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Control the Activity of Erwinia Amylovora Bacterium by Magnetic Field

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Abstract: ***We study the effect of promoting low magnetic field exposure on antimicrobial activity against Erwinia amylovora (E. Amylovora) bacterium. For this purpose, we treated E. amylovora at 28°C with two intensities (0.2T and 0.5T) of the magnetic field for one hour to see the intensity of bacterial growth inhibition. Bacterial growth was measured in experimental samples. The results indicate that exposure to E. amylovora to 0.2T and 0.5T for 1 hour reduced bacterial growth. Dielectric relaxation studies of treated and untreated alive bacterium showed changes of the surface charge distribution of the bacterium which indicates changes in the receptor properties and hence cell communications. The study showed bacteria treated with the magnetic field which was exposed for an hour, adjusted its cellular activity, slowing the rate of growth and affecting microbial pathogenicity. The effect was higher at 0.5 T than 0.2 T. We concluded that magnetic field formation is a promising technique for treating this type of bacteria.***

Keywords: ***Magnetic field, bacteria, Dielectric relaxation, E. amylovora***

I. INTRODUCTION

This is an International reputed journal that published research articles globally. All accepted papers should be formatted as per Journal Template. Be sure that Each author profile (min 100 word) along with photo should be included in the final paper/camera ready submission. It is be sure that contents of the paper are fine and satisfactory. Author (s) can make rectification in the final paper but after the final submission to the journal, rectification is not possible. In the formatted paper, volume no/ issue no will be in the right top corner of the paper. In the case of failure, the papers will be declined from the database of journal and publishing house. It is noted that: 1. Each author profile along with photo (min 100 word) has been included in the final paper. 2. Final paper is prepared as per journal the template. 3. Contents of the paper are fine and satisfactory. Author (s) can make rectification in the final paper but after the final submission to the journal, The gram-negative E. amylovora bacterium is a pathogen bacteria that destroys plant especially Rosaceae family species, and is the leading cause of plant fire blight disease [1].

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Infected parts of the plant have sticky droplets, soaked in water, which turn dark green and eventually turn brown and then black [2]. Fire blight disease not affected by currently synthetic compounds and therefore fire blight control is difficult. The use of antibiotic methodology carries the risk of improving pathogen strains [3,4].

Researchers are seeking to discover new ways [5] especially environmentally friendly methods with no residue [6].

It has been determined the effects of magnetic fields on bacteria. For example, Escherichia coli had no effect when exposed to weak electromagnetic waves.

Additionally, in studies of bacteria, acceleration of hydrogen peroxide by catalase has been reported in the 8 T magnetic field, and this can dissolved oxygen and affect catalase indirectly [7].

Bacterial strains have a different effect depending on the shape and properties of Gram staining. This finding can help to develop new ways to maintain global food production. Low-frequency electromagnetic field induce the production of free radicals in organic systems.

These free radicals can alter enzymatic pathways within cells by interfering with some metabolic processes.

This may interfere with DNA preparation or replication mechanisms, also it can overlap with protein and fat structures as external MF can provide protein alignment.

Protein activity can be changed with small differences in protein structure , the aromatic rings, β-sheet, α-helix and even peptide bonds [8] [9].

The purpose is to reduce the negative effects of conventional antimicrobial agents and their significant adverse effects on both humans and the environment. At this study, the effect of alternating magnetic field was studied.

We used a field strength of 0.2T and 0.5T on the E. Amylovora bacterium, then we evaluated the effect on its growth. Possible mechanisms underlying toxicity were examined by measuring dielectric relaxation and bacterial pathogenicity testing.

II. MATERIALS AND METHODS

A. Erwinia Amylovora growing conditions

The *E. amylovora* strain was gently given by the Brown Potato Rot Project (PBRP). Bacteria were encapsulated on medium King's B agar.

It was added in the incubator at 28 °C for 60 hours. For long-term storage, Luria-Bertani broth (LB) is ideal for storing bacteria, as it is improved by 20% glycerol and stored at -80 °C [10], also, plated on King's B broker (KB) to revive.

B. Magnetic Field Exposure Source

The magnetic field is provided alternately by a vertical coil with an internal diameter of 9.5 cm, and it is connected to an adjustable insulation transformer operating at 50/60 Hz, the electric current 2A and the main supply (220-230 V). Suspension of samples placed inside the coil. The system, locally manufactured at the Physics Laboratory at VACSERA, Egypt (Fig. 1 schematic diagram).

Fig. 1.The system that provided the magnetic field.

C. Determination of growth inhibition strength

E. amylovora suspension was freshly prepared using a 3 ml sterile broth with 100 µl of bacterial suspension in a closed glass test tube with a rubber stopper. Three groups (*E. amylovora* suspension with 15 tube samples per group), one control group (untreated) and two groups were treated with 0.2T and 0.5T of the LMF source for an hour exposure period at 25 °C. At the end exposure, all samples were added to the incubator at 28 °C for 60 hours during which the optical density (OD) was measure for the bacterial suspension every 6 hours interval at wavelength 600 nm (the reference was the sterile king's B broth medium) using a spectrophotometer (Jenway 6300 UK). Three replicated were carried out for each treatment and the mean average was calculated. After that, bacterial suspension OD was created as a function of incubation time to obtain distinct growth curves for all treatments, after that different dilutions (10^{-6} and 10^{-7}) from each treatment were plated on a king's B agar plate to set the count of cells that revealed the field strength caused maximum growth inhibition by plate counting technique [11].

D. Dielectric relaxation measurements for the bacterial cells

Dielectric relaxation of the samples was measured by Loss Factor Meter (HIOKI 3532 LCR Hi TESTER, version 1.02,1999, Japan) over the 10Hz-MHz frequency range. Using sample cell (PW 9510/60, Philips) contains two square platinum electrodes of 0.64 cm2 areas and separated by 1cm. The temperature of the sample was kept at (25 ± 0.1ºC) during the experiment. For each frequency, the samples' capacitance and resistance were measured. The run was done three times then the mean was calculated. For each sample, the relative permittivity (έ), loss tangent (tan δ), dielectric loss (ε″), conductivity (S) and relaxation time (τ) were calculated for every frequency using the following equations.

$\varepsilon' = C\, d / \varepsilon_o A$ Eq. (2-1)

$(\delta) = 1/2\pi f C$ Eq. (2-2)

$\varepsilon'' = \varepsilon'(\delta)$ Eq. (2-3)

$\tau = 12\pi f_c$ Eq. (2-4)

$\sigma = 2\pi f\, \varepsilon'' \varepsilon_o$ Eq. (2-5)

Where f_c is the critical frequency corresponding to the dispersion curves at the mid-point.

E. Statistical Analysis

The statistical software SPSS Version 16.0 was utilized to execute the statistical analysis. The P ≤0.05 was used to be statistically significant.

III. RESULTS AND DISCUSSION

A. Characteristics Growth of *E. Amylovora*

We exposed *E. Amylovora* bacterium to 0.2 and 0.5T until one hour to evaluate the magnetic field effect on *E. Amylovora* growth, and as shown in Fig. 2, the growth was found to be inhibited, compared to control, depending on magnetic field strength. Result indicates that magnetic field has antimicrobial effect for *E. Amylovora*. At the log phase (at 18 hours of culture), the treatment reduced the number of viable bacteria by 5% and 18% in magnetic strength 0.2T and 0.5T, respectively.

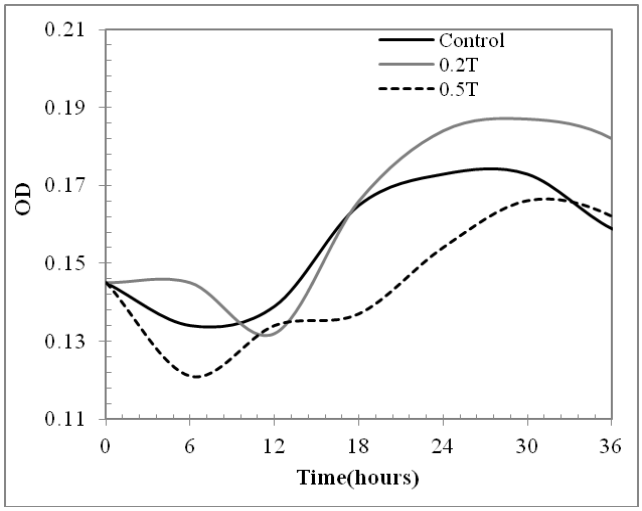

Fig. 2.The growth curves of a) the control *E. Amylovora* bacterium, b) The exposed *E. Amylovora* bacterium to 0.2 T for one hour, and c) The exposed *E. Amylovora* bacterium 0.5 T for one hour.

B. Dielectric relaxation

Figures 3 and 4 showed the relaxation curves of the *E. amylovora* bacterial suspension for control and treatment of 0.5 T LMF for one hour. The two figures represented the alteration of the relative of permittivity (ε′). Relative permittivity is drawn on the y-axis at left and electrical conductivity (S) on the y-axis at right with the change values of the frequency. Samples (control and treatment) have a dispersion of phenomena in the apparent frequency range. Conductivity (S), Relaxation time (τ) and dielectric increment (Δε') were calculated in the two samples. The mean values of S, τ, Δε′ and are shown in Table 1. The obtained data show changes in the dielectric properties in the treated sample of 0.5 T LMF for one hour compared to the control.

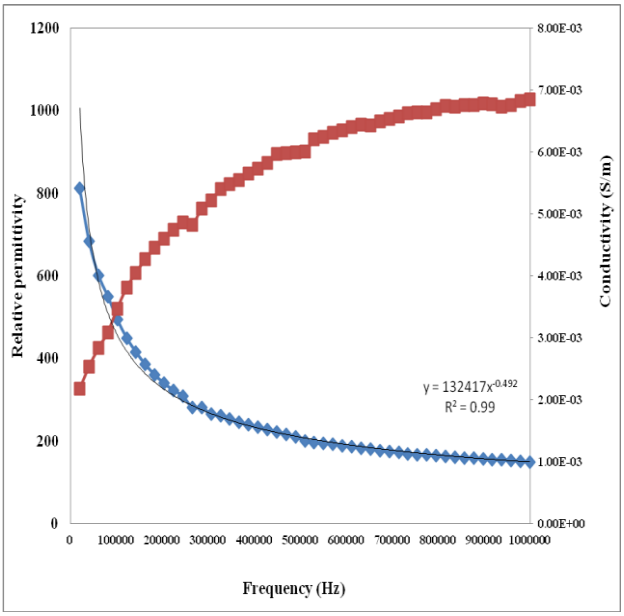

Fig. 3.The change in relative permeability ε' and electric conductivity S with respect to the frequency in the range10Hz-1MHz for control bacterial suspension.

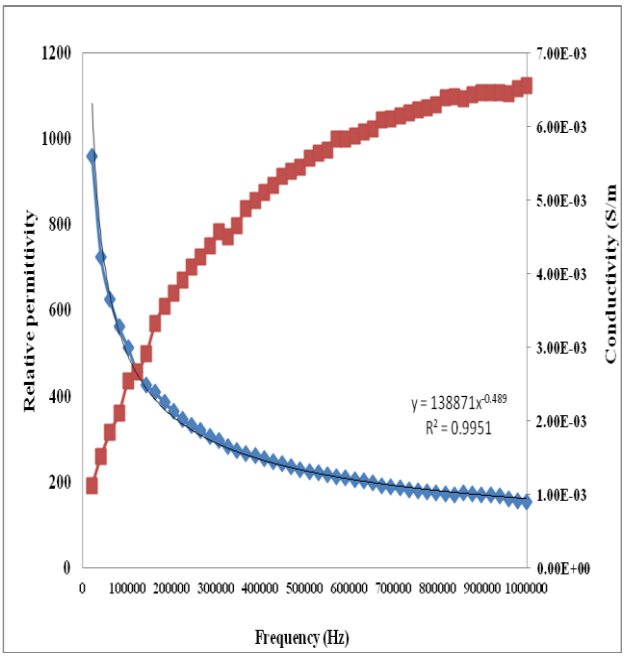

Fig. 4.The change in relative permeability ε' and electric conductivity S with respect to the frequency in the range10Hz-1MHz for bacterial suspension exposed to 0.5 T for 1hr.

Table- I: The difference in Conductivity (S), Relaxation time (τ) and dielectric increment (Δε') between the control and sample which exposed to 0.5 T for 1hr

Sample/Dielectric parameter	Critical frequency fc(Hz)	Relaxation time (τ) (x10^{-6}sec)	Δè=(èo - è∞)	Conductivity (S) At 1 MHz (x10^{7} S/m)
Control (unexposed)	(194419.499±4.5)	(5.14±0.026)	(662.55±0.86)	(331.28±0.001)
0.5 T L-MF for 1hr	(118906.06±2.5)	(8.4±0.02)	(804.61±0.57)	(402.30±0.0015)

Exposure to 0.5 T LMF may be assumed to interfere with biomedical signals resulting from the physiological functions of bacterial cells [12]. The ion channels in the cell membrane have been shown to have higher permeability when exposed to the magnetic field, it is accompanied by free radicals formation, cell wall dissociation, ejaculation of cytoplasm contents, and bleeding [13-17]. Changes in electrical charges on the surface of the treated cells may cause defects in the permeability of the cell wall and thus the ionic pumping mechanism. This leads to a loss of the common component of the cell which leads to a limited increase in the electrical conductivity (S) of a sample of treated bacteria, a limited increase in the mean values dielectric (Δέ) and an increase in the relaxation time (τ).

This study presented a novel method to help control fire blight disease by inhibiting the growth of *E. amylovora* using low-magnetic fields (L-MF). Current results indicated that exposure to 0.5 T of L-MF for one hour can inhibit *E. amylovora* growth (Fig. 2). The effect of L-MF inhibition can be explained by the interruption of the physiological functions of bacterial cells. This produce by interference of this field (with special strength) with the bacterial cells electrical biological that may inhibit or improve the ongoing physiological process [17]. The bacteria that were exposed to

L-MF were biologically affected. They have changes in cell division [16], changes in DNA expression and genetics [19,20] protein structure and synthesis [21] and transfer of different ions across the membranes of cell [22]. To highlight the alterations in the cell membrane that may occur as a result of exposure to the L-MF, dielectric properties and vivo study were preformed. Table 1 showed the measurements of dielectric relaxation, they indicate a clear increase in mean values of dielectric increment (Δέ), relaxation time (t) and electrical conductivity of the treated bacteria samples with L-MF. Since L-MF has the ability to adjust charge motions on the membrane, L-MF can influence cell membrane functions through ligand binding to receptors, ions flows and / or changing the membrane distribution of the intra membrane proteins [23]. These results conclude that *E. amylovora* exposed to 0.5 T of L-MF has changes in its structure that may change the activity of bacterial cell and the cells communication. In recognition of these results, we can suggest that using the L-MF exposure system in plant fields can be very useful in combating fire blight disease. Our results confirmed that L-MF had influenced the pathogenicity of E. amylophor by affecting the behavior of bacteria and the physiological processes.

IV. CONCLUSION

We concluded from the present results that the L-MF technique can successfully inhibit *E. amylovora* growth. It helps control fire blight disease without using of traditional agricultural techniques.

It enhanced structure changes in cell wall through altering the balance in electric charges of the membrane components. This is a new perspective that may help produce healthy crops and save the environment.

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