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SCANNING ELECTRON MICROSCOPIC STRUCTURE OF THE *BACILLUS* ORGANISMS ISOLATED FROM SOIL

E.Samjeeva Kumar[1], Dr.M.Ravikumar[2], P.Venkataramana[3], Shaik-Reshma[3], M. P. Naveen Kumar[3].

[1]Research Scholar, JNTUH, Hyderabad.
[2]Principal, Geetanjali College of Pharmacy, Hyderabad.
[3]Department of Pharmacy Practice,PRRM College of Pharmacy,Kadapa.

Abstract:

Soil has been defined as the region on the earth's crest where geology and biology meet. From a functional view point the soil may be consider as the land surface on the earth which provides substratum for plant and animal life. The genous bacillus consists of aerobic bacilli forming heat resistance spores they are gram positive but tend to be decolourised on gram staining. A scanning electron microscope (SEM) is a type of electron microscope that produces images of the sample by scanning with a focused beam of electron. easily so that it is gram variable or may be gram negative. the soil is collected from the mountain regions of south india, the primary screening and secondary screening were done and isolated organism is subjected to morphological and biochemical studies. Colonies where fixed in formalin vapour for a period of 24hr. After fixing the outer edge of the colonies were marked with dissecting needle. The agar was removed. The colonies were then coated with gold –palladium alloy to a thickness of 500A^0 by means of vacuum evaporation. Then the colonies were viewed using scanning electron microscopy. After that the chamber was evacuated to 10$^{(-5)}$ torr the specimens were scanned using electron beam the electrons liberated from the surface of the specimen were detected using a scintillation photo multiplier system. The resulting image was formed on a cathode ray tube. Photo graphs were taken with the Polaroid camera. At 3000x a small rode shaped, non- uniform distribution of organism were seen. At 5000x the rods were more elongated and the arrangement of colonies were non uniform. At 10,000x the colonies showed a length of 1.46micrometer and 1.64 micrometer and the width of the colonies were found to be 630.1 nanometre and 752.9 nanometre and irregularly arranged colonies. At 20,000x the rods were found to be more elongated. Scanning electron microscope can be used to view the organism isolated from the soil. The examination depends on the concentration of cells in the soil. This limits the use of sem technique in soils with more than 10^{-7} organisms per gram of the soil.

Keywords: *Soil, Bacillus organisms, Scanning electron microscopy.*

Corresponding author:
E.Samjeeva Kumar,
Research scholar,
JNTUH,
Hyderabad.
samjeevakumar@Yahoo.co.in.
9566972274.

QR code

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INTRODUCTION:

Soil has been defined as the region on the earth's crest where geology and biology meet. From a functional view point the soil may be consider as the land surface on the earth which provides substratum for plant and animal life. The characters of the soil environment vary with the local and climate. Soils differ in depth, physical properties, chemical composition and origin. The dominant mineral particles of most soils are silicon, aluminium and iron and lesser amount of other minerals including calcium, magnesium, titanium, manganese, sodium, nitrogen, phosphorus, sulphur. The mineral constituents of the soil vary in size from small clay particles to large pebbles and gravel. The physical structure aeration, water holding capacity are determined by the proportion of these particles which are formed by the weathering of the rock and the degradative metabolic activities of the micro organisms[1].

Soils can be classified as mineral soils which are solid matter that is largely inorganic and organic soils which have very little inorganic material. The plant and animal remains deposited on the soil contribute organic substances. The decomposition of plants and animal remains in the soil forms the humus; the dark colour amorphous substance composed of residual organic matter, but readily decomposed by micro organisms. No doubt the microbial population, both death cells, and living cells is of such a magnetive that contributes significantly the organic matter of the soil [2].

Certain agriculturally important proportion of the soil is contributed by the humus which improves the texture and structure of the soil: Contributes to its buffering capacity and increased in water holding capacity [3-5].

Fertile soil is inhibited by the root systems of fire plants by many iron forms. E.g.: Rodents, Insects and by tremendous members of micro organisms. The waist differences in the composition of the soil together with their physical characteristics and agriculturer practices by which they are cultivated resulting corresponding large differences in microbial population both in numbers and in kinds.

The above conditions influence the growth of the organism under laboratory cultivation or also applicable to the soil. The conditions which influence the growth of the micro organisms are

1. The amount and type of nutrients
2. Available moisture
3. Degree of variation
4. Temperature
5. PH
6. Practices and occurrences which contributes large number of organisms in the soil.

The existence of roots and extensiveness of the root system also influence the numbers and the kinds of micro organisms present.

The great diversity of microorganisms present in soil makes it extremely difficult to determine the total number of organisms present. Cultural methods will reveal only those nutritional and physiological types compatible with the cultural environment .Direct microscopy is used to identify all types of microorganism except viruses. This technique has the limitation of distinguishing living from dead microorganism. Very often the microbiological analysis of the soil involves isolation and identification of specific physiological types of microorganism.

Sporogenious rod shaped bacteria are classified into 2 genera the aerobic bacilli and the anaerobic clostridia. The genous bacillous consists of aerobic bacilli forming heat resistance spores they are gram positive but tend to be decolourise easily so that it is gram variable or may be gram negative. They are generally mortile with peritrichous flagella. Members of this group have great diversity in properties. The genous includes psychotropic, mesophilic and thermophilic species. The maximum temperature for vegetative growth is about 25^0C to above 75^0C and minimum of about 5^0C to 45^0C.

A scanning electron microscope (SEM) is a type of electron microscope that produces images of the sample by scanning with a focused beam of electron. The electrones interact with the atoms of the sample producing various signals that contain information about sample topography and composition. The electron beam is generally scanned in a raster scan pattern and beams position is combined with the detector signal to produce an image.

The scanning electron microscope is a valuable adjuvant to light and transmission electron microscopy for studying gross cell morphology.

Attempts have been made to observe the major images of the bacterial colonies present in the soil. This technique is superior to previous techniques because of grater magnification less distortion of the species and less disruption of the soil environment.

MATERIALS AND METHODS:

The soil is collected from mountain region of south india.

Primary Screnning:

1% of the soil solution is inoculated in the nutrient agar media.

Ingredients:

Peptic digest of animal tissue	5g
Beef extract	1.5g
Yeast extract	1.5g
NaCl	5g
Agar	15g
Final pH (at 25^0C)	7.4 + or – 0.3

SELECTIVE MEDIA FOR THE ISOLATION OF BACILLUS:

Di potassium phosphate	1.00g
Magnesium sulphate	0.200g
Sodium chloride	0.200g
Ferrous sulphate	trace
Soils extract	5.000g
Mannitol	20.000g
Agar	15.000g
Final pH (at 25^0C)	8.3(+/ –) 0.6

The isolated colonies were tested for morphological, biochemical characteristics.

SCANNING ELECTRON MICROSCOPY:

Colonies where fixed in formalin vapour for a period of 24hr. After fixing the outer edge of the colonies were marked with dissecting needle. The agar was removed. The colonies were then coated with gold –palladium alloy to a thickness of 500A by means of vacuum evaporation. Then the colonies were viewed using scanning electron microscopy. After that the chamber was evacuated to $10^{(-5)}$ torr the specimens were scanned using electron beam the electrons liberated from the surface of the specimen were detected using a scintillation photo multiplier system. The resulting image was formed on a cathode ray tube. Photo graphs were taken with the Polaroid camera.

RESULTS:

By using simple staining and examining under light microscope a rode shaped bacteria was seen. On gram staining gram positive species was seen. Catalase, oxidase, nitrate all the biochemical reactions which showed positive. Indole, methyl red, vogus proshkar, citrate showed negative reaction. The minimal number of microorganisms that can be detected using scanning electron microscope is between 10^{-7} and 10^{-10} per grams of the soil. The results were obtained using 3000x, 5000x, 10,000x, 20,000x.

The following figure shows the scanning electron micrographs of the bacillus microorganisms in different magnification. At 3000x a small rode shaped, non- uniform distribution of organism were seen. At 5000x the rods were more elongated and the arrangement of colonies were non uniform. At 10,000x the colonies showed a length of 1.46micrometer and 1.64 micrometer and the width of the colonies were found to be 630.1 nanometre and 752.9 nanometre and irregularly arranged colonies. At 20,000x the rods were found to be more elongated.

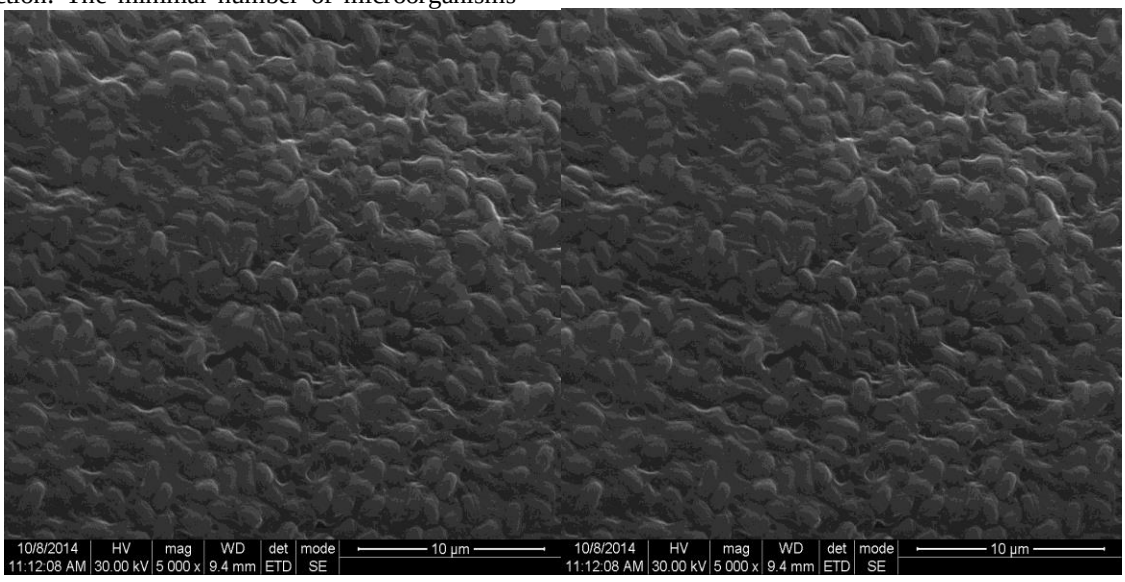
DISCUSSION:

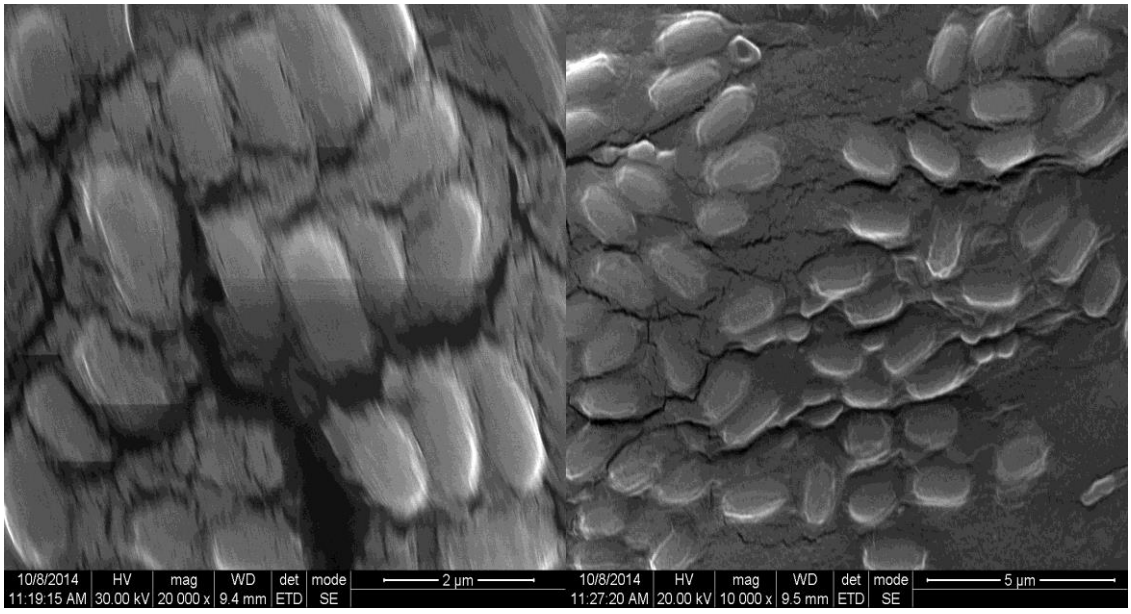
The scaning electron microscopy is a valvable adjuvent to light and transmission electron microscopy. The scaning electron microscope produces images comparable to light, ultraviolet, fluorescence and X-ray microscopes.

sing scaning electron microscopye morphological using of bacteria and surface views of spores at high magnification can be obtained. The alternative method for obtaining surface views of the electron dense structures is using transmission electron microsccope.

The scaning electron microscope has less resolving power than transmission electron microscope. But, has the advantage of reveiwing a three dimensional picture. The surface topography can be evolved clearly which is not possible by any other method.

In bacillus colonies branching occur which may be due to extracellular matrix thin intracellular bridges are also seen which may be due to genine extensions of the adherence sline.

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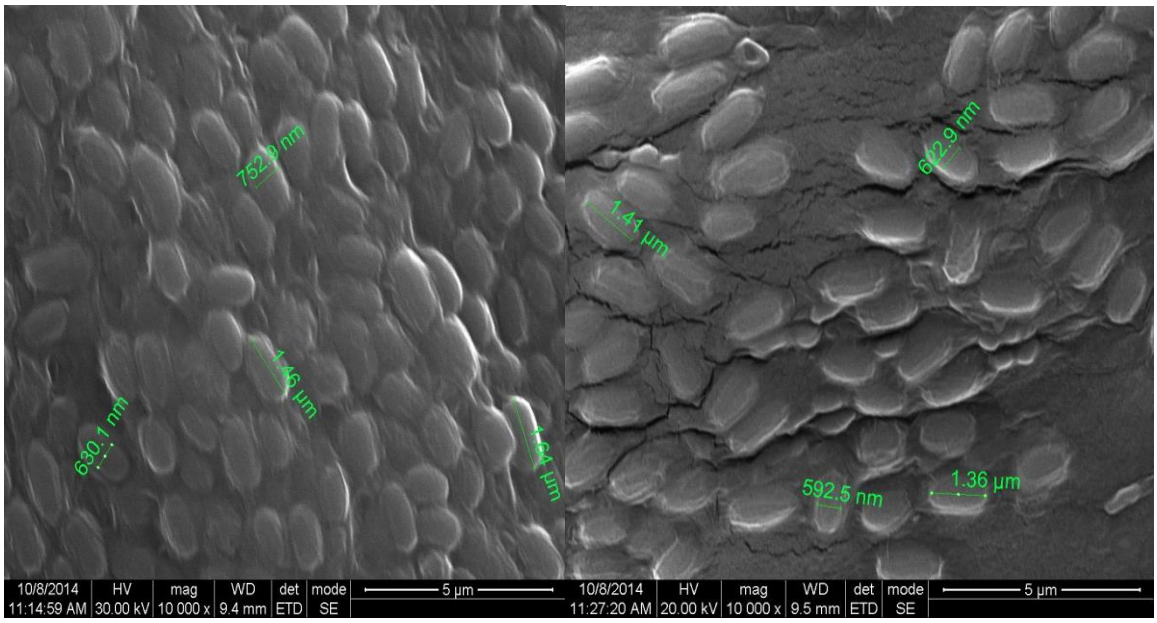
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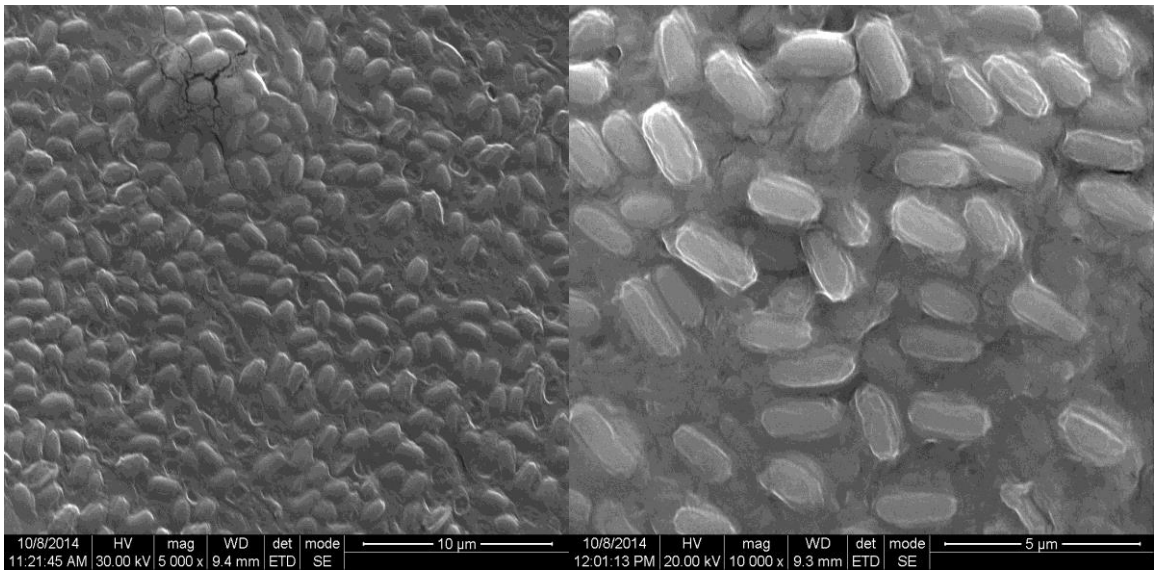
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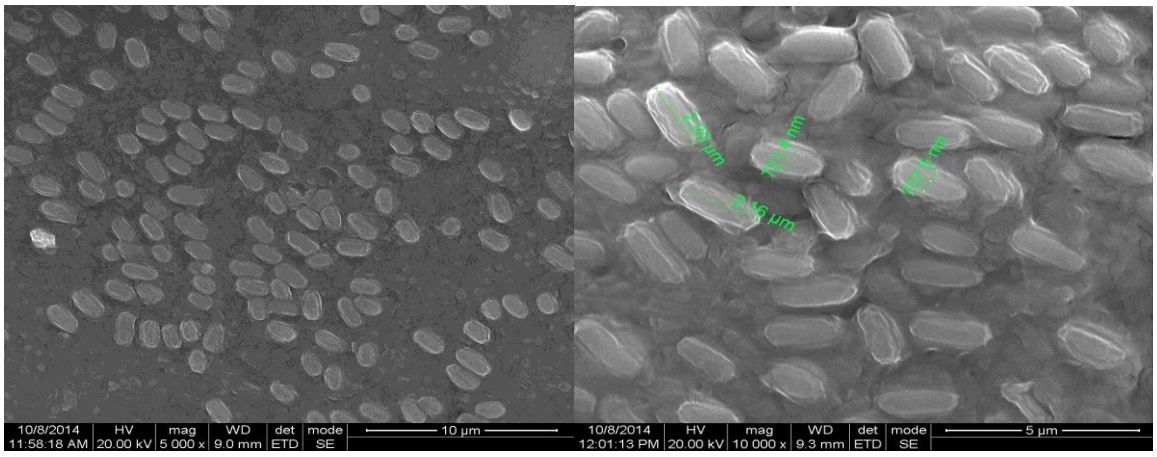
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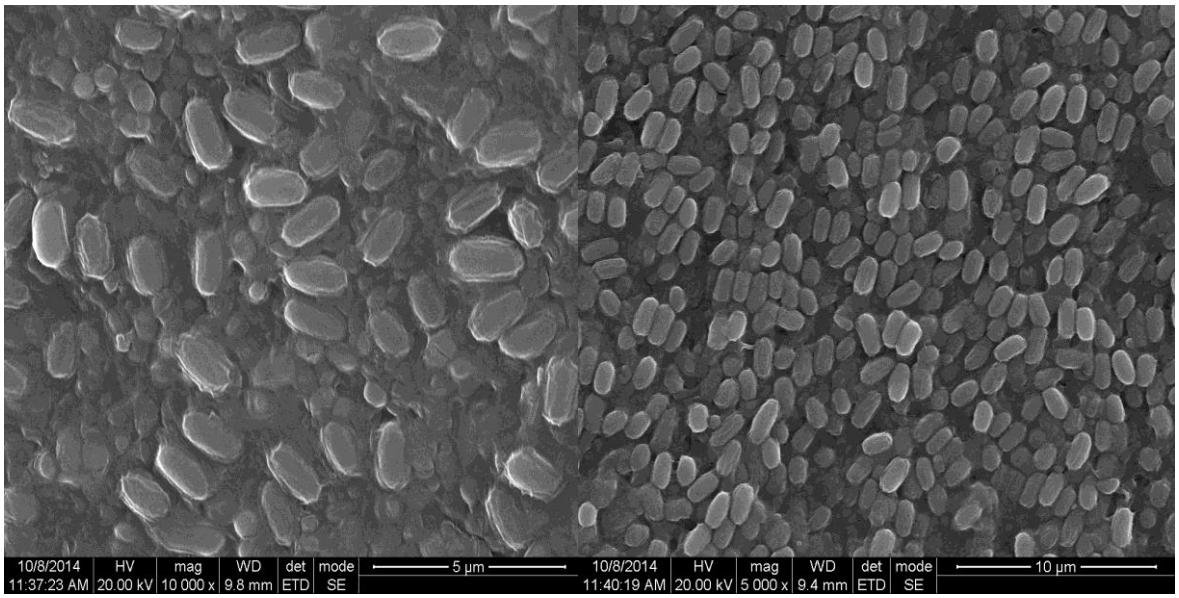
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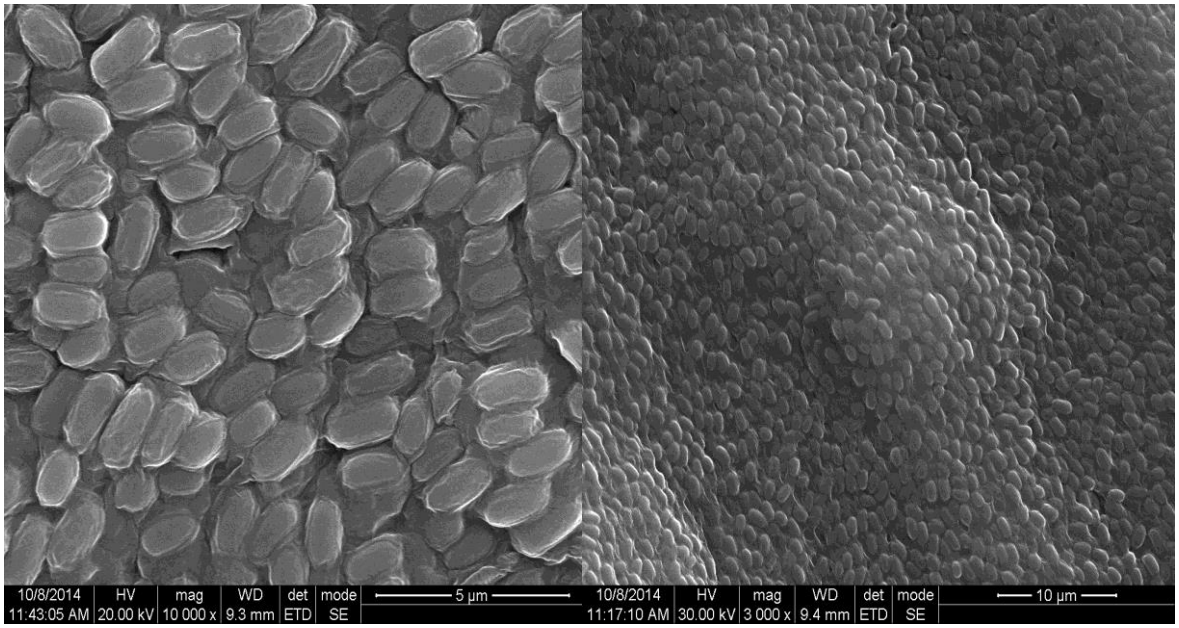
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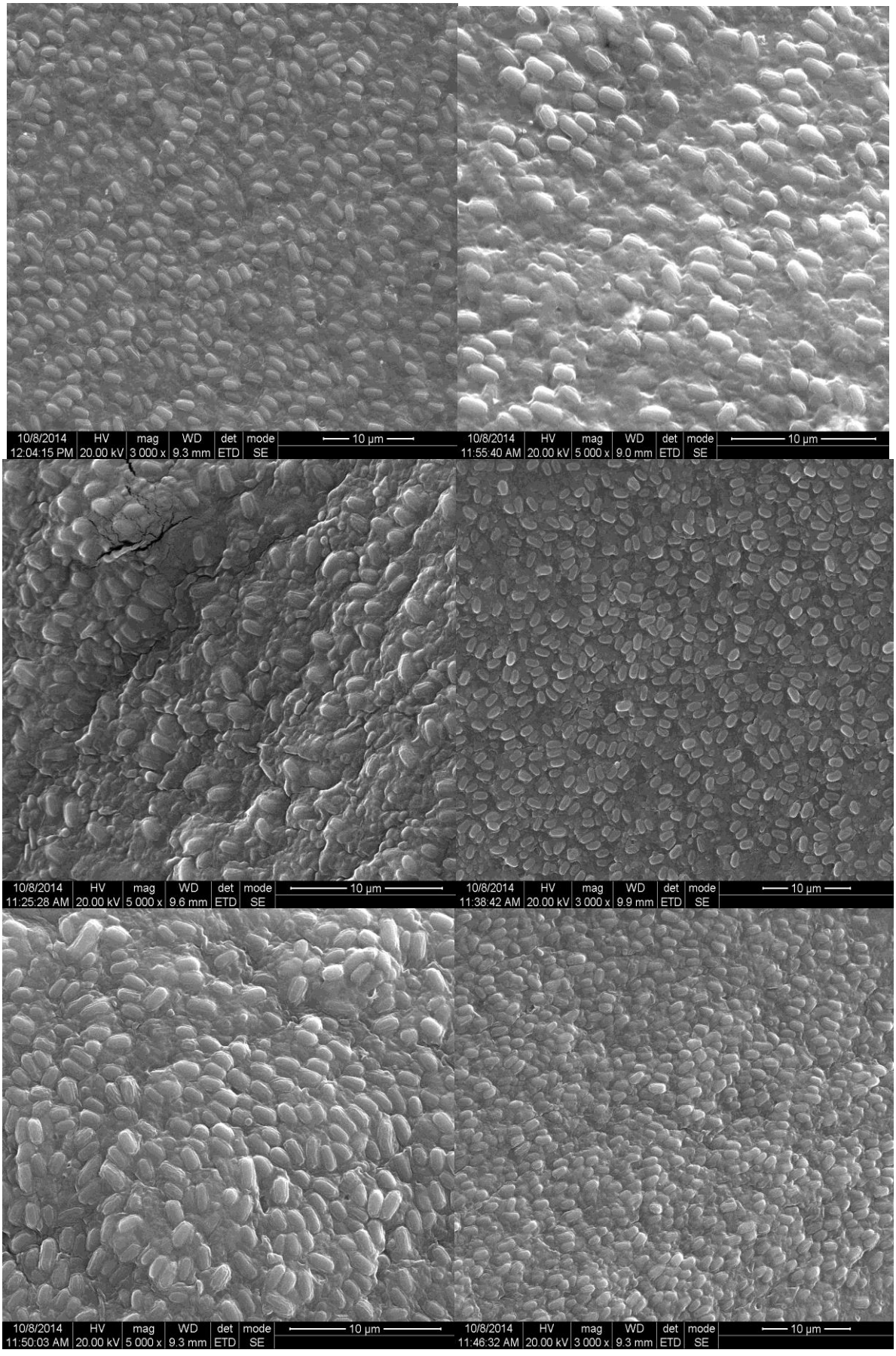
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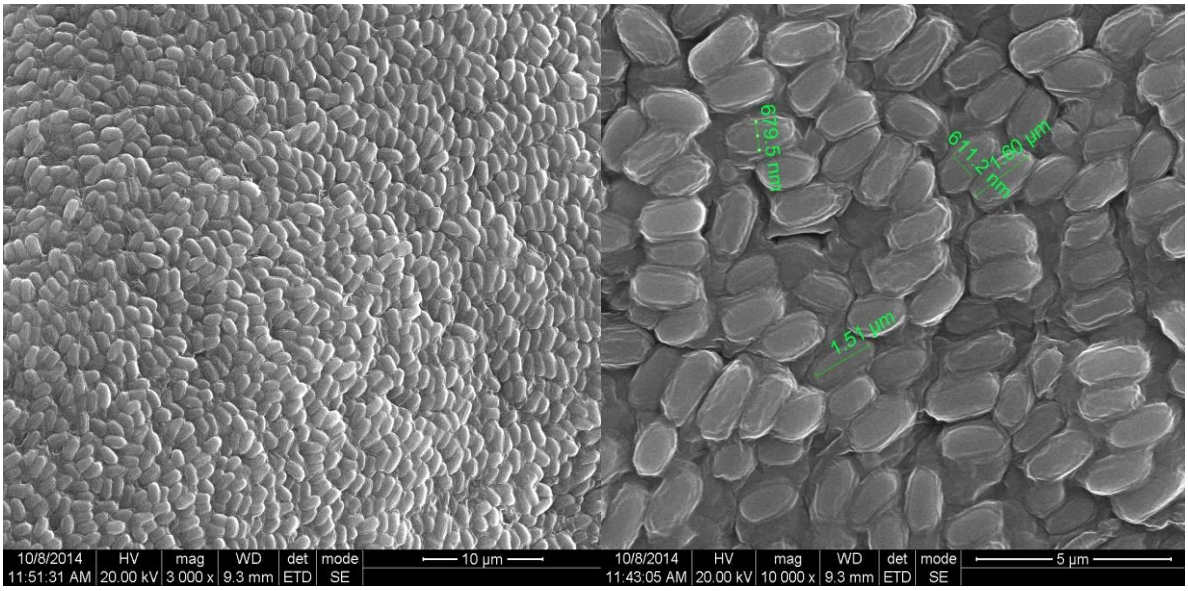

CONCLUSION:

Scanning electron microscope can be used to view the organism isolated from the soil.The examination depends on the concentration of cells in the soil. This limits the use of SEM technique in soils with more than 10^{-7} organisms per gram of the soil.

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