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Role of Electric and Magnetic Energy Emission in Intra and Interspecies Interaction in Microbes

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ABSTRACT

It is known that electromagnetic energy emission mediate the non-chemical distant cell to cell communication. However, its role in the intra and inter species interaction in microbes is not clear. This investigation has been initiated to study the possible role of electric and magnetic energy in the interaction between separated microbial cultures. Cultures of *Bacillus subtilis* and *Bacillus thuringiensis* were exposed to each other with glass barriers and their growth was measured. With improved DEI Meridian Energy Analysis Device (DEI MEAD) and Superconducting Quantum Interference Device (SQUID) we aim to understand the effect of energy emission on the growth of microbial population. We were able to record electrical and magnetic energy from each of the cultures with DEI MEAD which suggest that it may be a possible mode to exchange information. Further, it was observed that while the culture grew, the intensity of the electrical energy emission intensified. Also, there was significant growth in the population when two similar species of bacteria kept together in comparison to control. Whereas,

the growth reduced when two different species were exposed to each other. With SQUID, very weak magnetic energy from the culture could be recorded. However, it was observed that both species were diamagnetic in nature. We could infer here that the intra and inter-species energy exchange between the two cultures have a significant role in regulation of biological functions and hence, their growth. This could be further investigated with different species of microbes as it may be an aid to study primary and secondary infections in biomedical science.

Keywords: SQUID, microbes, electric energy, magnetic energy; intra and interspecies

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INTRODUCTION

Every living organism possesses a measurable subtle energy field around them¹. Different endogenous forms of energy including electrical and magnetic energy manifest themselves as a part of subtle field surrounding the cell^{1,2}. It is also evident from studies that such endogenous energy is responsible for regulation and organization of biological functions³⁻⁵. Microbial interactions have been a significant attribute responsible for their harmonized growth which in turn is a very important aspect in biomedical science³. Thus, all the factors affecting the population dynamics in microbes should be explored with holistic approach. It may aid to

understand the basic nature of primary and secondary infections enabling development of effective control strategies.

Communication being a vital characteristic of interaction in every living microorganism, contributes dynamically in their growth. It enables them to interact efficiently with both biotic and abiotic entities of ecosystem⁶⁻⁸. The non-chemical and non-contact cell to cell communication overcomes the limitations of chemical communication allowing the single cellular organisms to exchange information at a distance⁹⁻¹³. Significance of distant communication in microbial culture is controversial and warrants extensive research⁵. In the present study we attempted to investigate the non-chemical distant interaction in intra and interspecies bacterial cultures. Cultures of *Bacillus subtilis* and *Bacillus thuringiensis* were exposed to each other in a glass barrier to study communication. Their growth was assessed to observe the neighboring effect. To investigate how electrical and magnetic energy emissions from the cultures mediate communication, improved Meridian Energy Analysis Device (DEI MEAD) and Superconducting Quantum Interference Device (SQUID) were used.

In the present study, we observed that bacterial culture could affect the growth of the other culture placed at a distance. Keeping similar species adjacent to each other promoted their growth. Assessment of energy emission from cultures validates the role of electrical and magnetic in cell to cell interaction helping exchange of information with each other. Also, we could observe that with increase in their population, the measured energy intensified. With this study we were able to understand that microorganism's energy emission carry significant information and facilitate intra and inter-species interaction. They can identify their own kind and can regulate biological functions even at a distance. It may also be also noted that the bioradiation emission from cultures was observed as fingerprint emission of the bacterial species

under observation. However, this hypothesis needs further research and experimental authentication with other microbes.

MATERIALS AND METHODS

In this investigation, we have attempted here to study distant communication between two bacterial cultures separated by double walled normal silica glass to check chemical contact. Also, electric and magnetic emission was assessed to study its possible role in communication with improved Meridian Energy Analysis Device (DEI MEAD) and SQUID. In a self-designed experiment, we have studied the neighboring effect on growth (cfu/ml) of both *B. subtilis* and *B. thuringiensis* with DEI MEAD and SQUID. The cultures were exposed to each other for a given time period. The negative and positive controls were empty test tubes and sterilized nutrient medium paired with each microbial cultures. The cultures have been further subjected for the SQUID analysis and assessment for validation of the observations recorded. All the experiments were repeated five times to minimize error.

Bacterial culture

Pure culture of *B. subtilis* (MTCC, 441) and *B. thuringiensis* (MTCC, 4714) were procured from Microbial Type Culture Collection & Gene Bank (MTCC), Chandigarh, India. It was then subcultured in soyabean casein digest medium (HIMEDIA) with pH 7.2. To exclude chemical signaling, small test tubes were placed in bigger test tubes. Seven pairs were prepared with description in Table 1.

Table 1. Experimental setup of bacterial cultures to study physical communication

Set No.	A	B	Description
1	Blank	<i>B. subtilis</i>	An empty test tube paired with <i>B. subtilis</i> to study the growth (cfu/ml) without a neighbor.
2	Sterilized Media	<i>B. subtilis</i>	Sterilized media with <i>B. subtilis</i> to see possible effect of sterilized media on culture growth.
3	<i>B. subtilis</i>	<i>B. subtilis</i>	<i>B. subtilis</i> coupled with <i>B. subtilis</i> to investigate whether the presence of same microbial species affects each other's growth or not.
4	<i>B. subtilis</i>	<i>B. thuringiensis</i>	<i>B. subtilis</i> with <i>B. thuringiensis</i> to study the growth in presence of a different microbial culture.
5	Blank	<i>B. thuringiensis</i>	An empty test tube paired with <i>B. thuringiensis</i> to study the growth (cfu/ml) without a neighbor.
6	Sterilized Media	<i>B. thuringiensis</i>	Sterilized media with <i>B. thuringiensis</i> to see if sterilized media have any effect on culture growth.
7	<i>B. thuringiensis</i>	<i>B. thuringiensis</i>	<i>B. thuringiensis</i> with <i>B. thuringiensis</i> in neighbor.

Each of the sets closely held in pair was placed in a glass beaker. The beakers were separately incubated for 120 h at 37 °C in dark to check neighboring effect on each other (Fig.1).

**Figure 1: Maintenance of *B. thuringiensis* and *B. subtilis* in B.O.D.**

After every succeeding 24 h all these sets were subjected for total cell count and energy assessment with DEI MEAD. Later, small samples of 01 ml were taken from each culture for SQUID assessment after the total cell count. The experiment was done in five replicates and average value of bacterial population was used to plot growth curve.

Cultures and total cell count:

For the viable cell count, at time point 0 and after every 24 hour growth (cfu/ml) was estimated for 120 h. 100 µl from each culture was serially diluted to 10 fold and pour plated with plate count agar in duplicate. It was then incubated at 37°C for 24 h. The number of vegetative bacteria was estimated by calculating the average colony count of two agar plates per dilution.

Experimental setup & design

1. Biofield measurement devices and software

The improved Meridian Energy Analysis Device energy measurement setup was developed in electrical engineering lab of Dayalbagh Educational Institute, Dayalbagh, Agra, India^{14,15}. A block diagram is accompanied with MEAD assembly (Fig. 2).

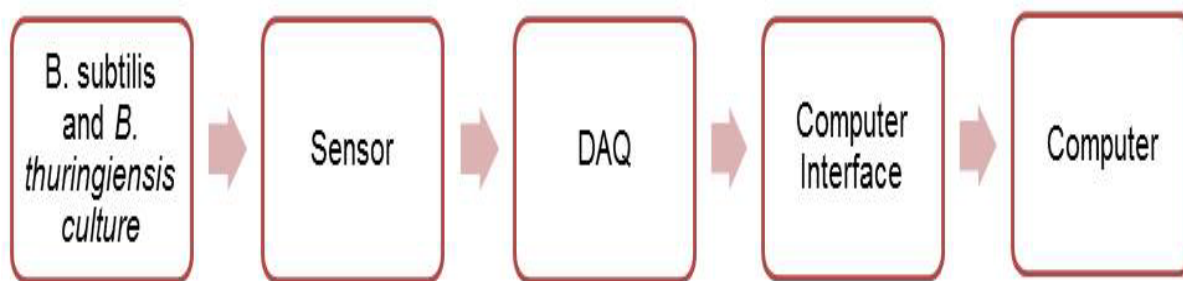


Figure 2: Block diagram of experimental setup for improved DEI Meridian Energy Analysis Device (DEI MEAD) an electromagnetic device assembled for electrical energy measurement of microbial cultures.

This technology has also acknowledged in to traditional complementary medicine system known as Electro Meridian Analysis System (MEAD Analyzer). The stability and repeatability of the data was examined before the initiation of this study. Also, the graph was plotted with the average value of total four replicates of the experiment.

Meridian Energy Analysis Device (DEI MEAD)

i. Sensor

The sensor is responsible for measuring the electric field of each bacterial culture. The sensor comprises of a hollow cylindrical copper electrode. The bacterial culture in test tube is placed into the core of this electrode. The energy emission from the cultures is recorded with the help of setup. In this assembly, the sensor is connected to computer through DAQ card (National Instrument USB 6009) and to measure the biofield of bacterial culture LabVIEW software has been used in our experiments^{14,15}.

ii. DAQ

The signals (analog waveforms) from the microbial culture were sampled by the process of data acquisition system (DAQ) and converted it into digital numeric values. The values were then processed by the DEI MEAD system^{14,15}.

iii. Computer interface

The interface used here is a bus-powered National Instruments USB 6009 B Series multifunction data acquisition (DAQ) module with built in signal connectivity. It has 8 analog inputs; 48 kS/s sample rate; two analog outputs; 12 digital I/O lines^{14,15}.

iv. Software

National Instruments provides a development environment and system design platform “Laboratory Virtual Instrumentation Engineering Workbench (LabVIEW) for visuals. The

advantage of LabVIEW is its extensive compatibility for accessing instrumentation hardware. It includes drivers and abstraction layers for other type of instruments. Several bus powered devices are also included or are available for inclusion. These present themselves as graphical nodes. The compatibility of hardware devices are interfaced with the standard software offered by abstraction layers. Also, the provided driver interfaces are fast enough to save program development time¹⁶. After the measurement of energy emitted by the microbial cultures the data were analyzed and interpret by MATLAB R2008b^{14,15}.

2. Superconducting quantum interference device (SQUID) measurement

The cultures of *B. subtilis* and *B. thuringiensis* were subjected for assessment of their magnetic energy emission at conventional SQUID magnetometer system (model Quantum Design ever cool MPMS XL-7) at Department of Physics, IIT Delhi, India (Fig 3).



Figure 3: Superconducting Quantum Interference Device facility.

The field range of SQUID device is ± 7.0 Tesla with stability of 1ppm/hour and range of magnetic moment measurement is ± 5.0 . This SQUID magnetometer consists of two superconductors separated by thin insulating layers to form two parallel Josephson junctions enabling to measure extremely low magnetism in living organisms also. A sample of 01ml from each Sterilized medium, *B. subtilis* and *B. thuringiensis* culture were transferred in a polycarbonate capsules and then subjected to sample rod separately. The assessment of magnetic moment was done at room temperature for eight hours in SQUID. The upright movement of sample produces an alternating magnetic flux in the pickup coil. The magnetic signal generated by sample is obtained via a superconducting pickup coil. This coil in turn with a SQUID antenna, transfers the measured magnetic flux to an rf SQUID device. This device acts as a magnetic flux to voltage converter. This voltage is then amplified and read out by the magnetometer's electronics. The experiment was repeated thrice and the average values have been depicted herewith. The graph was plotted with ORIGIN[®] 8 software.

Statistical analysis

Data from all replicates of the microbial cultures were tested for its significance with variance analysis (ANOVA) and are reported at a significance level of $p < 0.05$. The results are summarized in Table 2 and 3 depicting neighboring effect in sets of microbial culture.

Table 2. Neighboring effect in the three sets of microbial culture (*B. subtilis* + *B. thuringiensis*, *B. subtilis* + blank and *B. subtilis* + *B. subtilis*)

Source of Variation	SS	df	MS	F	P-value	F crit
Time (hours)	2.4523E+13	2	1.2261E+13	6.7974132	0.002327921	3.16824597
Paired microbial culture	2.9037E+15	5	5.8074E+14	321.953966	6.87462E-39	2.38606986
Interaction	3.2172E+13	10	3.2172E+12	1.78353859	0.086035644	2.01118092
Within	9.7406E+13	54	1.8038E+12			
Total	3.0578E+15	71				

(ANOVA: df=degree(s) of freedom; SS=sum of squares; MS=mean sum of squares)

Table 3: Neighboring effect in the three sets of microbial cultures (*B. thuringiensis* + *B. subtilis*, *B. thuringiensis* + blank and *B. thuringiensis* + *B. thuringiensis*)

Source of Variation	SS	df	MS	F	P-value	F crit
Time (hours)	2.5938E+13	2	1.2969E+13	24.883093	2.19343E-08	3.16824597
Paired microbial culture	3.1005E+15	5	6.201E+14	1189.75667	6.40152E-54	2.38606986
Interaction	2.3987E+13	10	2.3987E+12	4.60222632	9.92526E-05	2.01118092
Within	2.8145E+13	54	5.212E+11			
Total	3.1786E+15	71				

(ANOVA: df=degree(s) of freedom; SS=sum of squares; MS=mean sum of squares)

RESULTS

Previous studies reports that living microbial and other cellular culture systems influence and regulate biology of neighboring cultures by various means of physical communication^{6,17,18}. In the present investigation, both bacterial culture exhibited significant growth when incubated with similar species i.e., *B. subtilis* with *B. subtilis* (Fig. 4) and *B. thuringiensis* with *B. thuringiensis* (Fig. 5) as compared to their control counterparts (blank and sterilized media). Whereas, in case of the two dissimilar species (*B. subtilis* with *B. thuringiensis*) less growth was observed. The statistical analysis of microbial population indicates that the duration of the experimentation (hours) and the neighboring effect both had significant effect on the growth of microbial population (Table 2 and 3).

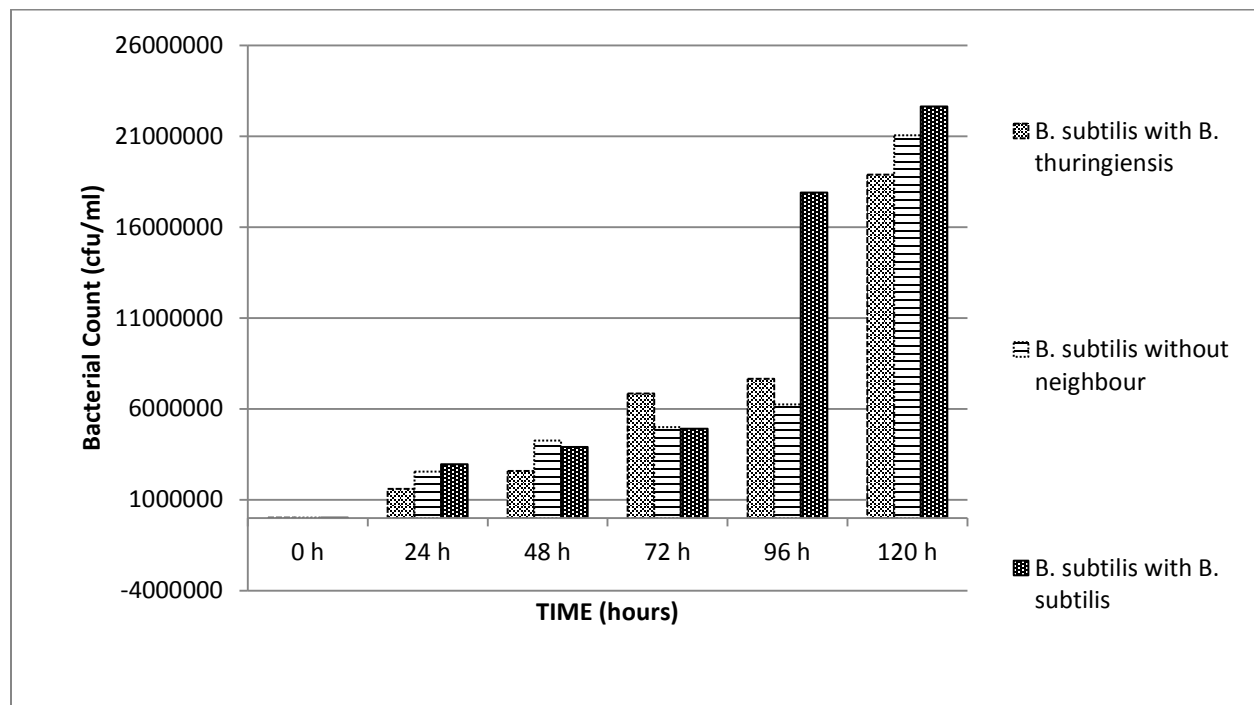


Figure 4: Comparative growth pattern for *B. subtilis*.

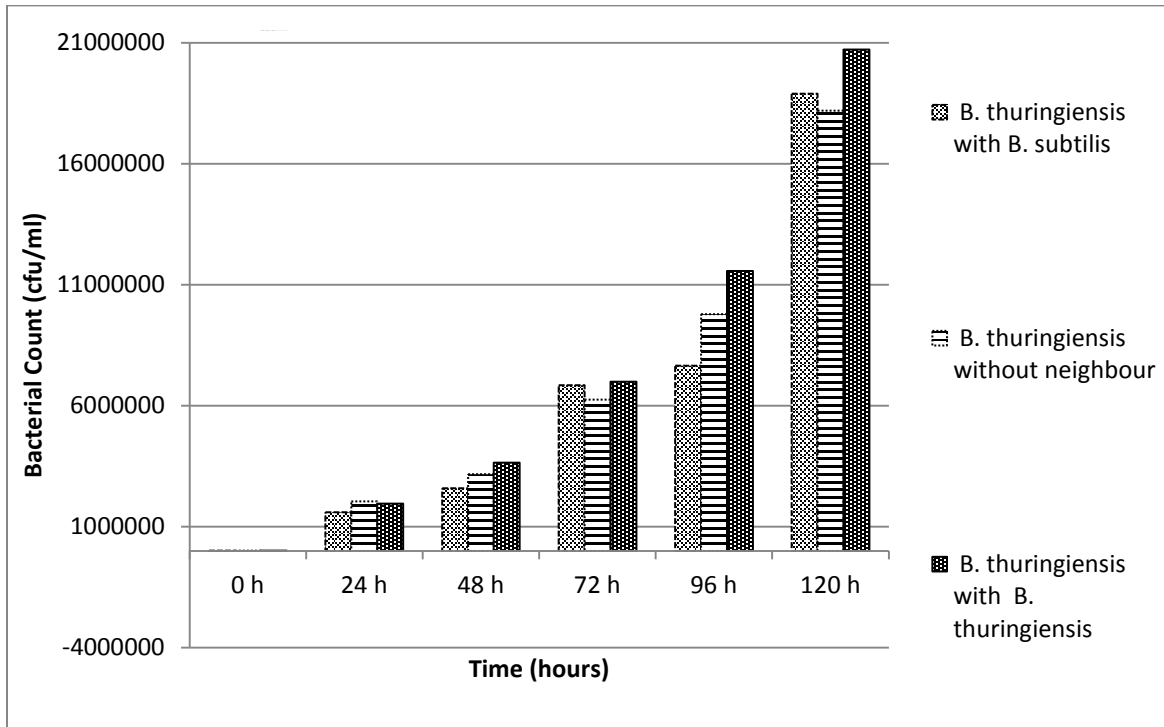


Figure 5: Comparative growth pattern for *B. thuringiensis*.

With DEI MEAD system we could record electrical energy emission during growth which got intensified with subject's population size (24 hr, 48 hr and 72 hr) (Fig.6 and 7) and time period. This further strengthens the proposed objective that the bacterial cultures can sense the presence of other organism in the environment and are able to exchange information without chemical contact.

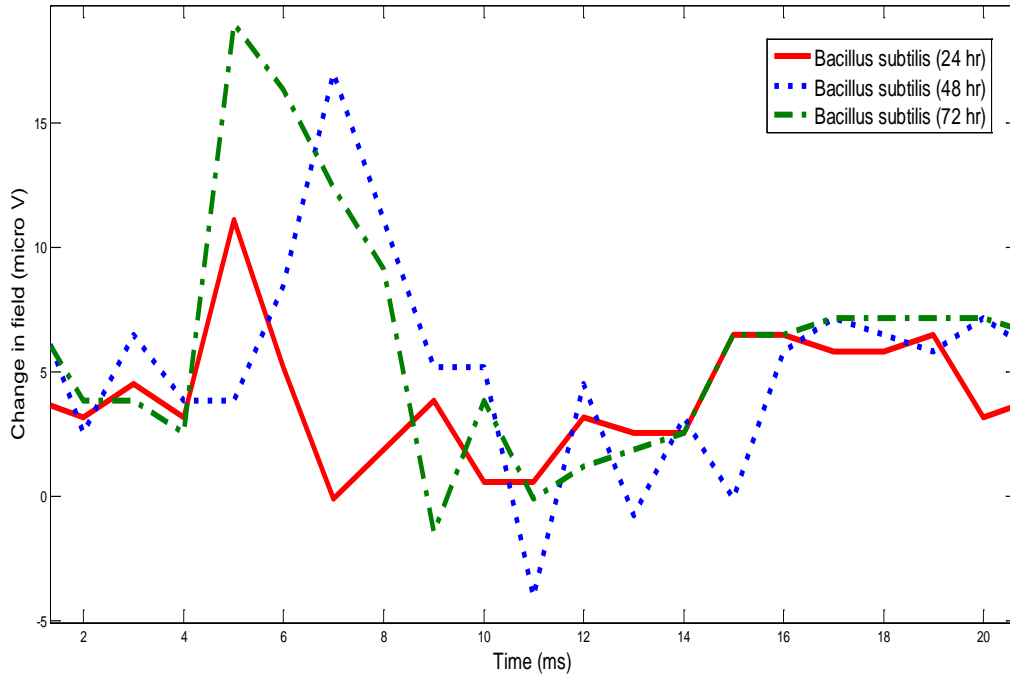


Figure 6: A comparative graph between electric field and time of *B. subtilis* after 24 h, 48 h and 72 h of incubation depicting the increase in magnitude of their electric field with its growth in population size from DEI MEAD.

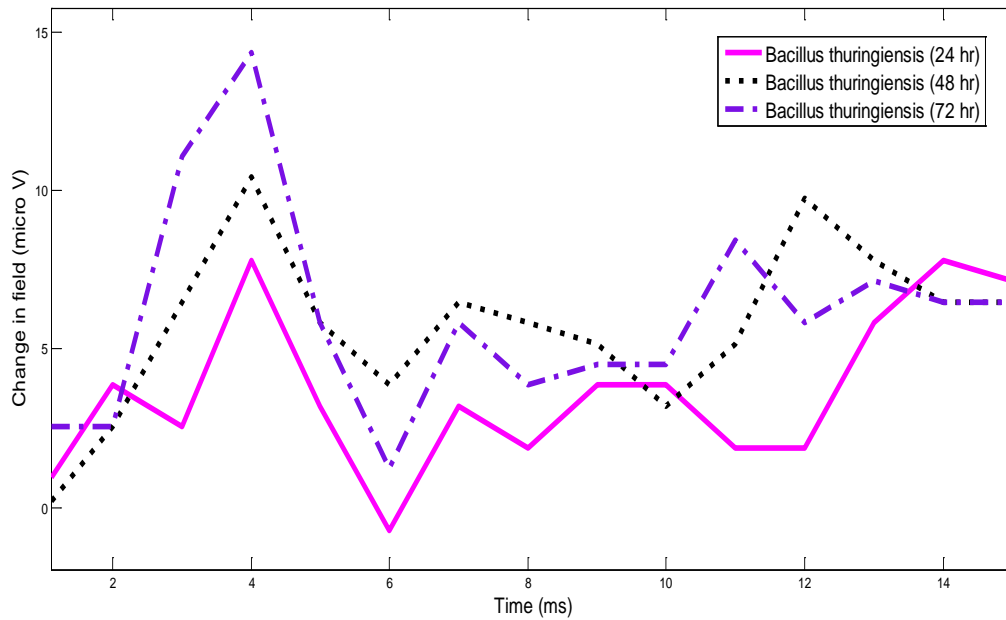


Figure 7: A comparative graph between electric field and time of *B. thuringiensis* after 24 h, 48 h and 72 h of incubation depicting the increase in magnitude of their electric field with its growth in population size from DEI MEAD.

Subsequently, assessment of magnetic energy emission was done with SQUID. Significant magnetic moment could be measured from both cultures. It shows the magnetic flux variation in *B. subtilis*, *B. thuringiensis* and sterilized medium respectively (Fig.8).

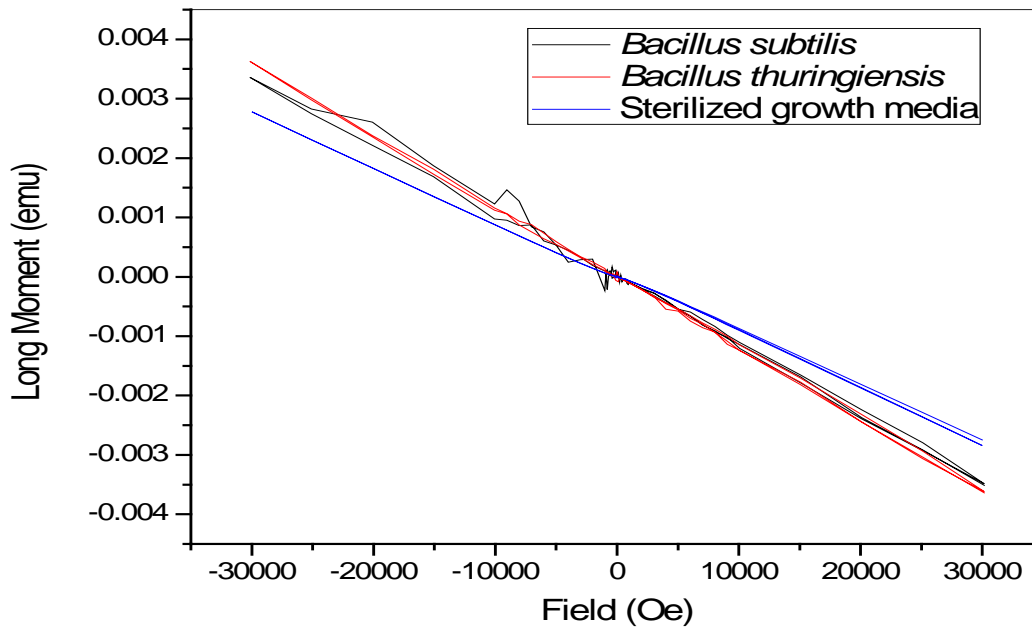


Figure 8: A comparative graph of measurement of magnetic moment from *B. subtilis*, *B. thuringiensis* and sterilized media with SQUID.

In a steady field of the SQUID magnetometer (Oe), it can be seen that all of them are diamagnetic. Further, it may also be noted that *B. thuringiensis* is slightly diamagnetic in comparison to *B. subtilis*. This implies that even magnetic energy may be responsible for mediating information exchange between the cultures, affecting their biological functions and thus growth.

Moreover, species specific energy emission from the cultures was also observed. The pattern obtained from *B. subtilis* was different from *B. thuringiensis* (Fig. 9 and 10).

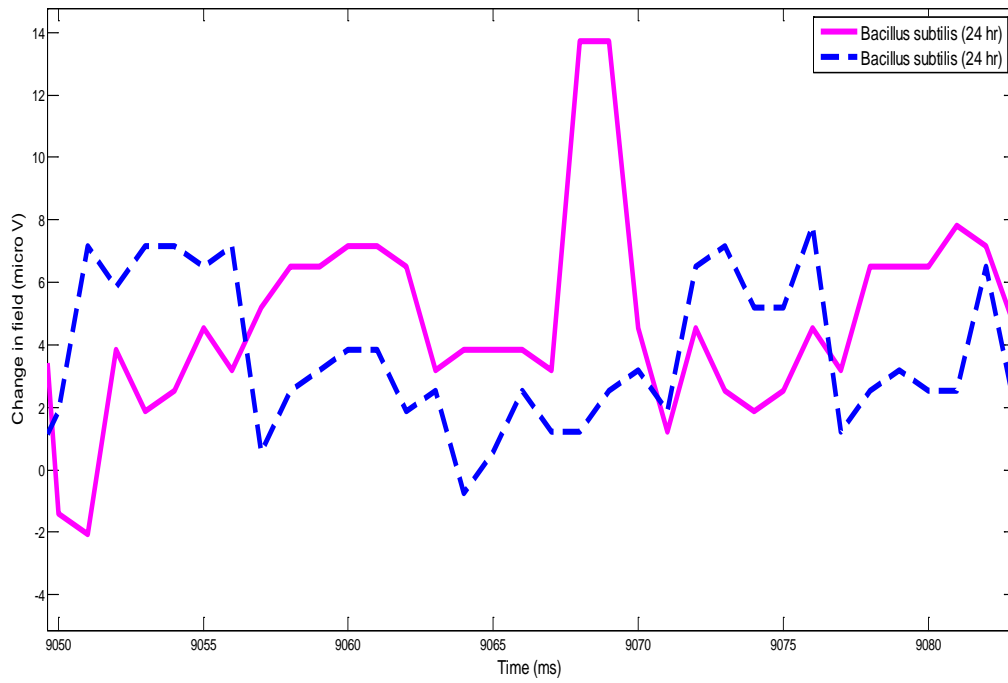


Figure 9: Species specific radiation recorded from *B. subtilis* with DEI MEAD at 24 h of growth.

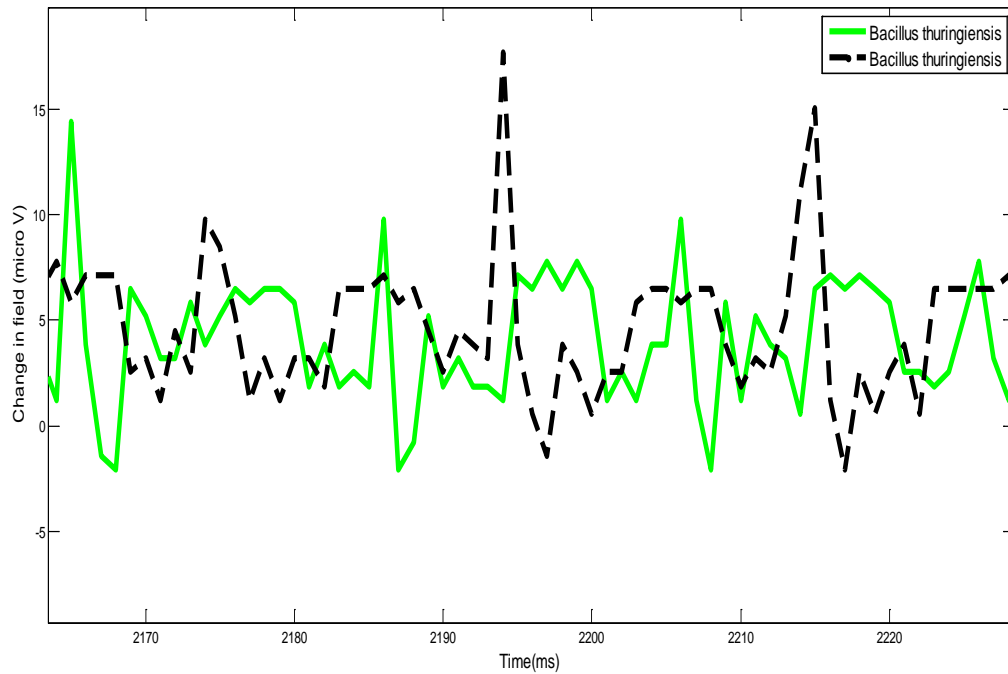


Figure 10: Species specific radiation recorded from *B. thuringiensis* with DEI MEAD at 24h of growth.

A tool may be developed based on unique energy emission to ease the extensive process of identification of microbes. However, it needs further validation with other microbes in future.

DISCUSSION

There are evidences for widespread importance of physical communication in bacterial cultures^{19,20-22} for cell division²³, adaptation of microorganisms to stress conditions²⁴, and adhesive capacities of cells²⁵. The observations in the present investigation support the existence of non-contact non-chemical distant cellular communication among the microbial cultures which supports earlier reports of similar phenomenon^{26,27}. Moreover, in present investigation, the two cultures were able to regulate each other's biology from a significant distance (double wall glass). The SQUID measurement of the culture's magnetic moment validates microbial ability to communicate to neighboring cultures with their electromagnetic energy emission, also validated by the statistical analysis of the population growth. Previous studies also provide evidence for neighboring effect on the growth rate of the organism. The increase and decrease in the population of *Paramecium caudatum*⁷ and *E.coli*²⁸ were also induced by the neighboring culture.

We could also observe that the pattern of energy emission from each cultures have species specificity in them. This indicates toward fingerprint energy emission of microbes. Likewise, infrared (IR) spectrum of *Lactobacillus species* was studied to develop a software for identification²⁹. In future, the electromagnetic energy pattern may decipher the fundamental question of distant communication that how the living organisms are able to identify self and non-self organisms existing in their vicinity. However, it needs extensive research at laboratory at individual species level which can be further validated on other microbial organisms. The concept of microbial biofield can explain this phenomenon with a new approach towards this

phenomenon²⁻⁵. Electromagnetism is an integral part of the biofield in each living cell. With a holistic and systemic approach and different ideology other than conventional science to such fundamental study, it can be an aiding tool in taxonomic identification.

The nature of complexity in the dynamic physical communication system in microbes here contributes further in social interaction and evolution of microorganisms like they collectively decide to make biofilm. The bacterial cultures even are separated by a distance were able to identify self and non-self cultures in the neighbor and were found communicating with each other regulating their growth. We therefore can conclude here that separated culture of *B. subtilis* and *B. thuringiensis* are involved intra and interspecies interaction. Also, it was found that the energy emissions from the cultures are involved in non-contact and non-chemical or physical communication in microbes validated by both DEI MEAD^{30,31} and SQUID magnetometer³².

CONCLUSION

In conclusion, we could say that the cultures of *B. subtilis* and *B. thuringiensis* were able to sense and identify self and non self cultures at a distance. It was also observed that electrical and magnetic energy emission have the potential to mediate intra and interspecific communication between the cultures. This mode of communication was strong enough to regulate the growth in neighboring culture. Further, the pattern of energy recorded from the subjects showed uniqueness towards the source. With future studies on the reported phenomenon, it can be developed as a potential aid for non-invasive diagnostics of microbes existing in living system.

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