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ANTIMICROBIAL ACTION OF A BACTERIA (SAKBNC-4) ISOLATED FROM SAUERKRAUT & ITS CHARACTERIZATION

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ABSTRACT: Sauerkraut is a fermented product of cabbage caused by the action of Lactic Acid Bacteria (LAB) naturally present in air. Lactic acid fermentation increases shelf life of fruits and vegetables. It also enhances beneficial properties including nutritive value, flavor and reduces toxicity. Fully cured sauerkraut may be kept for several months in an airtight container at room temperature. Fermented fruits and vegetables can be used as a potential source of probiotics as they harbor several LAB such as *Lactobacillus plantarum, L. brevis, L. acidophilus, L. fermentum* etc. In the present study the main aim is to isolate LAB from sauerkraut produced in our laboratory which can be used as potent bio-control agent to control the fruit and vegetables spoiling pathogenic fungi. In our study we have isolated one lactic acid producing bacteria. The isolate is gram positive, rod shaped and grow optimally at pH 4.5 in MRS broth media. The bacteria was tested against the fungal pathogens isolated from rotten fruits and vegetables like Apple, pear, cucumber, bitter guard, Mango and lemon. From the study it was observed that bacterial soup is able to inhibit the growth of some fungi in agar plate.

KEYWORDS: Lactic Acid Bacteria (LAB), antimicrobial compound, sauerkraut, spoilage fungi, fugal pathogen.

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1.INTRODUCTION

Sauerkraut, a fermented food made primarily from cabbage, is one of the most well-known varieties of fermented food historically centuries behind. It served as a source of nutrients during the winter months when fresh food was scarce since long. The proper fermentation preserves the nutritive value of cabbage while creating desirable sensory properties [1-2]. Its popularity declined

beginning in the 1930s as consumer preferences has been changed and a lack of product uniformity [1-3]. But advances in food fermentation science and modern consumer interests have brought sauerkraut renewed popularity in recent years. Today sauerkraut is widely sold in the United States due to mass production and artisanal preparations. Sauerkraut production and characteristics are largely dependent on the resident microbial community and the fermentation conditions [4]. Though the microbial composition of sauerkraut can vary during the initial stages of fermentation, appropriate fermentation conditions such as temperature and relative ingredient concentration ensure that lactic acid bacteria (LAB) are the dominant microorganisms in the final fermented product. LAB produces many compounds like organic acids, bacteriocins, vitamins, and flavor compounds responsible for many of the characteristic sensory qualities of fermented foods. These have very critical importance for successful fermentation including extended shelf life, flavor, and nutritional content [5-8]. Probiotics that contribute to human health and microbiome stability are also produced by certain LAB which is reported earlier [9-10]. One possible source of probiotic candidates is sauerkraut, a vegetable product resulting from the spontaneous fermentation of cabbage in anaerobic conditions after the addition of salt [11]. These claims have contributed to recent increased consumer popularity and consumption in the United States [12] though it was not fully scientifically substantiated. Sauerkraut fermentation begins with the initial proliferation of *Leuconostoc mesenteroides*, which rapidly produces acid and carbon dioxide and quickly lowers the environmental pH, inhibiting the growth of undesirable and spoilage causing microorganisms. After about 1 week of fermentation, the heterofermentative LAB die off. They are replaced by the more acid-tolerant homofermentative microorganisms. Heterofermentative microorganisms also use fructose as an electron acceptor, converting it to mannitol [13]. This biphasic pattern of growth and death can be seen by plating total LAB using MRS agar, with anaerobic growth at 30°C [14-15]. Pederson and Albury[16] described the traditional composition of the microbiota present in sauerkraut fermentation. The initial heterolactic stage of the fermentation results in production of both acetic acid and lactic acid. The volatile acetic acid is the key product for the flavor and aroma of the final product. There are also some other microorganisms present in lower numbers which may be important, mainly other species of *Leuconostoc* and *Lactobacillus*, as well as *Pediococcus* and*Weissella* [17-18]. There have been reports of putative probiotic bacteria isolated from sauerkraut, as well as from related products [19-22], which underlines that it may be a prospective source of probiotic microorganisms. Cabbage (*Brassica oleracea L.* var. capitata) is a good source of carotene, vitamin B and C, and minerals. India ranked second in cabbage production in the world while West Bengal ranked first in production and consumption as well among Indian states [23]. During harvesting about 20-40 per cent of the product is lost due to improper post harvest handling and preservation [24]. Some of the metabolites of these bacteria have an antimicrobial effect against many food spoilage and

pathogenic bacteria, include lactic acid, diacetyls, hydrogen peroxide and proteinaceous substances bacteriocins [25-26]. LAB are known for their production of antimicrobial compounds, including bacteriocins or bacteriocin – like peptides. Bacterocins of LAB are defined as ribosomally synthesized proteins or protein complexes usually antagonistic to genetically closely related organisms [27-28] which possess activity against other bacteria, either of the same species or across genera [29-31]. These bacteriocins are produced by both Gram negative and Gram positive bacteria [32]. Gangopadhyay and Mukherjee studied the different salt concentrations on the microflora and physicochemical changes in sauerkraut fermentation [33]. In this study, the sauerkraut was prepared using local variety cabbage and 3 % NaCl. Juice samples were taken at day 0, 7, 14, 21 and 28 of fermentation for the inhibition experiments. The gradual lowering of pH was recorded at weekly intervals. The amount of lactic acid was also estimated by titration method. One bacteria isolated from the sauerkraut and named sakbnc-4. It was found Gram + and rod shaped. The antifungal effect of the isolated bacteria from the fermented cabbage (sauerkraut) juice was determined against the fruit and vegetable spoilage fungi. In this case spoilage fungi isolated from rotten fruit like Apple, pear, cucumber, bitter guard, Mango and lemon were used.

2. MATERIALS AND METHODS

Sauerkraut Production

In the present investigation cabbage were collected from local market. Cabbage heads were trimmed, outer leaves removed including all bruised and soiled tissue. Cabbage heads were trimmed and washed thoroughly with tap water. Cabbage heads were cut in half and the hard, central core removed. Cabbage was shred using a knife. Shredded cabbage was weighed and distributed equally in five different beakers and 3% NaCl was added in each beaker. NaCl and cabbage were thoroughly mixed. The cabbage was compressed using a pressing object until the cabbage was covered by juice. Beakers were then sealed with plastic covers to create an anaerobic condition. The beakers were then kept for four weeks at room temperature for observation. After the experimental setup one beaker was opened at every week's interval. The cabbage juice was taken for different physical, chemical and microbiological analysis.

Physical Analyses

After opening the beaker, the colour of the cabbage was observed and then the odour was checked by having a smell of the beaker. To analyse the texture, the cabbage was taken and pressed between two fingers.

Chemical Analyses

The pH of the undiluted juice collected in the test tube was measured for every week's beaker using a pH meter. Strength of lactic acid for each week was measured by titration using 0.1 N NaOH with phenolphthalein as indicator. Prior to this, standardization of 0.1 N NaOH solution was done using oxalic acid and phenolphthalein as an indicator. Ten ml of the fermenting and

10ml distilled water was taken into a 100ml conical flask. Prior to titration the soup was boiled to drive off the carbon dioxide. It was cooled and 5 drops of 1% phenolphthalein was added to the distilled juice. The persistent sample was titrated with 0.1N NaOH till light pink coloration was observed and ml of alkali consumed was noted down. A titration of the juice to determine the percentage of lactic acid present was done as follows:

Percentage Lactic Acid = vol. of NaOH × Normality of NaOH / vol. of Sample (i.e., 10ml).

Microbiological Analyses

Isolation of bacteria

To isolate the lactic acid bacteria, the undiluted juice was taken in 0.1 ml volume and then spread in an MRS agar plate and was kept in an incubator. After 48 hours of incubation at 37 °C, the single colonies observed were then streaked on a fresh MRS agar plate and kept for incubation at 37 °C for 24 hours. The colonies observed were then maintained in MRS agar slants for future use.

Isolation of Fungi

For the isolation and purification of fungi, some rotten portion of fruits like Apple, pear, cucumber, bitter guard, Mango and lemon were placed in separate Czapek Dox agar plate and kept for 72 hours incubation._MRS broth medium and Czapek Dox agar medium were prepared in culture tubes containing 5ml media each. MRS broths were inoculated with 7 morphologically and culturally different organisms isolated from cabbage soup. The tubes were incubated under shaking condition for about 18 hours at 37^{o}C. The supernatant was then collected by centrifugations at 10000 rpm for 10 min. Czapek Dox agar plates was prepared and isolated fungal cultures (0.1 ml) from rotten fruits were spread on agar plate. Paper disc containing bacterial soup was placed in the centre of the agar plate. Then the plate was incubated at 37^{o}C for 3 to 5 days. Observations were made and the effects of the supernatants on the spoilage organisms were recorded.

MRS Broth preparation and inoculation

To check the pH where the bacterial growth is maximum MRS Broth was prepared of varying pH of 4.0, 5.0 and 6.0. Then a loop of bacteria was inoculated into one tube and mixed well and the same was repeated to inoculate 5 tubes each of pH 4.0, 5.0 and 6.0. The tubes were incubated in a shaker for 24 hrs and the bacterial growth was observed.

3. RESULTS AND DISCUSSION

On day 7 of incubation, putrid odour indicative of initiation of fermentation was observed. A slight slimy texture indicated rapid growth *Lactobacillus sp.* Microscopic appearance of flora indicated presence of both cocci and bacilli indicating initiation of lactic acid production by *Lactobacillus mesenteroides* as well as *Lactobacillus_plantarum.* On day 14 of incubation, acidic odour indicative of increase in lactic acid concentration was observed. This can also be seen in % lactic acid which increased from 0.729% to 0.783%. Change in the flora was observed to be only bacilli

and absence of any cocci as was seen on Day 7 indicative of the ceased fermentative activity by *L. Mesenteroides*. This also gave soft texture. On day 21 of incubation, odour slightly changed to earthy smell and % of lactic acid or acidity increased to 0.924% indicative of the product reaching the desired finished stage. No change in the flora was observed. On day 28 of incubation, the odour finally changed to spicy smell. Percentage of lactic acid increased to 1.253% indicating the product has finally reached desired state. No change in microbial flora was observed. Texture was almost dry and soft (Pic. 1 A,B,C,D,E & Table – 1).

Table 1. Study of different physical parameter of the sauerkraut

Result	**O Days**	**7 Days**	**14 Days**	**21 Days**	**28 Days**
Odour	**Normal cabbage odour**	Putrid	Acid	Earthy	Spicy
Colour	**Pale yellow**	Pale yellow	Pale Yellow	Light Brown	Deep Brown
Texture	**Slimy**	Slimy	Soft	Soft	Soft

Fig. 1 A

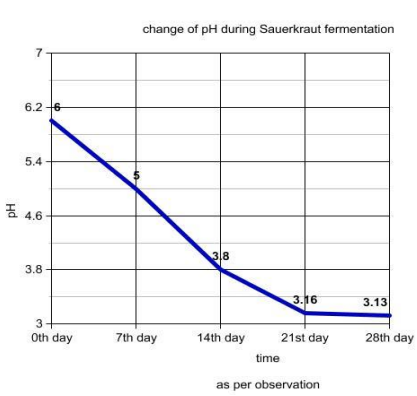
Fig. 1 B

Fig.1C

Fig. 1 D

Fig. 1 E

Fig 1: A-1E – (Colour and Texture changes of the cabbage at weekly interval, week 0-week4)



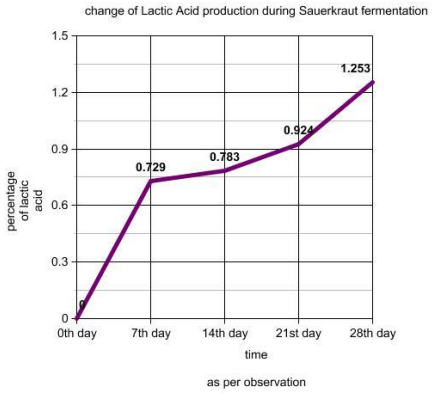


Fig. 2 A Fig 2 B

Fig-2 Study of pH change (Fig 2 A) and lactic acid production (Fig.2 B)

Gram Staining

The gram staining was performed on the bacteria isolated from the sauerkraut and it was found Gram + and rod shaped. The bacteria isolated was named as sakbnc-4 (Fig.3).

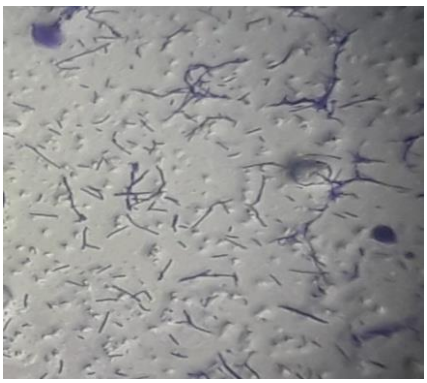


Fig . 3 Gram positive, rod

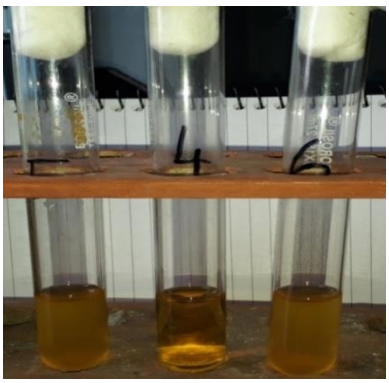


Fig . 4 Bacterial Growth under Different pH

Growth of the isolated bacteria sakbnc-4 (from sauerkraut) under different pH studied. It was observed that sakbnc-4 grow well in pH 5.0 and 6.0 as the solution turned turbid. At pH 4 no bacterial growth was observed (Fig.4). Inhibitory effect of isolated bacteria sakbnc-4 was studied. It was observed that among the six spoilage fungi, the isolated fungi from Mango, Apple, Bitter guard and cucumber were found to be sensitive against the isolated bacteria sakbnc-4. Fungi isolated from apple were found to be most sensitive (Table -2, Fig. 5)

Table 2. Inhibitory effects of isolated bacteria against fruit/ vegetable spoilage fungi

Serial No.	Source of spoilage fungi	Diameter of zone of inhibition (cm)
1	Mango	2.0
2	Lemon	-
3	Apple	3.2
4	Pear	-
5	Bitter guard	2.5
6	Cucumber	2

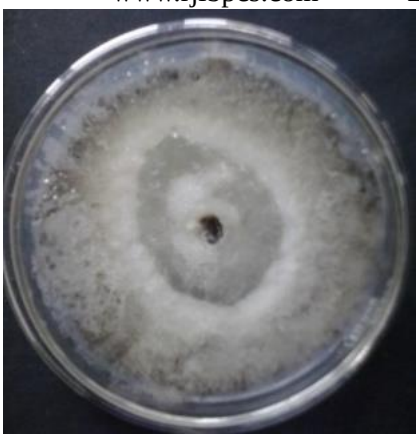


Fig. 5: Inhibition zone of spoilage fungi isolated from apple after inoculated with the cell free extract soup (soaked in filter disk) isolated bacteria (sakbnc-4) from sauerkraut.

4. CONCLUSION

The preliminary study showed that it is possible to prepare sauerkraut using local variety cabbage and 3% NaCl in a laboratory. The colour of the cabbage turned from green to brown by the end of week 4 (Fig.1). From week 1 to week 4, there was a gradual decrease in pH showing that there was lactic acid production (Fig.2 and table 1) When gram staining was performed on the isolated culture, purple, gram positive rod shaped bacteria could be observed, hence, it may be a *Lactobacillus* sp(Fig.3). The isolated bacteria were named sakbnc-4. It was observed that sakbnc-4 grow well in pH 5.0 and pH 6.0 (Fig.4) A clear zone was observed when a filter disc dipped in isolated bacterial culture soup was placed in a fungal plate. This was caused by the inhibitory effect of bacteria against fruit/vegetable spoilage fungi (Table-2 and Fig. 5).

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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