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Effect of Temperature on the Ability of *Pseudomonas stutzeri* Bacteria Isolated from Different Sources to Fix Nitrogen

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ABSTRACT

Background Research has tended to increase the number of nitrogen-fixing microbes in the ground to increase the rate of Stabilization and supply plants with their needs of Nitrogen while reducing nitrogen fertilization and the surrounding problems of pollution, loss, and cost, so a type of microorganism was used in this degree to fix Nitrogen and its use as an alternative to chemical fertilizers.

Methodology, the isolated *Pseudomonas stutzeri* bacteria from different sources, were planted on a nitrogen-free medium after sterilization in an autoclave device, and the medium was distributed in 10 ml tubes. Then the bacteria were cultured in this medium and incubated for five days at a temperature of 37 °C, after which the growth was measured by a spectrometer and using the Nessler reagent detector. The same nitrogen-free medium was used, but the bacteria were incubated at different degrees. They were divided into three groups, the first incubated at 25 °C, the second at 35 °C, and the last at 45 °C.

The effect of isolating *Pseudomonas stutzeri* bacteria from different sources on nitrogen fixation was isolated from urine, feces, and sputum. The nitrogen concentration in the medium containing the bacteria isolated from urine was 3.8%. In contrast, the Nitrogen installed in the medium containing the bacteria isolated from the feces reached the highest concentration of 6.2. The nitrogen fixation in the medium containing the bacteria isolated from the sputum reached the lowest concentration of 2.4%, As in Figure 1 This case can be explained by the fact that the presence of bacteria in the intestine led to the gene expression of the genes that encode the enzyme Nitrogenase. Effect of temperature difference on nitrogen fixation in the culture media. The treatments were divided into three treatments, with five replications for each treatment after sterilization of the culture media. They were distributed into 10 ml tubes and inoculum with bacteria for each tube. The first group was incubated at 25 °C, the second at 35 °C, and the last at 45 °C. For five days in a vibrating incubator, the results showed that the concentration of Nitrogen installed in the first treatment (25 °C) was 2%, while the highest concentration in the second treatment (35 °C) was 5.2%, and in the last treatment (45 °C) 3.4%, as shown in Figure 2. Interpretation of this is that the ideal degree for bacterial growth is 37 °C.

Keywords- temperature on the ability, to fix Nitrogen, *Pseudomonas stutzeri*, chemical fertilizers, isolate bacteria.

I. INTRODUCTION

Pseudomonas stutzeri It is a genus of Gram-negative bacteria. [1][2][3] *Pseudomonas stutzeri* bacteria is a bacteria that grows and multiplies inside the stool and intestines. Still, despite that, it does not cause any harm while it is in the intestinal area. Still, when it moves to other body parts, such as the bladder, lung, and brain, it leads to severe infections, so who are the people most susceptible to *Pseudomonas stutzeri* bacteria? [4][5]. At the outset, it is necessary to note the importance of obtaining medical advice as soon as possible if one of the symptoms of a urinary tract infection is observed; To get the appropriate treatment early to avoid any unexpected problems [6][7][8]. The enzyme that helps fix Nitrogen is known as nitrogenase [9]. Nitrogen fixation is the method by which the ammonium present and available in some form in the atmosphere can be converted from Nitrogen and its chemical symbol N2, which chemists consider to be an element that is considered inert, and this characteristic that it is characterized by inertness was acquired by the

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absence of any kind of reaction Between it and other chemical substances, until it becomes a component of any other new compound [10][11]. When Nitrogen is fixed, it changes from its binary form known as N2 to become possible to deal with it using other different and changing methods [12][13]. And there is nitrogen gas in the atmosphere in general around us, so it is seventy-eight percent of the gas components in our atmosphere, which means that it is the main component of air and not oxygen as some know, but this amount of nitrogen gas, there is no direct possibility of it Whether for humans or animals, there is a way to deal with it in a beneficial way to turn it into used items [14].

II. METHODOLOGY

Isolate Bacteria

Isolation of bacteria from different sources where they were isolated from samples (urinary, feces, and sputum) grown on the elective media with the medium of Maconkey agar and purified on the same medium to obtain pure colonies and diagnosed by biochemical methods to obtain a high percentage of purity.

Activation of isolates on nutrient broth medium

The components of the medium were prepared according to the manufacturer's instructions. The isolates were activated, using tubes containing 10 ml of the medium, inoculated with the isolates, and incubated at 37 °C for 24 hours. [3]

Nitrogen-free broth

Its components are prepared according to the following table [4]

The components	Concentration g/L
Glucose	10
K_2HPO_4	0.52
KH_2PO_4	0.41
$CaSO_4.2H_2O$	0.20
$Na_2MoO_4.2H_2O$	0.002
$MgSO_4.7H_2O$	0.16
$FeSO_4.7H_2O$	0.005
NaCl	0.20
$MgSO_4.7H_2O$	0.1

The isolated *Pseudomonas stutzeri* bacteria from different sources were planted on a nitrogen-free medium after sterilization in an autoclave device, and the medium was distributed in 10 ml tubes. Then the bacteria were cultured in this medium and incubated for five days at a temperature of 37 °C, after which the growth was measured by a spectrometer and using the Nessler reagent detector. The same nitrogen-free medium was used, but the bacteria were incubated at different degrees. They were divided into three groups, the first incubated at 25 °C, the second at 35 °C, and the last at 45 °C.

III. RESULT

Table 1: The effect of different sources of *Pseudomonas stutzeri* isolation on nitrogen fixation in the culture media

Descriptive								
Fixed Nitrogen Concentration %								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
urine	5	3.80	.837	.374	2.76	4.84	3	5
faeces	5	6.20	.837	.374	5.16	7.24	5	7
sputum	5	2.40	.548	.245	1.72	3.08	2	3
Total	15	4.13	1.767	.456	3.15	5.11	2	7

Nitrogen is what is considered one of the chemical elements, and it follows in its division the group that belongs to the divisions of metalloids. It is available in natural life and is found in a gaseous form. It has no color, no taste, and no smell. The atmosphere in it consists of Nitrogen only, more than seventy-five of the composition of air, and the abundant presence of Nitrogen in the universe. Still, we do not find human use for it, nor do the rest of the animals benefit from it clearly. And it needs Nitrogen to exist in the form of ammonia, which allows the organism to use it. Thus, it becomes in a state that allows it to be used by fixation, and it is one of the necessary parts of the cycle that belongs to Nitrogen. Without all of that, there will be no ability for the organism that uses Nitrogen. Table 1 shows the effect of isolating *Pseudomonas stutzeri* bacteria from different sources on nitrogen fixation, as it was isolated from urine, feces, and sputum. The nitrogen concentration in the medium containing the bacteria isolated from urine was 3.8%. In contrast, the Nitrogen installed in the medium containing the bacteria isolated from the feces reached the highest concentration of 6.2.

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The nitrogen fixation in the medium containing the bacteria isolated from the sputum reached the lowest concentration of 2.4%, As in Figure 1 This case can be explained by the fact that the presence of bacteria in the intestine led to the gene expression of the genes that encode the enzyme Nitrogenase.

Table 2: ANOVA table of the effect of different sources of *Pseudomonas stutzeri* isolation on nitrogen fixation in the culture media

ANOVA					
Fixed Nitrogen Concentration %					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	36.933	2	18.467	32.588	.000
Within Groups	6.800	12	.567		
Total	43.733	14			

Nitrogen fixation is the method by which the ammonium present and available in some form in the atmosphere can be converted from Nitrogen and its chemical symbol N2, which chemists consider to be an element that is considered inert, and this characteristic that it is characterized by inertness was acquired by the absence of any kind of reaction Between it and other chemical substances, until it becomes a component of any other new compound. When Nitrogen is fixed, it changes from its binary form known as N2 to become possible to deal with it using other different and change methods Table 2 shows the analysis of variance that there were significant differences between all treatments, but the treatment in which bacteria isolated from feces were used gave the highest significant difference.

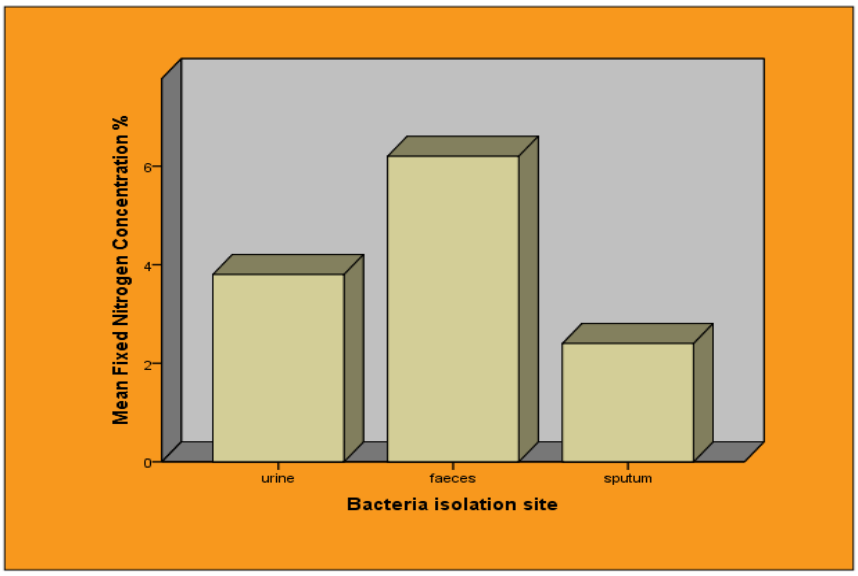

Figure 1 Effect of different sources of *Pseudomonas stutzeri* isolation on nitrogen fixation in the culture media

Table 3: Effect of different incubation temperatures of *Pseudomonas stutzeri* isolation on nitrogen fixation in the culture media

Descriptive								
Fixed Nitrogen Concentration %								
	N	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
					Lower Bound	**Upper Bound**		
25 c	5	2.00	.707	.316	1.12	2.88	1	3
35 c	5	5.20	.837	.374	4.16	6.24	4	6
45 c	5	3.40	.548	.245	2.72	4.08	3	4
Total	15	3.53	1.506	.389	2.70	4.37	1	6

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Temperatures also affect the rate of nitrogen fixation in the planting medium, at degrees Celsius. Low temperature The rate is low and increases with raising the temperature until it reaches higher Its rate is at 25-30 m and then decreases rapidly by raising the temperature above the optimum degree. Temperatures lower than the glass transition temperature of aqueous polyol solutions, which is about −136 °C (137 K, −213 °F), are considered acceptable to maintain significant biological activity. In contrast, the boiling point of liquid Nitrogen (which is −196 °C or 77 K) is considered acceptable. Or -321 degrees Fahrenheit), which is the optimum temperature for storing important samples. Table 3 shows the temperature difference effect on the culture media's nitrogen fixation. The treatments were divided into three treatments, with five replications for each treatment after sterilization of the culture media. They were distributed into 10 ml tubes and inoculum with bacteria for each tube. The first group was incubated at 25 °C, the second at 35 °C, and the last at 45 °C. For five days in a vibrating incubator, the results showed that the concentration of Nitrogen installed in the first treatment (25 C) was 2%, while the highest concentration in the second treatment (35 C) was 5.2%, and in the last treatment (45 C) 3.4%, as shown in Figure 2. Interpretation of this is that the ideal degree for bacterial growth is 37 °C

Table 4: ANOVA table of the effect of different incubation temperatures of *Pseudomonas stutzeri* isolation on nitrogen fixation in the culture media

ANOVA					
Fixed Nitrogen Concentration %					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	25.733	2	12.867	25.733	.000
Within Groups	6.000	12	.500		
Total	31.733	14			

Table 4 shows the analysis of variance for the effect of temperature difference on nitrogen fixation, where the table showed that there were no significant differences between all treatments and that the treatment in which the incubation degree of *Pseudomonas stutzeri* bacteria gave the highest significant difference to increasing the concentration of Nitrogen in the culture media and this is due to the optimal degree of bacterial growth is 37 pm

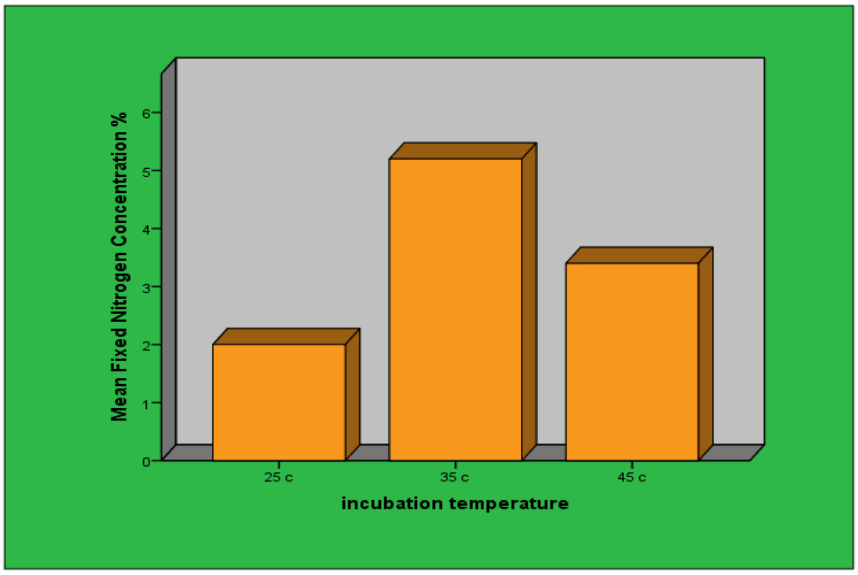

Figure 2: Effect of different incubation temperatures of *Pseudomonas stutzeri* isolation on nitrogen fixation in the culture media

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