

## Antifungal Activity of Bacteria Isolated from Sauerkraut and Its Partial Characterization

Abul Kalam<sup>1</sup> and S Rehan Ahmad<sup>2\*</sup>

<sup>1</sup>Assistant Professor, Bidhannagar College, Kolkata, W.B, India

<sup>2\*</sup>Assistant Professor, H M M College for Women, Kolkata, W.B, India

\*Corresponding Author E-mail: [zoologist.rehan@gmail.com](mailto:zoologist.rehan@gmail.com)

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### ABSTRACT

Sauerkraut is known to be the fermented product of a cabbage that is caused by an action of lactic acid bacteria or LAB which is naturally present in the air. The fermentation of lactic acid increases the shelf life of any vegetables and fruits. The valuable properties that includes the flavour, nutritive value and decreases the toxicity is also augmented. A completely cured sauerkraut can be kept for few months in a container which is airtight in room temperature. The fermented vegetables and fruits can be used as capable source of the probiotics as they harbour few LAB like *L. Fermentum*, *L. Acidophilus*, *Lactobacillus Plantarum*, *Lactobacillus brevis* etc. The core objective of current study is to isolate the LAB from the sauerkraut which is produced in the laboratory that can be used as the potent bio-control mediator to control vegetables and fruit spoiling the pathogenic fungi. In present study, a lactic acid producing bacteria has been isolated (ISAK-2), which is rod shaped, gram positive and breed optimally at a pH 4.5 in the MRS broth media. Bacteria was tried against fungal pathogens that is isolated from rotten fruits like Custard Apple (*Annona reticulate*), Papaya (*Carica papaya*), Pomegranate (*Punica granatum*) and rotten vegetables like Capsicum (*Capsicum annum*), sponge gourd (*Luffa cylindrica*) and Pointed gourd (*Trichosanthes dioica*). It was noticed from the study that the bacterial soup was able to constrain growth of fungi in the agar plate.

**Keywords:** Bio-control, Sauerkraut, Vegetable, Rotten fruits, Antifungal activity.

### INTRODUCTION

A fermented food which is made chiefly from cabbage known as Sauerkraut is one of the most famous variations of a fermented food factually centuries behind. It is famous for being a basis of nutrients during winter months when resources for fresh food were scarce for

a long time. The appropriate fermentation conserves cabbage's nutritive value along with creating the desirable sensory feature (Trail et al., 1996). Since the beginning of 1930's its popularity declined as the preferences of consumers changed and because of lack of uniformity in products (Varzakas et al., 2017).

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However, with the development in the consumer interests and science of food fermentation have resulted in renewed popularity of sauerkraut in current years. In recent years, sauerkraut is mainly available for purchase in the US because of its artisanal preparations and mass production. Production and features of sauerkraut are mostly dependent on community of resident microbial and conditions of fermentation (Stamer et al., 1971). Even though sauerkraut's microbial composition may differ in the initial stages of its fermentation, proper fermentation conditions like concentration of relative ingredient and temperature confirm that the LAB or lactic acid bacteria are central microorganism in ultimate fermented product. There are many compounds that are produced by LAB such as vitamins, bacteriocins, flavor compounds and organic acids which are responsible for various characteristic sensory qualities of the fermented foods. These have crucial significance for the successful fermentation that includes prolonged shelf life, nutritional content and flavor (Hong-Sik et al., 1994; Settanni et al., 2008 & Lee et al., 2011). Probiotics that add to the microbiome stability and human health are also harvested by a certain LAB which is stated earlier (Ljungh et al., 2006 & Ji et al., 2013). Sauerkraut is one of the possible sources of the probiotic candidates, a vegetable commodity that results from the immediate fermentation of a cabbage in the anaerobic conditions after addition of the salt (Harris 1998). These claims have added to a current increase in the popularity in consumers and consumption in US (Clarke et al., 2015) however, it was not completely substantiated scientifically. The fermentation of sauerkraut starts with primary proliferation of the *Leuconostocmesenteroides* that swiftly manufactures carbon dioxide and acid also lowers environmental pH that inhibits growth of the unwanted and decomposition causing microorganisms. After a week from the process of fermentation, hetero fermentative LAB dies. Moreover, more acid-tolerant homo fermentative microorganisms replaces it. Heterofermentative microorganisms make use

of fructose as the electron acceptor, altering it to a mannitol (McFeeters et al., 1986). The biphasic design of development and death can be noted by plating the aggregate LAB with the help of MRS agar, anaerobic growth at 30<sup>0</sup> C (Fleming et al., 1995). Pederson and Albury (Pederson et al., 1969) explained traditional structure of microbiota that is present in the sauerkraut fermentation. The primary heterolactic stage of fermentation concludes in manufacture of lactic and acetic acid. The instable acetic acid is the chief product for aroma and flavor of the ultimate product. There is also presence of various microorganisms in few numbers which are significant, chiefly other species of the *Lactobacillus* and *Leuconostoc* and *Pediococcus* and *Weisslla* (Holzapfel et al., 2008). There are reports that showcase the putative probiotic bacteria is isolated from the sauerkraut and from the related products (Plengvidhya et al., 2007 & Chang et al., 2010) that highlights the potential source of the probiotic microorganisms. Cabbage is known to be an efficient source of vitamin B and C, carotene, and minerals (*Brassica oleracea* L. var. *capitata*). India was ranked second among nations in production of cabbage and West Bengal, ranked first among states for production and consumption (De et al., 2014). During the process of harvest around 20-40% of the products are lost because of the improper preservation and handling of the post the harvest (Thakur & Kabir, 2015). Various metabolites of such bacteria have an effect which is antimicrobial against many spoilages of food and pathogenic bacteria that includes diacetyls, lactic acid, proteinaceous substance bacteriocins, hydrogen peroxide (Barefoot Klaenhammer 1983 & Daeschel 1989). LAB is famous for the production of their antimicrobial compounds that includes bacteriocins or the bacteriocin like the peptides. Bacteriocins of the LAB are defined as the ribosomal synthesized proteins or the complexes of the protein that is usually antagonistic to the genetically intimately related organism (De Vuyst Luc & Vandamme 1994 &

Klaenhammer, 1998) that possess activities against the other bacteria of the same species or across genera (Bowdish et al., 2005; Cotter et al., 2005 & Riley et al., 2002). Gram positive and Gram negative bacteria produces these kind of bacteriocins (Aly et al., 2006). Mukherjee and Gangopadhyay has studied the various concentrations of salt on microflora and the physicochemical alterations in the sauerkraut fermentation (Gangopadhyay & Mukherjee, 1971).

The present research work was done in dept. of Microbiology, Bidhannagar College, Salt lake, Kolkata in which the sauerkraut was prepared by making use of various types of cabbage and 3 percent of NaCl. Samples of the juice were taken on the day 0, 7, 14, 21, 28, 35 and 42 of the fermentation for inhibition experiments. The eventual decrease in the pH (from 6.5 to 3) was noted on weekly intervals. Titration method was used to measure the quantity of lactic acid. A bacterium was isolated from sauerkraut and was named ISAK-2. It was seen that the Gram positive and rod shaped. The isolated bacteria's antifungal effect from the sauerkraut or fermented cabbage juice was determined against vegetable and fruit spoilage fungi. In such case, the spoilage fungi that is isolated from rotten fruits like Custard Apple (*Annona reticulate*), Papaya (*Carica papaya*), Pomegranate (*Punica granatum*) and rotten vegetables like Capsicum (*Capsicum annum*), sponge gourd (*Luffa cylindrica*) and Pointed gourd (*Trichosanthes dioica*) were used.

## METHODS AND MATERIALS

### Formation of Sauerkraut

Cabbages were collected from the local markets, in the current experiment. The heads of the cabbages were trimmed the outer leaves were detached that includes the soiled and bruised tissue. The cabbages were thoroughly washed with tap water after the heads were trimmed and washed. The heads were cut into two halves and the central hard core was removed. The cabbage was shredded using a knife. This shredded cabbage was then weighed and equally distributed in six

different beakers / sterilized sealed polythene bag (sspb) and 3% NaCl was then added in each of these beakers / sspb. Then these two ingredients were thoroughly mixed together. The cabbage was compressed with the help of a pressuring object until it released the juice it held. The beakers / sspb were then sealed with the plastic covers in order to create an anaerobic condition. Furthermore, these beakers / sspb were kept under observation for six weeks in room temperature. Followed by this experimental set up, one of these beakers/ (sspb) was opened after weekly interval. The cabbage juice was then taken for various chemical, microbiological and physical analysis.

### Physical Analyses

The color of cabbage was detected and the odor was checked after the beaker / sspb was opened. In order to examine the texture of the cabbage, the sample was taken and compressed between two fingers.

### Chemical Analyses

With the help of a pH meter, the pH of the undiluted juice that was collected in test tube was measured every week. Strength of the lactic acid for each week was then measured by the titration using 0.1 N NaOH with phenolphthalein as an indicator. Erstwhile to this, standardization of the 0.1 N NaOH solution was done with the help of oxalic acid and phenolphthalein as an indicator. 10 ml distilled water and the fermenting water with the same quantity was collected into a 100 ml conical flask. Preceding to the process of titration, the soup was boiled in order to drive off the carbon dioxide. It was then cooled and 1% of phenolphthalein in 5 drops were added to the distilled juice. This persistent sample was then titrated with 0.1 N NaOH till the light pink coloration was noticed and quantity of alkali consumed was stated down. A titration of the juice for determining the percentage of the lactic acid present was conducted as follows:

**Percentage Lactic Acid= vol. of NaOH\* Normality of NaOH / vol. of Sample (10 ml).**

### Microbiological Analyses

#### Isolation of the bacteria

For the purpose of isolating the lactic acid bacteria, the undiluted juice was collected in 0.1 ml volume and later spread in the MRS agar plate and was then kept in the incubator. Succeeding to the 48 hours of the incubation, at a temperature of 37<sup>0</sup> C, the sole colonies that were observed were marked on a fresh MRS agar plate and then kept for incubation at the same temperature for 24 hours. The colonies that were observed were maintained in the MRS agar slants for future purpose.

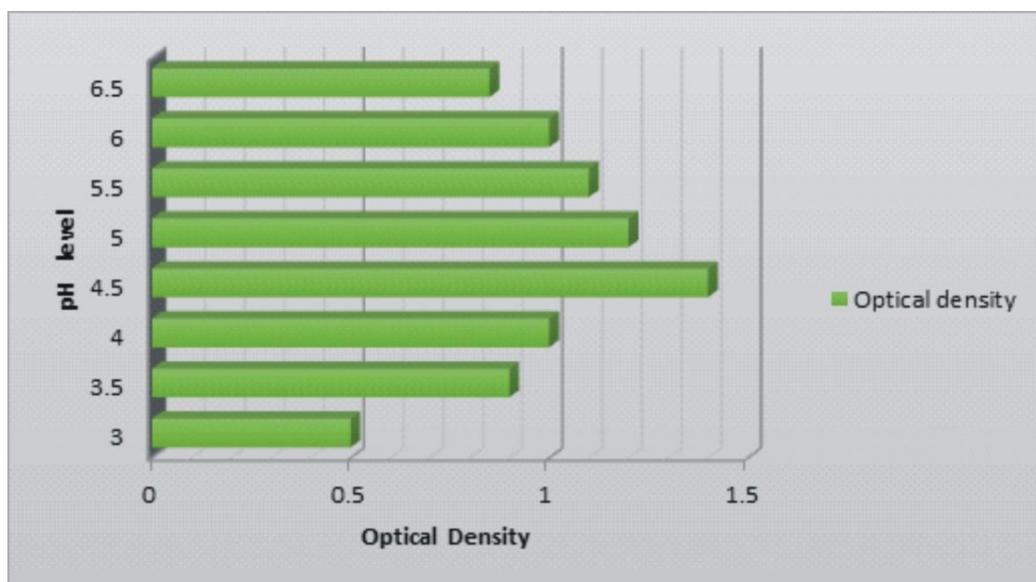
### Isolation of the Fungi

Some rotten portions of fruits like Custard Apple, Papaya, Pomegranate and some rotten portion of vegetable like Sponge gourd, Bell Capsicum and Pointed Gourd were placed in a distinct Czapek Dox agar plate and was kept aside for 72 of hours incubation for the purification and isolation of fungi. Czapek Dox agar medium and MRS broth medium were prepared in the culture tubes that contained 5 ml media each. MRS broth was inoculated with six culturally and morphologically different organisms that are

isolated from the cabbage soup. Tubes were incubated under the shaking condition for about 18 hours at a temperature of 37<sup>0</sup> C. Furthermore, the supernatant was collected by the centrifugations at 10000 rpm for 10 mins. Czapek Dox agar plates was then prepared and isolated cultures of fungi (0.1) from the rotten fruits were spread on the agar plate. Paper disc that contained bacterial soup was placed in the middle of agar plate. Further, this plate was incubated at a temperature of 37<sup>0</sup> C for around 3 to 5 days. Observations were conducted and effects of supernatants on the spoilage organisms were noted.

### Study of Optimum pH for growth

Optimum pH for the growth of isolated bacteria 3.5 to 6.5 was determined. Isolate was inoculated in the different pH and MRS broths (3.5, 4.0, 4.5, 5.5, 6.0 and 6.5) and incubated for the 24 hrs in shaker. The optical density was measured at 540 nm and growth was determined. The result shows that the organisms grows best at pH 4.5. (Bar Chat 01).



Bar Chat 01: Different OD at different pH level

### RESULTS AND DISCUSSION

On the 7<sup>th</sup> day of the incubation period, the putrid odor suggestive of the initiation of fermentation was noted and observed. The rapid growth *Lactobacillus sp.* Microscopic was indicated by the slimy texture and appearance of the flora specified the

occurrence of both bacilli and cocci which further led to an indication of lactic acid production by the *Lactobacillus mesenteroides* along with *Lactobacillus plantarum*. On the 14<sup>th</sup> day (2<sup>nd</sup> week) of incubation, the acidic odor indicated the increase in concentration of lactic acid. Thus, it can be noted in the

percentage of lactic acid which increased 0.62% to 0.54%. Change in the flora was then noted to be the only bacilli and the absence of any cocci was noted on the 7<sup>th</sup> day that indicated the ceased fermentative activity by *Leuconostoc mesenteroides*. Moreover, this also gave a soft texture. On the 21<sup>st</sup> day (3<sup>rd</sup> week) of the incubation, the odor slightly changed to the earthy smell and the percentage of lactic acid or the acidity augmented to 0.752% that indicated the product reaching continuously. Still changes observed on 35<sup>th</sup> day of incubation as it reaches up to 0.795. On

the 42<sup>th</sup> day (6<sup>th</sup> Week) of incubation, the odor changed into a spicy smell. The amount of lactic acid increased to 0.831% that indicated the product has eventually reached the desired state. No alteration in the microbial flora was noted. The texture was then almost dry and soft. The changes of pH during Sauerkraut fermentation have observed for the period of 6 (six week) and it was found that the pH changes drastically to 3 pH at 6<sup>th</sup> week from 6.5 pH at 0 week. (Fig. 01, 02 & 03; Table-1).

**Table 1: Study of different physical parameter of the sauerkraut**

Result	0 Days	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
Odour	Normal Odour	Putrid	Acid	Earthy	Spicy	Spicy	Spicy
Colour	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Deep brown	Deep brown
Texture	Slimy	Slimy	Soft	Soft	Soft	Soft	Soft



**Fig. 1 A**



**Fig. 1 B**



**Fig. 1 C**



**Fig. 1 D**



**Fig. 1 E**



**Fig. 1 F**

**Fig. 1: A-1 F – (Color and Texture changes of the cabbage at weekly interval of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> week)**

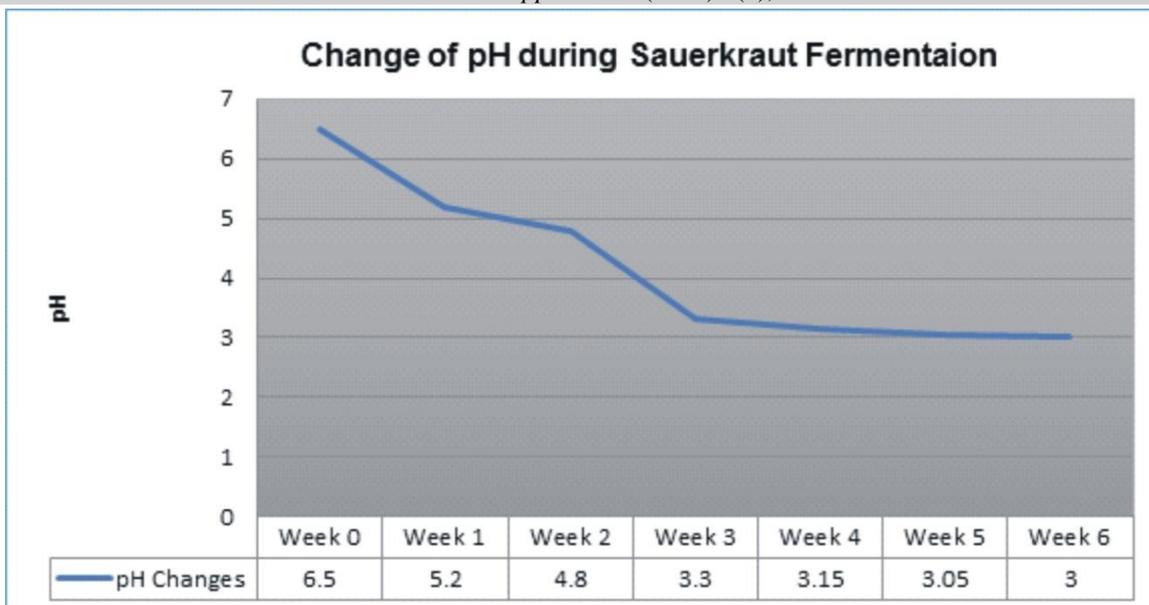


Fig. 2: (A): Study of pH change

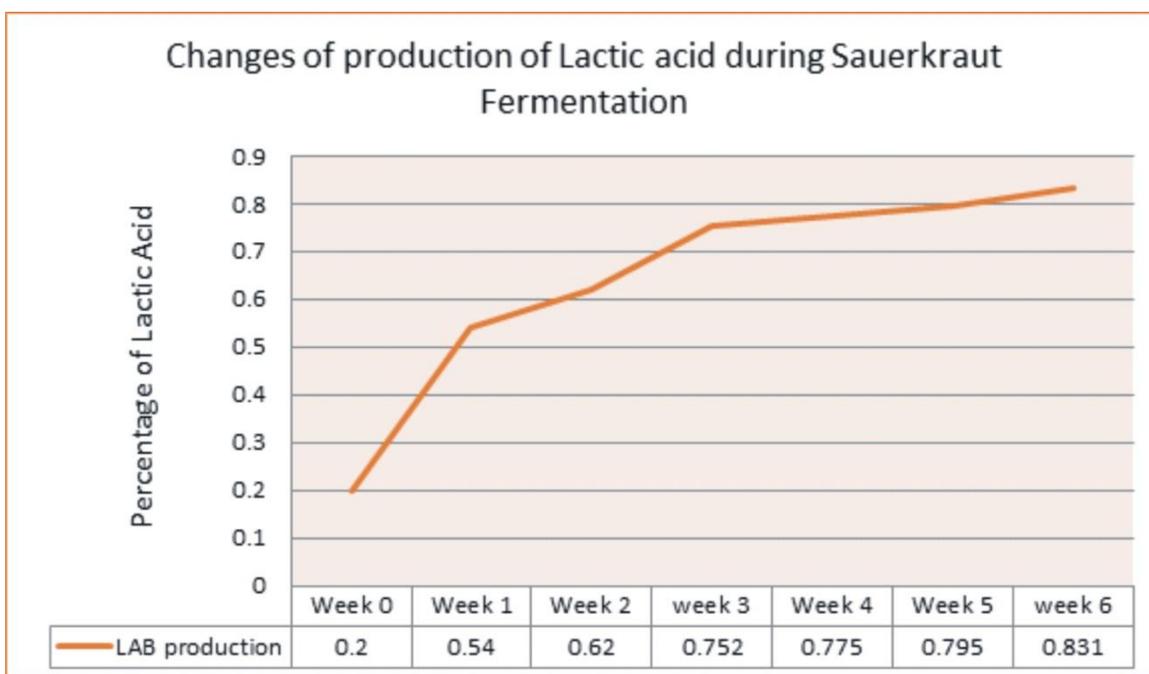


Fig. 2 (B): lactic acid productions (Gram Staining)

Table 2: Inhibitory effects of the isolated bacteria against fruits or vegetables spoilage fungi

Serial No.	Source of spoilage fungi	Diametoro of zone of inhibition (cm)
<b>Fruits</b>		
1	Custard Apple	1.7
2	Papaya	3.3
3	Pomegranate	1.2
<b>Vegetables</b>		
4	Sponge gourd	-
5	Pointed gourd	2
6	Bell Capsicum	2.5



Fig. 4:

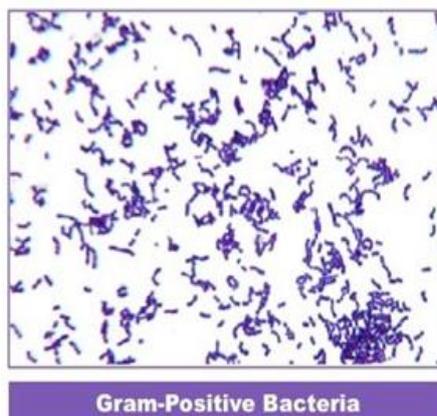


Fig. 5:

Fig. 4-5: Inhibition zone of spoilage fungi isolated from apple Papaya inoculated with the cell free extract soup (soaked in filter disk) isolated bacteria (ISAK-2) from sauerkraut & Fig. 5 Gram Positive bacteria

### CONCLUSION

There is a possibility to prepare the sauerkraut making use of the general variety cabbage and 3% of NaCl in the laboratory was showed by the initial study. The colour of cabbage transformed from green to brown by the end of the 6<sup>th</sup> week (Fig. 1). From 1<sup>st</sup> to the 6<sup>th</sup> week there was an eventual decrease in the pH showcasing that there was production of lactic acid (Fig. 2A and table 1). When the gram staining was completed on isolated purple, culture, gram+ rod shaped bacteria could be noticed. Hence, it might be a *Lactobacillus sp* (Fig. 5). The bacteria that was isolated were named as ISAK-2. It was noticed that ISAK-2 grew well in the pH 5.0 and pH 6.0. The clear zone was noticed when the filter disc was immersed in the isolated bacterial culture soup and was placed in the fungal plate. The reason behind this was the inhibitory effect of the bacteria against the vegetable/ fruit spoilage fungi (Table-2 and Fig- 4-5).

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### Declaration

#### Contribution by Principal author

The conception or design of the work was done by Principal author only. Data collection, Data analysis and Interpretation, Drafting the

manuscript, critical revision of the manuscript and final approval of the version was done by Principal author too.

#### Contribution by Corresponding author

Data collection, Data analysis and Interpretation, Drafting the manuscript, critical revision of the manuscript and final approval of the version was done by Corresponding author too.

#### Conflict of Interest

The author declare that there exist no commercial or financial relationship that could, in any way, lead to potential conflict of interest.

#### Funding Declaration

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**Ethical Approval:** This study has nothing to do with human and animal testing.

**ORCID Id:** <https://orcid.org/0000-0003-0796-5238>.

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