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Isolation and Identification of the Microorganism from Hot Water Spring Soil Sampleof Lasundra Village

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ABSTRACT: Soil sample was collected from hot water spring of Lasundra. Lasundra Village is located in Kathlal Taluka, Kheda District in Gujarat State, India. It is 59 KM away from District headquarters Kheda and 71 KM from State capital Gandhinagar.After drying with the help of sieving method collected soil sample was screened to remove unwanted particles. Aseptically dilutions were performed by using screened soil sampleserial dilution process was followed up to 10^{-6} dilution. Diluted sample was inoculated by spreading method in starch agar media plates and inoculated plates were incubated at 37 ° C for 24 hours. After completion of the incubation period well isolated colony was selected and sub cultured was performed 2 more times with selected colonies to get pure cultured colony.After completion of sub cultured activity, well isolated colonies were selected.As it was pure cultured colonyand identified as Bacillus Spp. by different types of identification process like cultural identification process, morphological identification process, biochemical tests.

KEYWORDS: Microorganism, Isolation, Hot water springs, cultural characteristics, Morphological characteristics, Biological characteristics

I. INTRODUCTION

Approximately three billion–four billion years ago Single-celled microorganisms were the first forms of life which are main responsible to start live cycle on earth.[1]But Further study was very slow. So, in case of the history of life on the earth is one and only forms of microorganisms. Microorganisms are present in all around us, inside us, in our food, in the air and in the water.At the end of the 19th century scientists were started to study about the diversity of microbial world. And also started to study to explain how life was begin on the earth and how it develops. Microorganisms are capable to live in every condition in almost every habitat like desert, geysers, rocks, in deep ocean etc. Some are adapted to extremes such as very hot or very cold conditions, others to high pressure and a few such at to high radiation environments. There are so many microbes, but from that scientist have identified only 0.5 % of them and 80 to 99 % are remain unidentified.[2]About 9300 prokaryotes species are known and it includes bacteria and archaea.

Our planet's undiscovered biodiversity is soil and it is probably the most complex microbial environment on earth with respect to species richness and community size.[3]In one gram of garden soil there are over 10,000 microbes.[4]The belowground microbial community structure is highly diverse. Soil biodiversity is generally high because it contains all the major groups of

microorganisms which include fungi, green and blue-green algae, bacteria also and a great number

of animal phyla. Microorganism numbers different between different soil types and conditions, with bacteria being the most numerous.

Extremophiles have been isolated from rocks as much as 7 kilometers below the Earth's surface. Hot water spring is one of the best sources for extremophiles.

A very highly accepted definition of a hot spring is that a naturally occurring water springs which temperature is more than 98 degrees Fahrenheit (36.7 degrees Celsius) when it passed through the ground. However, this is not a scientific definition and these phenomena can also be defined as a water spring is hotter than the surrounding ground and air temperatures.[5]Hot springs will also be referred to as geothermal or thermal springs.

Current research was carried out to isolate the organism from soil sample from hot water spring of Lasundra. Lasundra is located in Kathlal Taluka which is in Kheda District of Gujarat State, India. It is located 59 KM towards East from District headquarters Kheda and 71 KM from State capital Gandhinagar.

Lasundra hot water spring is a natural wonder where both hot and cold-water springs are adjacent with each other. Since hundreds of years

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all people are visit this wonder with faith and science also. In this case irrespective of summer and winter season, from that one spring can easily get cold and another one can get easily hot water. November to February is the best time to take visit at Hot Water spring. Morning time is good time to take Bath in Winter is the best time ever.

II. MATERIALS AND METHODS.

Sampling:

3 different soil samples were collected from hot water spring which is located in Lasundra village.At the time of sampling temperature was 102.2 º F (39º C).It was transferred in clean plastic bagand stored in refrigerator at 2 to 4 º C up to the further use. I was initiated my study by drying and carrying out extensive screening work in order to isolate organisms from soil sample.

Media Preparation:

1) Starch agar media:

10.0 gm of soluble starch was taken in 500 ml purified water containing beaker with the help of weighing balance. After starch was properly dissolved 3.0 gm of beef extract was added. After proper mixing volume was make up to 1000 ml with purified water and pH was adjusted to 7.0. After pH adjustment 15.0 gm of agar powder was added and mixed well. After mixing, 250 ml of media was distributed in 500 ml conical flask and it was sterilized in autoclave at 121 º C temperature for 15 minutes. After autoclave, aseptically plating was performed with sterilized media and it was kept on flat surface up tosolidifying. Solid media plates were stored in incubator for future use.

2) Normal saline:

9.0 gm of sodium chloride was taken in 100 ml of purified water containing 250 ml of conical flask. After proper dissolving, 9 ml of solution was distributed in individual test tubes and it was sterilized in autoclave at 121 º C temperature for 15 minutes. After sterilization it was stored at room temperature for future use.

Isolation of culture:

1 g soil sample was added into 9 ml sterile normal saline containing tube. Sample was mixed well to homogenize. Serial dilutions were made aseptically from each tube up to 10^{-6} dilution.[6]0.1 ml of suspension from each dilution of different samples was aseptically spreaded on sterile starch agar media. The plates were incubated at 37 º C for 24 hours.[7]After the completion of incubation period isolated colonies were selected and reinoculated on sterile starch agar media. Inoculated media plates were incubated at same temperature at 37 º C for 24 hours. This process was repeat two more time to get pure culture. After incubation only well isolated pure cultured colonies were taken for further study such as identification test and it was stored at refrigerator at 2 to 4 º C for future use.

Isolated colonies were identified with different morphological, physiological and biochemical studies.[8] This study is also provided in the Bergey's manual of systematic bacteriology.[3]

Identification of the isolated culture:

1) Identification by cultural characteristics:

The isolated colonies were observed for its different characteristics like size, shape, texture, opacity, color, consistency etc.

2) Identification by morphological characteristics:

Gram staining was performed for isolated culture and observed it under microscope.

Procedure for Gram staining:

Aseptically thin smear was prepared on a glass slide and it was heat fixed after air dried. First of all smear was covered with crystal violet for 1 minute. After 1-minute slide was washed by running water using wash bottle. After washing smear was covered with Grams iodine for 1 minute. After 1-minute slide was washed by running water using wash bottle. After washing it wasdecolorizedwith alcohol for 1 minute and again smear was washed with water. Then after smear was counterstained with safranin for 30 seconds and washed under running water. Slide was put in dry surface for air dried and it was observed under microscope for morphology of bacteria.[9]

This whole process is summarized in below mentioned Table 1

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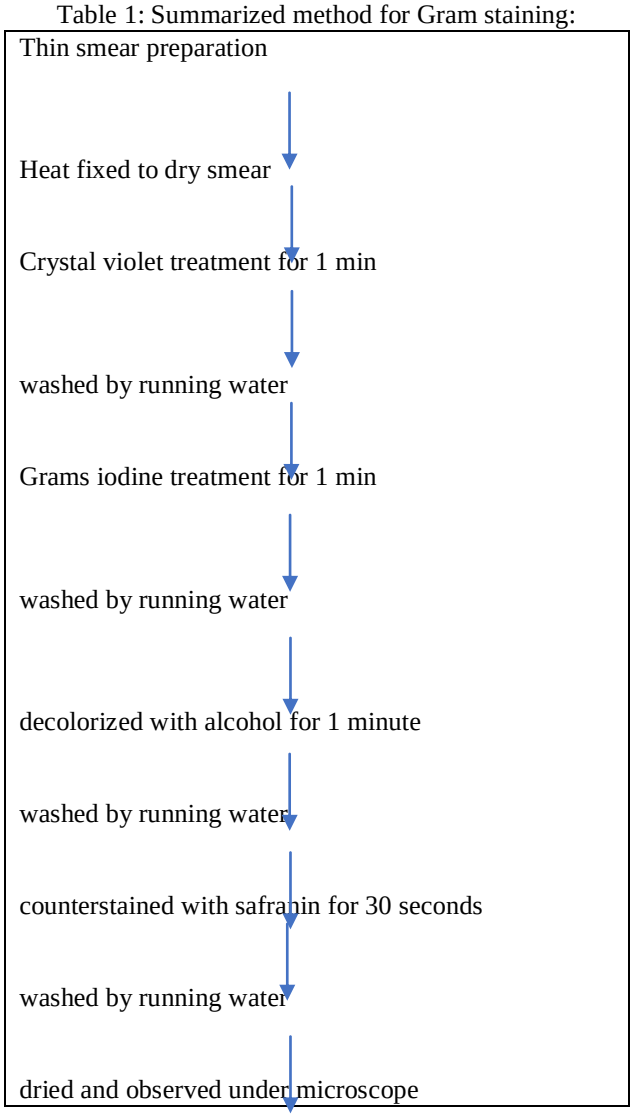
Table 1: Summarized method for Gram staining:



3) Identification by biochemical characteristics:

The isolated cultures were characterized biochemically by different biochemical identification tests like MSA test, Catalase test, Oxidase test, Methyl red test, Indole test, Voges Proskauer test, Starch hydrolysis test, Urease test, Citrate utilization test and Fermentation test.[7]

III. RESULTS AND DISCUSSION

Screening is defined as the detection and isolation of only desired microorganisms from a large microbial population by using highly selective methods.

The starch agar media was used for isolation of microorganism. In the present study 3 different samples were taken at same time from same location with some distance and proceed by screening, serial dilution was performed up to 10^{-6} dilution and inoculated on starch agar plates. Inoculated plates were transferred to incubator for 24 hours at 37 ° C temperature. After getting well isolated colonies sub cultured process was followed for 2 more times to get pure culture.

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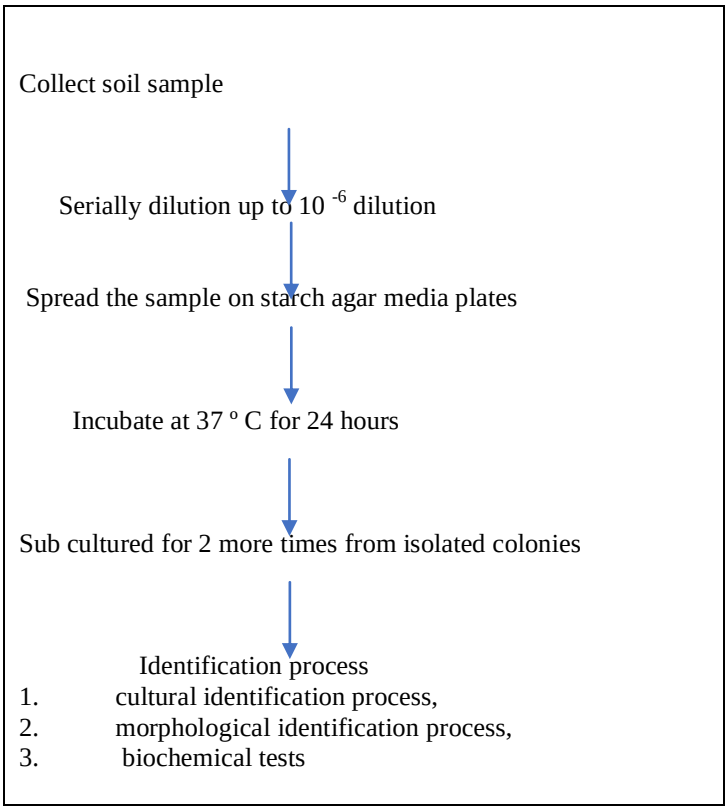
Table 1: Summarized method



From all samples well isolated colonies were selected for identification process and their colony characteristics (Table 2), morphology characteristics (Table 3) and biochemical test (Table 4) results were as in below mentioned tables.

Table 2: Colony characteristics

Characteristics	Observations		
	Sample 1	Sample 2	Sample 3
Size	Medium	Medium	Medium
Shape	Circular round	Circular round	Circular round
Texture	Rough	Rough	Rough
Consistency	Rough	Rough	Rough
Opacity	Opaque	Opaque	Opaque
Color	White to light yellow.	White to light yellow.	Whitish

Table 3: Morphological characteristics.

Characteristics	Observations		
	Sample 1	Sample 2	Sample 3
Grams reaction	Gram positive	Gram positive	Gram positive
Size under microscope	About 4 µm	About 5 µm	About 8 µm
Shape	Rod shape	Rod shape	Rod shape
Arrangements	Single and in clusters	Single and in clusters	Single and in clusters

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Table 4: Biochemical characterization:

Characteristics	Observations		
	Sample 1	Sample 2	Sample 3
MSA	Yellow to cream colored colonies	Yellow to cream colored colonies	Yellow to cream colored colonies
Catalase	Positive	Positive	Positive
Oxidase	Positive	Positive	Positive
Methyl red	Negative	Negative	Negative
Indole	Negative	Negative	Negative
Voges Proskauer	Positive	Positive	Positive
Starch hydrolysis	Positive	Positive	Positive
Urease test	Negative	Negative	Negative
Citrate utilization	Negative	Negative	Negative
Fermentation tests			
Glucose	Acid	Acid	Acid
Fructose	Acid	Acid	Acid
Lactose	Acid	Acid	Acid
Sucrose	Acid	Acid	Acid

IV. SUMMARY AND CONCLUSION

The present study was undertaken to isolate to microorganisms from soil sample from Hot water spring, located Lasundra. A total 3 sample was analyzed and bacterial isolates have been isolated. All the bacterial isolates colonies were medium in size, circular rounded, whitish to light yellow in color, Gram's positive and rod shaped. With the help of biochemical tests results (MSA test, Catalase test, Oxidase test, Methyl red test, Indole test, Voges Proskauer test, Starch hydrolysis test, Urease test, Citrate utilization test and Fermentation test.) it can be concluded that all the isolates were Bacillus spp.

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