

# The Ability of Some Thermo-Alkalophilic *Bacillus* Species to Fix Nitrogen, Isolated from Madinah Munawwarah Soils, Saudi Arabia

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ABSTRACT. Trials were made to detect the presence of Thermo-alkalophilic that has ability to nitrogen fixation, in certain soils at Madinah Munawwarah province, Saudi Arabia. Soil samples were obtained from two different localities, and they were chemically analyzed.

The microorganisms used were *Bacillus alvei*, *B. brevis*, *B. megaterium*, *B. circulans*, and *B. pumilus*, and they prefer to grow at high temperature 60°C in media adjusted at pH 10.

The result shows that these isolations have the ability to fix nitrogen. The biomass and the nitrogenase activity after 24 h at 60°C were: (*Bacillus alvei*, 1.5 µg/ml; *B. brevis*, 1.9 µg/ml; *B. megaterium*, 1.4 µg/ml; *B. circulans*, 1.6 µg/ml; and *B. pumilus*, 1.3 µg/ml).

## 1. Introduction

Microorganisms play an important role in chemical and biological transformations in soil and maintain soil fertility. The major biological elements, carbon, nitrogen, oxygen and sulphur are subject to comparable cyclic processes, and the most important, from both ecological and economic viewpoints, is the nitrogen cycle.

The ability to fix nitrogen is restricted to the most primitive living things, the bacteria. In the last few years, a number of alkalophilic bacteria have been isolated by some investigators *e.g.*<sup>[1-4]</sup>, from saline environments.

Very high ( $> 12$ ) and very low ( $< 3$ ) pH values are inhibitory to most microorganisms especially at high temperature degrees, but some are nevertheless, to be found in environments where pH values approach zero or exceed 12. Even in normally natural environments, wide fluctuations of pH are often observed. Under such conditions, certain bacterial populations may become subject to pH fluctuation<sup>[5]</sup>.

The present work aimed to study the ability of some thermoalkalophilic *Bacillus* species isolated from Madinah Munawwarah soils, Saudi Arabia to fix nitrogen.

## 2. Material and Methods

### **Location**

Two saline localities were chosen: Tareq Algameat (5 km north of Madinah) and Alkhaleal (10 km north of Madinah). Soil samples were collected from 4 sites, two at each locality. Samples were collected according to the method described by<sup>[6]</sup>. These samples were taken at random from each site, brought together into one composite sample, which was mixed thoroughly and kept in polyethylene bags.

Moisture content, total water soluble salts, organic matter, organic nitrogen and pH values were determined in replicate according to the techniques quoted by<sup>[7]</sup>. The dilution plate method, which was described by<sup>[6]</sup> was used for isolation and purification of alkalophilic bacteria.

### **Organisms**

The organisms used in the experiment were *Bacillus alvei*, *B. brevis*, *B. megaterium*, *B. circulans*, and *B. pumilus*, were identified by<sup>[4]</sup>.

### **Media**

The basal medium used in this study was Czapek-Dox N-free liquid medium adjusted to pH 10 with 2N NaOH, dispensed into Erlenmeyer flasks (100 ml in 250 ml flasks) and sterilized by autoclaving at 120°C for 20 minutes. After cooling the flasks were then inoculated with a single disc (11 mm) cut from a 24 h culture of the microorganisms grown on N-free Czapek-Dox solid medium.

The inoculated flasks were then incubated in triplicate at different temperature (10, 20, 30, 40, 50, 60, and 70°C) on a reciprocal shaker (125-rev min<sup>-1</sup>). A triplicate of the flasks was removed and the contents filtered through pre-dried and pre-weighed Waterman No. 1 filter papers. The filter papers were dried to constant weight at 46°C and the biomass calculated.

### ***Nitrogenase Activity***

Assay of nitrogenase was achieved according to<sup>[8]</sup> where 10% of the gas-phase from the nitrogen fixing system, in gas tight container, was replaced by acetylene (C<sub>2</sub>H<sub>2</sub>) using a disposable gas-tight syringe. The production of ethylene(C<sub>2</sub>H<sub>4</sub>) was measured at convenient intervals by injecting 1 ml samples into a GC (Pye Unicum 4500) equipped with flame-ionization detector.

### **3. Result and Discussion**

The characteristics of the collected soil samples are given in Table 1. It was found that for all collected soil samples, both organic matter, organic nitrogen, moisture content, and total water soluble salts (% , W/W) are almost of same level.

TABLE 1. Soil properties of samples collected from Tareq Aljameat (A&B) and Alkhaleal (C&D) localities.

Soil samples	*Mean values (% , W/W) of				
	Organic matter	Organic nitrogen	Moisture content	Total soluble salts	pH
A	0.44	0.05	4.30	0.19	9.0
B	0.63	0.11	5.73	0.63	8.5
C	0.45	0.09	3.02	0.45	8.7
D	0.37	0.06	4.00	0.37	8.2

\*Mean values of 3 determinations.

The obtained data revealed that, the collected soil samples (arid soils of, 0.37-0.63% organic matter; 0.05-0.11% organic nitrogen; 0.19-0.63%, total soluble salts; 3.02-5.73%, moisture content; and pH values ranges from 8.2-9.0).

As for the pH values of the used soil samples, the obtained results reveal that alkalinity is one of the characteristic features of such soils. The pH values ranged between 8.2-9.0. This finding is in accordance with the findings obtained by<sup>[9]</sup> who stated that in warm dry climate, soil are usually neutral to strong basic due to insufficient rainfall to leach away the bases as soon as they are released, while few acidic materials are produced by natural processes of decay.

This finding is also in accordance with that obtained by<sup>[10]</sup> in their finding concerning soil properties of samples collected from different sites at Sheaiba and Al-Kakeyah localities, Makkah Al-Mukarramah, Saudi Arabia.

The populations of microorganisms in desert soils were found to be lower than in soils of other climatic zones. This was attributed mainly to low organic matter content, low soil moisture and high temperature, particularly during summer season. Table 2 shows the ability of isolated microorganisms to produce nitrogenase at different temperature; the best production was at 60°C.

TABLE 2. Nitrogenase activity and biomass (mg) of *bacillus* species at different temperature, after 24.

Organisms species	*Nitrogenase activity and biomass (mg) of bacillus species at different temperature after 24 hr													
	10°C	B.	20°C	B.	30°C	B.	40°C	B.	50°C	B.	60°C	B.	70°C	B.
<i>Bacillus alvei</i>	0.1 ±0.1	0.3 ±0.1	0.3 ±0.1	0.5 ±0.1	0.4 ±0.2	0.6 ±0.1	0.6 ±0.1	1.0 ±0.2	1.2 ±0.2	1.9 ±0.2	1.5 ±0.2	2.1 ±0.2	0.9 ±0.1	1.7 ±0.1
<i>B. brevis</i>	0.2 ±0.1	0.3 ±0.2	0.4 ±0.2	0.7 ±0.1	0.7 ±0.3	0.9 ±0.1	1.0 ±0.1	1.5 ±0.1	1.5 ±0.1	1.8 ±0.2	1.9 ±0.1	2.7 ±0.1	1.0 ±0.2	1.4 ±0.1
<i>B. megaterium</i>	0.1 ±0.0	0.3 ±0.1	0.2 ±0.1	0.5 ±0.1	0.5 ±0.2	0.9 ±0.1	0.7 ±0.1	1.1 ±0.2	1.0 ±0.2	1.8 ±0.1	1.4 ±0.3	2.2 ±0.2	0.8 ±0.1	1.1 ±0.1
<i>B. circulans</i>	0.1 ±0.1	0.3 ±0.1	0.3 ±0.1	0.5 ±0.1	0.6 ±0.3	0.8 ±0.1	0.7 ±0.1	1.2 ±0.2	1.1 ±0.2	1.7 ±0.2	1.6 ±0.2	2.4 ±0.1	1.1 ±0.2	1.6 ±0.1
<i>B. pumilus</i>	0.1 ±0.1	0.3 ±0.1	0.2 ±0.1	0.4 ±0.1	0.4 ±0.2	0.6 ±0.1	0.6 ±0.1	1.1 ±0.1	1.0 ±0.2	1.8 ±0.2	1.3 ±0.3	2.3 ±0.2	0.8 ±0.1	1.1 ±0.1

\* = Mean values of 3 determinations.

± = Standard deviation.

B. = Biomass.

This result is in accordance with the results obtained by<sup>[11]</sup>, on desert soils of Saudi Arabia, and<sup>[12]</sup>, on desert areas of the South-West U.S.A.

Table 3 shows the biomass of different microorganisms that can produce highest nitrogenase activity at 60°C after 24 h. In general this work shows that some *Bacillus* species, which were isolated from Saudi Arabian soils, can fix nitrogen at high temperature and alkaline conditions.

TABLE 3. The best nitrogenase activity and biomass of *bacillus* species grown at 60°C after 24 h.

Organisms species	*Yield of	
	Nitrogenase activity (µg/ml)	Biomass (mg)
<i>Bacillus alvei</i>	1.5 ± 0.2	2.1 ± 0.2
<i>B. brevis</i>	1.9 ± 0.1	2.7 ± 0.1
<i>B. megaterium</i>	1.4 ± 0.3	2.2 ± 0.2
<i>B. circulans</i>	1.6 ± 0.2	2.4 ± 0.1
<i>B. pumilus</i>	1.3 ± 0.3	2.3 ± 0.2

\*Mean values of 3 determinations.

±Standard deviation.

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قدرة بعض أنواع الجنس باسيلوس المحبة لدرجات الحرارة  
والقلوية العالية على تثبيت النيتروجين الجوي،  
والمعزولة من ترب المدينة المنورة بالمملكة العربية السعودية

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المدينة المنورة - المملكة العربية السعودية

المستخلص. تهدف هذه الدراسة إلى التعرف على قدرة بعض أنواع الجنس باسيلوس المحبة لدرجات الحرارة والقلوية العالية على تثبيت النيتروجين الجوي، والمعزولة من تربة المدينة المنورة بالمملكة العربية السعودية. ولقد جمعت عينات التربة من موقعين مختلفين بالمدينة المنورة، وتم تحليلها كيميائياً.

والبكتيريا العضوية المستخدمة في هذه الدراسة تفضل النمو عند درجة حرارة ٦٠ م وعند pH10، وهي:

*Bacillus alvei*, *B. brevis*, *B. megaterium*, *B. circulans*, and *B.*

*pumilus*. ولقد أثبتت الدراسة أن البكتيريا موضوع الدراسة لها القدرة

على تثبيت النيتروجين الجوي على النحو التالي:

*Bacillus alvei*, 1.5 ug/ml; *B. brevis*, 1.9 ug/ml; *B. megaterium*, 1.4 ug/ml; *B. circulans*, 1.6 ug/ml; and *B. pumilus*, 1.3 ug/ml.