



# ANTIBACTERIAL ACTIVITY OF ORGANISMS WITH PROBIOTIC POTENTIAL ISOLATED FROM VEGETABLE VINEGAR PICKLES ON MRS MEDIA AND FRUIT PEELS

<sup>1</sup> Sharvani. K, <sup>2</sup> Roselin. P\*

<sup>1</sup> Student, <sup>2</sup> Asst. Professor

<sup>2</sup> Department of Microbiology

<sup>2</sup> St. Francis College for Women, Hyderabad, Telangana State

**Abstract:** The potential health benefits of probiotics have long been elucidated since Metchnikoff and his co-workers postulated the association of probiotic consumption on human's health and longevity. Pickle is a popular condiment throughout the world. The organisms isolated from olive, jalepeno and chilly Vinegar pickles were evaluated for their probiotic potential. The identification of the isolated organism was carried out on the basis of physiological, biochemical and enzymatic characterization based on Bergey's system of classification. The antibacterial activity of lactic acid bacteria was tested against indicator organisms such as E coli, *Staphylococcus aureus* and *Bacillus subtilis*. The antibacterial activity of the pH neutralised samples was tested. The influence of nutrients derived from the peels of fruits such as orange, banana and wood apple in enhancing the growth of lactic acid bacteria was studied by incorporating the fruit peel powders in the growth media. The organisms showed highest growth when they were provided with fruit peels as a substrate containing additional nutrients and showed highest activity against indicator organisms studied by comparative analysis. This investigation demonstrated that small amount (2%) of apple, banana and mango peel powder could be successfully utilized as prebiotics to enhance the growth of organisms with probiotic potential. This indicates that fruit peels may act as Prebiotics to support the growth of Probiotics.

**Index Terms:** Probiotics, fruit peels, pickles, antibacterial activity

## 1. Introduction

Probiotics are the live microorganisms which when administered in adequate amounts confer health benefits and helps in preventing and treating some illness [1]. *Lactobacillus*, a food fermenting bacteria helps in preventing the spoilage of food and increases the nutritive value of the foods. Probiotics can be obtained by various sources such as fermented foods like kefir, cheese, yoghurt, pickles and dietary supplements [2]

Pickles are the important diet of many cultures. Pickles are produced through spontaneous or controlled fermentations. Some Pickles are produced by the addition of vinegar solution by decreasing pH below 4.5. They are rich sources of Probiotic bacteria, Vitamin K, A, C and low in calories, fat free. Pickles contain antioxidants and help in preserving and extending the shelf life of the food [2]. Vinegar based pickling is a type of traditional pickling which helps in increasing the haemoglobin levels in the human body [3] Jalapeno's pickle has great antioxidant properties, helps in weight loss, prevents ulcers. Green Chilli pickle is a natural source of iron, helps in balancing blood sugar levels. Olive pickles are rich in antioxidant properties and may protect against osteoporosis and cancer.

Peels are the non- edible part of the fruits. Among the waste by-products generated by the fruit industry (peels, seeds, and skins), fruit peel constitutes the major component. It is estimated that fruit peel accounts for at least 20% of the fresh fruit weight. Fruit peels are considered as major sources of dietary fiber and anticipated to be successfully utilized as prebiotics. [4] Banana fruit peels rich in fructo oligosaccharides can be used as a prebiotic source for stimulating the growth of *Lactic acid bacteria*. Orange peel and pulp waste can be used for the production of *lactic acid bacteria* Peels contain many vital nutrients and non-nutrient compounds which plays an important role in wellbeing and used for the production of various valuable compounds. Probiotics are non-toxic and safe to consume [5].

This study examined the prebiotic effects of three fruit peels (orange, banana and wood apple). The prebiotic effect was tested using the probiotic strains isolated from vegetable (chilly, olives and jalepeno) vinegar pickles. The evaluation of antibacterial potential of these organisms against *E.coli*, *Staphylococcus aureus* and *Bacillus subtilis* was tested using Agar well diffusion method.

## 2. Materials and Methods

### 2.1. Raw material processing

Three different samples Olives, Jalapenos and Chilli vinegar-soaked pickle samples were collected and packed in bags and brought to the laboratory. Olives and Jalapenos pickle samples were brought from the shop and chilli pickle was prepared in the Laboratory.

### 2.2. Preparation of chilli pickle

Vinegar based Chilli pickle was prepared by collecting the chillies from the market, washed, dried to remove moisture, chopped into pieces and transferred to an air tight container. Vinegar and salt were added and was then allowed to ferment.

### 2.3. Olives and Jalapenos pickle

Olives and Jalapenos pickle samples were collected from the local market. All the samples were collected in clean bags and brought to the laboratory.

### 2.4. Isolation of probiotic organisms

For the isolation of Probiotic organisms, the samples were homogenised in peptone solution.

#### 2.4.1. Homogenization in peptone solution

20g of pickle samples were homogenized in 5ml of 1% w/v sterile peptone solution. After homogenization, serial dilutions were made in 1% sterile peptone solution and plated on MRS agar and kept for incubation under anaerobic conditions (gas pack) at 30<sup>0</sup>C for 3 days. Colonies were observed and these colonies were purified by sub culturing. [6,7]

### 2.5. Identification of probiotic organisms

The isolated Probiotic organisms were identified by morphology, gram staining, endospore staining, catalase test, Arginine Hydrolysis test, Carbohydrate fermentation tests, growth at different temperatures etc. The organisms were further identified based on Bergey's Manual system of classification. [7]

## 2.6. Effect of different substrates on probiotic isolates

To determine the activity of Probiotic isolates on different substrates, Banana, orange and wood apple fruits were collected from a local market. The fruits were peeled and the peels were air dried, ground and autoclaved [8]. 5g of powdered sample of wood apple, banana and orange were added to the MRS agar. The culture was streaked on the agar plates and kept for incubation at 37<sup>0</sup>C for 24h. Sterile Powdered Samples of wood were also added to the broth along with the culture inoculum and was incubated at 37<sup>0</sup>C for 24 h [9]. After 24h of incubation, Colonies were selected on the basis of cultural characteristics and gram staining. The isolated organisms were further tested for their antibacterial activity

## 2.7. Antibacterial activity

### 2.7.1. Preparation of culture supernatants

Pure cultures isolated from pickle samples were inoculated in MRS broth and kept for incubation at 37<sup>0</sup>C for 24h. After 24h of incubation, bacterial cells were separated by centrifugation for 20mins, and pH was adjusted to 7 for neutralising the sample by using 1M NaOH in order to prevent organic acid effect. After neutralising, the supernatant was collected and used for determining the Antibacterial activity [8]

### 2.7.2. Antibacterial activity

The antibacterial activity of the isolated Probiotic organism was tested against indicator organisms including Gram positive and gram-negative organisms such as E coli, *Staphylococcus aureus*, *Bacillus subtilis* by using agar well diffusion method. Nutrient agar plates were seeded with indicator organisms such as E coli, *Staphylococcus aureus* and *Bacillus subtilis* and wells were cut into the agar plates by using sterilized tips. After preparation of wells, 100µL of the cultured supernatant fluid was placed into wells. Non centrifuged samples were also added to the other wells to check its activity. After inoculation of the sample into the wells, the plates were kept for incubation overnight at 37<sup>0</sup>C. After 24h of incubation, inhibition zones were observed and recorded [7]

Isolates were examined for their antagonistic activity against test organisms. The nutrient agar plates were seeded with E coli, *Staphylococcus aureus* and *Bacillus* spp. Isolates from the MRS broth were added to the wells containing nutrient agar with the test organism and kept for incubation at 37<sup>0</sup>C for 24h. After 24h of incubation, the results were recorded and tabulated. The isolates were further compared for the growth and activity on Biowastes [10].

## 3. Results and Discussion

### 3.1. Isolation and characterization of organisms isolated from Vinegar pickles

3 pickle samples Olives, Jalapenos and Chilies samples were homogenized in peptone solution. Serially diluted pickle samples were plated on MRS agar and incubated at 30<sup>0</sup>C for 3 days under anaerobic conditions i.e., gas pack system. After 72h of incubation the plates were removed from the gas pack and observed for the colonies. Probiotic colonies were isolated from the pickle samples. The isolates of the samples were gram positive rods, negative to the endospore staining and catalase test. The isolates have an ability to hydrolyse arginine. Glucose, Mannitol, mannose, lactose, fructose, Galactose fermentation were seen in all the isolates. Most of the isolates showed growth at different temperatures such as all the isolates of Probiotic organisms grew at 30<sup>0</sup>C by showing turbidity and some at 45<sup>0</sup>C Based on Bergey's Manual of Systematic Bacteriology, the strains isolated on MRS were identified as *Lactobacillus casei*, *Lactobacillus delbruckii*. Bacteriocin producing LAB originally isolated from pickle samples shows that pickles are rich sources of Probiotics. These isolates from pickle samples were tested for their antibacterial activity [11].

Table 1: Cultural characteristics of the isolates

S.No	Sample	Cultural characteristics	Identified organism
1	Chillies	Gram Positive rods, endospore negative, catalase negative, acid production in carbohydrates fermentation, positive Arginine hydrolysis test	<i>Lactobacillus</i> spp
2	Jalapenos	Gram Positive rods, endospore negative, catalase negative, acid production in carbohydrates fermentation, positive Arginine hydrolysis test	<i>Lactobacillus</i> spp
3	Olives	Gram Positive rods, endospore negative, catalase negative, acid production in carbohydrates fermentation, positive Arginine hydrolysis test	<i>Lactobacillus</i> spp

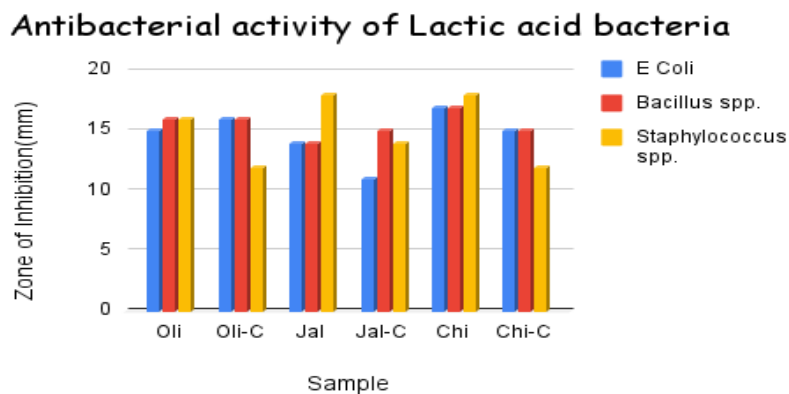
### 3.2. Antibacterial activity of Lactic acid bacteria against test organisms

*Lactic acid bacteria* showed antibacterial activity against the 3 test organisms *E coli*, *Bacillus* spp, *Staphylococcus aureus* and it is indicated by inhibition zones ranging from 10-20mm. Diameter of inhibition zones against *E coli*, *Bacillus subtilis* and *Staphylococcus aureus* ranged from 10-17mm, 15-17mm, 12-18mm respectively. Inhibitory activity was higher against gram positive organisms.

On further study, using neutralized cell free supernatants of *Lactic acid bacteria* by agar well diffusion method, the isolates showed inhibitory activity against test organisms *E coli*, *Bacillus subtilis* and *Staphylococcus aureus*. This indicates that pH is not responsible for the activity against the organisms. The study by Arindam Roy and Chandan Rai [12] showed pH neutralized culture supernatant of isolate from jackfruit has showed antibacterial activity against *B. cereus*, *E. coli* and *S. typhimurium* by agar well diffusion method.

Table 2: Antibacterial activity of Lactic acid bacteria against test organisms

S.No.	Organisms isolated from	Zone Of Inhibition (mm)		
		<i>E coli</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>
1	Chillies	17	17	18
2	Chillies(C)	15	15	12
3	Jalapenos	14	14	18
4	Jalapenos(C)	11	15	14
5	Olives	15	16	16
6	Olives (C)	16	16	12



**Fig 1: Graphical representation of Antibacterial activities of *Lactic acid bacteria* against the test organisms.**

The graph represents the organisms isolated from chillies showed a high zone of inhibition against *E. coli* and *Bacillus subtilis*, whereas organisms isolated from Jalapenos and Chillies showed a high zone of inhibition against *Staphylococcus aureus*. Neutralized extracts of Olives showed higher zone of inhibition than Jalapenos and Chillies to *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*

The effect of different Biowastes (Fruit peels) on the growth and activity of the organisms was tested. The fruit peels of oranges, bananas, and wood apples were used. These fruit peels provided a high amount of nutrients for the growth of *Lactobacillus*. Media incorporated with the fruit peels were inoculated with the cultures and incubated. After incubation the plates were observed for growth. Gram staining was performed to identify the organisms.

The organisms isolated using Biowaste as substrates were further tested for its antibacterial activity against indicator organisms by using agar well diffusion method including both gram positive and gram-negative organisms to understand the effect of fruit peels on the activity of the organisms. A comparative analysis was performed to check the activity with and without the incorporation of the Biowaste and also to check which Biowaste had more impact on the growth and the activity of the organisms.

After incubation the Zones of Inhibition were measured. The Zones of Inhibition ranged from 14 to 28mm. Diameter of the inhibition zones against *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus* ranged from 14-20mm, 20-23mm, 14-28mm respectively. Inhibitory activity was highest against gram positive organisms. Isolates grown on Wood apple as substrate showed highest antibacterial activity against test organisms. Similar work is observed in the article published by Arekemase M.O *et.al.*, [11], the study shows that the *lactic acid bacteria* can be grown effectively by utilizing orange peels as a substrate than banana and mango peels, in another study by Pushparani Mudaliyar *et.al.*, [9], the growth of *Lactic acid bacteria* was more in media with mango and orange peel substrates but less in media with corn peel.

**Table 3: Antibacterial activity of *Lactic acid bacteria* with substrate against indicator organisms**  
Zone of inhibition in mm

Sample	Organism isolated from	<i>E. coli</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>
Wood Apple	Olives	15	23	21
	Jalapenos	17	24	22
	Chillies	20	22	28
Orange	Olives	-	23	17
	Jalapenos	-	22	14
	Chilies	-	22	16
Banana	Olives	16	21	23

Jalapenos	20	21	21
Chilies	19	20	20

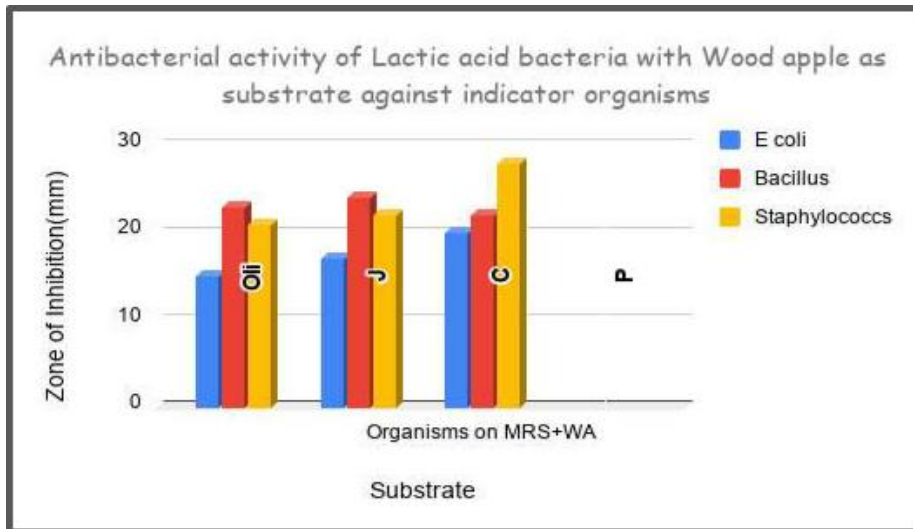


Fig 2: Effect of isolates on wood apple as a substrate

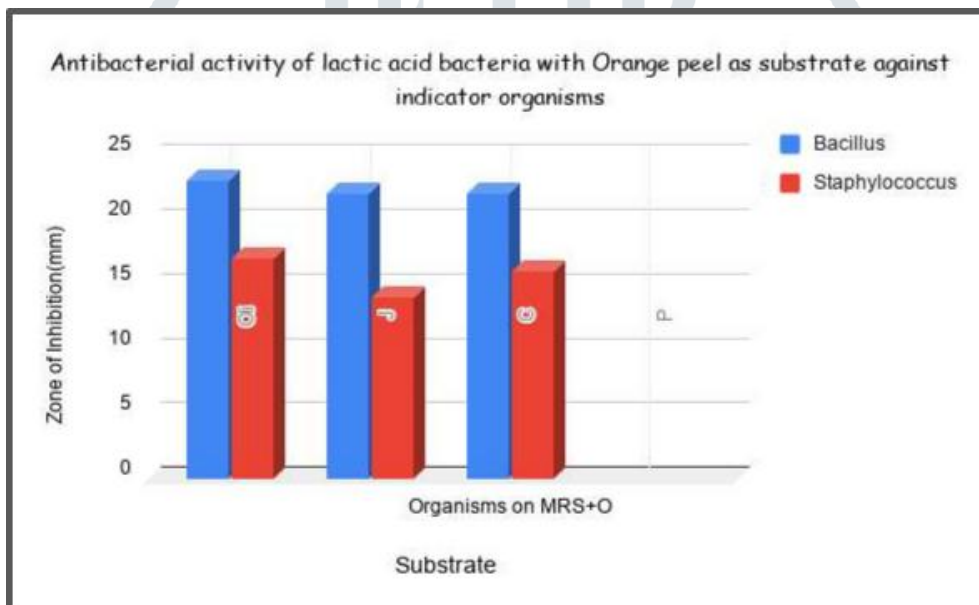


Fig 3: Effect of isolates on orange as a substrate

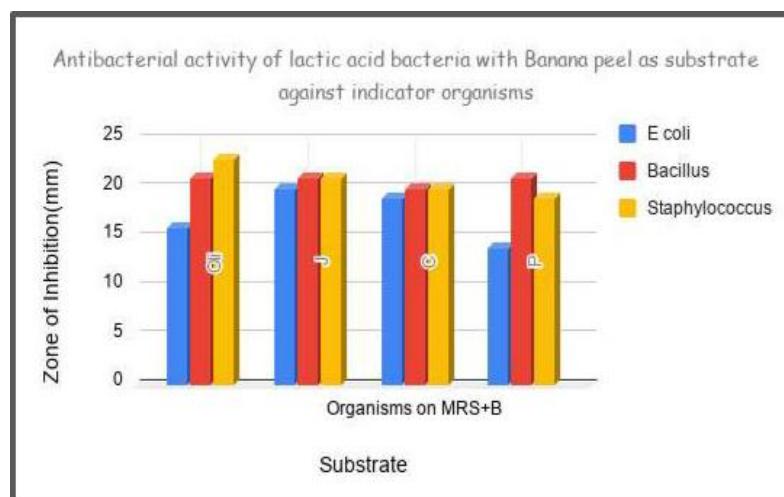
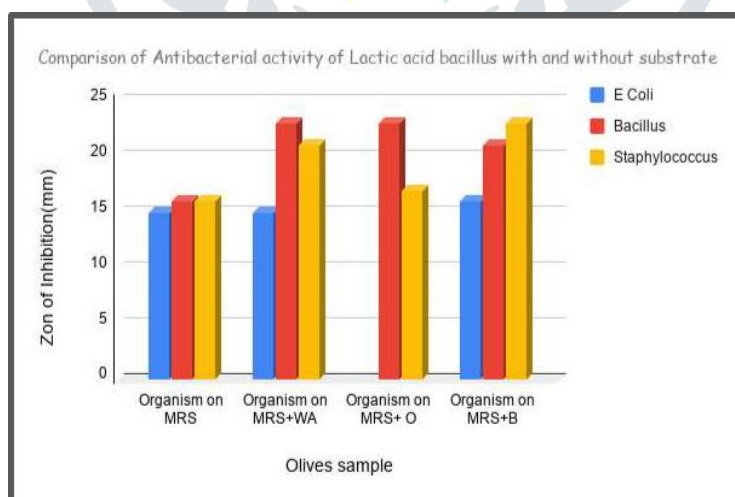


Fig 4: Effect of isolates on banana as a substrate

The Probiotic organisms showed maximum activity against *Staphylococcus* spp when they were provided with wood apple as a substrate. The organisms isolated from vinegar-soaked pickles showed less activity when compared with the organisms provided with biowaste (fruit peels) as a substrate. It shows that biowaste (fruit peels) can be used as a substrate for the growth of *Lactobacillus*.

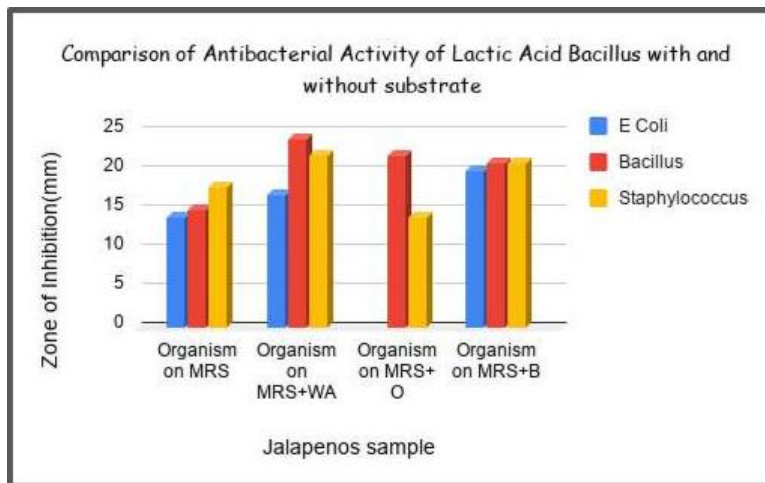
**Table 4: Comparison of Antibacterial Activity of Lactic Acid Bacteria**

S. No	Sample	Organism	Culture on MRS	Culture on MRS+Wood apple as a substrate	Culture on MRS+Orange as a substrate	Culture on MRS+ Banana as a substrate
1	Olives	E coli	15	15	-	16
2		Bacillus	16	23	23	21
3		Staphylococcus	16	21	17	23
4	Jalapenos	E Coli	14	17	-	20
5		Bacillus	15	24	22	21
6		Staphylococcus	18	22	14	21
7	Chilies	E Coli	17	20	-	19
8		Bacillus	17	22	22	20
9		Staphylococcus	18	28	16	20



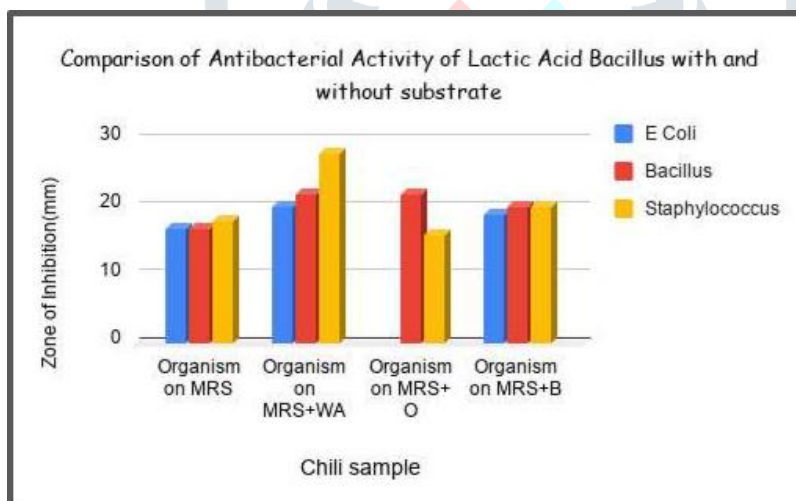
**Fig 5: Comparison of Antibacterial activity of Lactic acid bacillus isolated from Olives**

Isolated Organisms on MRS broth incorporated with Banana peel as a substrate showed highest zone of inhibition against *Staphylococcus* spp. Organisms on MRS broth incorporated with orange peel as a substrate showed highest zone of inhibition against *Bacillus* spp. MRS broth incorporated with Wood apple peel has a high inhibitory activity against *Bacillus* spp



**Fig 6: Comparison of Antibacterial activity of Lactic acid bacillus isolated from Jalepenos.**

Isolated Organisms on MRS broth incorporated with Banana peel as a substrate showed highest zone of inhibition against Staphylococcus spp and Bacillus spp. Organisms on MRS broth incorporated with orange p eel as a substrate showed highest zone of inhibition against Bacillus spp. MRS broth incorporated with Wood apple peel has a high inhibitory activity against Bacillus spp. Organisms on MRS broth isolated from Jalapenos sample showed highest zone of inhibition against Staphylococcus spp.



**Fig 7: Comparison of Antibacterial activity of Lactic acid bacillus isolated from chilli sample.**

Organisms were isolated from chili sample. Organisms on MRS broth incorporated with Banana peel as a substrate showed highest zone of inhibition against Staphylococcus spp and Bacillus spp. Organisms on MRS broth incorporated with orange peel as a substrate showed highest zone of inhibition against Bacillus spp. MRS broth incorporated with Wood apple peel has a high inhibitory activity against Staphylococcus spp. Organisms on MRS broth isolated from Jalapenos sample showed zone of inhibition against all lab organisms



#### 4. Summary

This study reports the isolation, characterization and identification of Probiotic organism isolated from vinegar-based pickles. Probiotic organisms were isolated from pickles and organisms were identified. The organism was identified as *Lactobacillus* spp. Based on Bergey's Manual System of classification, the identified *Lactobacillus* spp may be a *Lactobacillus casei*. The isolated Probiotic organisms showed a zone of inhibition against E coli, *Staphylococcus aureus* and *Bacillus subtilis*.

The effect of Biowaste (fruit peels) on the growth of Probiotic organisms was tested. The isolated Probiotic organism was tested for its growth and activity on different biowastes such as fruit peels which includes Orange, Banana and Wood apple. They showed maximum growth when they are provided with wood apple as a substrate than on Orange peels and banana peels.

#### 5. Conclusion

This study reports the comparison of the growth and antibacterial activity of organisms grown on fruit peels. Comparison of isolated Probiotic organisms on MRS agar isolated from the pickle samples and with the Probiotic organisms on MRS media agar incorporated with biowastes (fruit peels) as a substrate. The result shows that isolated Probiotic organisms when provided with biowaste (fruit peels) as a substrate showed maximum growth and inhibitory activity against indicator organisms such as E.coli, *Staphylococcus aureus* and *Bacillus subtilis* which indicates that the organisms absorbed nutrients provided by the fruit peels and showed maximum growth.

The present study concludes that Biowaste such as fruit peels can be effective substrates for the growth of Probiotic organisms and the vinegar-soaked pickles of Olives, Jalapenos and Chillies act as a good source for the isolation of the organisms.

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