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# Study of the Impact of Ecological Stress Factors on Growth and Survival of Isolates Exhibiting Rod-To-Coccus Morphogenesis Obtained from Soil and Sewage

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**ABSTRACT**

**Microbial life has to face devastating competition in environment. In their scuffle for growth and survival, bacteria adapt several responses to stress. The isolates A11 and A40 obtained from soil and sewage depicted typical rod-to-coccus morphogenesis. They were identified by morphology, cultural and 16SrRNA gene sequencing as Arthrobacter. The morphology helps them in surviving extreme ecological conditions. Arthrobacter are commonly found in soil and sewage environments because of their nutritional versatility. Several species have been found in sediments, subterranean caves, saline and water deficient environments. Some species have developed strategies to survive cold shock, high salt concentrations and oxygen limitation. The above isolates, were found to be potential biodegradors of pollutants qualitatively and quantitatively. They were checked for their ability to survive under environmental stress factors such as extreme pH, temperature, salt concentration, starvation, and desiccation. Standard strains Arthrobacter nicotinaemtccno. *2 and Arthrobacter chlorophenicolus*3706 were also studied simultaneously.The isolates and the standard strains were checked for growth at different pHs of 3-12 and at temperatures of 4to55$^0$C. They were also checked for growth in sodium chloride concentration of 2-12%. For effect of starvations, each of the isolates were grown with and without carbon source in medium. For desiccation effect the isolates were incubated in presence of desiccants. Survival percentage was calculated.**

**Both Arthrobacter isolates could grow in extreme conditions of pH 12, temperature 45 $^o$ C, salt concentration of up to 8-10%. They could survive without carbon source till 5daysand 72 hoursof desiccation. The standard strainsalso gave similar results. Also, they exhibit an extensive biodegradative profile. Thus, Arthrobacter can be explored to remediate subsurface pollution under extreme conditions of pH, temperature, starvation, water deficiency, and high salt concentration.**

**Keywords: Stress, deficient, starvation, desiccation, salt**

## INTRODUCTION

Bacteria adapt several diverse responses to environmental stress, in their struggle for survival These adaptations require sensitive monitoring of number of environmental factors for performance of various activities by organisms. Microorganisms in logarithmic phase of growth produce basic and acidic metabolic waste products. Often these wastes become inhibitory agents altering the pH of surrounding environment. Any change in the environmental pH may either enhance or inhibit the the enzyme activity. Thus, pH can dramatically affect the growth and activities of microorganisms. Every species of microorganisms shows specific pH growth range [1]. Environmental factor as growth temperature strongly impacts fatty acid composition of the cell membrane. Changes in fatty acids are thought to occur in order to maintain a functional bacterial cell membrane regardless of environmental stress.

The fluidity of the membrane needs to stay in a liquid-crystalline state to keep the cell membrane from cracking or melting. Arthrobacter can grow under extreme conditions of, low /high temperature. Their typical rod-to-coccus morphogenesis helps in surviving extreme environmental conditions [2, 3].Itcan grow under extreme conditions of salinity. In order to grow and reproduce in high-salt and low-water activity environments, organisms make basic biochemical adaptations in their proteins, nucleic acids, lipids and osmoregulation mechanisms. Effluents of different industries can have varying salt concentrations from low to very high. The proteins inside the microorganisms help make it possible for them to survive in extreme saline/salty environments [4].Nutrient limitation is one type of stress generally encountered by soil bacteria. Polluted niches may be nutrient deficient though high in percentage of chemicals. Many bacteria respond to starvation by reduction in cell size and a change to spherical shapes. For example, many Arthrobacter species convert from rod-shaped to coccoidal cell shapes. This results in an increase in the relative surface area of the cell, enabling a higher nutrient uptake and in some species, in the synthesis of unique cell wall peptidoglycans. Glucose limitation in the medium results in loss of 99% cell viability within 48 hrs [5,6].All types of microorganisms are not able to survive under desiccating conditions as water is required for metabolic activities. Arthrobacteria being desiccation resistant are generally very tolerant to drought, which may be the reason for their abundance in soil. Various dry soil environments do not support growth and activities of all organisms. It is believed that the spherical form of Arthrobacter predominates in soil and, therefore, it has become generally accepted as the more resistant of the two morphological forms [7].In this study impact of extreme pH, temperature and salt concentration on the growth of the two probable Arthrobacter isolates and two standards strains was studied. Also the effect of starvation (absence of carbon source) on the growth/survival response of the Arthrobacter isolates and standards strains were studied. The survival capability of Arthrobacter isolates and the standard strains was assessed under desiccating conditions created in laboratory [8].

## MATERIALS AND METHODS

### 1. Effect OF pH

**Materials**

Culture suspension of isolates A11 and A40 and
Standard strains of Arthrobacter- Arthrobacter nicotinaemtcc no.*2 and Arthrobacterchlorophenicolus*3706
Sterile 5 ml nutrient broth tubes - 5 with different pH (3, 5, 7, 9 and 11) – 4 sets
Sterile 5 ml nutrient broth tubes-pH 7 – 3 as positive, negative and media controls
Sterile 10- and 1-ml pipettes, Refrigerator
Sterile 5 ml nutrient broth tubes-5 - 04 sets
Sterile 5 ml nutrient broth tubes–03 as positive, negative and media controls
Sterile 10- and 1-ml pipettes, test tubes
Refrigerator $4^0$C, Incubators at RT, 37, 45 and $55^0$C
Sterile 5 ml nutrient broth tubes 6 with different sodium chloride concentrations (2, 4, 6, 8, 10 and 12 %) – 04 sets
Sterile 5 ml nutrient broth tubes– 03 as positive, negative and media controls
100 ml Sterile Mineral salts liquid medium in flask with glucose (0.02%) - 5
100 ml Sterile Mineral salts liquid medium in flask without glucose-5
E.colias control organism
Sterile nutrient agar plates
Ammonium chloride and Calcium chloride as desiccants ($NH_4Cl$ and $CaCl_2$)
Sterile saline, alcohol
Test tubes, spreader, Desiccator, Colorimeter at 660nm

## METHOD

**Effect of pH,temperature , salt (NaCl) concentration**

The above Arthrobacter isolates and the standard strains (O.D of 0·5 @ 660 nm) were inoculated in nutrient broth medium of different pH **of 3, 5, 7, 9 and 11** and incubated at RT (except –negative control). They were also grown in nutrient broth medium and incubated at respective temperatures of 4, RT, 37, 45 and $55^0$ C, for 24 hrs, (except –negative control) Positive, negative and medium/turbidity controls were used for comparison of growth. They were also inoculated in nutrient broth medium with varying concentration of sodium chloride as specified above and incubated at R.T for 24 hrs, (except –negative control). This was followed for both standard strains of Arthrobacteralso.

**Results were read as turbidity/growth in the medium after incubation. They were compared with positive, negative and medium control.**

**Effect of starvation:** Each of the Arthrobacter isolates and the standard strains (**O.D of 0·5 @ 660 nm**) were grown in mineral salts medium in two separate flasks. First flask with glucose which was used as control and second one was without glucose/ carbon source in medium.One pair of flasks was also kept as uninoculated control. Effect of **absence** of carbon source was measured in terms of growth in both media. Growth was recorded in terms of increase and decrease in turbidity/optical density at 660 nm. This was done every 24 hrs for 72 hrs.

**Effect of desiccation:** The saline preparations of the Arthrobacter isolates and the standard strains were placed in a cotton plugged test tubes, inside desiccator and incubated at room temperature. A combination of $NH_4Cl$ and $CaCl_2$ desiccants in the proportion of 1:1 was used for desiccation which were placed in desiccator. After three days entire diluent got dried. The isolates were resuscitated, appropriately diluted and spread plate count was performed. The plate count was also performed before exposure of isolates to desiccating conditions. Control used was culture suspension of Escherichia coli. The plates were incubated at RT for 24 hrs.

The plate counts were recorded and survival percent was calculated with the standard formula given below.
% Survival = B X 100/A and
% Reduction = 100 - % Survival

Where
A-Initial count of cells before exposure to desiccation
B- Count of cells after exposure to desiccation
These were compared with the % Survival of control

The selected potent Arthrobacter isolates and the Arthrobacter Standard strains were checked for their growth potential and survival ability under extreme conditions of stress.

## RESULTS AND DISCUSSION

The identified Arthrobacter isolates A11and A40, which were found to be potential biodegradors of a wide range of pollutants qualitatively and quantitatively were tested for their ability to survive under stress conditions such as extreme pH, temperature, salt concentration, starvation and desiccation.Standard strains namely Arthrobacter nicotinaemtcc no*2 (AS1) and Arthrobacter chlorophenicolus*3706 (AS2) were also studied simultaneously. The results obtained are given below in **table 1 to 6**

**Effect of pH:** The results of effect of different pH on the growth of A11 and A40, andtwo standards strains are given in **table 1**

**Table: 1 Effect of different pH on the growth of A11 andA40 and, standard strains of Arthrobacter**

| Isolate → / pH ↓ | A11 | A40 | AS1 | AS2 |
|---|---|---|---|---|
| 3 | - | - | - | - |
| 5 | + | + | + | + |
| 7 | + | + | + | + |
| 9 | + | + | + | + |
| 11 | + | + | - | - |
| 12 | + | + | - | - |
| Positive Control | + | + | + | + |
| Negative Control | - | - | - | - |
| Medium Control | - | - | - | - |

Positive control--- Medium inoculated with culture. Negative control---Medium inoculated with culture and incubated in refrigerator**.** Medium control----Uninoculated medium

| Ligand | + means Turbidity/growth |
|---|---|
| | - means No turbidity/growth |

As can be seen from the table, both Arthrobacter isolates A11 andA40could grow in extreme condition of pH of about 12, though at pH 3, no growth was seen. Both the standard strains of Arthrobacter could tolerate and grow in pH of 5 to 9.[9]

**Effect Of Temperature:** The effect of different incubation temperature on the growth of the identifiedisolates, as well asA40and, standards strains were studied and the results are shown in table**2.**

**Table: 2 Effect of various temperatures on the growth of A11as well as A40 and, standard strains of Arthrobacter**

| Isolate → / Temperature $^0$C ↓ | A11 | A40 | AS1 | AS2 |
|---|---|---|---|---|
| 4 | - | - | - | - |
| Room Temperature | + | + | + | + |
| 37 | + | + | + | - |
| 45 | + | - | + | - |
| 55 | - | - | + | - |
| Positive Control | + | + | + | + |
| Negative Control | - | - | - | - |
| Medium Control | - | - | - | - |

Both the Arthrobacter isolates A11 and A40could grow comfortably at room temperature, at 37 $^o$ C and also at 45 $^o$ C. However both isolates could not grow at 4$^o$C and 55$^o$C. With respect to Standard strains Arthrobacter nicotinaemtccno.*2 (AS1) could tolerate upto 55 $^o$ C and Arthrobacter chlorophenicolus*3706 (AS2) was very sensitive and could grow only at RT.

**Effect of Salt (Nacl) Concentration**

The effect of different concentrations of salt on the growth of identified Arthrobacter isolates A11, A40and on Arthrobacter standards strains were studied and results are given in **table 3**

**Table: 3. Effect of salinity on the growth of A11and A40and standard strains of Arthrobacter**

| Isolate → / Salt concentration (%) ↓ | A11 | A40 | AS1 | AS2 |
|---|---|---|---|---|
| 2 | + | + | + | + |
| 4 | + | + | + | + |
| 6 | + | + | + | + |
| 8 | + | + | + | - |
| 10 | - | + | - | - |
| 12 | - | - | - | - |
| Positive Control | + | + | + | + |
| Negative Control | - | - | - | - |
| Medium Control | - | - | - | - |

Both A11 and A40were able to tolerate and grow at a salt concentration of up to 8 % though A40could tolerate and grow in 10 % salt concentration as well. Standard strains Arthrobacter nicotinaemtccno. *2 (AS1) gave similar results and Arthrobacter chlorophenicolus*3706 (AS2) could grow at a salt concentration of up to 6 %.[9]

**Effect Of Starvation:** The effect of starvation (absence of carbon source in medium) on the growth of A11 and A40, **and** on Arthrobacter standards strains were studied and results are given in **table 4 and 5.** Typical growth of these Arthrobacter isolates in same medium with carbon source, was used as control. Concentration of glucose used in medium was 20 mcg/ml. Growth was recorded in terms of optical density at 660 nm.

**Table: 4 Growth (OD at 660 nm) of A11 and A40in presence and absence of sugar/carbon source in medium**

| Growth (OD at 660nm) | | | | |
|---|---|---|---|---|
| **No of days / Isolates** | **A11** | **A11** | **A40** | **A40** |
| | **Glucose (carbon source)** | **No carbon source** | **Glucose (carbon source)** | **No carbon source** |
| **Day 1** | 0.04 | 0.04 | 0.06 | 0.04 |
| **Day 2** | 0.21 | 0.09 | 0.05 | 0.04 |
| **Day 3** | 0.38 | 0.04 | 0.35 | 0.04 |
| **Day 4** | 0.45 | 0.04 | 0.4 | 0.04 |
| **Day 5** | **0.22** | **0.05** | **0.12** | **0.04** |

**Table: 5 Growth (OD at 660 nm) of Standard strains of Arthrobacter in presence and absence of sugar/carbon source in medium**

| Growth (OD at 660nm) | | | | |
|---|---|---|---|---|
| No of days / Standard Strains | **Std Arthrobacter nicotinaemtccno.*2** | **Std Arthrobacter chlorophenicolus* 3706** | **Std Arthrobacternicotinaemtccno. *2** | **Std Arthrobacter chlorophenicolus* 3706** |
| | **Glucose (carbon source)** | **No carbon source** | **Glucose (carbon source)** | **No carbon source** |
| Day 1 | 0.04 | 0.07 | 0.03 | 0.04 |
| Day 2 | 0.05 | 0.08 | 0.57 | 0.12 |
| Day 3 | 0.33 | 0.06 | 0.48 | 0.06 |
| Day 4 | 0.42 | 0.06 | 0.51 | 0.06 |
| Day 5 | **0.2** | **0.05** | **0.3** | **0.04** |

As can be seen from the table, both the isolates A11 andA40 could survive in the mineral saltsmedium without any carbon source till 5$^{th}$ day, though luxuriant growth was not seen.The standard strains of Arthrobacter also gave similar results[10]

**EFFECT OF DESICCATION:** The survival capability of both Arthrobacter isolates, A11 as well as A40**,** and the standard strains of Arthrobacter was checked under desiccating conditions created in laboratory. **Control used was culture suspension of Escherichia coli**. The plate counts were recorded and survival percentage was calculated with the standard formula. They were compared with the percentage survival of control organism. Results of percentage survival and percentage reduction are given below in **table 6** and same results depicted in **figure 1.**

**Table: 6. Effect of desiccation on the survival of A11, A40 and standard strains of Arthrobacter**

| **Isolate/ Parameter** | **A11** | **A40** | **AS1** | **AS2** | **Control (E. coli)** |
|---|---|---|---|---|---|
| **Plate Count before desiccation** | $8x10^8$ | $8x10^8$ | $8x10^8$ | $8 x10^8$ | $2x10^7$ |
| **Plate Count after desiccation (48hrs)** | $7x10^8$ | $6x10^8$ | $6 x10^8$ | $5x10^8$ | $2 x10^6$ |
| **% Survival** | **87.5%** | **75%** | **75%** | **62%** | **10%** |
| **% Reduction** | 12.5% | 25% | 25% | 38% | **90%** |

## CONCLUSIONS

Arthrobacter IsolatesA11 and A40were checked for their ability to survive under environmental stress conditions**.** BothArthrobacter isolates could grow in extreme condition of pH 12, temperature 45$^{o}$C and salt concentration of up to 8%-10%. They could survive in the mineral salts medium without any carbon source till 5 days and also could survive 48 hrs of desiccation. The standard strains of Arthrobacter also gave similar results.The percentage survival of the Arthrobacter isolates was as much 87.5 % compared to 75 % survival of standard organisms. Because of their nutritional versatility, Arthrobacters are commonly found in soil, sewage, food and several other environments. Several species of Arthrobacter have been found in subterranean caves, sediments, sewage, saline and/or water deficientenvironments.[11]Thus some species of Arthrobacter have developed oxygen independent growth strategies in order to survive periods of oxygen limitation, cold shock and cold acclimation proteins and choline oxidase enzyme to prevent the denaturation of soluble enzymes at high salt concentrations[12].Many conventional microorganisms are unable to operate as efficiently as Arthrobacterunder adverse conditions. This makes the genus's high abundance in soil very reasonable. Hence these microorganisms are potential candidates for the degradation of pollutants at even under extreme conditions of temperature, starvation, water deficiency and metal-concentrations.

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