in the fields of industry, agriculture and medicines. They both are gram positive, endospore forming rod shaped bacterium having peritrichous motility that is non-pathogenic found in plants roots that help in nitrogen fixation and increases root expansion and plant growth. They are also found in aerobic and anaerobic soil and in marine sediments. Besides this, these species are also important in medical and in agriculture fields (3).

Some strains of *A. Polymyxa* are capable of producing an antibiotic known as "Polymixin, paenibacillin, and fusaricidin"effective against gram positive and gram negative organisms and helps in removing biofilm

formation formed by *Escherichia coli, Streptococcus pneumonia* and *Staphylococcus aureus* (4, 5). Their wide range of applications in industries is due to the secondary metabolites produce by this specie (6). They are also used in bio preservation of foods due to their antimicrobial activity (2, 7).

*A. Polymyxa* became its own genus in 1993 as it was first classified under the genus *Bacillus*. This classification was done when the 16S rRNA of 3 different bacilli was analyzed comparatively which showed the phylogenetic difference enough to classify A. *Polymyxa*in its own genus(7)(8). *A. polymyxa* is a chemoorganoheterotrophs. It utilizes variety of carbon source for the energy i.e. glucose, mannitol, glycerol, xylan, xylose and arabinose and produces acetoin, lactate and ethanol as metabolites. Besides this, *A. Polymyxa*are also mesohphilic that grows around at 30˚C optimally maintaining the pH around 4-7. They also produce $H_2$ gas as a by-product. There are total 13 strains of this species (9, 5).

*B. megaterium* is the best cloning host. It is the largest Eubacteria than any other micro-organisms about 60 micrometers cubed hence the name "mega" means "relatively big". Due to its large size *B. megaterium* is well-suited for research on cell morphology, such as cell wall and cytoplasmic membrane biosynthesis, sporulation, spore structure and cellular organization, DNA partitioning, and protein localization (3, 6). *Bacillus megaterium* is one of the first bacteria's genome to be coded completely (7).

It can produce numerous plasmids remaining stable to its unique external proteases (3). This specie is able to produce variety of industrial proteins that due to the absence of alkaline proteases which allows for recombinant protein synthesis (8).

Besides this *B. megaterium* is a commercially available, non-pathogenic host for producing many biotechnological substances, including vitamin B12, penicillin acylase, and amylases (9). *B. megaterium* is known to produce poly-γ-glutamic acid (3, 9).Using the penicillin amidase produced in this organism many synthethic penicillin have been derived (10). Harvested glucose dehydrogenase is used in glucose blood tests; ß-Amylases which are often used in the bread industry; and neutral proteases which are used by the leather industry (3, 10). B. *megaterium* is a cell factory towards the production of Vitamin B-12.In addition to being a common soil bacterium and an endophyte, it can be found in various foods, including honey, in which most microorganisms do not grow and on a variety of surfaces, including clinical specimens, leather, paper and stone (23, 24). It is also able to survive in extreme conditions such as desert environments due to the spores it forms (11, 12).

*A. Polymyxa* and *B. megaterium* are fastidious organisms therefore they grow best on TGB media (15). They required 3 – 40°C to grow (3, 16). To isolate them from soil it requires 4 days of incubation period in an incubator (17). They appear as white, mucoid, opaque on cultural plates. They may appear as spreaded or pin-pointed colonies. Microscopically they may appear as long, scattered or diploid or in chains as well (25). They may be vegetative cells microscopically (18).

There many microorganisms found in soil i.e. bacteria, fungi, actinomyces, protozoa and algae. Up to 10 billion bacterial cells inhabit each gram of soil in and around plant roots, a region known as the rhizosphere (20).

In this research, I have studied about the isolation of *A. polymyxa* and B. *megaterium* from different sources of soil and other samples to study its cell morphology and biochemical reactions to differentiate it from other species (21). Isolating this organism was difficult due to the high chances of growth of *Bacillus* subtilis and other organism commonly found in soil and environment (22).

## Materials and Method

**Samples Collection:** Total 16 samples for *A. polymyxa* and 18 samples for *B. megaterium* were collected, for *A.* ploymyxa aerobic and 8 anaerobic soil samples from different sources were taken while for B. *megaterium*4 aerobic soil samples, 4 anaerobic soil samples, 4 milk powder samples and 4 distilled water sample were taken.

**Isolation of Cultures:** Samples were serially diluted upto $10^4$ dilution and were poured in the TGB and NA media. 8 samples were poured in TGB medium and 8 samples were poured in nutrient agar for *A. polymyxa*. 2 samples from each source were poured in TGB while 2 samples from each source were poured in NA for B. *megaterium*. Plates were then incubated at 37˚C and were allowed to

grow for 4 days. Plates were checked daily in order to check the growth.

**Identification of Cultures:** 5th day plates were observed for cultural morphology. The grams staining technique was applied on the cultures and observe the gram stained smear under the oil immersion lens (100x). The biochemical tests are also performed for the further identification of colonies.

## Results

16 out of 9 samples have showed positive results for *A. polymyxa*. 8 samples appear to be white, thick, opaque and mucoid colonies on TGB medium while 1 plate of nutrient agar showed clonies of *A. polymyxa* along with *Bacillus* subtilis. Other 7 plates of NA show colonies for *Bacillus* subtilis. These results were further confirmed by biochemical reactions. 16 out of 8 samples showed positive results for *B. megaterium*. From the anaerobic samples 2 plates of TGB media and 1 plate of NA showed positive results. The colonies appear to white mucoid thick colonies. Aerobic soil samples give positive results on both TGB media. Milk samples give positive results from 1 TGB media and 1 NA media. Water sample show no results for *B. megaterium* in any of the plates except for *Bacillus* subtilis. The remaining negative plates have sown the results for *Bacillus subtilis*, *Staphylococcus aureus* and Streptococcus.

**Table I:** The colony morphology of *A. Polymyxa* on TGB and NA

| S. NO. | SAMPLE | TGB | NA | Microscopy |
|---|---|---|---|---|
| 1. | Anaerobic soil sample | Large, white, thick, opaque, mucoid and round colonies | Large, thick, opaque, mucoid round colonies. | Small scattered rods and diplococcic. |
| 2. | Aerobic soil sample | White, thick and pin pointed, small, round colonies | - | Long and short scattered rods |

**Table II:** The colony morphology of B. *megaterium* on TGB and NA

| S. NO. | SAMPLE | TGB | NA | Microscopy |
|---|---|---|---|---|
| 1. | Anaerobic soil sample | Large, white, thick, opaque, mucoid and round colonies | Spreaded, mucoid, round white colonies | Thick chains of capsulated spore forming scattered rods. |
| 2. | Aerobic soil sample | White, thick and pin pointed, small, round colonies | - | Capsulated scattered spore forming rods |
| 3. | Powdered milk | Spreaded large and small, white, hard and pin pointed colonies | Spreaded, large and pin pointed white round colonies | Capsulated spore forming cocci in chains |
| 4. | Distilled water | - | - | - |

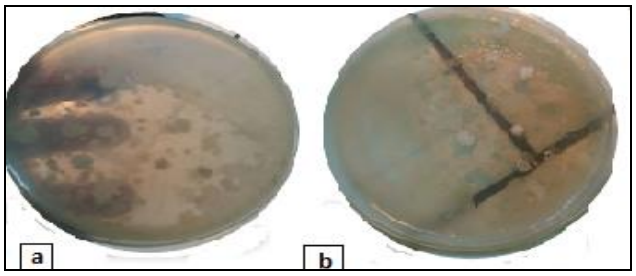


**Fig 1:** Colonies of a) *A.polymyxa*b) B. *megaterium*

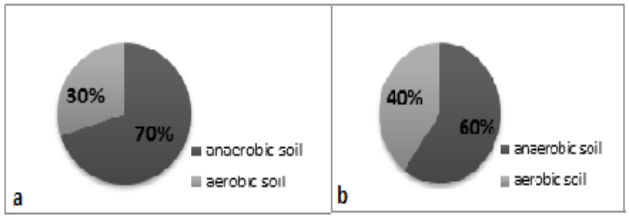


Chart: Proportion of a) *A. polymyxa* b) B. *megaterium*

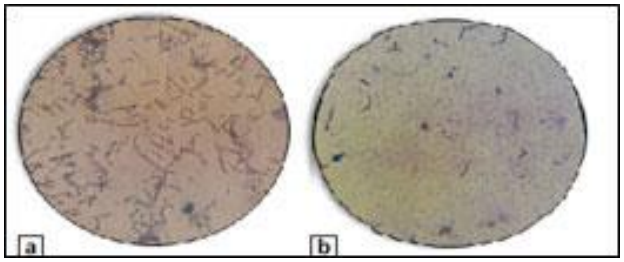


**Fig 2:** Microscopic examination of a) B. *megaterium* b) *A. polymyxa*

## Discussion

This article concludes two main points for both microorganisms. The ratio of *A. polymyxa* in anaerobic soil is greater (70%) as compared to aerobic soil i.e., the upper layer of soil (30%). These organisms are mainly involved in nitrogen fixation that also promotes the soil fertility. They protect plants from other harmful microorganisms by producing an antibiotic "Polymixin" that is also used medicinally. Second part of this research was to study the cell morphology and their arrangements on growth culture media as well as under microscope. They appeared as white, opaque, thick, mucoid, small spread, and pin pointed colonies on TGB media and NA. Under microscope these organisms appeared as long and small scattered rods. The ratio of B. *megaterium* in anaerobic soil is greater (60%) as compared to aerobic soil i.e., the upper layer of soil (40%). Likewise, these organisms are also helpful in nitrogen fixation, promote soil fertility. They are the best producers of proteins and synthetic penicillin. Isolating this specie can be useful in research for cell morphology and others, also being helpful in housing several plasmids. Second part of this research was to study the cell morphology and their arrangements in media as well as under microscope. They appeared as large and small spread, white, hard, and pin pointed colonies on TGB media and NA. Under microscope these organisms appeared as thick chains of capsulated spore, forming scattered rods.

## Conclusion

This research provide the information about the isolation method of *A. polymyxa* and B. *megaterium*, their habitat and their cell morphology. It also showed that they are mostly present in deep roots where they play an important role in fixing nitrogen and protecting plants. The antibiotic polymixin produce by *A. polymyxa* is highly efficient in removing biofilm formation. These organisms can be widely use in industries due to the activity involved in bioflocculation and biopreservation of foods. *B. megaterium* on other hand is highly involved in protein synthesis which is widely used in industrial processes and is helpful in making synthetic penicillins. Growing these two microorganisms in laboratory according to their growth conditions can be helpful for various purposes. They can be very helpful in today's environment.

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