

Synthesis and Antimicrobial Properties of Chitosan: A Case Study

Heet Chauhan¹, Gaurav Dave¹, Rushabh Patel¹, Maulin Shah^{1,2*}, Anjali Bishnoi^{1,3} and Sandeep Rai^{1,4}

¹Department of Polymer and Rubber Technology, Shroff S. R. Rotary Institute of Chemical Technology, Bharuch, Gujarat, India

²Department of Microbiology, Enviro Technology Limited, Ankleshwar, Gujarat, India

³Department of Chemistry, Indian Institute of Technology Hauz Khas, New Delhi, India

⁴GRP Limited, GIDC Estate, Panoli, Bharuch, Gujarat, India

***Corresponding Author:** Maulin Shah, Department of Polymer and Rubber Technology, Shroff S. R. Rotary Institute of Chemical Technology and Department of Microbiology, Enviro Technology Limited, Gujarat, India.

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Abstract

In the present study, an attempt was made to synthesis of chitosan from chitin extracted from crab shells. Chitosan is the second most abundant natural polymer. The different methods such as deproteinization, demineralization, and deacetylation respectively were used in synthesis of chitosan from chitin. Antimicrobial activity was studied and it was found that Chitosan is extremely good in inhibiting the growth of microorganisms; confirmed by the results obtained from experiments. In order to evaluate antimicrobial activity, the serial dilution method was used towards *Escherichia Coli*, *Staphylococcus aureus*, *Bacillus subtilis*.

Keywords: Chitin; Chitosan; FTIR Spectroscopy; Biodegradability; Microbial Activity

Introduction

Chitosan is a natural polymer and has numerous applications in biomedical and other industries. Due to high biodegradability and non-toxicity it has opened up many fields of application [1]. Studies have shown that it also has very good anti-microbial properties thus it can be widely-used as an antimicrobial agent either alone or blended with other natural polymers. Chitosan consists of β -(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine randomly distributed within the polymer (Figure 1). Chitosan is cationic in nature, while most of polysaccharides are either neutral or negatively charged in an acidic environment. This property of chitosan allows forming electrostatic complexes or multilayer structures with other negatively charged natural or synthetic polymers [2]. Chitosan is also having various biological properties, such as antitumor [3], antimicrobial [4,5], and antioxidant [6] activities. The degree of de-acetylation (Molar fraction of de-acetylated units or % de-acetylation) and the molecular weight of chitosan, significantly affect its biological properties [7]. Chitosan finds numerous biological and biomedical applications such as in water treatment [8], pharmaceutical excipient or drug carrier [9], and as a scaffold for tissue engineering [2,10]. Waste (crab, prawn shell) is generated from the seafood handling plant and the waste is directly dumps into the landfills and this have made a serious administration and natural issues. The production of chitosan from crab/prawn shells obtained from seafood handling plant is economically viable and environment friendly way to overcome this problem. The present work reports preparation of Chitosan using crab exoskeletons and subsequently this lab made Chitosan was evaluated for its antimicrobial properties against eleven most commonly found microorganism. In this study, we prepared a series of chitosan solution and checked them for antimicrobial activity against *Escherichia Coli*, *Staphylococcus aureus*, *Bacillus subtilis*. Our main goal of this work is to combine the antimicrobial activity of chitosan in order to design biocompatible antimicrobial biomaterials.

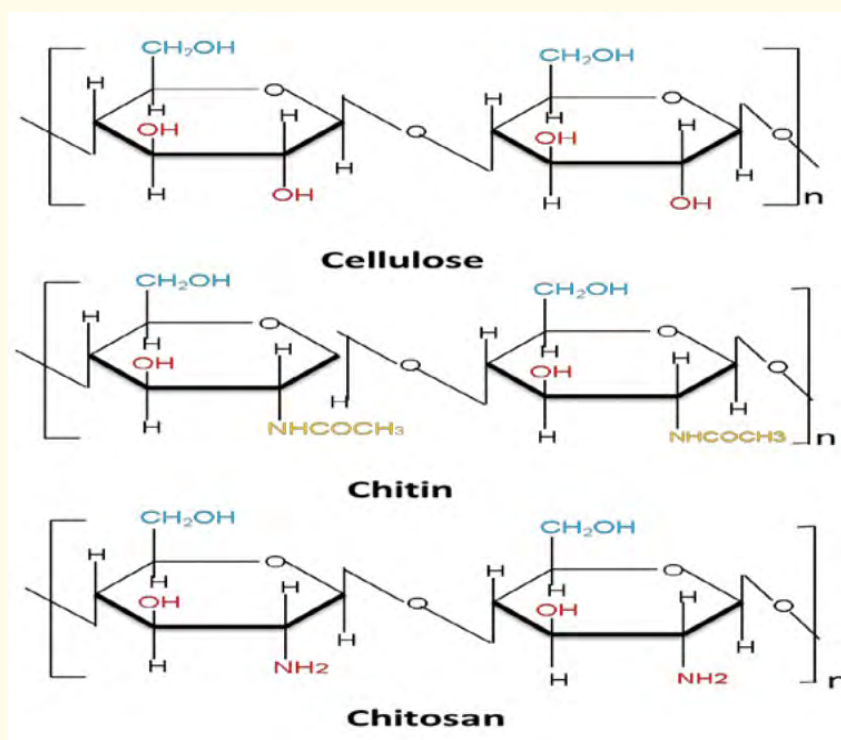


Figure 1: Chemical structures of cellulose, chitin, and chitosan [11].

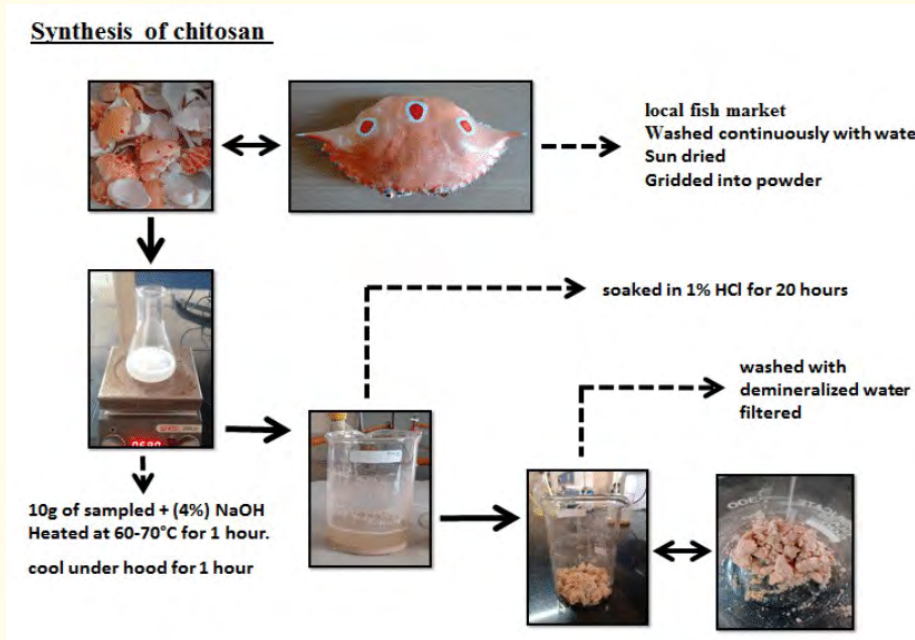
Materials and Method

Sample collection and preparation

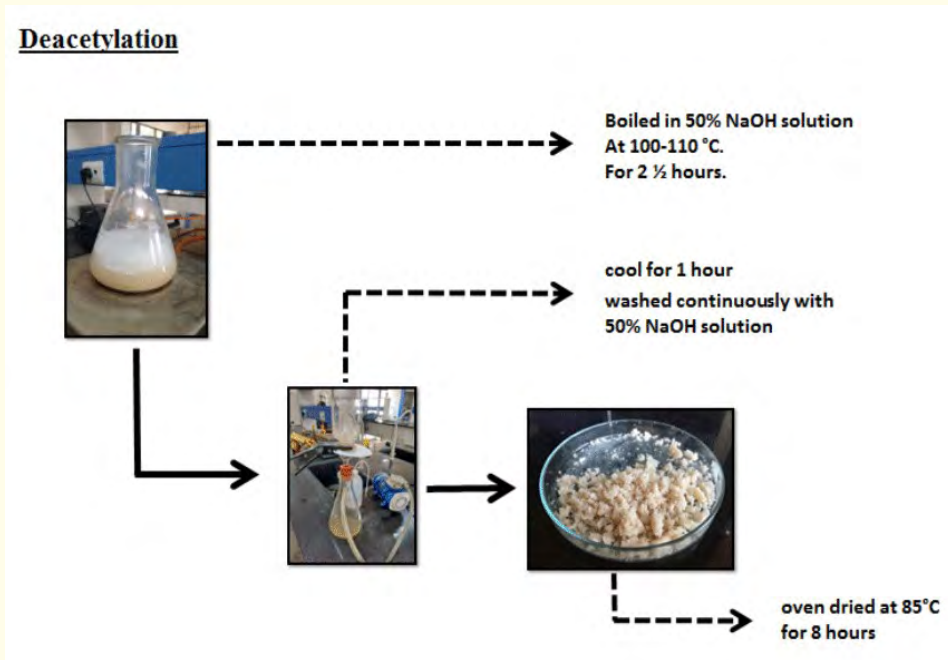
The crab exoskeletons were obtained from local fish market (Bharuch). The crab exoskeletons were washed continuously with water. And they were sun dried for two days in order to remove moisture present in the crab shell. After which shells were crushed into small pieces and again oven dried for 4-5 hours at 100°C. The small pieces were then gridded into powder and stored in zip-lock bag.

Synthesis

Initially crushed exoskeletons were heated in an oven at 100°C for 30 minutes. Sample was then taken in 500 ml beaker and 100 ml of (4%) NaOH solution was added into it. The solution was heated with the help of a hot plate at 60 - 70°C for 1 hour. And it was allowed to cool for 1 hour. Solution was then filtered and then washed with demineralise water. Then it was soaked in 1% HCl solution for 20 hours. After filtering the solution, the residue was washed with demineralised water and the process was followed by deacetylation (Figure 2,3).



(A)



(B)

Figure 2 A, B: Pictorial representation of Synthesis of Chitosan.

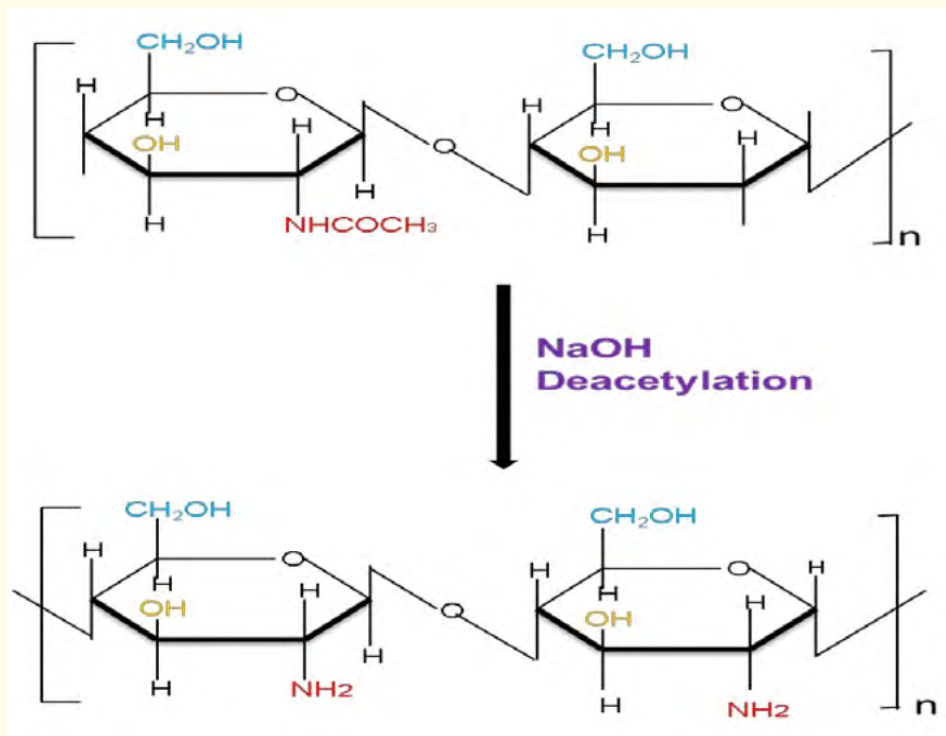


Figure 3: Chemical Reaction to synthesize Chitosan from Chitin [11].

The residue was boiled in 50% NaOH solution at 100 - 110°C and 70 - 80 RPM with the help of hot plate magnetic stirrer for 3 hours. Then the sample was allowed to cool for 1 hour. Then the sample was washed continuously with 50% NaOH solution. The chitosan obtained by above method was of pale white colour. After which it was oven dried at 85°C for 8 hours [12-14].

Characterization of Chitosan

Chitosan was characterized with help of Fourier Transform Infra-red spectroscopy and Nitrogen content. Experiments were carried out at Ankleshwar Research and Analytical Infrastructure Ltd., Ankleshwar.

Antimicrobial activity of chitosan

Test Organisms

The bacteria tested used (*Staphylococcus aureus*, *E. Coli*, *Bacillus subtilis*) in this study were obtained from Microbiology Lab of Enviro Technology Limited, Ankleshwar, India. All the cultures were incubated at 37°C and all were activated by incubation for 24 hours in nutrient broth (Hi-Media, India). *Staphylococcus aureus* is a gram-positive bacterium that is commonly associated with a food product as a result of human treatment. *Escherichia coli* is a gram-negative bacterium and has been selected based on its infectious characteristics than other genera. The choice of *Bacillus subtilis* (gram-positive bacterium) was made because it is frequently isolated and bearing in mind the increased resistance of Gram-positive bacteria to the bactericidal action of chitosan compared to Gram-negative ones excluding *E. coli*.

Antibacterial activity determination

Bacterial Cultures were emulsified in normal saline solution and turbidity was observed and matched with 0.5 McFarland opacity standards. The agar cup method [15] was followed to examine the antibacterial activity of the extracts. 0.1 ml broth culture of the test organisms were strongly seeded over the Mueller-Hinton agar plates. Wells of 6 mm diameter were punched over the agar plates with a sterile cork drill. The floors of the wells were formed by casting 50 - 100 μ L of molten muller-hilton in the broken wells. With a micro-pipette, solution of chitosan was added to various wells in the plate. These plates were then kept at low temperature (4°C) for exactly 3 hours and then incubated at 37°C for 24 hours. After the incubation period, formation of zones around the well, confirms the antibacterial activity of the respective extracts. All results were compared with the standard antibiotic slice.

Results and Discussions

Characterization of Chitosan

FTIR study of chitosan was performed to characterise the chemical structure. FTIR spectrums of chitosan are shown in Figure 4. In this spectrum, a band of 3467 cm^{-1} was obtained which corresponds to the peak of OH overlapped on N-H (strong). A 2923 cm^{-1} peak of C-H group was obtained. A 1578 cm^{-1} peak of N-H was obtained. A 1073 cm^{-1} peak of C-O-C was obtained.

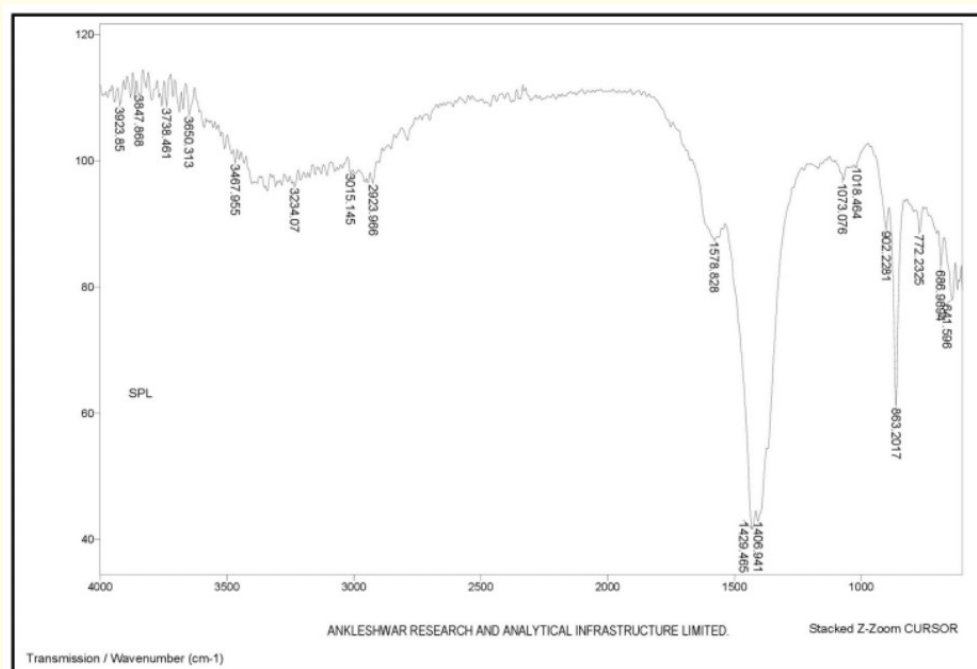


Figure 4: FTIR spectrum of Chitosan.

Nitrogen Content

Nitrogen content of the prepared chitin as well as chitosan was measured. Nitrogen content of chitin was found to be 7.96 and that of chitosan was 8.06.

Antimicrobial Activity of Chitosan

As per research reports available, the anti-microbial potential of chitin, chitosan and their derivatives were considered to be a bacteriocidal (kills the live bacteria or some fraction therein) or bacteriostatic (hinders the growth of bacteria but does not imply whether or not bacteria are killed). Several studies show that the effect of Degree of Acetylation and molecular weight is significant on the anti-microbial properties [5]. The effect of the required chitosan was evaluated against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* and the results are shown in Table 1.

	Concentration ($\mu\text{g/mL}$)				
	100	200	500	800	1000
Zone of inhibition in diameter (mm)					
Test Organisms					
<i>E. coli</i>	8	9	10	12	13
<i>S. aureus</i>	7	10	10	12	14
<i>B. subtilis</i>	8	10	11	12	14

Table 1: Antimicrobial activity and zone of inhibition of Chitosan (mm).

S. aureus can cause skin infections such as pimples, impetigo, boiling, cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, life-threatening diseases such as Pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis. It is still one of the five most common hospital infections [16]. *Escherichia coli* is one of the most common causes of numerous common bacterial infections including cholecystitis, bacteria, cholangitis, urinary tract infection and traveler's diarrhea as well as other clinical infections such as neonatal meningitis and pneumonia [17]. The most common inhibition zone against *S. aureus*, *B. subtilis* was observed at 12 mm and 14 mm at 800 and 1000 ppm/well. Many hypotheses have been proposed to determine the mechanism of antibacterial activity of chitosan. Chitosan contains three types of reactive functional groups, an amino/acetamido group, both primary and secondary hydroxyl groups, respectively, in C-2, C-3 and C-6 positions. Amine content is the main reason for differences in their structures and physico-chemical properties and their correlation with their chelation, flocculation and biological functions [15]. They are designed with three models that are the most beneficial interactions with the positively charged chitosan molecule and the negatively charged microbe cell membranes. In this model, the interaction is mediated by the protonated NH_3^+ and negative groups of electrostatic forces [18], probably compete with Ca^{2+} electronegative sites on the surface of the membrane [19]. Electrostatic interaction results in two interventions: (i) supporting permeability changes in the properties of the membrane wall, resulting in internal osmotic imbalance and hence reducing microorganism growth and ii) hydrolysis in the peptidoglycan wall to microorganisms that result in leakage intracellular electrolytes, Such as potassium ions, and other proteins with low molecular weight components [20]. Dislodging produces ions and water flow that causes a decrease in internal pressure bacteria [21]. The visual enhancement effective film lysis has also been reported against gram-negative and gram-positive bacteria [22]. Given that this mechanism is based on electrostatic interaction suggests that a higher amount of amines katonizovaných, the greater is the antibacterial activity [23].

Conclusions

This study revealed that crab chitosan could be used to inhibit *S. aureus*, *Bacillus subtilis* and *E. coli* in food products involving human handling where there is possibility of contamination of *S. aureus*, *Bacillus subtilis* and *E. coli*. Hence, we suggest that the chitosan matrix will be a promising material for biomedical as well as food science applications.

Bibliography

1. Marguerite Rinaudo. "Chitin and chitosan: Properties and applications". *Progress in Polymer Science* 31.7 (2006): 603-632.
2. Venkatesan J and Kim SK. "Chitosan composites for bone tissue engineering-An overview". *Marine Drugs* 8.8 (2010): 2252-2266.
3. Karagozlu MZ and Kim SK. "Chapter Twelve-Anticancer effects of chitin and chitosan derivatives". In *Advances in Food and Nutrition Research*. Academic Press: Waltham, MA, USA-72 (2014): 215-225.
4. Martins AF, *et al.* "Antimicrobial activity of chitosan derivatives containing N-quaternized moieties in its backbone: a review". *International Journal of Molecular Sciences* 15.11 (2014): 20800-20832.
5. Rejane C Goy, *et al.* "A review of the antimicrobial activity of chitosan". *Polímeros* 19.3 (2009): 241-247.
6. Ngo DH and Kim SK. "Chapter Two-Antioxidant effects of chitin, chitosan, and their derivatives". *Advances in Food and Nutrition Research* 73 (2014): 15-31.
7. Bagheri-Khoulenjani S, *et al.* "An investigation on the short-term biodegradability of chitosan with various molecular weights and degrees of deacetylation". *Carbohydrate Polymers* 78.4 (2009): 773-778.
8. Onsosyen E and Skaugrud O. "Metal recovery using chitosan". *Journal of Chemical Technology and Biotechnology* 49.4 (1990): 395-404.
9. Felt O., *et al.* "Chitosan: A unique polysaccharide for drug delivery". *Drug Development and Industrial Pharmacy* 24.11 (1998): 979-993.
10. Zhang Y and Zhang M. "Three-dimensional macroporous calcium phosphate bioceramics with nested chitosan sponges for load-bearing bone implants". *Journal of Biomedical Materials Research* 61.1 (2002): 1-8.
11. Sandeep Rai, *et al.* "Chitosan: Chemistry, Applications & Economics of Production -A Review". *International Journal of Analytical and Applied Chemistry* 2.1 (2016): 23-34.
12. Younes I and Rinaudo M. "Chitin and Chitosan Preparation from Marine Sources. Structure, Properties and Applications". *Marine Drugs* 13.3 (2015): 1133-1174.
13. Yuan Y, *et al.* "Deacetylation of Chitosan: Material Characterization and in vitro Evaluation via Albumin Adsorption and Pre-Osteoblastic Cell Cultures". *Materials* 4.8 (2011): 1399-1416.
14. Sneha Paul, *et al.* "Extraction and purification of chitosan from chitin isolated from Sea prawn (*fenneropenaeusindicus*)". *Asian Journal of Pharmaceutical and Clinical Research* 7.4 (2014): 0974-2441.
15. Barry AL. "Procedure for testing antimicrobial agents in agar media". In: *Antibiotics in laboratory medicine*. Williams Wilkins Co. Baltimore: USA (1980): 1-23.
16. Luytmans J., *et al.* "Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks". *Clinical Microbiology Reviews* 10.3 (1997): 505-520.
17. Kappeli U., *et al.* "Human Infections with Non-O157 Shiga Toxin-producing *Escherichia coli*, Switzerland, 2000-2009". *Emerging Infectious Disease* 17.2 (2011): 180-185.
18. Amiri A., *et al.* "Efficient method for functionalization of carbon nanotubes by lysine and improved antimicrobial activity and water-dispersion". *Materials Letters* 72.1 (2012): 153-156.

19. Aslan S., *et al.* "Carbon nanotube-based antimicrobial biomaterials formed via layer-by-layer assembly with polypeptides". *Journal of Colloid and Interface Science* 388.1 (2012): 268-273.
20. Aziz MA., *et al.* "Antimicrobial properties of a chitosan dextran-based hydrogel for surgical use". *Antimicrobial Agents and Chemotherapy* 56.1 (2012): 280-287.
21. Reinheimer JA., *et al.* "Inhibition of coliform bacteria by lactic cultures". *Australian Journal of Dairy Technology* 45.1 (1990): 5-9.
22. National Committee for Clinical Laboratory Standards (NCCLS). "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically". National Committee for Clinical Laboratory Standards (NCCLS), Villanova, Pa, USA (2006).
23. D Hernandez., *et al.* "Antimicrobial activity of lactic acid bacteria isolated from Tenerife cheese: Initial characterization of plantaricin TF711, a bacteriocin-like substance produced by *Lactobacillus plantarum* TF711". *Journal of Applied Microbiology* 99.1 (2005): 77-84.

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