Bioprospecting Of Halophilic *Nocardiopsis Sp.* From Saline Habitats

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ABSTRACT

Extremophilic microorganisms inhabiting saline and alkaline environments, as well as cold and high-pressure habitats, represent valuable sources of bioactive metabolites due to their unique adaptive mechanisms and significant biotechnological potential. Members of the genus *Nocardiopsis*, comprising salt-tolerant and alkaliphilic actinomycetes, exhibit remarkable adaptive capabilities and a strong capacity for the production of extremozymes. This review summarizes the diversity, ecological distribution, physiological adaptations, and amylase production and characterization of *Nocardiopsis sp*. Overall, the review provides insights into the biotechnological potential of *Nocardiopsis sp*. isolated from saline niches.

Keywords : Saline environments, Halophilic actinomycetes, *Nocardiopsis sp.*, amylase.

INTRODUCTION

EXTREMOPHILES & EXTREME ENVIRONMENTS

In 1974, Macelory introduced the term, ‘extremophiles’, (from Latin extremus meaning "extreme" and Greek philiā meaning "love"). An organism has the ability to reside in ecosystem which contains the high physical (e.g.: temperature, pressure and rock) and chemical environment (Salinity and pH, Oxygen) (Rampelotto, 2010*;* Rothschild and Mancinelli, 2001*;* Macelory, 1974; Gomes and Steinier, 2004). Salt act is essential element for the growth of the organisms known as halophlies (Cavicchioli and Thomas, 2000). The groups of bacteria that can grow under alkaline conditions in the presence of salt are referred as haloalkaliphiles. The dual extremity of halophiles and alkaliphiles is an interesting model for the fundamental research and exploration of biotechnological potential (Dodia *et al.,* 2005).The exploration of the natural saline and alkaline environments beyond the above boundaries is just the beginning (Purohit and Singh, 2011). Saline habitats act as notable sources of new natural products, including antimicrobial and anticancer compounds, enzymes, cytotoxic compounds and neurotoxic compounds (Cragg and Newman, 2005; Bull *et al.,* 2000).

SALT TOLERANT AND ALKALIPHILIC ACTINOMYCETES

Actinomycetales; Actinomyces is derived from the Greek word 'actis' for beam and 'mykes' for mucus, fungus, gram-positive bacteria, aerobic and mycelial, and are characterized by high guanine–cytosine (GC) contents, with colonies forming fungus-like branched networks of hyphae (Holt, 1994; Demain, 1999; Henis, 1986). The actinomycetes decompose recalcitrant organic materials, produce extracellular hydrolytic enzymes and recycle natural biopolymers (Goodfellow and Williams, 1983). Actinomycetes capable of producing antimicrobial compounds have been isolated from terrestrial habitats and saline environments (Zobell, 1944 ; Grein,1958). Streptomyces accounts for up to 80% market of antimicrobial compounds (Jensen *et al.*, 2005; Bull *et al.*, 2000). Genera and families have representatives of many different salt requirements and tolerance of halophiles and non-halophilic actinomycetes (Oren, 2008).

In 1976, new genus, *Nocardiopsis' (Nocardia* – a genus of the order Actinomycetales; *opsis*– appearance) as *Actinomadura Lechevalier* and then further in 1996 described based on the morphological and biochemical criteria of *Nocardiopsis* species, a new family for the genus referred to as *'Nocardiopsaceae'*. Actinomycetes are isolated from the marine ecosystems and act as potential source of antibiotics, drug and enzymes (Subramani and Aalbersberg, 2013). Other features of the genus *Nocardiopsis* include cell walls containing meso-2,6-diaminopimelic acid but lacking diagnostically important carbohydrates. Absence of madurose and nocardomycolic acids, phospholipid and menaquinone profiles used to distinguish intra species variations (Kroppenstedt and Evtushenko, 2006). Identification of actinomycetes was carried out by using classical microbiological approaches such as isolation, identification and characterization with morphological, biochemical, sugar utilization, salt and pH profile 16S rRNA gene amplification and sequencing of actinomycetes. The various molecular finger printing tools such RFLP, RAPD, AFLP and many more are used to captured the inter and intraspecies diversity.

Nocardiopsis salina sp. nov. YIM 90010^T was isolated from Xinjiang Province, China, using modified ISP 5 medium containing 10% (w/v) NaCl. The strain produces abundant aerial mycelia and fragmented substrate mycelia on most tested media. Optimal growth is at 28°C, pH 7.2, and 10% NaCl. It is negative for milk coagulation, milk peptonization, starch hydrolysis, urease activity, H_2S production, and melanin formation, while nitrate reduction is positive. The genomic G+C content is 73.1 mol% (Wen-Jun Li *et al.*, 2004).

Nocardiopsis fildesensis sp. nov. GW9-2^T was isolated from Fildes Peninsula, King George Island, West Antarctica, using Gause medium supplemented with 3% (w/v) NaCl. The strain is aerobic and Gram-positive, producing white aerial mycelium and white to yellowish substrate mycelium without diffusible pigments. Aerial mycelia differentiate into long spore chains with rough, rod-shaped, non-motile spores. Growth occurs at 16–37 °C (optimum 28 °C), pH 5.0–11.0 (optimum pH 9.0), and 0–12% NaCl. The strain reduces nitrate but is negative for milk coagulation, peptonization, gelatin, urease, and

H_2S production. The G+C content is 76.8 mol%.

Nocardia jiangsuensis sp. nov. KLBMP S0027^T was isolated from Lianyungang, Jiangsu Province, eastern China, using starch–arginine agar supplemented with 3% (w/v) NaCl. The strain is Gram-positive, aerobic, and non-motile, producing reddish to pink substrate mycelium and sparse white aerial mycelium. It degrades Tweens 20, 40, and 80 but not starch, casein, or cellulose, and does not produce H_2S. Growth is optimal at 28 ℃ and pH 7.0 with 0–9% NaCl. The G+C content is 70.5 mol%.

No.	*Nocardiopsis sp.* ; Site ; Location	Optimum Conditions
1.	*N.halophila* DSM44494 ; Salines oil ; Iraq	NaCl : 20% ; Temperature:30◦C
2.	*N. metallicus* DSM 44598 ; Alkalines lag dump ; Germany	NaCl : 10% ; pH:7.0 -10.5 ; Temperature : 30◦C
3.	*N.xinjiangensis* DSM44589 ; Saline soil ; Xinjiang, China	NaCl : 10% ; pH:7.2 ; Temperature : 28◦C
4.	*N. salina* YIM90010 ; Hypersaline soil ; Xinjiang,China	NaCl : 10% ; pH:7.2 ; Temperature : 28◦C
5.	*N. gilva* DSM44841, *N. baichengensis* DSM 44845 ; *N.rosea* DSM44842, *N.rhodophaea*DSM 44843, *N. chromatogenes* DSM44844 Hypersaline soil ; Xinjiang, China	NaCl : 5 -8% ; pH:7.2 ; Temperature : 28–30◦C
6.	*N.quinghaiensis* DSM44739 ; Saline soil ; Qinghai, China	NaCl : 3% ; pH :7 ; Temperature : 28◦C
7.	*N.valliformis* DSM45023 ; Alkalilake soil, Xinjiang, China	NaCl : 0-5%; pH : 9.5–13 ; Temperature : 28◦C
8.	*N.terrae* YIM90022 ; Saline soil , Qaidam Basin, China	NaCl : 3 -5% ; pH:8.5 ; Temperature : 30◦C
9.	*N. aegyptia* DSM44442; Marine sediment, Egypt	NaCl : 5% ; Temperature :10◦C
10.	*N .flavescens* CGMCC4.5723 ; Marine sediment, China	NaCl : 0-3% ; pH:7.2-7.5 ; Temperature : 35◦C
11.	*N.kunsanensis* KCTC9831; Saltern, Korea	NaCl : 10%; pH:9;Temperature : 37◦C
12.	*N. halotolerans* DSM44410 ; Salt marsh soil, Kuwait	NaCl : 0 -10%; Temperature : 28-35◦C

Table 1: Diversity of *Nocardiopsis* identified and summarized from hyper saline and marine environment.

AMYLASE

Starch is an abundantly available natural polymer of D-Glucose monomers linked via α (1→4)-glycosidic bonds. A variety of amylases (1,4- glucan-4 glucano hydrolases, EC 3.2.1.1) are a family of endo-amylases acting on the glucosidic linkages present in the biopolymer, degraded starch to glucose, maltose and maltotriose (Sunna *et al*.,1997). *Nocardiopsis* species inherently produce α-amylases under such adverse condition like cold, high temperature and salt (Cavicchioli *et al.*, 2002). *Nocardiopsis* strain 7326 obtained from this region yielded a cold- adapted α-amylase optimally active at 35℃ under the alkaline conditions. Another cold-adapted α-amylase was obtained from *Nocardiopsis aegyptia*, isolated from Egyptian marine sediment.

To enhance the production of enzyme, the optimization of various parameters and manipulations of medium based on one factor variable at time is essential. Optimization was achieved by monitoring all the factors by using one factor variable at time, factors including various carbon and nitrogen sources, pH, temperature, aeration and agitation with respect to SSF and SmF (Gohel and Singh 2012). The influence of temperature on the amylase production has been described in mesophilic and thermophilic organism. Salt tolerant and alkaliphilic microorganisms can grow at high salt and pH required for the grow and enzyme production. Halophilic actinomycetes, *Nocardiopsis sp.* grew optimally and produced amylase at pH 9. *Nocardiopsois sp.* isolated from India produced amylase and had maximum enzyme activity with 11% (w/v) NaCl. There are only limited studies on the purification and characterization of halophilic amylases from halophiles.

***Nocardiopsis sp.* 7326**

The first reported cold-adapted α-amylase was from *Nocardiopsis sp.* 7326, isolated from deep-sea sediment in Prydz Bay. The purified amylase had optimized temperature at 35℃ and pH 8.0. The activity was stimulated and inhibited by Ca^{2+}, Mn^{2+}, Mg^{2+}, Cu^{2+}, Co^{2+}, and Rb^{2+}, Hg^{2+}, EDTA, respectively. The molecular weight was found55 kDa. (Zhang JW, 2008).

Nocardiopsis aegyptia

The amylase production in *Nocardiopsis aegyptia* was optimized by Plackett – Burman statistical analysis. Potassium nitrate at 1.5 g/l and inoculums sizeof 1.5 ml /50 ml were the most dependent factor for enhanced production of amylase. The optimum activity of the amylase was obtained at 25℃ and pH 5.0 (Abou-Elela et al., 2009).

Nocardiopsis sp.

Nocardiopsis sp. an endophytic actinomycete isolated from yam bean (*Pachyrhizus erosus* L. Urban), produced thermostable α-amylase with maximum activity at pH 5.0 and 70℃. Ammonium sulfate precipitation purified the enzyme to a specific activity of 1130 Umg protein (28% yield, 2.7-fold purification) (Stamford *et al.,* 2001).

***Nocardiopsis sp.* Stain B2**

Nocardiopsis sp. strain from marine sediments produced α-amylase. The α-amylase was purified by gel filtration chromatography by using sephadex G-75 and the molecular mass determined by SDS-PAGE was 45KDa. The enzyme was immobilized by ionotropic gelatin technique using gellan gum (GG) and characterized by FTIR and SEM (Chakraborty *et al.*, 2014).

CONCLUSION

The unique diversity of *Nocardiopsis species* exhibits remarkable ecological specialization, enabling their survival in saline and alkaline environments. Their enzymatic functional and catalytic performance is closely linked to metabolic efficiency, which collectively enhances both survival and biotechnological potential. These adaptive traits are supported by distinctive genomic and physiological features such as high GC content, broad pH tolerance, and elevated NaCl tolerance. Amylase derived from these species act as efficient extremozymes, displaying exceptional operational stability and process compatibility. Consequently, they are well suited for applications in pharmaceutical, food, textile, and bioremediation industries. Advances in genomics and protein engineering are expected to further expand the industrial exploitation of these enzymes, including those from non-cultivable actinomycetes.

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