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CHARACTERIZATION OF ANTAGONISTIC EFFECT OF FERMENTED MILK PRODUCT MICRO-FLORA AGAINST DIFFERENT MDR PATHOGENS.

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ABSTRACT

Due to gaining resistivity against various antibiotics, the pathogenic organisms moving towards MDR categories. The removal of MDR pathogens are becoming a significant public health concern globally, and the widespread use of antimicrobials in health care environments leads to exposure to microbes. Rising pathogen's antibiotic tolerance associated with bacterial infections has now been a significant clinical problem for doctors. Thus the creation of alternative treatments is to be use, such as the extracted active metabolites from fermented milk products. It may help to fight troubling drug-resistant and multidrug resistant bacteria. Bacteria of the genus Lactobacillus are a good example of probiotic and considered useful for reducing the antimicrobial usage.

Key words: MDR, Lactobacillus, Antibiotics, Fermentation, Optimization.

INTRODUCTION

Multiple drug resistance (MDR) multi drug resistance or multi resistance is shown by a species of microorganism to multiple antimicrobial drugs. The type most threatening to public health is MDR bacteria that resist multiple antibiotics; other types include MDR viruses, parasites, (resistant to multiple antifungal, antiviral & antiparasitic drugs of a wide chemical variety). Recognizing different degrees of MDR, the terms extensively drug resistant (XDR) & pondrug-resistant (PDR) have been introduced. The definition was published in 2011 in the journal clinical microbiology & infection & is openly accessible. Organisms that display multidrug resistance can be pathologic cells, including bacterial & neoplastic cells. Multidrug-Resistance Organisms (MDROs) are defined as microorganisms that are resistant to one or more classes of antimicrobial agents.

EFFECTS OF MDR PATHOGENS

1) P*seudomonas* infections: - Pseudomonas infections are diseases caused by a bacterium from the genes pseudomonas. The bacteria are found widely in the environment, such as in soil, water, & plants. They usually do not cause infections in healthy people. If an infection does occur in a healthy person, it is generally mild. More severe infections occur in people who are already hospitalized with another illness or condition, or people who have a weak immune system. A pathogen is a microorganism that causes disease. Infection acquired in a hospital is called nosocomial infections. Infections can occur in any part of the body. Symptoms depend on which part of the body is infected. Antibiotics are used to treat the infections. Pseudomonas infection could be fatal in people who are already very ill.

A) What are the symptoms of pseudomonas infections:- Infections in the skin tend to be less severe than infections that occur in the blood or lungs.

a) Blood: - A bacterial infection of the blood is called bacteremia. A blood infection is one of the most severe infections caused by *Pseudomonas s*ymptoms are included: Fever, Chills, Fatigue, Muscle & joint pain. Bacteremia with pseudomonas can also cause very low blood pressure, known as hemodynamic shock, which can lead to failure of other organs including the heart kidneys & liver.

b) Skin: - When this bacterium infects the skin, it most often affects the hair follicles. This is called folliculitis. Symptoms are including: - Redness of the skin, Abscess formation in the skin, draining wounds.

c) Lungs: - Infections of the lungs is called pneumonia. Symptoms are including: - Chills, Fever, and Cough with or without sputum production, Difficulty breathing.

d) Ear: - An external ear canal infection may sometimes be caused by pseudomonas & result in swimmer's ear. Symptoms may include: - Swelling, Ear pain, Itching inside the ear, Discharge from ear, Difficulty hearing.

B) What causes pseudomonas infections: - *Pseudomonas* infections are caused by a free-living bacterium from the genus pseudomonas. They favor moist areas & are widely found in soil & water. Only a few of the many species caused disease. The most common species that causes infection is called *Pseudomonas aeruginosa.*

2) *Escherichia Coli* (EC):- Named by *Escherichia* wide group of bacteria on basis of biotyping & Serotyping. Produce infections in human & animals detections of *E. coli* in water indicates pollution & contamination. E. coli (Escherichia coli), is a type of bacteria that normally lives in your intestines. It's also found in the gut of some animals. Most types of E. coli are harmless and even help keep your digestive tract healthy. But some strains can cause diarrhea if you eat contaminated food or drink fouled water. While many of us associate *E. coli* with food poisoning, you can also get pneumonia and urinary tract infections from different types of the bacteria. In fact, 75% to 95% of urinary tract infections are caused by *E. coli.* Some versions of *E. coli* make you sick by making a toxin called Shiga. This toxin damages the lining of your intestine. The strains of E. coli that make the toxin are sometimes called STEC, which is short for "Shiga toxin-producing *E. coli."* It causes abdominal cramps, vomiting, and bloody diarrhea. It is the leading cause of acute kidney failure in children. It can also cause life-threatening symptoms such as: Adult kidney failure, Fever, Bleeding, Confusion, Seizures.

A) Fast facts on *E. coli:* - Here are some key points about E. coli. More information is in the main article. E. coli refers to a wide range of bacteria that can cause various diseases, including pneumonia, urinary tract infections, and diarrhea. Most strains of E. coli are harmless to humans.

Some strains of E. coli infection can include nausea, vomiting, and fever. In susceptible individuals, certain types of E. coli infection can lead to kidney failures.

B) How do you get infected: - You can become infected when you swallow even a small amount of E. coli bacteria? Among the ways this can happen:

a) Ground meat: - You eat ground meat that carries E. coli, and the meat wasn't cooked enough to kill the bacteria. When meat is processed, sometimes bacteria from the animals' intestines make their way into the meat. This happens more with ground meat because it comes from more than one animal.

b) Untreated milk: - You drink unpasteurized milk, which hasn't been heated to kill bacteria. E. coli can get into the milk from the cow's udder or from milking equipment.

c) Vegetables and fruit: - You might eat fresh vegetables or fruit that's been tainted by water that has the bacteria. This happens most often when manure from nearby animals mixes with the water supply.

d) Other foods and beverages: - You might also get E. coli from unpasteurized fruit juices and yogurt and cheese made from raw milk.

e) Water: - You swallow water that contains E. coli, perhaps while swimming in a pool, lake, or pond.

f) Other people: - You might get E. coli from another person who has it, such as a child. The bacteria can be passed to you if you clean up after an infected person and then don't wash your hands really well before you touch your mouth.

METHODOLOGY

Sample collection:

Different rotten fruit samples were collected from different location to obtain the desired species **[11]**.

Isolation of bacteria from milk sample:

The bacterial cultures were isolated from samples after serial dilution and spreading. These cultures were shortlisted on the basis of morphological parameters and then converted to pure cultures using streak plate method **[12]**.

Screening for antibacterial activity:

The broth culture were prepared and then loaded to well present in the MDR pathogen agar plates. The activity was screened out on the basis of zone of inhibition.

Identification of bacteria:

The culture which were expressive to showing their activity were go through various staining and biochemical tests for identification purpose **[15]**.

Selection and optimization of the production media:

For the production of antibacterial component against the MDR pathogens were done by the media selection and optimization. The best components were selected by observing the bacterial growth and the antibacterial property **[16]**.

Fermentation and purification of antibacterial component:

The culture was inoculated in sterilized fermentation media and then incubated for 72 hours for the production of sntibacterial component **[17]**.

The fermentation is based on shake flask method. Further downstream processing was performed by solvent extraction method by using polar and nonpolar solvents **[18,19,20]**.

RESULTS

Collection of soil sample:

Fermented milk was collected as sample in a sample specimen bag from local milk corners near Indiranagar, Lucknow to isolate the desired cultures.

Figure -1 Milk sample

Isolation of bacterial culture:

The milk allowed for fermentation after that the cultures were isolated by serially diluted in 0.87% NaCl solution and then spreading on nutrient agar plates. Further these cultures were converted to pure cultures by streaking, as shown in figure 2.

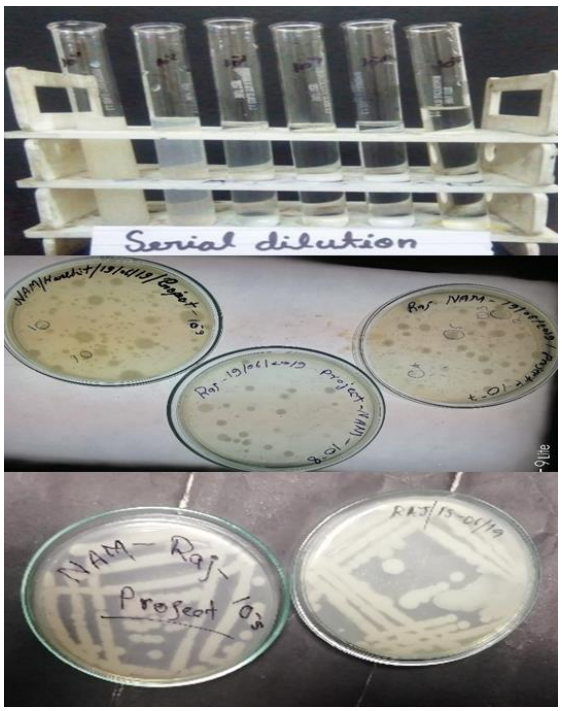

Figure 2: The pure cultures obtained after serial dilution and spreading of milk sample in nutrient agar plates.

Screening for antibacterial property:

The pure cultures were inoculated in nutrient broth and allowed to grow 24 hours. Further these cultures were loaded to the agar plates spread by MDR pathogens. The C2 was selected as it shows the clear zone by inhibiting the MDR pathogens growth as mentioned in Table 1 and figure 3.

Table 1: Antibacterial screening of isolated pure cultures.

Cultures	S aureus	P aeruginosa
C1	0	0
C2	12	15
C3	9.1	9.2
C4	9.8	0

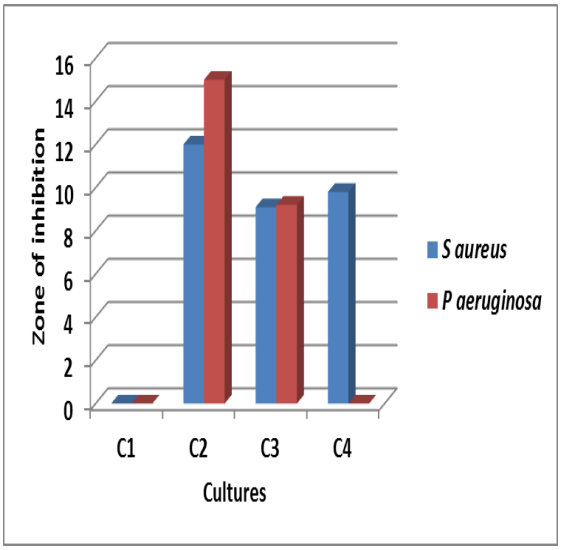

Figure 3: Graphical representation of antibacterial screening of cultures against the MDR pathogens.

Media selection and Optimization:

The media was selected randomly for the checking the growth of bacterial cultures and enhancement in the antibacterial property.

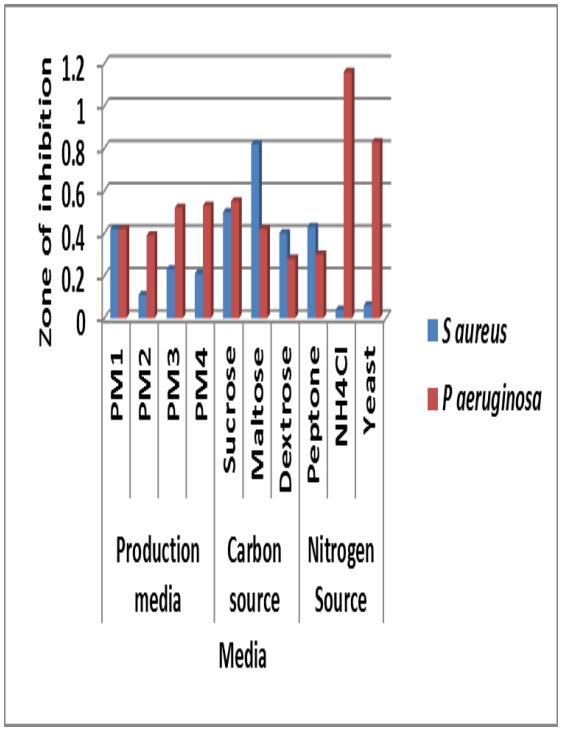

Figure 4: the selection and optimization of media components.

Fermentation and downstream processing:

The sterilized optimized media was prepared, then culture was inoculated and allowed for fermentation by shake flask method. The purification of the active metabolites was performed by solvent extraction method. These extracted metabolites were screened for the antibacterial activity.

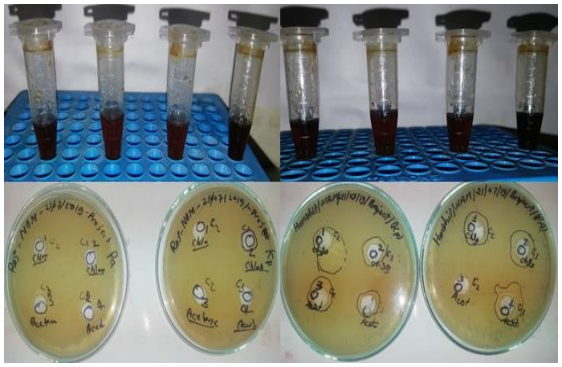

Figure 5: the antibacterial screening of extracted metabolites.

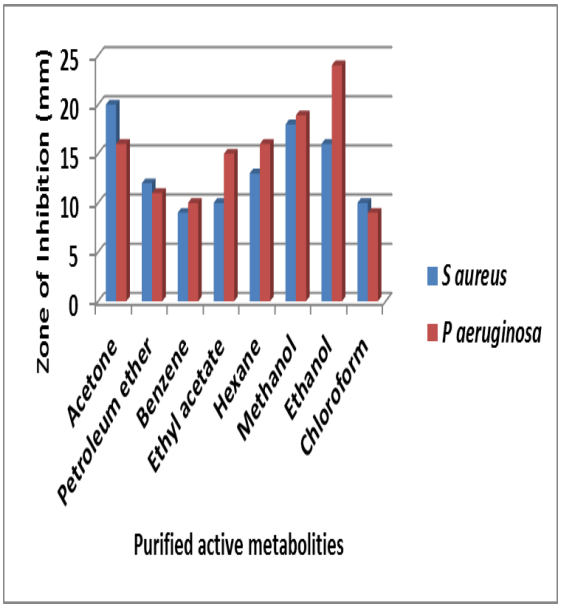

Figure 6: Graphical representation of the antibacterial activity of extracted metabolites against MDR pathogens.

Biochemical characterization of culture:

Table 2: Biochemical analysis of the cultures for identification of culture.

Tests	Results
Gram's staining	Positive, Bacillus
Endospore staining	Positive
Catalase test	Negative
MR test	Positive
VP test	Negative

DISCUSSION AND CONCLUSION

The milk sample was collected from the store, and the bacterial isolation was done by serial dilution and spread plate technique, after spreading the total 6 different types of strains were selected on the basis of different morphological parameters. Then the cultures were pure by streaking and then screening was done by antimicrobial sensitivity test by using agar well diffusion method. The antimicrobial effect of the cultures were done against the MDR pathogens. And the culture number 2 and 3 was selected as the positive culture, by showing the clear zone. Then media selection and optimization was done for the selected cultures. The different parameters was optimized for enhancing the growth of culture such as, carbon sources, nitrogen sources, pH temperature etc. when the media was designed then growth curve study was performed. For the production of secondary metabolites the cultures were incubated for fermentation and then the purification of metabolite was done by solvent extraction method. And last the antimicrobial assessment was performed to check the antimicrobial property. As a result it was found that the property was enhanced after the fermentation and the purification of the metabolites.

It was concluded that the microbial strains can be used as the probiotics formation. Which will help in the human welfare.

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