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THE EFFECTS OF ELECTRICAL SHOCK ON THE GROWTH OF THE BACTERIAL SPECIES SINORHIZOBIUMMELILOTI AND KLIBSELLA PNEUMONIAE AND CONJUGATION BETWEEN THEM

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Abstract

The main objective of this study was identifying the effect of Electrical shock 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 (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 an electric shock using voltages (200,250,300). Moreover, in seven-time periods.The results showed that the electrical treatments had appositive effect on increasing the number of bacterial colonies, as well as increasing the conjugation frequency between both types of bacteria.

Key words: Electric shock,Klibsella Pneumoniae, conjugation

INTRODUCTION

Klebsiella bacteria are Gram-negative bacilli belonging to the family Enterobacteriaceae, which are widespread in nature. They have two types of common habitats, as they are common in different environments, mainly surface waters. In this respect, Klebsiella bacteria are similar to Enterobacter and Citrobacter but not Shigella spp or coli. E, which is common in humans but not in the environment.The Klebsiella bacterium was known as a common cause of pneumonia, and it was named after the German scientist KlebsEdwan, who was the first to isolate it in 1834, then this bacterium was diagnosed for the first time by the scientist Friedlander in 1882 (John, Adam 2022) and (Jose and Joana 2019).

Klebsiella is a genus of bacteria. It is a rod-shaped, non-motile, non-spore-like bacterium with a prominent capsule composed of polysaccharides. It was named by the scientist Edwin Klebbs (Edwin Klebbs). It causes many diseases such as pneumonia, urinary tract infection, septicemia, etc. . Klebsiella is classified into three types, according to the clinical importance and biochemical interactions, which are as follows:K. pneumonia , K. ozaenae, K. rhinoscleromatis ,K. oxytoca,. planticolaK. , K. terrigena , K, ornithinolytica. Klebsiella is scientifically classified into the bacterial kingdom, the order of intestinal bacteria, genus Klebsiella, and the scientific name Klebsiella.. (Sylvain, et al., 2021). It grows in conditions up to 37°C and pH 7.2. This bacterium resides in the intestines of hosts, both animals and humans, and thrives on Maconkey medium containing lactose and bile salts, as well as nutrient agar medium.

Bacterial colonies of the genus Klebsiella are distinguished by their mucous, viscous texture

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and tend to merge after a period of incubation, so it is difficult to count the bacterial cells. pneumonia is of a great importance from the chemical point of view, as it gives a positive test for the consumption of citrate and a negative test for the methyl red and indole tests, and this is a detector of the presence of this type of bacteria (Salauddin et al.2019), The individuals of this species are frequently found in the waters of rivers, stagnant waters and in the soil.

Klebsiella bacteria are characterized by their ability to fix atmospheric nitrogen in a non-symbiotic manner, which means non sym biotic, and studies have confirmed the ability of these bacteria to have this vital ability .

(Christopher et al. 2021) indicated that this genus has an important role in nitrogen fixation and K. pneumonia and K. oxytoca are among the most important species of the genus Klebsiella enterica that are able to fix atmospheric nitrogen and provide it to the plant, and therefore they are called diazotrophs(Trinetra et al. 2021) showed through his research that these bacteria are of great importance from the agricultural point of view because of their effect on increasing the yield plant and its ability to adhere to plants in high numbers due to its lack of flagella.

RHIZOBIUM DEFINITION

"Rhizobium is a soil bacterium that fixes atmospheric nitrogen once it finds a base within the roots of 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.Nitrogen fixation helps in increasing soil productivity and soil fertility.

Classification of Rhizobium Bacteria : Rhizobium can be classified based on the host plant species as well as on the basis of growth rate into the following (Halima and Kawtar , 2014):

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

Possible role of electric shock in conjunction efficiency :

Electroporation is an important technology for genetic manipulation in prokaryotic and eukaryotic cells (Zhenkui Qin et al., 2016), it has been widely used to introduce various molecules such as proteins and plasmids into cells (Smith et.al, 2004) and nucleic acids (Fromn

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et., 1986). al) and ions (Kinosit & Tsang 1977) and their penetration into living cells, whether animal, plant, protoplast, fungal, bacteria, yeast cells, or hormones (Zhenkui Qin et al., 2016). Plasmid DNA was also introduced into human cells using electrophoresis (Gersch & Ray, 2002).The injection of these elements into these cells requires the removal of natural barriers represented by cell walls and plasma membranes that hinder or prevent the entry of the desired molecules into the recipient cells, during which the biological sample is subjected to an electric shock by applying certain voltages for very short periods of time (Purvis et.al, 2001), and the efficiency of the Technique depends on the intensity of the applied field, the time required, and the shape and size of the cell under experiment (Urszula et al. 2017). The efficiency of the technology depends on the intensity of the electric applied, the time required, and the shape and size of the cell under experiment (Urszula et al. 2017). Its mechanism of action is based on the principle that the electric field applied to the cell membrane increases its permeability (Bill, 2003, 2005; Heller), causing the formation of holes that look like openings temporarily (Lee, 1989).Some researchers (Joersbo & Brunstedt, 1991) have mentioned different explanations for the formation of these holes in the plasma membrane (Pavlin et.al, 2006).

Some sources indicate that this technique is commonly used with bacterial cell.The genetic transformation with the plasmid improved Streptococcus lactis and S. Cremori by adopting the electrical treatment (1988, Powell et.al).In another study, plasmid DNA was inserted into Staphylococcus,Propionibacter, Listeria, Enterococcus, Pediococcus, Lactobacillus, acidophilus (Luchanskyeal, 1988).The same study reported that the possibility of transferring DNA with this technique facilitates the methods of DNA recombination and the jumping technique in Gram-positive and Gram-negative bacteria (Fiedler & Wirth, 1988).

MATERIALS AND METHODS

The method of Winterbourne (Winterbourne, et al., 1988) was based on studying the effect of electric shock treatment on the preparation of bacterial colonies, where K. pneumonia bacteria were grown on McConkey medium supplemented with antibiotics, and YEM liquid medium was used to grow S. meliloti bacteria. The density of the suspensions for both types used was estimated spectrophotometrically. At a wavelength of 600 nanometers.

Serial dilutions of the bacterial suspensions under study (K. pneumoniae and S. meliloti) were carried out, and 22 ml of the inoculum grown in McConkey medium at the age of 24 hours was taken at the seventh dilution of the species (K. pneumoniae) growing in McConkey liquid medium supplemented with the two antibiotics (rifadin and carbenicillin (or kanamycin)), and a suspension of S. meliloti bacteria growing in YEM liquid medium, at the same dilution, and their intensities were estimated spectrophotometrically at 600 nm.

The bacterial suspension (for each type) was distributed to 22 samples of 1 ml per test tube were transferred with the contents of each sample to the sterile chamber of the electro puncture device. (Ting, et a 2019).

The three voltages 200, 250, and 300 volts were selected to expose the bacterial samples for the following periods: 0.5. , 1,2 , 3 , 4 , 10 , 20 milliseconds.After drying , the plates were

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incubated upside down in the incubator at 37 °C / 24 hours for K. pneumoni and at 28 °C / 24 hours for bacteriaS.meliloti in dark condition.

Testing the effect of electrical treatment on the occurrence of bacterial conjugation between S,meliloti and K.pneumoni:

The effects of electrical treatment were tested on the occurrence of conjugation between the two types of bacteria to be conjugated in two cases:

The first case: Exposing the coupling mixture to electrical treatment

Mix 25 ml of K. pneumoniae suspension with 25 ml of bacterial suspensionS. meliloti with 25 mL of YEM culture medium, then Dividing the total conjugation mixture (75 mL) directly into 5 sections, 15 mL/sample in a 25 mL vial. The samples were incubated in an incubator at 37 degrees Celsius for a period of time (0, 30, 60, 90, 180 minutes).

The suspension of the first section was taken at the time of zero, with a volume of 15 ml, and divided into fifteen samples, at a rate of 1 ml / sample in glass bottles of 5 ml, and divided into three groups, each group containing five samples, and treated as follows:

The first group: Itincludes 5 samples that were exposed to a voltage of 200 volts and for the periods of time 0.5,1.0, 2.0, 3.0, 4.0, 10, 20,milliseconds, respectively.

The second group: includes 5 samples that were exposed to a voltage of 250 volts and for the same exposure times

The third group: includes the last five samples that were exposed to a voltage of 300 volts and for the same exposure times as above.

As for the bacteria S. meliloti, 5 ml of the seventh dilution was taken and mixed with. pneumonia and 5 ml of YEM liquid medium were added to the mixture, then the conjugation mixture was divided into 15 sections of 1 ml/sample in a 5 ml vial. The first group, which includes 5 samples, was exposed to 200 volts and for exposure times of (0.5 ,1 ,2 ,3 ,4 ,10 ,20) milliseconds..

The five samples in the second group were exposed to a voltage of V250 and for the same durations of exposure. As for the samples of the third group, they were exposed to a voltage of V300 and for the same durations of exposure mentioned, where each sample was placed in the device room and exposed to the selected treatment in the electric perforation device (Ting, et al. 2019).

After exposing all the samples, they were placed in the incubator at 28 °C for three hours in the dark, then 0.1 ml of each sample was spread using a glass rod on the surface of 15 ml of solid YEM medium containing a final concentration of 5 µg / ml of rifadin and 100 µg/ml of carbenicillin (or kanamycin).The plates were incubated upside down in the incubator at 28°C for 24 hours in dark conditions. The comparison sample was represented by spreading 0.1 ml of the non-electrolytically treated conjugation mixture on the surface of the same medium and incubated under the same conditions indicated.

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The second case: pre-exposing the two types of bacteria to the electrical treatment before mixing them.

The suspension of each of the K. pneumoniae, and S. meliloti into 15 specimens per 5 ml vial was divided separately.

The samples of each bacterial species were placed in three groups, one group included to samples of the bacterial suspension, and each sample was transferred to the sterile room of the electro puncture device (Ting,et.a 2019) ,and exposing them individually by dividing them into three groups, as before, in terms of the voltages used and exposure times

The first sample previously treated at 200 V for 0.5 ms of K. pneumoniae was mixed with the first sample of S. meliloti previously treated with the same treatment and 1 ml of YEM medium was added to them, in the same way as the first sample previously exposed to the treatment 200 volts for 0.5 milliseconds of K. pneumoniae bacteria with the first sample of S. meliloti that had previously exposed to the same treatment, and the same method was adopted in mixing the rest of the electrically treated samples with the appropriate selected treatments. The all conjugation samples were incubated in the incubator at 28 °C conditions for three hours, then 0.1 ml was taken from each sample and spread on the surface of 15 ml of solid YEM medium in a 9 cm diameter plastic Petri dish (Sterilin U.K) containing streptomycin with a final concentration of 5 µg/ml and cephalexin with a final concentration of 100 µg/ml, and after drying the plates, it was incubated upside down in the incubator at 28 degrees Celsius for 24 hours.

RESULTS AND DISCUSSION

The Effect of exposing bacterial suspensions to electrophoresis

The results of exposing K. pneumoniae suspensions of intestinal bacteria to a set of electrical treatments shown previously, followed by culturing them directly on the appropriate nutrient medium(nutrient agar or Maconky)indicated that these bacteria tolerated the electric shock to which they were exposed, indicative of their growth on their nutrient media after 24 hours of cultivation, and a clear increase The numbers of bacterial colonies varied according to the treatments used(Table 1).

Table 1: the effect of exposing the intestinal bacterial suspension K. pneumonia to a set of electric treatments on the number of colonies formed when grown on appropriate nutrient media

Electric treatment v/msec	Total number of bacterial colonies formed
	K. pneumonia
Comparison (without exposure)	**65**
200/0.5	87
200/1.0	42
200/2.0	25
200/3.0	83
200/4.0	89
200/10.0	90

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200/20.0	110
250/0.5 250/1.0 250/2.0 250/3.0 250/4.0 250/10.0 250/20.0	78 95 115 125 115 139 137
300/0.5 300/1,0 300/2.0 300/3.0 300/4,0 300/10.0 300/20.0	90 67 78 122 144 110 114

The values in the table represent the rate of three replicate

Table 2: the effect of exposing the intestinal bacterial suspension Sino-meliloti to a set of electric treatments on the number of colonies formed when grown on appropriate nutrient media

Electric treatment v/msec	Total number of bacterial colonies formed
	Sino-meliloti
Comparison (without exposure)	**15**
200/0.5 200/1.0 200/2.0 200/3.0 200/4.0 200/10.0 200/20.0	18 22 20 27 35 28 30
250/0.5 250/1.0 250/2.0 250/3.0 250/4.0 250/10.0 250/20.0	30 22 26 34 38 24 18
300/0.5 300/1,0 300/2.0 300/3.0 300/4,0 300/10.0 300/20.0	20 23 37 35 30 20 23

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The values in the table represent the rate of three replicate

The results of exposing suspensions of bacteria (S. meliloti) that infect plants to the set of electrical treatments used above indicate an increase in the number of bacterial colonies at some voltages (Table 2).

Table 3: the effect of exposing bacterial suspensions (K.pneumoniaandSino-meliloti) to a set of electric treatments before orafter mixing them on the occurrence of conjugation and its return

v/msec	Colonies numbers	coupling frequency ($x10^{-7}$)	Colonies numbers	coupling frequency ($x10^{-7}$)
Comparison (without exposure)		9		0,31
200/0.5	13	0.38	14	0.43
200/1.0	1	0.01	0	0
200/2.0	1	0.01	0	0
200/3.0	21	0.35	22	0.36
200/4.0	22	0.51	24	0.38
200/10,0	17	0.58	18	0.29
200/20.0	16	0.34	16	0.27
250/0,5	15	0.34	17	0.56
250/1.0	14	o.32	16	0.44
250/2.0	16	0.35	15	0.34
250/3.0	18	0.40	17	0.48
250/4.0	20	0.34	21	0.35
250/10.0	16	0.35	14	0.36
250/20.0	14	0.32	16	0.35
300/0,5	6	0,21	3	0.20
300/1.0	10	0.35	9	0.35
300/2.0	24	0.34	23	0.37
300/3.0	19	0.33	19	0.48
300/4,0	17	0.48	18	0.50
300/10.0	12	0.36	11	0.43
300/20.0	10	0.35	9	0.25

The values in the table represent the rate of three replicate

(Table 3) shows the results obtained from exposing suspensions of bacteria that infect the plant, S. meliloti, and intestinal bacteria K.pneumoniae, at a ratio of 1:1, before mixing them afterwards, to a set of electrical treatments mentioned above, where exposure to electrical

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treatments encouraged coupling between the two types of bacteria and raised its efficiency due to the increase that occurred. In the conjugation frequency, this shows that the electrical treatment has clear and positive effects in increasing the conjugation frequency. To discuss these results, we explain that the increase achieved in the total numbers of colonies of the bacterial species used in the current study, which consists of cultivating their suspensions that were previously exposed to different electric fields, may be due to the stimulating effect of this treatment, which lies in increasing the building of proteins and the doubling of nucleic acidDNA leading to increased cell division (Panel et al., 2022) In addition to the effect of electric shock on increasing the permeability of bacterial cell walls, three possible hypotheses have been put forward to increase this permeability. A study (Chizmadzhev 1999) indicated that mechanical pressure on the cell membrane resulting from exposure to an electric field leads to its permeability.Another study (Shadeeb,Ahmed 2020) explained that the action of the electric shock is concentrated in the sites of charges and the dual polarization of the membrane, leading to a decrease in the degree of tolerance of the membrane to pressure and an increase in the level of defects in its structure, ending with the formation of holes. Additional explanations (Dimitrove 1984) suggested that the kinetic characteristics of the bilayer membranes and the movement of the monolayers that make up the membrane lead to slippage or ripples in the thickness of the membrane, and the electric shock increases these ripples in the structure of the membrane, causing their succession in the formation of these holes.The accompaniment of electric shock to the conjugation process between K. pneumoniae bacteria. And the bacterium S. meliloti led to an increase in the conjugation frequency compared to the comparison sample. This effect is likely to be attributed to the fact that the electric shock used accelerated the conjugation and increased its incidence due to the availability of holes formed by the action of the shock, achieving an increase in the chances of transferring plasmids from the donor cells to the recipient cells (Chloe et al. 2020). One study confirmed that the electric shock creates temporary holes in the plasma membrane or the walls of treated cells, which facilitates the entry of substances into the cell.(2003, Sugden), and that high voltages allow DNA molecules to be polarized from the solution into the cells because they make the membrane permeable for a short time, and this technique was also used to introduce peptides and amino acids (Olofsson et.al, 2003). and ions, drugs, and some proteins, antigens, and nucleic acids into bacterial cells (Gehl,2003).This technique may fail in some cases, and this may be due to the length of the electrical pulse used, which leads to the occurrence of large holes that are difficult to repair, causing physiological damage or tearing and death of the cell.

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