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Journal of
Microbial & Biochemical Technology

Research Article

Antibacterial Activity of Natural Preservatives

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ABSTRACT

Aim: To test the antibacterial activity of natural preservatives like vinegar, salt and sugar.

Objectives: To prepare the media for the bacteria to grow , To inoculate the bacteria on the agar plate through the spread plate technique and To make wells with T rod.

Methodology: First sterilize the glass ware , then prepare and sterilize the media , then pour the media into the petri plates and allow it to be cooled and then take a T rod and make 3 wells of 6 to 10mm diameter and inoculate bacteria on to the agar plate and incubate it for 2 days.

Result: After 2 days, we could observe the growth of the bacteria. In the 1st well which is filled with vinegar the zone of inhibition is 1.8 cm. In the second well which is filled with salt the zone of inhibition is up to 1cm. And in the 3rd well which is filled with sugar has no inhibition of the bacterial growth. **Conclusion:** The inhibition of the bacterial growth with vinegar is more as compared to other preservatives like salt and sugar and salt shows slight inhibition of bacterial growth than sugar as per the results obtained the sugar has no reactivity even though we have taken the preservatives with the same concentration so we can conclude that the reactivity of vinegar is greater than salt and the reactivity of salt is greater than sugar.

Keywords: Antibacterial Activity; Natural preservatives

INTRODUCTION

Background

Spices traditionally have been used as coloring agents, flavoring agents, preservatives, food additives and medicine in Bangladesh. The present work aimed to find out the antimicrobial activity of natural spices on multi-drug resistant Escherichia coli isolates. Anti-bacterial potentials of six crude plant extracts (Allium sativum, Zingiber officinale, Allium cepa, Coriandrum sativum, Piper nigrum and Citrus aurantifolia) were tested against five Escherichia coli isolated from potable water sources at kushtia, Bangladesh. All the bacterial isolates were susceptible to undiluted lime-juice. None of them were found to be susceptible against the aqueous extracts of garlic, onion, coriander, pepper and ginger alone. However, all the isolates were susceptible when subjected to 1:1:1 aqueous extract of lime, garlic and ginger. The highest inhibition zone was observed with lime -11 mm. Natural spices might have anti-bacterial activity against enteric pathogens and could be used for prevention of diarrheal diseases. Further evaluation is necessary.

This review discusses the status, antimicrobial mechanisms, application, and regulation of natural preservatives in livestock food systems. Conventional preservatives are synthetic chemical substances including nitrates/nitrites, sulfites, sodium benzoate, propyl gallate, and potassium sorbate. The use of artificial preservatives is being reconsidered because of concerns relating to headache, allergies, and cancer. As the demand for biopreservation in food systems has increased, new natural antimicrobial compounds of various origins are being developed, including plant-derived products (polyphenolics, essential oils, plant antimicrobial peptides, animal-derived products (lysozymes, lactoperoxidase, lactoferrin, ovotransferrin, antimicrobial peptide, and microbial metabolites (nisin, natamycin, pullulan, ε-polylysine, organic acid, and others). These natural preservatives act by inhibiting microbial cell walls/membranes, DNA/RNA replication and transcription, protein synthesis, and metabolism. Natural preservatives have been recognized for their safety; however, these substances can influence color, smell, and toxicity in large amounts while being effective as a food preservative. Therefore, to evaluate the safety and toxicity of natural preservatives, various trials including combinations of other substances or different food preservation systems, and capsulation have been performed. Natamycin and nisin are currently the only natural preservatives being regulated, and other natural preservatives will have to be legally regulated before their widespread use.

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Received Date: September 12, 2020; **Accepted date:** October 15, 2020; **Published date:** October 22, 2020

Citation: Gangadhar D (2020) Antibacterial activity of natural preservatives. J Microb Biochem Technol. 12:441 Doi: 10.35248/1948-5948.20.12.441

Several factors are investigated that normally cause variation in zone diameters in conventional disc plate diffusion assay procedures. Of these factors the most serious is the unequal exposure of the individual plates at top or bottom of stacks to temperatures above and below room temperature. This unequal temperature exposure is avoided by novel handling and incubation procedures. A major variable, but one which can be controlled, is the varying time interval between pouring seeded agar and the time of applying the pads with antibiotic to the plates. This influence of time of setting and the effects of several other sequential operations are combined into a composite variable. This variable is then accounted for and normalized by interposing "external" reference plates set with a reference solution in the sequence of approximately 100 plates. No "internal" reference zones are employed. Such factors as volume of agar poured, wedge shape of agar in a dish, volumetric errors in dilutions, and timing considerations are studied and discussed. The results of this study form the basis for a test protocol which is presented in a following paper.

The food industry has developed along with globalization, resulting in an increased risk of foodstuffs being contaminated with pathogens, chemical residues, and toxins. The proliferation of pathogenic and spoilage bacteria should be controlled to guarantee food safety. Conventional preservatives are a group of synthetic chemical substances including nitrates/nitrites, sulfites, sodium benzoate, propyl gallate, and potassium sorbate. The use of these conventional preservatives in food has known side effects [1]. Antimicrobial effectiveness of natural preservatives depends on the kind of preservatives, its composition and concentration, type and concentrations of the target microorganism, substrate composition, and processing and food storage conditions [2]. New families of antimicrobial agents will have a short life expectancy [3]. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multidrug resistant microbe strains [4]. During recent decades, investigation on food preservation have focused on more natural and healthier food [5]. Pure cultures of isolates were preserved at 4°C on nutrient agar slants. In order to confirm the identity of the test bacterial isolates morphological characteristics and conventional biochemical tests were performed according to Harley and Prescott [6]. The diffusion assay of antibiotics employing a disc plate method has great virtue of convenience, simplicity, sensitivity, efficiency, and dependability [7,8]. The relation of diameter of inhibitory zones to concentration of antibiotic in a solution applied in cups has been considered theoretically [9,10]. In practice, the diameter is directly responsive to antibiotic concentration, and the limitations on accuracy are the recognition and control of the many subtle variables. The applicability of the system is general, and materials and equipment for the conduct of the procedure are widely available. Paper discs and blow-molded plastic petri dishes are available. An instrument for accurate reading of zone diameters was described by Davis et al. [8] and is manufactured.

MATERIALS AND METHODS

Apparatus required: conical flask, measuring cylinder, beaker, boiling tubes, Petri plates and glass rod, T rod Instruments required: autoclave, laminar air flow chamber, weighing balance Chemicals required: beef extract, peptone, NaCl, agar, distilled water, vinegar, salt, sugar.

Sterilization of glassware

- Sterilizing glassware such as bottles, petri dishes and test tubes, dry heat is required, and this is carried out in a hot air oven
- First the hot air oven is to be wiped with ethanol
- Then clean the glassware and make them ready
- Temperature of the oven needs to reach is at least 160°C for atleast 40 to 50 minutes
- And the glassware needs to be cooled gradually because there might be a chance of formation of cracks on glassware

Preparation and sterilization of media

- Composition of media is

Peptone: 5 grams

Beef extract: 3 grams

NaCl: 5 grams

Agar: 1 - 2.5 %. (In 1000ml of water)

- Prepare the media for 100ml
- First weight the ingredients of the media
- Take 100ml distilled water in a conical flask
- Mix the media completely
- Then close the conical flask with a cotton plug
- Then sterilize the media in the autoclave for 15- 20 minutes at 121°C and 15 lbs pressure
- Then allow the medium to be in loop warm
- Then pour the media in the Petri plates and allow it to be solidify

Inoculation of Ecoli

- Use spread plate technique for the inoculation of bacteria
- First heat the rod and pour a drop of Escherichia coli broth and spread with the T rod
- Then go with well diffusion method

Well diff usion method

Take a T rod and make 3 holes of 6 to 8 mm diameter by sterilizing the rod. Then fill the agar wells with the preservatives for which the anti-microbial activity is to be tested. Here in our experiment we are using vinegar, salt and sugar. Then incubate it at 30 -37 °C for 1 to 2 days

Action of preservatives

Vinegar: vinegar is an effective natural preservative. The acetic acid in vinegar kills microbes and stops their growth.

Salt: The salt is a water absorbent and hence removes water from any culture. In absence of water bacteria don't multiply and hence acts as preservative.

Sugar: Sugar tends to draw water from the microbes (plasmolysis).

RESULTS

After incubation of the culture for 2 days. After calculating the zone of inhibition, the results are as follows, for vinegar the zone of inhibition is 1.8 cm. For salt, the zone of inhibition is 1 cm and for sugar, no reactivity is found (Figure 1).

Figure 1: Antimicrobial Activity of Natiral preservatives(*vinegar, salt and sugar)*

CONCLUSION

From the results obtained we can conclude that the growth of the bacteria can be inhibited by natural preservatives too. Vinegar has high inhibiting capacity than compared to salt and sugar. Sugar has no reactivity with the bacterial growth. Salt has less reactivity compared to vinegar. Hence by the results obtained we can conclude that the order of the anti-bacterial activity of the 3 solutions that are taken is Vinegar>salt>sugar.

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