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A Brief Study on Identification of Microorganisms from Jujube Pickle and Mirabilis Jalapa Flowering Seed

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Abstract- *The main research of this study is to isolate bacteria from jujube pickle and flowering seed by using a common media, nutrient agar media and accomplish all the staining processes to know the type of the organisms present, followed by the effect of antibiotics (ABST test). After the invention of antibiotics from bacteria, it has been so much significant to use antibiotic to kill bacteria [1]. Antibiotics are of 2 types, broad spectrum and narrow spectrum. Broad spectrum antibiotics kill or resist the growth of both Gram positive and Gram negative bacteria. Narrow spectrum antibiotic kill or resist the growth of only Gram positive or Gram negative bacteria. So, obviously broad-spectrum antibiotics would have much more greater effect than that of narrow spectrum. Here, we select 2 samples for isolating bacteria from them and checking the effect of antibiotics on them, they are homemade jujube pickle and flowering seed. Some according steps are to be followed to accomplish the experiment. First is to isolate the bacteria from the samples using one of the three conventional methods, spread plate, pour plate and streak plate technique on a nutrient agar plate. After plating the petri dishes are to be kept inside the incubator for 48 hours to allow the bacteria to grow and form visible colony. The next step that is to be done is staining of the organisms to know about the nature of the organisms present there. Both Simple and differential staining methods are done. The last step is to check the antibiotic effect by ABST test or Kirby Bauer method.by using the commercial antibiotics like Ampicillin, Amoxycillin, Meropenem, Ertapenem and Amikacin [2]. It is important to note that all the works are done in the laminar chamber to avoid contamination.*

Keywords: Jujube pickle, Isolation of bacteria, flowering seed, Kirby Baeuer method

1. Introduction

As we all know, microorganisms are present in everywhere, from the thermal spring to the coldest regions of the world, it is significant to isolate the microorganisms from a randomly selected sample and check the nature of the microorganisms present in the sample. In this experiment the microorganisms are isolated from the home made pickles and dry seeds. In India pickles are generally pickled with oil, lemon juice, vinegar and water. Indian pickles are often made into reddish chutney type, which provide the additional flavor to the food. Mangoes, Goose berries and lemons are the common examples of the Indian cuisine [3]. In India the types of pickles are amla pickle, assorted pickle, Carrot pickle, Garlic pickle, Green chilli pickle, Hibiscus leaf pickle, Onion pickle, Red chilli pickle, Sweet Mango pickle, Jujube pickle etc. In these experiment we were using Jujube Pickle which was home-made. A wide range of microorganisms have been already found in pickles, specially homemade pickle as generally they are not mixed with strong chemical preservatives. All the lactic acid bacteria (LABs) which produce exopolysaccharides have antibiotic susceptibility and possess activity of β-galactosidase, β-glucosidase, protease and amylase, while none of the isolates showed haemolytic activity [12].

We have also performed biochemical test for the identification of the organisms present in the sample and this already found organisms has helped us to predict the microorganisms present in our sample as per the results of the biochemical tests. From these two samples Different types of microorganisms were collected by some staining methods and the effect of the antibacterial drugs on these microorganisms were checked. We had to take several precautions to make these experiment succeeded.

2. Materials and Method

The materials used for the project work is Conical flask, petri dishes, test tubes, inoculation loop , Nutrient agar and Brain-heart infusion agar , Bunsen burner , L- shaped glass rod, Sterile swab, antibiotic discs, stains, pressure cooker, microwave oven**.**

2.1 Collection of Materials:

Jujube pickle had been made previously at home. It is made by jujube, molasses, table salt, chili, turmeric, cumin and other spices. After preparation it is kept in a jar. Some pickle is taken out from the jar using sterile forceps and taken into a sterile container.

Mirabilis jalapa, called 'Sandhyamalati' in Bengali, is a tree which grows naturally in India. It is a small, bushy tree, height 0.5 meter to 1 meter. The flowers are conical shaped, reddish pink in colour. The seeds are almost round, green when unripe and black when ripe. Some of the seeds of the flowers are taken using a sterile forceps.

2.2 Method:

Nutrient agar media was prepared in a conical flask by adding 2.3 gm of nutrient agar powder and 2 gm of agar powder in 100 ml water followed by sterilization of media in pressure cooker. Other glass wares were also sterilized. Melted agar was poured in the petri plate and put in UV ray for 5 minutes. A dilute solution of homemade pickle which had been prepared by serial dilution process (10^{-4} dilution) is introduced to the solidified agar plate by spread plate method. Incubated for 48 hrs. Bacteria grew on the agar plate. Thereafter, Monochrome staining, Negative staining, Gram's staining and spore staining were done [4].

2.2.1 Monochrome staining:

A clean grease free slide is taken. One drop of distilled water is taken and a colony of bacteria is taken from the agar plate using sterilized inoculation loop. A thin smear is made using the loop. Then the smear is air dried and heat fixed. Thereafter methylene blue stain is added and kept for 60 seconds. After that the stain in washed away with water and observed under microscope [5].

2.2.2 Negative staining:

A clean grease free slide is taken and one drop of nigrosin stain is taken on a side of the slide. After that, a colony is taken from the culture plate and mixed with the drop of stain. Thereafter, using another slide, a thin smear is made on the first slide. Then allowed to air dry and observed under microscope[6].

2.2.3 Gram's staining:

On a clean grease free slide a colony is taken along with distilled water drop and mixed followed by making of a thin smear and air drying and heat fixation of it. Thereafter, the smear is flooded with crystal violet stain and kept for 1 minute, then washed off. After that mordant or Gram's iodine is added which is a dilute solution of iodine and KI in alcohol, kept for 1 minute and washed off. Then, Gram's decolourizer (acetone-alcohol) is used for the washing of the stain. It is added drop by drop until the last drop looks colourless, then washed with water. At last, safranin (counter stain) is added and kept for 2 minutes and washed off with water. Then observed under microscope [7].

2.2.4 Spore staining:

Bacterial colony is taken on a clean, grease free slide with a drop of distilled water and a thin smear is made using the inoculating loop which must be sterilized before using. Then the smear is air dried and heat fixed. After that malachite green stain is added on the smear and the slide is kept inside the hot water bath for 15 minutes. The smear is replenished with stain when the stain dries up. After 15 minutes the stain is washed away with distilled water and safranin stain is added and kept for 2 minutes. Next, the stain is washed with distilled water and observed under microscope.

2.25 ABST Test:

Brain heart infusion agar was prepared by adding 5.2 gm of BHI powder and 3 gm of agar powder in 100 ml water and sterilized in the same way and poured in a petri dish and waited for solidification. The Petri plates were kept in UV ray for 5 minutes. The culture from the first petri plate was swabbed on the BHI plate and 5 antibiotic discs, ampicillin (10 mcg), amoxicillin (30 mcg), meropenem (10 mcg), ertapenem (10 mcg), amikacin (10 mcg) along with a test which has no antibiotic effect was placed on the BHI plate. The plate was kept for 48 hrs.

2.2.6 Biochemical Test:

Test kit is a kit or plate like thing embedded with a number or holes, where the reagents of biochemical tests for the identification of organism are present in separate holes in semi-solid state. From the culture plate, one colony is taken up using a sterile swab and it is swabbed on all the reagents. All the works should be done in the laminar air flow in laminar chamber. Then, the kit is kept inside the incubator for the growth of the organisms and accomplishment of the biochemical reactions happened for metabolism [8].

3. Result:

At the end of the experiment we have seen a series of diverse microorganisms were present in the following two sample. After all the staining procedures we were seen that monochrome stainable bacteria, negative stainable bacteria, spore forming bacteria, Gram positive and Gram negative bacteria were present in the following two samples. Results of biochemical tests are given in Table 3 and Table 4. After done the antimicrobial activity of the following drugs here is the measure of the antimicrobial effect of those drugs and the result also can be seen in Figure 1.

4. Discussion:

So, as per the result of the experiment it is clear that in the sample of homemade jujube pickle a lot of bacteria were present. As it is a food sample, it should be free from microorganisms as possible. But the number of microorganisms present in the sample was notable. It is also significant that why some people suffers from digestive problem along with stomach pain after eating those types of pickles [10]. The problem is not only with jujube pickle, but also other homemade pickle made with other things like mango, chilli, olive etc mentioned earlier. As per observation, pickles which were made long before and had been left for a long time, affects more in comparison to recently made pickle [9]. The reason is clear from this experiment, the more the microorganisms present, the greater the effect. Little children and old men are affected comparatively greater. Some methods can be used to lessen the number of bacteria in those pickles, like keeping in sunlight for a long time after preparation of the pickle, using vinegar while preparing the pickles, etc. As per the result of the ABST test, it can be predicted that, for the cure of the diseases caused by consumption of homemade pickles, mainly the digestive problems, using ertapenem or other antibiotics having similar structure like ertapenem is most effective in comparison to ampicillin or amoxicillin. While coming to the seed, it is natural to be a large number of bacteria there, as they are old dry. But it is also notable that there could be some type of bacteria which prevents the germination of the seed, or after the germination, they can infect the seedling which can lead it to death [11]. Or some bacteria could also be helpful to the plant. Needless to say, it is not possible to sterilize all the seeds of all plants before germination, also, sterilization methods are not applicable for this case as heating or chemical treatment would spoil the seed and the seed would lose its viability. Even though, if we want to make a seed germ free, the most effective antibiotic to kill the bacteria would be Ertapenem or other antibiotics having structural similarity with Ertapenem.

5. Conclusion:

The experiment was done successfully using the required solution and the bacteria were clearly visible. From antibacterial drug test we found that for the microorganisms present in the pickle sample the most effective drug was Ertapenem and the least effective drug was Ampicillin and for seed sample microorganisms also the most effective drug was Ertapenem and least effective drug was Ampicillin. The found microorganisms are already discussed in the result part.

Tables:

Class	Species
Lactic acid bacteria	Lactobacillus plantarum Lactococcus lactis L. brevis Leuconostoc mesenteroides Pediococcus cerevisiae Pediococcus pentosaceus
Enterobacteria	Escherichia coli Enterococcus faecalis
Other bacteria	Shigella dysenteriae Staphylococcus aureus

Table 1: Already reported microorganisms in homemade pickle

Type of Microorganisms	Species
Pathogenic	Fusarium tricinctum Fusarium oxysporum
Non pathogenic	Xanthomonas sp. Azospirillum sp. Streptococcus sp. Staplylococcus aureus Anadenathera colubrina.

Table 2: Earlier report of microorganisms present in seed

Sl no	Indole Test	Methyl red test	Voges Proskauer's test	Citrate utilization	Lactose test	Possible Microorganisms (genus)
1	+ve	+ve	-ve	-ve	-ve	Shigella
2	+ve	+ve	-ve	-ve	+ve	Escherichia
3	-ve	+ve	+ve	-ve	+ve	Lactococcus
4	+ve	+ve	+ve	-ve	+ve	Enterococcus
5	-ve	-ve	-ve	-ve	+ve	Lactobacillus

Table 3: Biochemical tests for microorganisms obtained from jujube pickle sample

Sl no	Indole Test	Methyl red test	Voges Proskauer's test	Citrate utilization	Lactose test	Possible Microorganisms (genus)
1	-ve	+ve	+ve	-ve	-ve	Staphylococcus
2	-ve	+ve	-ve	-ve	-ve	Xanthomonas

Table 4: Biochemical tests for microorganisms obtained from *Mirabilis jalapa* seed

Figures:

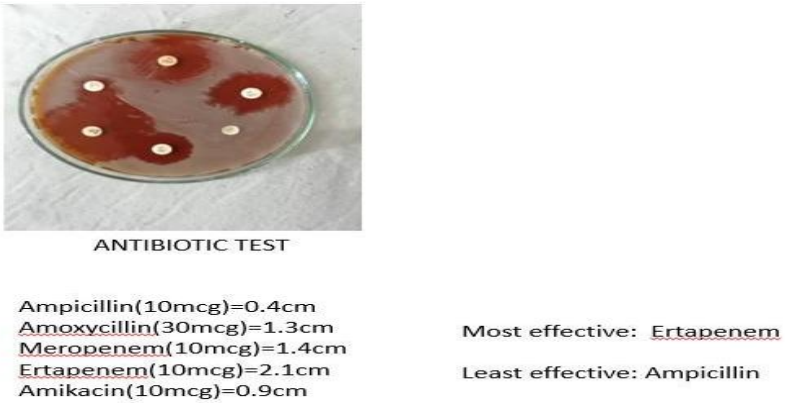

Figure 1: Antibiotic sensitivity test

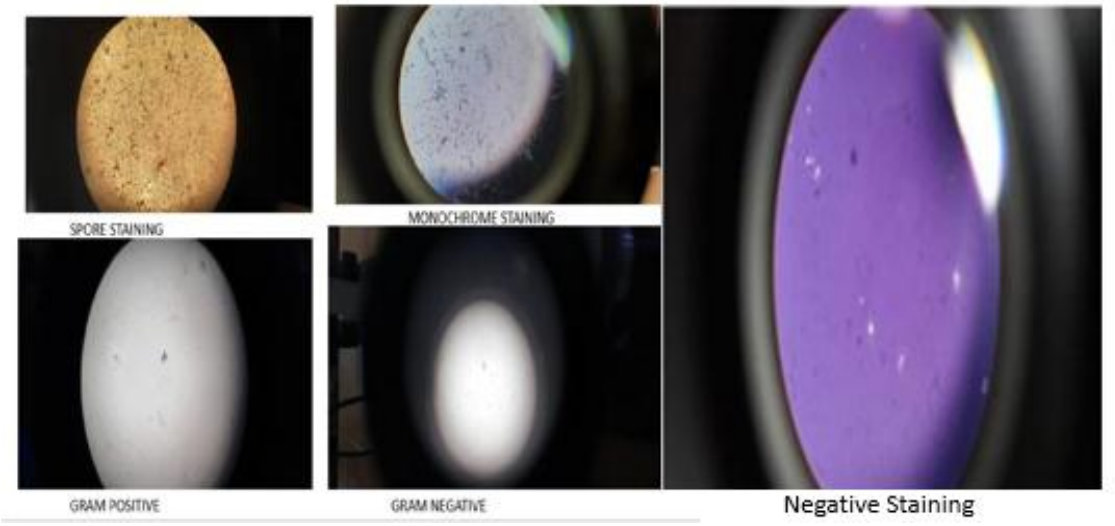

Figure 2: Negative, Gram, Spore and Monochrome Staining

Figure 3: Sample Collection

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