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THE EFFECTS OF HEAT SHOCK ON THE GROWTH OF THE BACTERIAL SPECIES *SINORHIZOBIUMMELILOTI* AND *KLIBSELLAPNEUMONIA*E AND CONJUGATION BETWEEN THEM

Rezan Esa Mosa[1]* and Tofeeq Basheer Al-salman[2]
[1]College of Agricultural Engineering Sciences, University of Duhok, Iraq
[2]College of Agriculture and Forestry, University of Mosul, Iraq
*Email: Rezan.mosa@uod.ac

ABSTRACT
The main objective of this study was identifying the effect of temperature on the growth and efficiency of the types of bacteria infecting the plant host .Two types of bacteria were used in this study, *(Klibsella Pneumonia*), which is an intestinal bacteria, and bacteria (*Sinorhizobiummeliloti*), which infect leguminous plants . The first type was obtained from Media Medical Center / Erbil, and the second was isolated from the root nodes of the jet plant and diagnosed in terms of the family specialization. The bacterial suspensions under study were subjected to heat shock using temperatures (40, 45.50.55.60,65,70)^{0}C, and for a period of five minutes/treatment using the water bath. The results of the study showed that the intestinal bacteria (*K. Pneumonia*) did not show significant differences in the number of bacterial colonies at a temperature of 40 C compared to the control sample, as it gave 189 colonies compared to the control sample 200 colonies. The results also showed that the bacteria showed a state of adaptation to the temperatures 45 and 50 C, where a good number of bacterial colonies appeared, and giving 164 compared to 200 colonies compared to the control Sample. This confirms that it is the optimum temperature for the growth of these bacteria, as it showed that bacterial colonies do not grow after this temperature, and after that, it is considered a lethal or fatal degree for bacteria. As for the root nodules bacteria *S. meliloti*the results showed that there is growth and formation of bacterial colonies at temperatures 40 and 45 compared to the control sample, it gave (168,164) bacterial colonies compared to the control sample 170 colonies. The results showed that high temperatures of 60 and 70 degrees Celsius are lethal for these bacteria.

Key words: Heat shock, Conjugation, *inorhizobiummeliloti* and *KlibsellaPneumonia*e

INTRODUCTION
Klebsiella is a Gram-negative bacilli belonging to the Enterobacteriaceae family, and is widespread in nature. It has two types of common habitats, the first is environmental, as it spreads in various environments, especially surface waters, and the other habitat is the mucous surfaces of the plant.

In this respect, Klebsiella bacteria are similar to Enterobacter and Citrobacter but unlike spp Shigella or coli. E, which are common in humans but not in the environment. Klebsiella bacteria are known to be a common cause of pneumonia Klebsiella bacterium was known to be one of the main causes of pneumonia, and was named by that name according to. The German scientist

Klebs Edwan, who was the first to isolate it in 1834, then diagnosed this bacterium for the first time by the scientist Friedlander in 1882, from which it was called Friedlander's bacilli (Bengoechea and Sa Pessoa, 2019). Klebsiella is a genus of bacteria, a non-motile, non-motile, rod-shaped bacterium with a prominent capsule made of polysaccharides. Named according to the scientist Edwin Klepps (Edwin Klepps). It causes many diseases such as pneumonia, urinary tract infection, septicemia and others. Klebsiella is classified into three types, according to the clinical importance and biochemical reactions, which are as follows: K. pneumonia, K. ozaenae, K. rhinoscleromatis ,K. oxytoca,. planticolaK. , K. terrigena , K, ornithinolytica.
Klebsiella is scientifically classified within the bacterial kingdom, the order of intestinal bacteria, the genus Klebsiella, and the scientific name is Klebsiella (Trevisan ,1886). It grows in conditions of 37 degrees Celsius and PH 7.2. This bacteria is present in the intestines of the animal and human host and is active in its growth on the McConkey medium containing lactose sugar and yellow salts, as well as the nutrient agar medium. The bacterial colonies of the genus Klebsiella are mucous and sticky in texture and tend to merge after a period of incubation, so it is difficult to count bacterial cells (Galvão , et al. ,2018).
Bacteria of this genus are characterized by possessing the enzyme urease, so they have the ability to decompose urea and produce ammonia (Juarez, et al.,2020), and it is indicated that K. pneumonia is of great biochemical importance, as it gives a positive test for citrate consumption and a negative test for the methyl red and indole tests. This indicates the presence of this type of bacteria, (Paulsen, et al., 2019). This species is most abundant in river water, stagnant water, and in the soil.

The technique of using heat shock shock heat for bacterial colonies is one of the means affecting the living mass and numbers of bacterial colonies due to the possibility of its effect in increasing the building of proteins and the replication of DNA. The mechanism of the electric shock technique is based on exposing the biological sample to a thermal shock at multiple thermal levels and for very short times. The results depend on the intensity of the level applied and the duration of time used (Copp, et al., 2020).
Klebsiella bacteria are characterized by their ability to fix atmospheric nitrogen in a non-symbiotic manner (Free living nitrogen fixing bacteria), which means Non symbiotic. Studies have confirmed the susceptibility of these bacteria to this vital ability, and (Christopher et al. 2021) indicated that this genus has an important role in nitrogen fixation (Biaosheng, et al., 2019) also referred to the same results above . K. pneumonia and K. oxytoca are the most important species of the genus Intestinal Klebsiella, which are capable of fixing atmospheric nitrogen and supplying it to plants. They are called Diazotrophs(Mukherjee, et al.,2021) showed through his research that these bacteria are of great importance from the agricultural point of view because of their impact on increasing plant yield and their ability to stick to plants in high numbers due to their lack of flagella.

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Rhizobium Definition

"Rhizobium is a soil bacteria that fixes atmospheric nitrogen once it finds a base inside the roots of the leguminous plants**"**. Rhizobium is the bacteria that live in symbiotic association with the root nodules of the leguminous plants. Fixation of nitrogen cannot be done independently. That is why rhizobium requires a plant host. Rhizobium is a vital source of nitrogen to agricultural soils being toxic in nature. Is rapidly absorbed into organic compounds.

Rhizobium

Nitrogen fixation helps in increasing soil productivity and soil fertility. The various behavioural factors such as drought stress, nutrient deficiency, salt stress, fertilizers, pesticides of nitrogen-fixing systems are reviewed.

Classification of Rhizobium Bacteria

Rhizobium can be classified on the basis of the types of the plant they are associated with and also the rate of growth. Few species of Rhizobium bacteria include (Wekesa, et al.,2022) :

- *Rhizobium leguminosarum*
- *Rhizobium alamii*
- *Rhizobium lantis*
- *Rhizobium japonicum*
- *Rhizobium trifolii*
- *Rhizobium phaseolii*
- *Rhizobium smilacinae*

Materials and methodology

(Harry and Paul, 1991) method was used to study the effect of heat treatment on the preparation of bacterial colonies. K. pneumonia bacteria were grown on MacConkeye medium supplemented with antibiotics, and YEM liquid medium was used to grow S. meliloti bacteria, and the density of the suspensions for both types used was estimated spectrophotometrically at the wavelength of 600 nm.

Sequential dilutions of the studied bacterial suspensions, K. pneumoni and S. meliloti, 8 ml was taken from the seventh dilution of suspensions, then each volume of suspension was distributed in eight tubes at a rate of 1 ml / 5 ml sterile test tube. The tubes were closed with their caps and the temperatures were selected (0.40,45,50,55,60,65,70) degrees Celsius and a duration of exposure of 5 minutes to treat K. pneumoni bacteria.

Suspensions of (S. meliloti) bacteria were also exposed to temperatures (30,35,40,45,50,55,60) degrees Celsius for 5 minutes, and a water bath was used to choose temperatures for heat shock by immersing the base of the test tube containing the bacterial suspension in The water bath, whose temperature was previously fixed at the required temperature in each treatment, for a period of 5

minutes. After the completion of exposing each bacterial sample to thermal shock, the bacterial sample tube is lifted from the water bath and its base is immersed in a beaker containing water at laboratory temperature, and this continues for all samples under study.

After the completion of exposing all samples, 0.1 ml of each bacterial sample was taken and spread on the surface of 15 ml of the selected MacConkey solid environment medium for bacteria K. pneumoni and on the surface of 15 ml of solid YEM medium suitable for bacteria S. meliloti in 9 cm petri dishes available in the laboratory. The samples were kept inverted in the incubator at 37°C conditions for 24 hours in the case of K. pneumoni and S. meliloti in the incubator at 28 °C for 24 hours in complete darkness. The samples were monitored and the data obtained from this study were recorded.

To test the effect of heat treatments on the incidence of bacterial conjugation, the study included two cases:

The first case: Pre-exposure of the bacterial suspensions to heat treatments before conjugation. The same bacterial suspensions were used, as a 6 ml suspension of K. pneumoni was prepared and another similar one from S.meliloti, each of which was divided into 6 samples at 1 ml/sample. Samples of each bacterial species were subjected to the required heat treatment by immersing the base of the test tube containing the bacterial culture in the water bath for 5 minutes and then immersing it in a container containing room temperature water. A sample was taken from the suspension of K.pneumoni bacteria and another from the suspension of S.meliloti bacteria and the two samples were mixed after exposure and 1 ml of liquid nutrient medium was added to them, and so on for the rest of the samples.

After completing the preparation of the conjugation mixture, the samples were incubated at 280 °C for 3 hours in dark conditions, then (0.1) ml of each conjugated mixture was taken and spread on the surface of 15 ml of solid YEM nutrient medium supplemented with the final concentrations of antibiotics 5 µg/ml rifadin. 100 g/ml of cephalexin were added to a 9 cm diameter plastic petri dish. The sample dishes were kept upside down in the incubator at 280 °C for 24-48 hours in dark conditions.

Second case :

After preparing the conjugation mixture and subjecting it to the thermal coefficient, the seventh probe was selected from the suspension of S.meliloti bacteria and the suspension of K.pneumoni bacteria from each of them for the conjugation procedure, where 6 ml of the suspension of S.meliloti bacteria was mixed with 6 ml of the suspension of K.pneumoni bacteria and then 5 ml of YEM liquid nutrient medium were added to them.

The conjugation mixture was divided into six samples of 3 ml/sample in a 5 ml test tube. The samples of the conjugation mixture were exposed to thermal treatment temperatures of (30,35,40,45,50) degrees Celsius for 5 minutes, in addition to the control sample (without exposure) three hours.

Each tube was lifted and placed in a container containing water at room temperature, then the samples were kept in the incubator at a temperature of 28 degrees Celsius for three hours. Then

0.1 ml of each sample was taken and spread on the surface of 15 ml of solid YEM medium containing the final concentrations of rifadin and pyrroxene in a plastic Petri dish (sterilin U.K.) with a diameter of 9 cm. The dishes were incubated upside down in the incubator at a temperature of 28 °C for 24-48 hours in the dark.

RESULTS AND DISCUSSION :

It was clear from the data of the treatment of exposing the seventh dilution of the bacterial suspensions of both types under study, K pneumoni and S. meliloti to different levels of temperatures and grown on solid culture medium selected for each type , The effect of both sexes on their tolerance of electric shock varied in terms of the difference in the growth rate and the formation of colonies at some temperatures and not growing at all "in others.

Table (1)the effect of exposing the intestinal bacterial suspension *K. pneumonia* to a set of heat treatments (for a period of 5 minutes) on the number of colonies formed when grown on appropriate nutrient media.

Heat treatment Celsius degree/5mintes	**Total number of bacterial colonies formed**
	K. pneumonia
control	220
40	225
45	157
50	70
55	18
60	2
65	0
70	0
0:No growth The values in the table represent the rate of duplicates	

The results in Table (1) for K pneumoni bacteria indicated a variation in the growth of bacterial colonies, which was inversely proportional to the increase in temperature and when the exposure time was fixed (5 minutes), the number of K. pneumoni colonies at the temperature (400) was 196 bacterial colonies and at the temperature (650) was (3) bacterial colonies, while no growth appeared after this temperature, which is consistent with what was found by (Wang , et al., 2020).

Table (2)the effect of exposing the intestinal bacterial suspension *Sino-meliloti* to a set of heat treatments (for a period of 5 minutes) on the number of colonies formed when grown on appropriate nutrient media.

Heat treatment Celsius degree/5mintes	**Total number of bacterial colonies formed**
	Sino-meliloti
control	170

40	175
45	169
50	122
55	80
60	20
65	10
70	0
0:No growth **The values in the table represent the rate of duplicates**	

The results in Table (2) also indicated that S. meliloti bacteria showed resistance to many low and medium temperatures according to its growth in good numbers on its selected culture medium YEM and its growth is inversely proportional to the increase in the temperatures used in the exposure during the exposure period, as the growth completely disappeared" at the maximum temperatures used as referred to in Table (2).

Table (3) the effect of exposing bacterial suspensions (*K. pneumonia* and*Sino-meliloti*) to a set of heat treatments before or after mixing them on the occurrence of conjugation and its return

Heat treatment	**before mixing**		**After mixing**	
Celsius degree	**Colonies numbers**	**coupling frequency ($x10^{-7}$)**	**Colonies numbers**	**coupling frequency ($x10^{-7}$)**
control	**5**		**------**	**------**
30	**7**	**0.032**	**10**	**0.087**
35	**9**	**0.038**	**9**	**0.035**
40	**8**	**0.037**	**8**	**0.032**
45	**3**	**0.030**	**4**	**0.019**
50	**2**	**0.02**	**2**	**0.014**
55	**1**	**0.015**	**1**	**0.01**
60	**0**	**0**	**0**	**0**
70	**0**	**0**	**0**	**0**

The data in Table (3) also indicates an increase in the conjugation frequency between S. meliloti and K pneumonia when exposed to heat treatment after mixing them compared with the conjugation frequency between these two types of bacteria without exposing them (comparison) to heat treatments.

The highest conjugation frequency for the two types of bacteria mixture was 36.9 x 10-1 at a temperature of 30 degrees Celsius, and the lowest conjugation frequency was for the conjugation mixture 19 10-1 at a temperature of 45 degrees Celsius. These results are higher than the conjugation frequency value of the comparison treatment, which was 5.1.

The results of exposing the conjugate before mixing the bacteria indicated that the achieved frequency was 32.3 x 10-1 compared to the comparison factor 7 x 10-1.

The results obtained as a result of exposing the two types of bacteria involved in the conjugation and increasing the number of conjugated bacteria as well as its reflection on the conjugation frequency produced between K.pneumonia and nitrogen-fixing bacteria S. meliloti, could be explained by the increase in cellular root permeability (Zeng, et al. ,2019). Which gives a greater chance of obtaining the requirements from the culture medium, and that the decrease in the number of conjugated bacteria and the frequency of conjugation at high temperatures is due to the fact that high temperatures caused the death of cells due to the damage of proteins or the destruction of enzymes.

The use of heat shock technology aims to subject the bacterial species under study to a range of temperatures as heat shock, as many studies indicated that these treatments have positive effects for some bacterial types. In this study, it was found that the effect of some short-term treatments with pathogenic bacteria K. pneumoni and nitrogen-fixing bacteria S. meliloti was reflected in the increase in the number of bacteria in both genus . This may be due to the fact that heat shock leads to an increase in cellular root permeability, and this is what was obtained by (Rocha, et al. ,2021). This encourages cells to obtain nutritional requirements from the culture medium and thus to increase the division of bacterial cells and increase their numbers, and heat shock has stimulated the construction of proteins called Heat Shock Proteins. This is indicated by (Zhang, et al. 2020), as the stability of the cell's internal environment and its physiological state is due to the proteins synthesized by heat shock genes, and that high temperature has an effective role in the formation of these proteins, in addition to the positive effect on the growth and division of bacterial cells (Zhang, et al., 2020).

One of the requirements for growth at high temperatures is the presence of these genes, and (Uddin, et al. 2020) indicated that temperature and cultur media are basic requirements for the physiological activity of bacteria. The negative effect of heat shock and its effect on the decrease in the number of bacterial colonies depends on the temperature used and its duration.

The heat shock mechanism is controlled by three genes, which lead to an overproduction of the heat shock protein (Kmiecik and Mayer, 2022), and this supports our obtaining an increase in the number of bacterial colonies at a non-excessive temperature. The death of bacterial colonies is attributed to the effect of high temperatures on protein damage and enzyme breakdown (Yu, et al., 2021). We recommend researchers to use the appropriate temperature for each genus when growing these colonies.

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