

Antibacterial potential of inulinase enzyme obtained from *Nocardiopsis* sp.

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ABSTRACT

The enriched nutritional and functional properties of inulinase with wide attention are considered commercial/industrial food enzymes. It can be produced by many microorganisms such as yeasts, fungi, and bacteria. *Nocardiopsis* is a genus under Actinomycetes, which has biotechnologically important microorganisms. This study aims to isolate and identify marine Actinomycetes *Nocardiopsis* species and to evaluate the antibacterial potential of the inulinase enzyme obtained from it. Marine actinobacteria (*Nocardiopsis* sp.) were isolated from sediment samples on YM agar. The isolate was identified by biochemical analysis of cell walls (amino acid and sugar). Enzyme screening assay was performed with temperature and pH influence in the production inulinase enzyme production. Antibacterial activity and minimal inhibitory activity of inulinase enzyme were performed with *Staphylococcus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Antimicrobial testing revealed that with higher concentrations of inulinase enzyme, the zone of inhibition of bacterial growth increased, and the minimum inhibitory concentration of inulinase enzyme that prevented the growth of bacteria was close to the standard tetracycline. Inulinase enzyme obtained from *Nocardiopsis* species shows good antibacterial activity against *Staphylococcus aureus*, *K. pneumoniae*, and *P. aeruginosa* in comparison to the standard, tetracycline.

Key words: Actinomycetes, antibacterial, inulinase, *Nocardiopsis*, novel enzyme

INTRODUCTION

The inulinase enzyme is considered commercially important food enzyme with wide attention. Its systemic name is 1-beta-D-fructan fructanohydrolase. Inulin is a naturally occurring water-soluble polysaccharide and it is one of

the most frequently used carbohydrates like starch.^[1] It is a nondigestible carbohydrate called fructans and has wide applications in the food industry as dietary fiber and fat and sugar replacer. Inulin consumption enhances the absorption of minerals and prevents gut problems like constipation. It also stimulates the immune system.^[2] Inulinases target the β -2,1glycosidic linkage of inulin and break the inulin by hydrolysis to produce fructose, inulo-oligosaccharides, and glucose.^[3] Inulinase can be of two types; endoinulinase, which hydrolyzed lengthy chains into tiny and exoinulinase will obtain the fructose through the hydrolysis.^[4] It has various industrial applications in the production of protein, mannitol, sorbitol, citric acid, lactic acid, cell oil, and biofuel. Furthermore, it improves digestion,

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lowering cholesterol, bone health, blood sugar control, and immunomodulation activity.^[1,5] *Nocardiopsis* a genus under the order *Actinomycetes*. The organisms which are under the order *Actinomycetes* have huge importance in agriculture through the decomposition of organic matter in the soil system.^[6] *Nocardiopsis* species are Gram-positive, aerobic, catalase positive, and nonacid-fast organisms will form both aerial and substrate mycelium with a long spore chain.^[7-10] Most of the species are halophilic or halotolerant which is associated with the production of various metabolites.^[11] Especially the species of *Nocardiopsis* are economically resourceful and biotechnologically significant compounds which have various therapeutic activities. In addition, diverse enzymes, such as chitinase, β -glucanases, xylanase, amylase, cellulose, protease, and inulinase, have been reported by the species of *Nocardiopsis*^[12] with potential agriculture and food application. Natural products remain to be the most propitious source of antibiotics.^[13] Previously, our team has had extensive experience in working on various research projects across multiple disciplines involving the use of various compounds obtained from natural products and evaluating their biological importance.^[14-23] In industries, microbes like actinobacteria have been used for high yield of enzyme inulinase due to easy growth and high yield in a short time.^[24-27] Furthermore, marine actinobacteria have extensive bioactive applications such as antibacterial, antioxidant, anticancer, antifungal, antiviral, insecticidal, and antidiabetics has drawn global attention in the past several decades.^[28] In the phylum of actinobacteria, the species of *Streptomyces*, *Nocardiopsis*, *Nocardia*, *Marinospora*, *Rhodococcus*, *Micromonospora*, and *Salinospora* are well known to contribute to the maximum level of potentially bioactive compounds in the commercial.^[29] Further, our team has extensive knowledge and research experience that has translated into high-quality publications (30–49). This study aims to isolate and identify marine Actinomycetes *Nocardiopsis* species and to evaluate the antibacterial potential of the inulinase enzyme obtained from it against a few important bacterial strains.

MATERIALS AND METHODS

Sample collection and preparation

Samples were collected from the Gulf of Mannar biosphere reserve, Tamil Nadu. Samples were collected by van veen grab in the offshore region, and the collected sediment materials were processed as per the previous reference.^[8]

Isolation of actinobacteria

Marine actinobacteria were isolated on Kuster's agar

medium prepared with seawater. The medium was supplemented with antibiotics (10 μ g/ml of cycloheximide and nalidixic acid) to inhibit the contamination of bacteria and fungus. A serially diluted sediment sample was inoculated on KUA medium and incubated for a week at ambient temperature. Well-grown distinct pure cultures were used for further studies.

Identification of marine actinobacteria

Identification of marine actinobacteria was made by certain characteristic features of bacteria. These include aerial mass color (white), spore chain morphology (long chain), and the absence of melanoid, reverse side, and soluble pigment.

Chemotaxonomical characteristics

Whole-cell amino acid analysis and sugar pattern were analyzed through the hydrolysis of strain with the respective procedure. The actinobacterial isolate was well cultivated and harvested through the centrifuge and rinsed twice with sterile distilled water then part of the samples was hydrolyzed with 6N HCl for amino acid analysis, remaining samples were hydrolyzed with 1N H₂SO₄ for sugar pattern. The hydrolyzed samples were placed on the TLC plate to notice the presence of amino acid and sugars in the cell wall of the strain.

Carbon source utilization

The carbon source utilization of each strain was analyzed with different carbon sources with the proper method given by International Streptomyces Project (Shirling and Gottlieb, 1966).

Inulinolytic potential

The isolate was spotted on Czapek-Dox agar supplemented with 1% of inulin and incubated for 24 h in room temperature. After growth, the plates were treated with lugol's iodine solution for 3 to 5 min and rinsed with sterile distilled water and incubated for an hour at room temperature. The colorless clear zone around the isolate indicated the hydrolysis of inulin.

Inulinase enzyme assay

Well-grown culture was centrifuged, and the aqueous phase has been considered the source of enzyme. The substrate inulin was prepared (2%) with phosphate buffer (pH 7). An equal volume of enzyme source (1ml) and substrate (1ml) was added and kept at 50°C for half an hour. Then, the reaction was stopped by the addition of 2 ml of DNS

Table 1: Cell wall sugars and amino acids released upon hydrolysis

Cell wall amino acids	MesoDAP	Glycine	Cell wall sugar	Galactose	Cell wall type	Index
LL-DAP			Arabinose			
-	+	-	-	±		<i>Nocardiopsis</i>

LL-DAP: LL-diaminopimelic acid

solution, then allowed it to boil for 15 min. After the color developing from red to brown, the absorbance was measured at 540 nm.

Impact of pH and temperature

The potential strain was inoculated in Czapek-Dox broth at a pH range from 6 to 8. Similarly, the culture medium was prepared and incubated at various temperatures ranging from 25°C, 30°C, 35°C, 40°C, and 50°C. After incubation, the enzyme production was measured by the previous method, and the cell growth was measured at 600 nm.

Antibacterial and minimal inhibitory concentration

Antibacterial potential of the Inulinase was done by following the method of disc diffusion. The 5mm diameter discs were used for the assay and different concentration such as 50, 100, 150, 200 and 250 µg/ml of Inulinase samples with oral antibiotic tetracycline and DMSO as a negative control. Further, the plates were kept in incubator for 1 day and maintained the room temperature. The zone of inhibition was considered as better results and measured the zone and calculated the activities. The Minimal Inhibitory Concentration (MIC) of the Inulinase was analyzed using different concentrations such as 50, 100, 150, 200 and 250 µg/ml of melanin with tetracycline (Standard), DMSO was negative control. The bacterial suspension (in test tubes) was kept in incubator for 1 day in room temperature and optical density was analyzed.

RESULTS

Assessment of the antibacterial potential of inulinase

The different concentrations of inulinase were loaded in media containing *Staphylococcus*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*; the plates were incubated for 24 h at room temperature. After the incubation time, the zone of inhibition of bacterial growth was measured.

In this study, inulinase-producing marine actinobacteria *Nocardiopsis* was isolated from sediment sample. The isolate was identified through cell wall amino acid, sugar pattern, and micro-morphology.

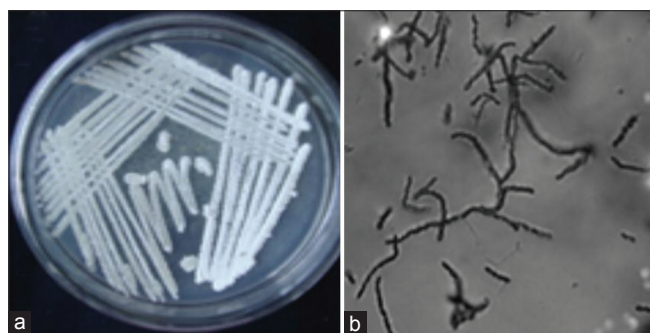


Figure 1: Aerial mass colour (white) and long spore chain morphology obtained for *Nocardiopsis* sp

The aerial mass color and spore chain morphology are recorded in Figure 1. Cell wall sugars and amino acids released on hydrolysis are given in Table 1. Assimilation of carbon source for metabolic activity of actinobacteria is given in Table 2. The enzyme production was confirmed in various pH and temperatures to optimize the enzyme production up to 147 IU/mg. The temperature and pH have an impact on enzyme production on the Czapek-Dox medium [Tables 3 and 4]. Enhancing the temperature improved the production of enzyme production with lower stability. Finally, antibacterial testing was done by incorporating the enzyme in different concentrations into the media plates containing each bacteria, and the zone of inhibition was measured. Table 5 reveals that, with increasing concentrations of Inulinase enzyme, the zone of inhibition of bacterial growth increased for all the bacteria, providing a higher range of inhibition in *Klebsiella pneumoniae* and *P. aeruginosa* when compared to *Staphylococcus* sp and minimum inhibitory concentration of Inulinase enzyme that prevented the growth of the

Table 2: Assimilation of carbon source

Color of aerial mycelium	White
Melanoid pigment	-
Reverse side pigment	-
Soluble pigment	-
Spore chain	Long chain
Assimilation of carbon source	
Arabinose	+
Xylose	+
Inositol	+
Mannitol	-
Fructose	+
Rhamnose	-
Sucrose	+
Raffinose	-

Table 3: Effect of temperature on enzyme production

Temperature	IU/ml±SE
25	9.27±2.1
30	12.08±2.9
35	14.25±2.4
40	10.36±2.7
50	8.21±2.2

SE: Standard error

Table 4: Effect of pH on enzyme production

pH	IU/ml±SE
6	8.24±2.4
6.5	11.35±1.9
7	16.38±2.8
7.5	12.07±2.5
8	10.25±2.2

SE: Standard error

Table 5: Zone of inhibition exhibited by different concentrations of inulinase

Concentration ($\mu\text{g/ml}$)	<i>Staphylococcus</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
50	3 \pm 1.8	5 \pm 1.7	4 \pm 1.6
100	5 \pm 2.1	11 \pm 1.9	9 \pm 1.2
150	9 \pm 1.9	16 \pm 1.6	13 \pm 1.9
200	13 \pm 2.4	20 \pm 1.7	22 \pm 1.8
250	18 \pm 2.1	23 \pm 1.2	26 \pm 1.7
300	25 \pm 2.4	27 \pm 1.9	31 \pm 1.4

Table 6: Minimum inhibitory concentration of inulinase that prevents the growth of bacteria in relation to the positive control

	0	10	20	30	40	50	MIC ($\mu\text{g/ml}$)
<i>Staphylococcus</i>	+	+	+	+	-	+	40
Positive control	+	+	-	-	-	-	20
<i>Klebsiella pneumoniae</i>	+	+	+	-	-	-	30
Positive control	+	+	-	-	-	-	20
<i>Pseudomonas aeruginosa</i>	+	+	+	-	-	-	30
Positive control	+	+	-	-	-	-	20

MIC: Minimum inhibitory concentration

bacterial strains was very close to that of the standard tetracycline [Table 6].

DISCUSSION

A similar study done by Aziz *et al.* purified the inulinase enzyme from *K. pneumoniae* and assessed its antibacterial potential. They found that inulinase obtained from *K. pneumoniae* exhibited broad-spectrum bioactivity against microbial pathogens and also observed that inulinase had increased the activity of ceftazidime against bacteria when a combination between this enzyme and the antibiotic was used.^[30] Muslim *et al.*, in their study, purified inulinase enzyme from *Staphylococcus aureus* S3, and their findings, showed that anti-staphylococcal activity of ceftazidime antibiotic was increased in the presence of inulinase enzyme, and the inulinase may serve as a useful adjuvant for the treatment of Staphylococcal infections in combination with this antibiotic.^[31] Vimal *et al.*, in their study, assessed the antimicrobial potential of different extracts and compounds derived from *Nocardiosis* species and found that the petroleum ether extract exhibited antibacterial activity against many Gram-positive as well as Gram-negative bacteria, ethyl acetate extract showed good antifungal activity, chloroform extract was very effective against yeasts, and the compounds present within these extract showed broad-spectrum antimicrobial activity.^[32] Siddharth and Rai, in their study, isolated 4-bromophenol and Bis (2-Ethylhexyl) phthalate from genus *Nocardiosis*, and derived their antibacterial and antioxidant activities and concluded that marine actinobacteria are promising sources of therapeutically important bioactive metabolites.^[33]

Bennur *et al.*, in their study, assessed the biological properties of secondary metabolites of *Nocardiosis*, namely Nocardipyrene (A and B), and found that Nocardipyrene A was active against *P. aeruginosa*, *Enterobacter aerogenes*, and *Escherichia coli*. Nocardipyrene B was active against *P. aeruginosa*, *E. aerogenes*, *E. coli*, *S. aureus*, and *Candida albicans*. Many studies have found the antibacterial and antimicrobial potential of different strains of *Nocardiosis* such as *Nocardiosis* sp. TFS65-07,^[34] *N. dassonvillei* (MAD08),^[35,36] Many studies have also reported that *Nocardiosis* species produce polyketide-based structures and peptides that display anticancer and antitumor activities.^[37]

CONCLUSION

Inulinase enzyme obtained from *Nocardiosis* species shows good antibacterial activity against *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* in relation to the standard tetracycline. Further studies could be done to evaluate the antimicrobial potential of inulinase against other microorganisms.

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Conflicts of interest

There are no conflicts of interest.

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