ISOLATION, IDENTIFICATION, ANTIMICROBIAL SCREENING OF MICRO-ORGANISM FROM THE NATURAL SOURCE.

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ABSTRACT: In natural ecosystem ,micro-organism play a highly specialized role in the recycling of resource and water purification.Bacteria and fungi predominate in aquatic system.

Lactobacilli have recently been found to be useful in combating various pathogens,limited data exists on their therapeutic potential for saureas infection. The increasing prevalence of methicillin-resistance staphylococcus aureus has become a major threat to public health.

Lactic acid fermentation is a natural method of antimicrobial food protection. Antagonistic activity of lactobacillus sp. Bacteria ,taking part in this process, is directed mainly against the same or other micro-organisms . In this work we determine the impact of presence of xylitol and galactosyl-xylitol on the antagonistic activity of 60 lactobacillus sp. Strain against indicator molds (Alternarialternata, Altertnariabrassicicola, Aspergillusniger, Fusariumlaternata, Geotrichumcandidum, and Mucorhiemalis) and yeasts (Candida vini). We used double-layer method to select antifungal strains of fungistatic properties. Additionally, we examined the inhibition of Alternariabrassicicola by Lactobacillusparacasei LOCK 0921 cultivated with xylitol or galactosyl-xylitol directly on wild cherries. The presence of xylitol and its galactosylderivative led to increase of spectrum of antifungal activity in most of the studied plant-associated lactobacilli strains. However, no single strain exhibit activity against all the indicator micro-organisms. The antifungal activity of lactobacillus bacteria againstmolds varied considerably and depended on both the indicator strain and the composition of medium. The presence of xylitolandgalactosyl-xylitol in the growth medium is correlated with the antifungal activity of the studied Lactobacillus sp. Bacteria against selected indicator molds.

INTRODUCTION -:

The amphibian framework are generally overwhelmed by micro-organism and organism in the common habitat. Miniature organic entities play quite certain part with respect to reusing of material and cleansing of water pressure of coliform microbes in the water demonstrates the waste contaminated in the water.

As per the world welling association the mortality of water related sickness surpasses 50,00,000 individuals each years in which microbial digestive contamination are over half with cholera hanging out in any case.

Expanding weight of anti-microbial opposition around the world wide is of grave concern. Many engineered science approaches and levelheaded medication configuration have been not able to pace up and handle this problem. Natural assest all the more explicity the microbial variety then again make a conventional despite everything the stage to look for new synthetic framework and mixture.

The proposed work is to finding normal wellspring of miniature living being use against pathogenic microscopic organism and parasites. Water is considered as vehicle for the spread and dispersal of human related bacteria. Safe drinking water is an essential basic freedom and whenever polluted with apportunistics pathogenic natural microbes it might have wellbeing suggestion for consumers. Feacal coliform, Aeromonas, Pseudomonas are utilized as sign of waste defilement in water and the presence of this micro-organism have wellbeing suggestion on buyers.

While lactobacilli have recently been found to be useful in combating various pathogens, limited data exist on their therapeutic potential for S. aureas infection. The increasing prevalence of methicillin-resistance staphylococcus aureus has become a major threat to public health.

Infectious disease caused by resistant micro-organism is accountable for increased health costs as well as high morbidity and mortality, particularly in developing countries. Multi-drug resistance bacteria are the cause of numerous clinical problems worldwide. Increased resistance among pathogens causing nosocomial and community-acquired infections is known to be related to widespread use of antibiotics.

According to the most recent report from the food and agriculture organization and world health organization, probiotics are live micro-organisms whose adequate intake causes beneficial effect on health. Several studies demonstrated their effects on the prevention and treatment of many disease. Probiotics are special micro-organism with important characteristics. Diarrhoe is one of the causes of childrens illness and mortality in developing countries.

Proteomic analysis of theL. Salivaniussecretome revealed a total of five secreted proteins, including a LysM-containing peptidoglycan binding protein and protein peptidase M23B. These proteins may represent potential novel anti-staphylococcal agent that could be effective against S. aureus biofilms. Compared to pH-neutralized supernatant alone, cell-free supernatant that was pH neutralizes and heat inactivated.

Sugarcane, also known as Saccharumofficinarum L., is derived from the Arabic word Sakara, which means white sugar, and the Latin word officinal, which means used as a medicine. It was thought that sugarcane originated in southern Asia, where it

was used for healing purposes. Sugarcane is the world largest crop by production quantity, and its common name comes from the cane-like appearance of the stalk and the excessive amount of sugar it naturally produces (Sharma andTara, 2015). Mold are common spoilage organisms in various food and feed products. Worldwide, these molds that go bad cost a lot of money.

They can tolerate stomach acid and bile salts. These micro-organisms can be a part of the gut microflora and complete with pathogen micro-organism by binding to intestinal cells. Antimicrobial compounds like lactic acid, bacteriocin, and others can be produced by them. The well-known friendly bacteria known for their probiotics activity against pathogens are lactobacilli. Clinical isolates of Pseudomonas aeruginosa were tested to see how different strains of lactobacilli, either commercially produced or isolated from human feces, inhibited them. When confronted with anti-pseudomonal antibiotics that were already in use in clinical settings, the isolated were chosen because they were the most resistant strains.

One of the probiotics that is generally accepted as safe (GRAS) is lactobacillus, which is considered to be a biological therapeutic and an immune-modulating biological. Recent research revealed that Lactobacillus has a number of stimulation, production of inhibitory compounds, and competition for nutrients. Additionally, Lactobacilli can reduce intestinal pH through the production of lactic acid, acetic acid, formic acid, and other acids. This could be the most significant mechanism. Lactobacilli are Gram-positive, non-spore-forming bacilli that produce antibacterial peptidase and small proteins called bacteriocins, which have a beneficial effect on the host when administered as live organisms in adequate amounts.

To exert the antimicrobial activity, these bacteria can also secrete certain antimicrobial molecules, such as ethanol, fatty acid, hydrogen peroxide and bacteriocins. Many lactobacilli strain are known to have probiotics effect against S. aureus, but oral strains like lactobacillus salivarius have largely been ignored. It has been hypothesized that their probiotic activity may be due to (1) Direct inhibition of microbial growth, (2) Competition for space or nutrients, (3) Immune modulatory activity or (5) Modulation of the intestinal barrier.

MATERIAL AND EQUIPMENTS -:

❖ List of Material -:

Drug and material used in dissertation work is listed in table.

Table : List of drug and material used in dissertation work

Sr. No	Material
1.	Ethanol
2.	Agar
3.	Yeast Extract
4.	Peptone
5.	Sodium Chloride
6.	Distilled Water

❖ List of Equipments -:

Equipment used in dissertation work is listed in table.

Table : Equipments used in dissertation work

Sr. No.	Equipments
1.	Petri Plate
2.	Nichrome loop
3.	Measuring Cylinder
4.	Conical flask
5.	Test tube
6.	Test tube holder

EXPERIMENTAL WORK -:

A} Bacterial Inoculation -:

1. Assemble all chemicals in the work area before beginning.
2. Accurately weight the media base calculated powders by electronic balance.
3. Add the powder into the flask.
4. Add distilled water to the flask, for making the correct volume.
5. Heat and stir (agar will be burned if is not stirred) until all of the ingredients go into the solution, when the media boils, it is ready for sterilization.
6. For sterilization autoclave should be used and covered by aluminium foil to prevent contamination.
7. Media are sterilized in the autoclave at 121℃ for 15 lbs of pressure.

- Procedure for preparation of sample solution -:

1. Prepare two sample solution one from the panchaganga river and another from rankala lake.
2. Prepare 10 dilutions from both panchaganga and rankala lake.
3. First 5 dilution P1, P2, P3, P4, P5.
4. Label all 5 test tubes (Panchaganga)
   P1=1ml (Sample solution) + 9ml normal saline =10 ml
   P2=1ml (Sample solution) +14ml normal saline =15ml
   P3=1ml (Sample solution) +19ml normal saline =20ml

P4=1ml (Sample solution) +24ml normal saline =25ml
P5=1ml (Sample solution) +29ml normal saline =30ml

5. Another 5 dilutions R1, R2, R3, R4, R5.
6. Label all 5 test tubes (Rankala)
   R1=1ml (Sample solution) +9ml normal saline =10ml
   R2=1ml (Sample solution) + 14ml normal saline =15ml
   R3=1ml (Sample solution) +19ml normal saline =20ml
   R4=1ml (Sample solution) +24ml normal saline=25ml
   R5=1ml (Sample solution) +29ml normal saline=30ml

- Procedure for streaking method -:

1. Sterilize laminar air flow with the help of ethanol.
2. After some time inoculate sample solution into petri plate with the help of nichrome loop.
3. Place petri plate into the incubator at about 28℃.
4. After completion of 24 hrs observe the bacterial growth.
5. Identify type of bacteria either it is harmful or useful.

B} Antimicrobial Screening -:

Well diffusion method was used to check the antimicrobial activity of sample against Gram-positive and Gram-negative bacteria. For this sterile nutrient agar plate was prepared. Then 24 hrs old culture of test organisms were spread aseptically on sterile nutrient agar plate after that wells were prepared aseptically having 0.7 cm diameter and then 100µl sample was added into the well. Kept for diffusion in Refrigerator for 5 min. the plates were incubated at 37℃ for 24 hrs.

RESULT AND DISCUSSION -:

Identification of micro-organism -:

Table : Identification of micro-organism

Sr. No.	Water Source	Identified micro-organisms
1.	Rankala (R1)	Gram-Positive Staphylococcus
2.	Rankala (R2)	Gram-Positive Staphylococcus
3.	Panchaganga (P1)	Escherichia Coli

Fig.: Growth of micro-organisms from water sample of Panchaganga and Rankala

Antimicrobial Screening -:

The Antifungal study of P2 sample showed antifungal activities against Candida Albicance and Aspergillus Niger.

Table : Zone of Inhibition of sample

Sr. No.	Organisms	Sample P2 (Zone of Inhibition in mm)
1.	Aspergillusniger	1.0
2.	Candida albicans	2.0

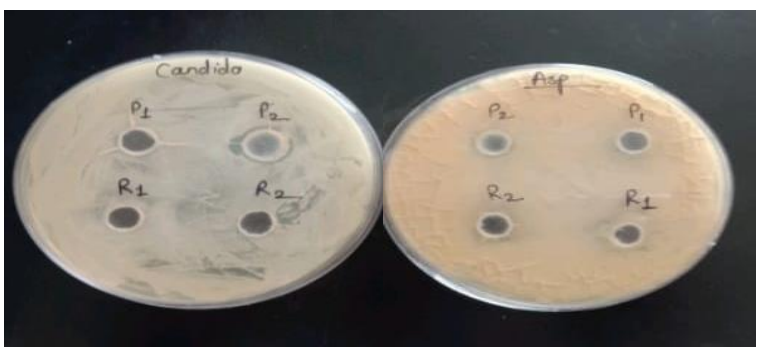

Fig. : Growth of micro-organisms from water sample of Panchaganga and Rankala

SUMMARY AND CONCLUSION -:

Summary -:

1. From Rankala and Panchaganga water source isolated bacteria found such as Gram-Positive Staphylococcus cocci, E-coli.
2. The given isolated bacteria is shows antifungal activity against Candida Albicance and Aspergillus Niger.
3. Isolated bacteria further research studies as research of novel synthesis of antifungal agent.

Antimicrobial Screening -:

The antifungal study of P2 sample showed antifungal activity against Candida Albicance and Aspergillus Niger.

Table : Zone of Inhibition of sample

Sr. No.	Organisms	Sample P2 (Zone of Inhibition in mm)
1.	Aspergillus niger	1.0
2.	Candida albicance	2.0

Fig. Antifungal activity against Candida Albicance

Fig. Antifungal activity against Aspergillus Niger

CONCLUSION :

The result of present study provide a basis for the development of effective and eco-friendly management stratergy against candida albicance and aspergillus niger however further studies are required to employ the isolated staphylococcus cocci bacteria may development of novel synthesis of antifungal agent.

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