Production, purification and biochemical characterization of dextran sucrase from Lactic acid bacteria isolated from sauerkraut

Abstract
In this study Lactobacillus sp. has been isolated form Sauerkraut for the production of dextran sucrase. The fermentation process is continued by the mixed activity of lactic acid bacteria (LAB) present in the medium. Morphological, physiological and biochemical analysis deciphered the presence of acid producing bacteria belongs to the genera Lactobacilli. Characteristically the isolates were catalase producing but oxidase negative significant to the Lactobacilli genera. The isolated bacterium was tested to be positive in the production of dextran sucrase in Tsuchiya medium. Maximum protein production containing dextran sucrase was achieved to be 400µg/ml which is significantly high in terms of concentration. Partially purified enzyme exhibited V_{max}=2.9±0.2µmole/min, K_{cat}=1.1±0.2mg/ml and K_{m}=2.75s^{-1} and the enzyme followed first order rate kinetics. Better exploration strategy for higher dextran sucrase activity from Lactobacillus sp. by inducing operon to higher dextran production can be used later as potent nutraceuticals.

Keywords: sauerkraut, Lactobacillus sp., Tsuchiya medium, dextran sucrase

Introduction
Saurkraut or sour cabbage is a fermented plant product of cabbage in the presence of 2 to 3 % (w/v) salt in which natural population of lactic acid bacteria (LAB) plays a major role. It is also one of the greatest prebiotic foods. The carbohydrates present in cabbage are fermented by the mixed activity of (LAB) like Leuconostoc mesenteroides, Lactobacillus brevis and L. plantarum. Presence of 2.2 to 2.8% (w/v) NaCl in sauerkraut preparation plays important role which does not allow the growth of initial spoilage by microorganisms. Pseudomonas, through the procedure of osmosis removes moisture lead to dampness and the destroy the cabbage from the frame the saline solution in which maturation of the organism happens whereas, LAB (L. brevis and L. Plantarum) helps in keeping up the surface of the cabbage by pulling back water and repressing endogenous pectolytic compounds which mollify cabbage followed by maturation.

The extracellular and cell bound compounds introduce in the microorganisms to create a scope of exopolysaccharide which is assumed to be a noteworthy part in security maker of cells from ominous microorganisms to create a scope of exopolysaccharide which is one of the greatest prebiotic foods. The carbohydrates present in cabbage in the presence of 2 to 3 % (w/v) salt in which natural population of lactic acid bacteria (LAB) plays a major role. It is also one of the greatest prebiotic foods. The carbohydrates present in cabbage are fermented by the mixed activity of (LAB) like Leuconostoc mesenteroides, Lactobacillus brevis and L. plantarum. Presence of 2.2 to 2.8% (w/v) NaCl in sauerkraut preparation plays important role which does not allow the growth of initial spoilage by microorganisms. Pseudomonas, through the procedure of osmosis removes moisture lead to dampness and the destroy the cabbage from the frame the saline solution in which maturation of the organism happens whereas, LAB (L. brevis and L. Plantarum) helps in keeping up the surface of the cabbage by pulling back water and repressing endogenous pectolytic compounds which mollify cabbage followed by maturation.

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Identification of bacterial culture
Identification of bacterial isolates was done by recording the various biochemical activities as mentioned in following methods. The different biochemical tests with the bacterial culture by inoculating it into different media, slants and broth. The physiological and morphological identification of bacterial isolate was done using gram staining and endospore staining. The bacterial was tested for its ability to ferment carbohydrates as described earlier by Kandler and Weiss. From the overnight grown culture in MRS broth containing 2% (w/v) Glucose as carbon source, 50µl was transferred to 5.0ml liquid MRS medium lacking glucose but containing phenol red and other hydrophilic polymer which specifically encourages high sub-atomic weight or total type of protein purification. In this study we unveiled the isolation of lactic acid bacteria from sauerkraut, making its pure culture and production and characterization of dextran sucrase.

Materials and methods
The culture (sample) was isolated from sauerkraut prepared by fermenting the fresh cabbage collected from local market of Guwahati, Assam, India. The Chemicals and ingredients required for preparing MRS (DeMan, Rogosa and Sharpe) media are from Hi-Media Pvt. Ltd. All other reagents and chemicals required for the maintenance and enzyme production and biochemical characterization were procured from Hi-Media India Pvt. Ltd. India.

Isolation of microorganisms
Fermented cabbage of 100gm sauerkraut was ground to paste and mixed in 10ml of saline (0.9% w/v) homogenously in test tubes. Serial dilutions methods were made till the dilution factor 10^{-5}. One hundred microliter from all the dilutions of sauerkraut from 10^{-5} to 10^{-1} dilution was taken and spread plated on MRS agar plates with glucose as carbon source. The micro-aerophilic condition atmosphere was created by nitrogen purging and tightly packed the plated using parafilm and the plated were then incubated at 25°C for 24hours.

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test carbohydrates 1% (v/v) were supplemented in final inoculums. Carbohydrates utilization test was performed to investigate whether the isolates can ferment lactose, sucrose, mannitol, and dextrose. The test media were incubated for 2 days at 30°C without shaking. The acid production was recorded between 24hr to 48hr. The acid production was indicated by a change in colour of the phenol red indicator from red to yellow. Amylase is an exo-enzyme that hydrolyses starch. Amylase production to degrade starch was tested by inoculating the bacterial isolate in MRS agar plating containing 1% (w/v) starch. The test is performed for the utilization of starch by the bacterial isolate isolated from sauerkraut in a starch agar media by using iodine as an indicator. Other biochemical viz. Gelatin liquefication, indole production, citrate utilization, catalase and oxidase tests were performed and bacterial genus was identified using Bergey’s manual of systematic bacteriology.¹

**Production and purification of dextranase**

The present knowledge on the characteristics of dextranase and its mode of action is based primarily on the important investigations of Hehre.² The production of dextranase in sucrose broth cultures of LAB and its role in dextran synthesis was thoroughly explored in Tsuchiya medium.³

For the production of dextranase from the media components (w/v) Sucrose (5%), glucose as co-substrate, Tween 80 (0.1%), yeast extract (1.5%), peptone (2.0%) and KH₂PO₄ (1.5%) was mixed properly in 250ml Erlenmeyer in 150ml of media and sterilized by autoclaving. The isolate of the previous MRS broth culture of 1.5µl was then inoculated and incubated for 36hours at 30°C.

**Purification of dextranase**

The Dextranase enzyme catalyzes the synthesis of exopolysaccharide, dextran from sucrose. Majority of the dextran are synthesized from sucrose by dextranase secreted mainly by bacteria belonging to genera *Leucanostoc, Streptococcus* and *Lactobacillus*. Among all the purification method, Polyethylene glycol (PEG) is an effective, rapid and single step purification method for dextranase.⁴ Dextranase was purified using PEG 8000Da. Polyethylene Glycol 25% (v/v) was prepared and allowed to mixed properly in microwave for about 2mins. The prepared PEG was allowed to cool down and kept in refrigerator for about 30minutes to make it ice cold. Enzyme samples were taken in a centrifuge tube and allowed to cool down and kept in refrigerator for about 30minutes to properly in microwave for about 2mins. The prepared PEG was used for the purification of dextranase from the isolate of LAB and its role in dextran production was thoroughly explored in Tsuchiya medium.³

The supernatant (cell free extract) was analyzed for enzyme activity. The enzyme activity was carried out with 5% (w/v) sucrose as substrate in the 1ml reaction mixture. 1ml enzyme was taken and mixed with the above mixture. The enzymatic reaction was performed at 30°C for 30minutes in water bath. 2ml of the prepared DNS (3,5-Dinitrosalicylic acid) reagent was added to the above mixture. The solution was mixed and heated for 10minutes in boiling water bath. The absorbance of colour developed was measured at 540nm on a UV–visible spectrophotometer. Enzyme activity was measured in standard SI unit (U/ml). One unit (U) of enzyme activity is the amount enzyme catalyzes 1µmol of substrate per minute under standard conditions. Kinetic properties of the Vₘₐₓ, Kₘ and Kₘₛₚ were determined for dextranase from the isolate under optimum pH and temperature following above mentioned DNS method.

### Results

**Isolation of microorganisms**

Initial 36hours of incubation the colonies on MRS agar medium appeared as large clear opaque colonies in an enhanced CO₂ environment. The colonies appeared to be slimy, convex, entire white, opaque and no pigment shown producing with 1.2x10⁸ CFU/ml. Eight colonies (SC1-SC8) were further pure cultured in MRS agar slant supplemented with 1% (w/v) glucose. Morphologically and physiologically, the pure colonies observed under 100X magnification were gram positive, long rod shaped non spore forming bacteria (Table 1) (Figure 1). Pure colonies were taken further for biochemical analysis. Biochemical analysis of carbohydrate utilization, starch hydrolysis (amylase production), gelatin hydrolysis, indole production, citrate test, catalase and oxidase production were listed in Table 1. Test results were verified using Bergey’s manual of systematic bacteriology⁵ decipher all the pure colonies were belong to the genera Lactobacillus. Therefore, the pure colony was identified as *Lactobacillus* sp.

| Isolate | Gram staining | Endospore staining | Lactose | Sucrose | Mannitol | Dextrose | Starch hydrolysis | Citrate utilization | Indole production | Gelatin liquefication | Catalase | Oxidase |
|---------|---------------|--------------------|---------|---------|----------|----------|------------------|--------------------|-------------------|---------------------|----------|--------|
| SC1     | +             | -                  | +       | +       | +        | +        | +                | -                  | -                 | +                   | -        | -      |
| SC2     | +             | -                  | +       | +       | +        | +        | +                | -                  | -                 | -                   | -        | +      |
| SC3     | +             | -                  | +       | +       | +        | +        | +                | -                  | -                 | -                   | -        | +      |
| SC4     | +             | -                  | +       | +       | +        | +        | +                | -                  | -                 | -                   | -        | +      |
| SC5     | +             | -                  | +       | +       | +        | +        | +                | -                  | -                 | -                   | -        | +      |
| SC6     | +             | -                  | +       | +       | +        | +        | +                | -                  | -                 | -                   | -        | +      |
| SC7     | +             | -                  | +       | +       | +        | +        | +                | -                  | -                 | -                   | -        | +      |
| SC8     | +             | -                  | +       | +       | +        | +        | +                | -                  | -                 | -                   | -        | +      |

+ = indicates positive and - = indicates negative outcome

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**Table 1** Morphological, physiological and biochemical characteristics of pure isolated colonies from sauerkraut

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Production and purification of dextranucrase

The enzyme Dextranucrase production was carried out as described by Tsuchiya et al. Sucrose was supplemented instead of Glucose for enzyme production. Among the nutrients carbon source sucrose was chosen as it induces the dextranucrase production and is also a substrate for dextran production from Lactobacillus. The cell free supernatant containing crude dextranucrase from Lactobacillus was purified by polyethylene glycols and the protein concentration was found to be 400g/l. The partially purified dextranucrase displayed 2.5±0.2U/ml enzyme activity. All the enzyme activities were measured in triplicates. Temperature and pH were optimized for dextranucrase and were found to be 30°C and 4.0, respectively (Figure 2A & 2B). Kinetic parameters were calculated using Sigma Plot statistical tool (www.sigmaplot.co.uk/products/sigmaplot/sigmaplot-details.php). MM plot and Lineweaver burk plot (LB plot) displayed distinct hyperbolic curve and regression line y=0.97 and the kinetic parameters were measured from the LB plot was found to be $V_{max} = 2.9±0.2\mu$ mole/min, $K_m = 1.1±0.2mg/ml$ and $K_{cat} = 2.75s^{-1}$ (Figure 3A & 3B). The production of dextranucrase from Lactobacillus spp. is increased with an increase in Sucrose concentration. With increase in substrate concentration the enzyme activity has been increased up to certain level where zone of saturation occur and stationary phase appears.
Discussion

The physiological and biochemical characterization were carried out to distinguish the isolate from other closely related Lactic acid bacteria (LAB). Based on biochemical and physiological studies the isolate showed relation with other Lactobacillus spp. The isolates were gram positive, non-sporo forming, non-motile, rod shaped, short-medium chain. By performing biochemical test the Catalase, Oxidase, Methyl-red, Voges-proskauer and Indole were negative. The isolates were able to ferment the Carbohydrates like Lactose, Sucrose, mannitol and Dextrose. The isolates cannot hydrolyse starch and in Gelatin liquefaction test the Gelatin could not be liquefied. Generally, the cultural and biochemical properties of the isolates agreed with the description of Kandler and Weiss and confirmed the Bergey’s Manual of systematic bacteriology. The colonies on MRS Medium appeared to be slimy, convex, white, opaque and without pigment. Production of dextransucrase from Lactobacillus species is induced by the presence of sucrose. The production of dextransucrase could be increased by optimizing the fermentation process. As described earlier among all the purification methods, polyethylene glycol (PEG) is an effective, rapid and single step purification method for dextranucrase. MM plot exhibited the enzyme dextranucrase followed first order rate kinetics. Kinetic parameters of the dextranucrase isolated from the sauerkraut Lactobacillus has significant V_{max}, K_{m} and K_{cat} which can be utilized for higher dextran production. Dextran has wide applications in food, Pharmaceuticals, and cosmetics industries.

Conclusion

Sauerkraut is a popular fermented food product of South East Asia made up of raw cabbage after fermentation by lactic acid bacteria. In this study we have implemented strategies to explore the potential of Lactobacillus sp. in the production of dextranucrase with significantly good enzyme activity. This source of enzyme production can serve the purpose of dextran synthesis by progressively increasing demand of natural polymers for various industrial applications.

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Conflict of interest

Authors declare that there is no conflict of interest.

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