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# Effect of Temperature, pH and NaCl on the Isolates of Actinomycetes from Straw and Compost Samples from Savar, Dhaka, Bangladesh

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#### **Abstract**

The effect of physiological parameters *viz.*, temperature, pH and NaCl on actinomycetes strains belonging to the genus *Streptomyces* (JUBM-35-NS-1) and *Nocardia* (JUBM-35-NS-2), isolated earlier from straw and compost materials used in commercial mushroom cultivation, was studied. Growth maxima were observed at 30°C and 35°C respectively in *Streptomyces* sp. and *Nocardia* sp. Arrest in growth for both isolates was found to be at 4°C and 50°C temperatures. The optimum growth was recorded at pH range of 6.0-6.5 for the isolate JUBM-35-NS-1 while it was at 6.5-8.0 for isolate JUBM-35-NS-2. Maximum growth was shown at NaCl concentration of 0% and 0.75% respectively by the isolate JUBM-35-NS-1 and JUBM-35-NS-2. The NaCl tolerance for growth of isolate JUBM-35-NS-1 was up to 8% and that for the isolate JUBM-35-NS-2 was up to 2.0%. A lower concentration of NaCl was found stimulatory with decline over 1.5% and complete cessation at 2.5% for the isolate JUBM-35-NS-2 (*Nocardia* sp.). The isolate JUBM-35-NS-1 (*Streptomyces* sp.) was found to be salt tolerant showing a stable growth from 0.5% to 8.0% with complete arrest of growth at 8.5% of NaCl concentration in culture medium.

#### **Keywords**

Actinomycetes, Growth, Temperature, pH, Salinity

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#### 1. Introduction

Actinomycetes are Gram-positive bacteria frequently filamentous and sporulating, exhibiting a wide range of morphological forms from coccoid through fragmenting hypha to permanent and highly differentiated branched mycelium. Actinomycetes are present in various ecological habitats such as soil, fresh water, black water, lake, compost, sewage and marine environment [10]. Actinomycetes are the most fruitful source for production of bioactive secondary metabolites [5]. This is the largest group of microbes producing antifungal, antibacterial, and antiparasitic drugs. They also play very important role in producing wide range

of bioactive compounds. Other biotechnological applications include the exploitation of their capacity of producing various enzymes which degrade complex organics such as cellulose, lignin, chitin, and proteins.

They play an important part in the cycle of life in nature by bringing about the decomposition of complex plant and animal residues and the liberation of a continuous stream of available elements, notably carbon and nitrogen, essential for fresh plant growth. Some of the biochemical activities of the actinomycetes are now being utilized for the large-scale production of chemical substances essential for public health [38]. The increased waste production is of great concern globally at different levels of population. Various alternatives

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are in exercise to address this crisis by elimination, purification and/or recycling. The modern concept of environmental management is based on the recycling of wastes. In this context, composting appears to be a safe form of treatment of some wastes and the reclamation of the nutrients [1]. Degradation of straw, the most abundant agricultural by-products with high amounts of cellulose and lignin, by a number of microbes would result in production of good quality compost containing higher amounts of total nitrogen, total potassium and total phosphorus content [36].

Microorganisms are specific to temperature, pH, and salinity for their growth, development and for the production of specific metabolites. For the production of bioactive secondary metabolites it is essential to maintain the optimum temperature, pH and salinity otherwise production could be failed or no growth also be observed. Isolation and identification of strains of *Streptomyces* sp. and *Nocardia* sp. were reported earlier from straw and compost samples [16] which were found to degrade rice straw. We report hereby the effect of temperature, pH and salinity on *Streptomyces* sp. (JUBM-35-NS-1) and *Nocardia* sp. (JUBM-35-NS-2) recovered previously.

# 2. Materials and Methods

#### 2.1. Actinomycetes Isolates

The isolate JUBM-35-NS-1 previously identified as Streptomyces sp. was recovered from the straw sample [16]. During isolation chloramphenicol and nystatin was added to the medium and initially isolated at 45°C temperature in actinomycetes selective medium. The isolate JUBM-35-NS-2 identified as Nocardia sp. was recovered from compost sample at 45°C temperature in actinomycetes selective medium [16]. For the isolation from compost sample no antibiotic was used. In case of isolation of Streptomyces strain JUBM-35-NS-1 a small piece of straw was placed on selective agar plates; and for *Nocardia* strain JUBM-35-NS-2 a small amount of compost used in mushroom cultivation was directly inoculated by spreading over the solid surface of the selective medium. Subcultures were repeated until the colonies were found pure by visual and microscopic observation. Identification was made according to Bergey's Manual of Determinative Bacteriology [13] based on morphological, biochemical physiological and characterization.

#### 2.2. Effect of Temperature

0.1 ml of freshly developed seed cultures of the actinomycetes isolates in nutrient broth was inoculated into test tubes containing nutrient broth medium with the help of a micro pipette. Optical density (OD) was measured by a

colorimeter at 600 nm light spectrum which was treated as initial density or density of control. Incubation was made at temperatures of 4°C, 25°C, 30°C, 37°C, 40°C, 45°C and 50°C for 48 hours in a shaker bath. After 48 hours, the optical density of each treatment was measured at 600 nm. Each treatment was replicated thrice. Initial OD was subtracted from that after 48 hours to calculate the growth obtained by each treatment.

#### 2.3. Study on pH Effect

Nutrient broth was used to determine the effect of pH on the growth of actinomycetes isolates. Various pH values used were 4.0, 5.0, 5.5, 6.5, 7.0, 7.5, 8.0, 9.0, 10, and 11 adjusted with 1N HCl and 1N KOH solutions as per requirement. 0.1 ml seed cultures were inoculated in each tube with a micro pipette and the initial optical density was recorded. Incubation was made at 35°C temperature for 48 hours and the growth was measured by using optical density. Three tubes were incubated for each pH treatment.

#### 2.4. Effect of NaCl

Nutrient broth in test tubes with NaCl concentrations of 0%, 0.5%, 0.75%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5%, 5.0%, 5.5%, 6.0%, 6.5%, 7.0%, 7.5%, 8.0%, 8.5%, and 9.0% was prepared. Three replications of each treatment were used. After inoculation with seed cultures the initial growth was detected by measuring optical density at 600nm. Then the treatments were incubated for 48 hours at 35°C.

### 3. Results and Discussion

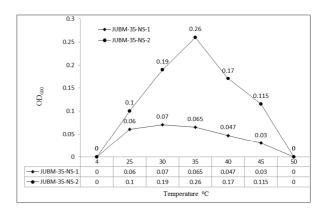
Microbial growth is directly dependent on how temperature affects cellular enzymes. With increasing temperatures, enzyme activity increases until the three-dimensional configuration of these molecules is lost because of denaturation of their protein structure. On the other hand, as the temperature is lowered toward the freezing point, enzyme inactivation occurs and cellular metabolism gradually diminishes. At 0°C temperature biochemical reactions cease in most cells. For normal growth and maintenance the temperature at a range of 25-30°C are usually used for incubation of the great majority of Streptomyces, Nocardia, and Micromonospora. Frequently somewhat different temperatures are used for seed production and for antibiotic formation under factory conditions. Pathogenic organisms require 37°C, and thermophiles need 50-60°C or even higher temperatures [39]. Figure 1 represents the different growth patterns of two actinomycetes isolates viz., JUBM-35-NS-1 (Streptomyces sp.) and JUBM-35-NS-2 (Nocardia sp.). The temperature range for growth was found from 25°C to 45°C with respective  $OD_{600}$  values of 0.06 and 0.03 for isolate

JUBM-35-NS-1 and it was 0.1 and 0.115 for isolate JUBM-35-NS-2. The maximum growth was observed at 30°C and 35°C respectively for the isolates JUBM-35-NS-1 and JUBM-35-NS-2. At 4°C and 50°C temperatures growth of both isolates was found to be ceased completely. At 30°C temperature the isolate JUBM-35-NS-1 showed better growth with an OD<sub>600</sub> value of 0.07 while at temperature 35°C the isolate JUBM-35-N-2 showed better growth with an OD<sub>600</sub> value of 0.26. Thus the strain JUBM-35-NS-2 is more vigorous at its optimum temperature than the strain JUBM-35-NS-1. Waksman [37] reported a number of species isolated from soil with their temperature optimum for growth varied from 25°C to 55°C, the maximum for most species being at 40°C and the minimum below 18-20°C. Another study by Haines [11] on measurements of growth rate of Actinomyces from both soil and cold stores showed that the majority of the strains had their optimum temperature at 37°C, capable of growth from 40 to 50°C. One strain, however, had its optimum temperature for growth less sharply defined at 20-30°C. Tawfik and Ramadan [33] studied in Saudi Arabia and reported that the optimum temperature for growth and antibiotic production by two strains of Streptomyces was 30°C.

Growth and survival of bacteria are also greatly influenced by the pH of the environment. The specific range for bacteria is between 4 and 9, with the optimum being 6.5 to 7.5. Different bacteria prefer different pH range. Streptomyces are neutrophilic showing better growth in between pH 6.0 and 8.0 [18]. The pH range for growth of the isolate JUBM-35-NS-1 was detected from 5.5 with OD<sub>600</sub> of 0.04 to 7.5 with OD<sub>600</sub> of 0.055 and it was found ranging from pH 5.5 with  $\mathrm{OD}_{600}$  of 0.075 to pH 8.0 with  $\mathrm{OD}_{600}$  of 0.085 in case of isolate JUBM-35-NS-2. In case of isolate JUBM-35-NS-1 the best growth was recorded at pH 6.0 and 6.5 (OD value 0.065) and in case of JUBM-35-NS-2 the best growth was recorded at pH 6.5 (OD value 0.14). The optimum growth was observed in between pH 6.0 to 7.5 with OD<sub>600</sub> range of 0.065 - 0.055 for isolate JUBM-35-NS-1, and it was in between pH 6.5 to 8.0 with  $OD_{600}$  range of 0.14 - 0.13. Thus it has been evident that the isolate JUBM-35-NS-2 is more vigorous with wide ranging pH adaptability (Figure 2). Farmer [8] reported that Nocardia favours an alkaline pH 8.0 for their growth. Ten species of Streptomyces from Finland have been reported to show maximum growth at a pH range of 4.0-5.5 [20].

James et al. [17] reported an optimum temperature of 45°C and that of pH in between 5.5 to 6.5 for highest growth rate of *Streptomyces thermoviolacus*. Kim et al. [19] reported a new species of *Streptomyces* growing abundant at a pH range of 4.3-7.3 and between a temperature of 25 and 37°C but not at 45°C. An antifungal *Streptomyces* has been found to show its optimum activity at 30°C and pH 6.5 [15]. Palanichamy et

al. [25] has reported *Streptomyces* species from Chennai coastal region with optimum temperature of 28-30°C and pH of 7.6-8.0. Bhavana *et al.* [4] have reported that optimum mycelial growth and antibiotic production by *Streptomyces carpaticus* from the Vishakhapatnam sea coast of Bay of Bengal occurred at pH 7.2 and at 30°C. Higher production of bioactive metabolites by *Streptomyces* species SRDP-TK-07 isolated from Talakaveri, Western Ghats of Karnataka, India was observed at temperature 45°C, initial pH 7.0 and salinity of 2% [28].



**Figure 1.** Effect of temperature on the growth of Actinomycetes isolates JUBM-35-NS-1 and JUBM-35-NS-2.

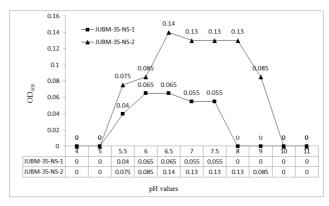


Figure 2. Effect of pH on Actinomycetes isolates JUBM-35-NS-1 and JUBM-35-NS-2.

The isolates were also evaluated for their ability to withstand salt stress by growing at NaCl concentrations of 0%, 0.25%, 0.5%, 0.75%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5%, 5.0%, 5.5%, 6.0%, 6.5%, 7.0%, 7.5%, 8.0%, 8.5% and 9.0%. The best growth (OD<sub>600</sub> value 0.095) showed by isolate JUBM-35-NS-1 was in absence of NaCl and it was observed in case of JUBM-35-NS-2 at 0.75% NaCl with OD<sub>600</sub> value of 0.2 (Figure 3). Growth arrest was recorded at NaCl concentration of 8.5% and 2.5% respectively for the isolate JUBM-35-NS-1 and JUBM-35-NS-2. Thus the NaCl tolerance limit for growth of JUBM-35-NS-1 was up to 8% (OD value 0.0325) and that for the isolate JUBM-35-NS-2 was 2% (OD value 0.03). The growth of isolate NS-1 dropped from OD<sub>600</sub> 0.095 at 0% NaCl to OD<sub>600</sub> value of

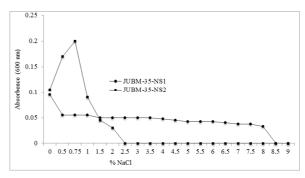
0.055 with increased NaCl concentration to 0.5%. Then it continued till the NaCl concentration of 8.0% without further declination. It testifies the isolate's capacity to withstand salinity up to that level and thus this is a halotolerant strain. However, in case of isolate JUBM-35-NS-2 NaCl at lower concentration exerted a stimulatory effect on its growth up to 0.75% NaCl and thereafter growth of the isolate declined sharply to complete cessation at NaCl concentration of 2.5%. Hence, the isolate JUBM-35-NS-2 is not a halotolerant strain though its maximum growth is dependent on low salt concentration (Figure 3). Tresner et al. [34] reported that Streptomyces strains can tolerate NaCl concentration from 4% up to 13%. They also reported that in evaluating the relationships of NaCl tolerance to various taxonomic features, higher tolerance was statistically associated with the "vellow" and possibly the "white"-spored *Streptomyces*. The isolate of this present study is in agreement with Tresner et al. [34]. Moreover, Salle [30] noted that minute amounts of metallic ions or salts stimulate growth of microorganisms whereas concentrations produce an inhibitory effect. In addition to affecting osmotic pressure, high concentrations tend to denature proteins and obligate halophiles possess specialized enzymes that are in their active configuration only at high salt concentrations [2].

Streptomyces spp. VITSVK9 showed the maximum growth with highest biomass at 30°C and at 5% of NaCl concentration in the medium and at pH 7.0; and the growth of the strain was inhibited in the absence of NaCl in the medium [31].

Moreover these halophilic microorganisms can be conveniently grouped according to their NaCl requirements for growth [35]. Extreme halophiles have been shown to grow in saturated NaCl concentration and fail to grow below 12% NaCl. Larsen [22] has defined as moderate halophiles are organisms growing optimally between 5 to 20% NaCl concentrations.

Ripa *et al.* [29] recovered a new *Streptomyces* strain in 2009 from Bangladesh soil with its activity maxima at temperature range of 25-45°C and at NaCl concentration range of 0.5-3.0% at alkaline pH.

A strain of *Streptomyces grancidicus* with optimum activity at 40°C, pH 7.0 and 1.5% NaCl has been reported in 2015 from Indian soil [21]. Effect of different physico-chemical parameters included pH, temperature, incubation period and NaCl concentration on the growth of a *Streptomyces* isolate from India showed growth optima at 45°C, pH 7.0 with failure of growing beyond 6.0% NaCl concentration [27]. Three species of *Streptomyces* from Malaysia have been found to show optimum growth at temperatures of 25, 30 and 37°C at pH 7.0 with most preferred NaCl concentration of 3.0% by all three isolates [12].



**Figure 3.** Effect of NaCl on the growth of *Streptomyces* sp. (JUBM-35-NS-1) and *Nocardia* sp. (JUBM-35-NS-2).

Nocardia strains reported from South Africa showed growth at pH range of 7.0-10.0; at 30 and 37°C, but not at pH 4.3 and 45°C; and in the presence of 4% NaCl; some strains grow weakly in the presence of 7% NaCl [7]. The investigation on optimal growth temperature and NaCl tolerance of a Nocardiopsis alba strain isolated from bioaerosol of a mushroom compost facility revealed growth optimum at temperature range of 28-37°C and NaCl tolerance up to 10% without any growth at temperature below 25°C and above 45°C [26].

# 4. Conclusion

Population of microorganisms during composting indicates the performance and quality of the compost. Reports on microbial community during composting reveal wide variations caused by the feedstock differences and the vast variety of composting conditions (i.e., type of raw material, facility design, aeration rate, pH, C/N ratio, temperature and moisture content). Therefore, monitoring of the microbial succession during composting process may provide important information of compost quality [3].

Proper composting promotes the development of a number of saprophytic soil microorganisms. Among others, species of *Streptomyces* and *Nocardia* have been reported by several authors in various parts of the world from compost and straw samples [1], [6], [9], [14], [21], [23,] [24], [32]. In the present study optimum levels of culture conditions were determined for *Streptomyces* and *Nocardia* isolates which have been evident playing effective role in degrading rice straw and compost development for mushroom cultivation (data not shown here).

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