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Study of Magnetic Field Treatment on Rhizosphere

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Abstract **- Bacterial isolates screened from rhizosphere of common arid vegetation growing at Rajasthan arid zone showed potential plant growth promoting regulators (PGPR) based on 16S rRNA gene sequence. The isolate Cubc1 species was identified as Bacillus sp. under IASE University project. The strain Cubc1 produced IAA, siderophore, ACCdeaminase. Magnetic field treatment studies on the production of IAA, siderophore and ACC deaminase were performed and it was observed that the magnetic field±1700 µT resulted in a significant increase in the production of IAA, siderophore, ACC deaminase. On the other hand Cubc1 bacterial growth studies under different stress like Nacl, pH and temperature were carryout and it was observed that magnetic field treatment causes significant stress tolerance in the bacterial strain.**

Keywords - Magnetic field, rhizosphere, ACC, IAA, Stress adaptation, Siderophore

1. INTRODUCTION

Soil bacteria partaking valuable effect on plant health are generally referred to plant growth promoting rhizobacteria (PGPR). PGPR promote directly and indirectly plant growth but the exact mechanisms involved have not all been well characterized [6]. PGPR fix atmospheric nitrogen, produce siderophore, phytohormones, solubilize phosphate, potassium and zinc, alleviate the various stress by secreting ACC (1-aminocyclopropane-1- carboxylate) deaminase enzyme and control disease by suppressing or killing the phytopathogens. Generally 2–5% of rhizosphere bacteria are PGPR.Manmade chemical combinations used to inhibit plant pest and disease symptoms or to fertilize plants can be harmful to human health and they may also endure in natural ecosystems[7, 8, 16]. In the previous decade there has been a push to use organic agents such as micro- organisms (bacteria and mycorrhizal fungi) to switch conventional chemical products. Indeed, inorganic agriculture arrangements the use of synthetic chemical products is outlawed[14,15].Bacillus spp. is studied to be the harmless microorganisms that hold amazing abilities for producing a vast range of beneficial substances [20].The influence of the magnet is one of the utmost basic influences in nature. We recognize that magnetism itself was an element in the primitive soup from which the universe and our planet originate downward. Magnetism is the energy that keeps order in the galaxy, allowing stars and planets to spin at significant velocities. And in a sense, our own planet's magnetic field is responsible for protecting all life on earth [17].Numerous living microbes contain tiny amounts of ferromagnetic material utmost commonly magnetite that position the host in the geomagnetic field [3, 19].The influence of magnetic field was mutable depending on the nature of the microbes and field. Noraketal explain that magnetic field has important effect on bacteria's cell as well as on its life and they added that the influence of magnetic field surrounded in cell membrane [13].Modification in properties of solutions being candidly exposed to the effect of the constant magnetic field (CMF) are linked, including other things, with modification in their polarization, molecular structure and ordering of particles as well as with a alteration in the electric charge [11].The magnetic field of 0.42 [T] caused significant changes in the analyzed parameters of the methane fermentation process. The analysis of biogas composition demonstrated that there were significant differences between control and Magnetic field facility digester production. A positive effect of the Magnetic Field was established in respect to the sedimentation process of anaerobic slurry andreduction of COD concentration in the effluent[9].

This study aimed to investigate the effects of magnetic field on the metabolites of Bacillus sp (Cubc1) bacterium as well as bacterial growth studies under different stress like Nacl, pH and temperature.

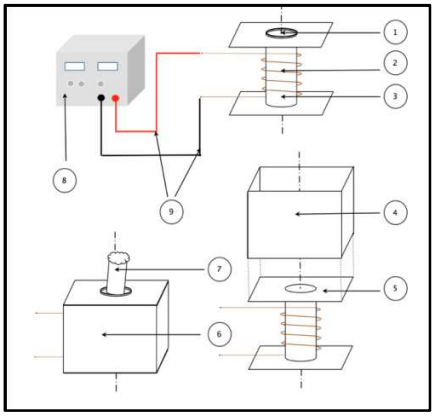
2. MATERIALS AND METHODS

Isolated cultures maintained at the microbiology laboratory at IASE Deemed University have been used during this investigation. All bacterial strains were cultivated over night on nutrient broth at 37 ° C. Inoculums of these strains were used to inoculate nutrient agar plates and incubated at 37 ° C till used.

2.1 Magnetic field exposure

The selected bacterial strains need their exposure to magnetic field of various strength for which a device (Machine) was develop by the author having following components. (Figure 1) (a) Cylindrical hollow core (Ductile iron pip) around which copper winding of 300 turns was coiled. (b) The cylindrical core & copper winding were housed in the iron frame and the frame was covered by iron plates to increase the magnetic strength. (c) The central hollow was used for keeping the sample in 100 ml test tube. Where it can be exposed to magnetic field of 100μT to 10000μT. (d) The magnetic field may be regulated by DC power supply.

In the instrument we do need homogeneous but variable magnetic field strength according to bacterial strain. For that we have constructed a machine in which we use cylindrical hollow core (Ductile iron pipe).Around the cylindrical hollow core we place copper winding of around 3000 turns. To support cylindrical core we constructed a plated iron frame on the edges of cylindrical hollow core and to increase magnetic strength in free cylindrical hollow core we cover this frame using iron plates. Iron frame decreases the loss of magnetic flux in outside of cylindrical core. The sample loading site posses space for 100 ml test tube and 100 μT to 10000μT homogeneous magnetic field. Magnetic field can be adjusting by varying the current and voltage using variable D.C power supply.



1. sample loading site
2. Copper winding
3. Cylindrical hollow cores (Ductile iron pipe)
4. ductile iron plates for medium
5. ductile iron plate frame
6. unit after assemble
7. 100ml Test Tube
8. D.C. powersupply (1-35v)9 connecting wire

Fig. 1 Schematic illustration of the apparatus

2.2 Analysis method

15 minutes at 2 hour intervals for 36 hours. The bacterial strains were given magnetic treatment called MCUbc1.The growth rates of magnetic field treated (Mcubc1) and control (Cubc1) were studied through measuring the absorbance at wavelength 600 nm of the viable cells and then plotted as a function of time. Best-performed MCubc1 under selective magnetic field intensity were subjective to compare with the control using different stress factor like Growth studies under salt (NaCl) stress, Growth studies at different pH.Stress tolerance of MCubc1 for NaCl, pH and temperature was studied in relation to its growth afterwards IAA production by bacterial isolates was determined following the methods of Gordon and Weber (1951), ACC deaminase enzyme (EC 4.1.99.4) activity was assayed according to method of Glick et al. (1995), Siderophore production was determined on Chrome-azurol S (CAS) medium following the method of Schwyn and Neilands (1987).

3. RESULT AND DISCUSSION

3.1 Experiment 1- Growth studies

Growth curve characteristics of both strains (MCubc1 and control Cubc1) by inoculating equal volume (10^7cells/ml) of overnight grown cultures in nutrient broth and incubated in shaker incubator at 30^0C for 36 hour. Three different magnetic field ranges 500μT,1700μT and 3000 μT were used. The magnetic field treatment was given at intervals of 2 h till 36h and the Growth was determined at various time intervals by measuring absorbance at 660 nm.

MCubc1 exposed to 1700 μT (magnetic field) bacterialstrain showed increased growth as compare to the control (Cubc1) this growth was also higher when we compare it with the strain exposed to a magnetic field range 500μT to 3000μT and other magnetic field range shown in Fig 2.

Fig. 2 illustrates the changes in the absorbance of the bacterial suspension as a function of incubation time for 36 hr. It is clear from the fig 2 that the lag phase ended after two hours followed by exponential growth period ended after 14 hr and followed by the stationary phase.500μT, 1700μT and 3000μT magnetic field treated strain preserved on fresh prepared nutrient agar plates.

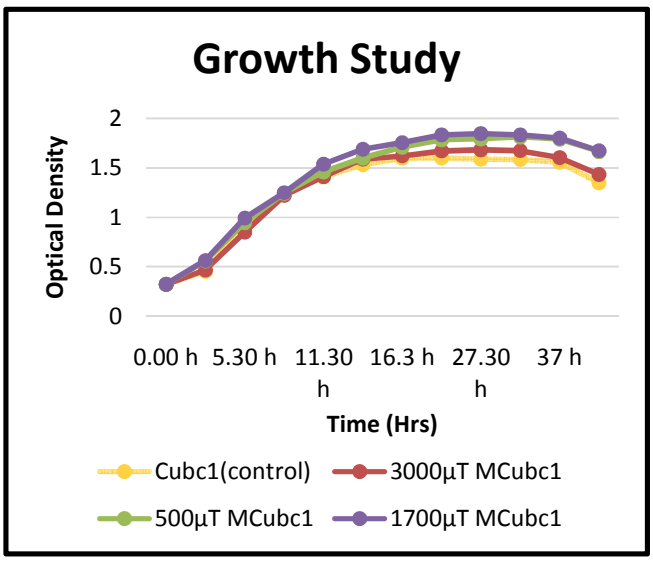


Fig 2 showing growth study

3.2 Experiment 2-Growth studies under salt stress (Nacl)

The tolerance of MCubc1 and Cubc1 (control) to salt (Nacl) was measured by their growth on nutrient broth medium at 0, 1, 2, 4, 5, 8, 10 and 12% NaCl (wt/vol).Growth curve characteristics of cultures in the presence of high salt concentration were compared by inoculating equal volume (107cells/ml) of 32Hrs grown cultures in nutrient broth, containing extra 1% to 12% NaCl. The magnetic field (1700 μT) treatment was given to MCubc1 in an intervals of 2 h till 32h.Preserved strains of Experiment 1 (1700 μT MCubc1) were used in this experiment. Growth determined at intervals of 2 h till 32Hrs by measuring absorbance at 660 nm. MCubc1 showed improved growth compare to Cubc1 (Control) shown in Fig 3 and 4. In 1% to 5% extra added Nacl the MCubc1 and Cubc1 show growth but MCubc1showed higher growth as compare to Cubc1.

When 6% to 12% extra salt was added to cubc1 and MCubc1 (1700μT magnetic field treated strain) It was recorded that no growth was found in Cubc1 while significantly higher growth was reported in MCubc1.

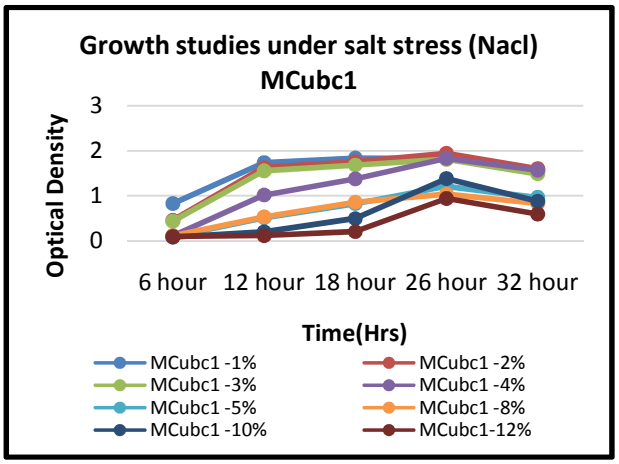


Fig 3 – Growth Studies under salt stress Nacl (MCubc1)

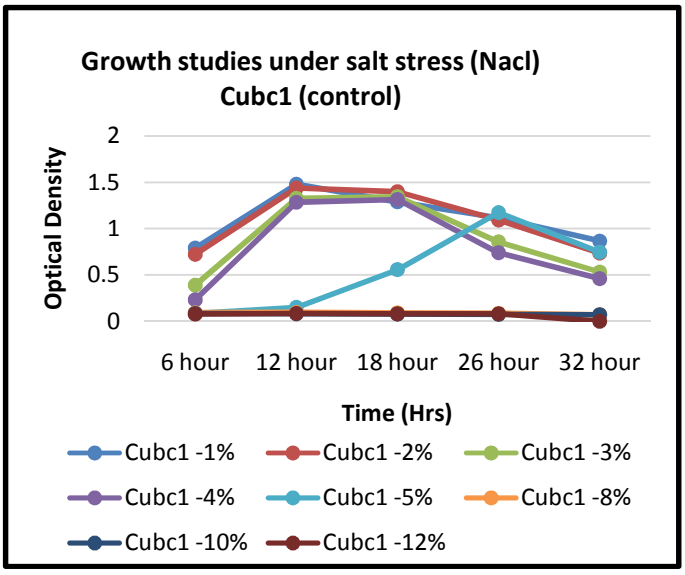


Fig 4 – Growth Studies under salt stress Nacl (Control)

3.3 Experiment 3-Growth studies at different pH

To study magnetic field effect on pH stress (pH >7–11) the nutrient medium was buffered with AMPD buffer, while for the low pH range between 4 and 7 it was buffered with 25 mM HOMOPIPES. To adjust the medium pH from 4.5 to 7, 20 mM MES was added as described by Priefer et al. 2001.MCubc1 and Cubc1(control) were compared by inoculating equal volume (107cells/ml) of 24Hrs grown cultures in nutrient broth, containing pH range 5,6,7,8,11. Experiment 2 preserved 1700 μT MCubc1used in experiment. Growth determined after 16 Hrs by measuring absorbance at 660 nm 1700 μT magnetic field treatment was given to Mcubc1 in intervals of 2 h till 32h. Result shows (bar column chart 5) marginal pH stress tolerance in MCubc1 over Cubc1 (Control).

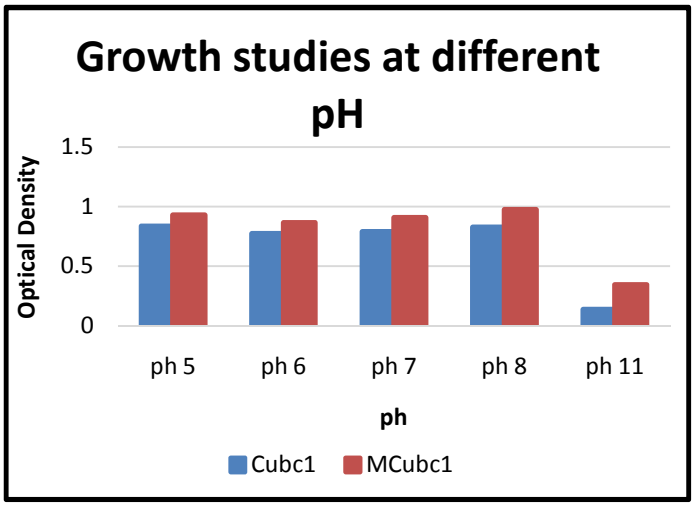


Fig.- 5 Growth studies at different pH

3.4 EXPERIMENT 4-Growth studies at different temperature

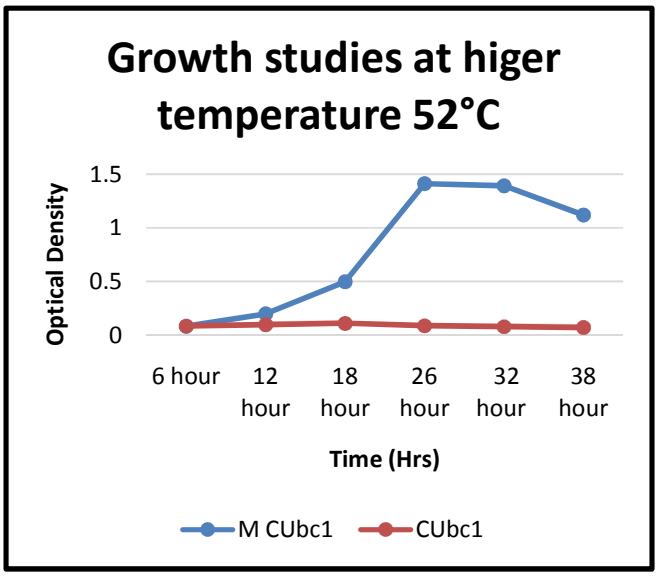


Fig.- 6Growth studies at 52^0 C

To test temperature tolerance, MCubc1 and Cubc1(control) were compared by inoculating equal volume (107cells/ml) of 24Hrs grown cultures in nutrient broth in shaking incubator with the temperature adjusted to 52^0C compared with the optimum of 30^0C.Experment 3 preserved 1700 μT MCubc1used in experiment. 1700 μT magnetic field treatment was given to Mcubc1 in intervals of 2 h till 32h and Growth was determined at intervals of 2 h till 32Hrs by measuring absorbance at 660 nm. Result shows marginal good temperature tolerance in MCubc1 bacterial strain over Cubc1 (control) strain shown in Graph 6.

3.5 EXPERIMENT 5-ACC Deaminaseactivity

To measure ACC deaminaseactivity, bothMCubc1 and Cubc1(control) were grow in 5 ml of nutrient broth medium at 30°C for 32 Hrs until they reached stationary phase. To induce ACC deaminase activity, the cells were collected by centrifugation, washed twice with 0.1 M Tris-HCl (pH 7.5), suspended in 2 ml of modified M-9 minimal medium supplemented with 5 mM final concentration ACC, and incubated at 30°C with shaking for another 36-40 hours. ACC deaminase activity was determined by measuring the production of α-ketobutyrate generated by the cleavage of ACC by ACC deaminase[10]. The concentration of α-ketobutyrate in each sample was determined by comparison with a standard curve of α-ketobutyrate.MCubc1 *showed* ACC deaminase activity (OD≒0.208) *while CUbc1* (OD0.117).

3.6 EXPERIMENT 6 -IAA Production

To observe IAA production, 2 ml supernatant {3.5 EXPERIMENT 5-ACC Deaminase activity&3.2 EXPERIMENT 2-Growth studies under salt stress(Nacl)} of each culture was mixed with 100 μl of 10 mM*O*-phosphoric acid and 4 ml of Salkowaski's reagent was added and the absorbance of pink colour developed was read at 530 nm. IAA concentration in culture was determined by a standard curve prepared with known concentrations of pure IAA. Maximum IAA production was recorded in MCubc1 (21 μg ml^{-1}) broth culture on the other hand 16μg ml−1 in control CUbc1. The IAA productionby MCubc1arebetter in case of salt (Nacl) stress over control Cubc1 shown bar fig-7.

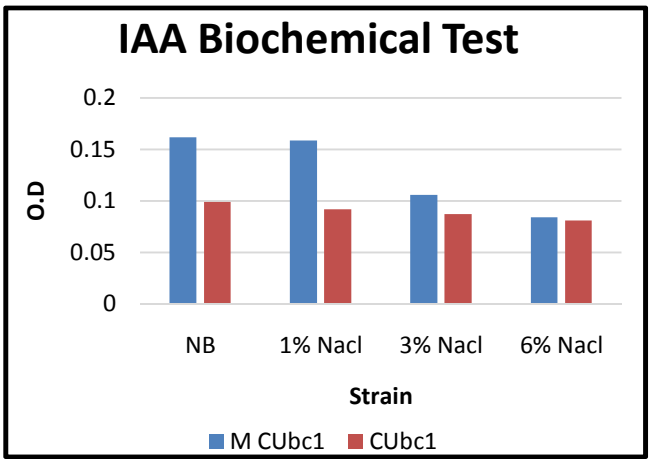


Fig.- 7 IAA Biochemical Test

3.7 EXPERIMENT 7 -Siderophore Production

Siderophore production was determined on Chrome-azurol S (CAS) medium [18]. The preserved bacterial strains MCubc1 from experiment 1,2,3 and control

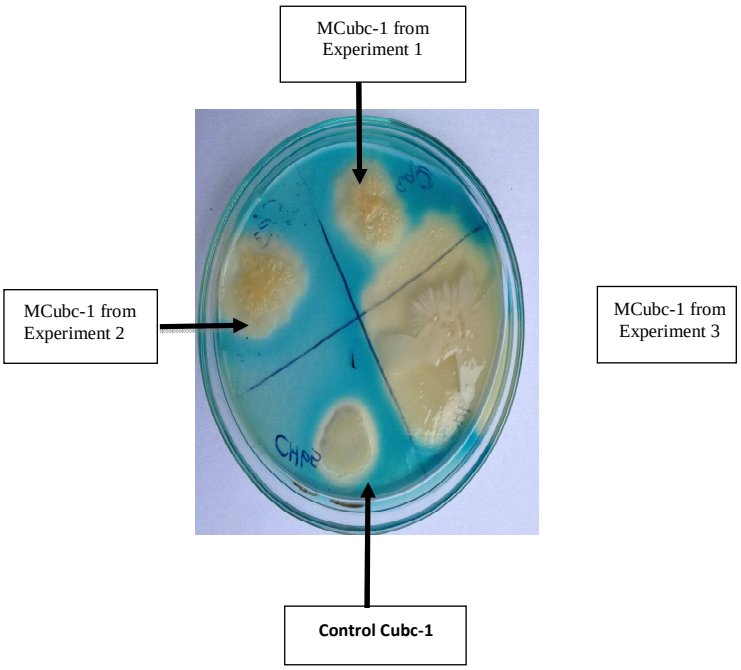


Fig.8- Siderophore Production

Cubc1 (24 h old cultures) spotted separately on CAS medium. Plates were incubated at 28 ± 10^0C for 48 h. Formation of orange to yellow halo around the colonies showed the production of siderophore. MCubc1 magnetic treated bacteria compare to control Cubc1 produce siderophore in short time (48h) and formation of orange to yellow halo around the colonies in a big scale.

All the results of the study indicated that the studied microorganisms could switch on new pathways to adapt themselves against stress induced by exposure to Magnetic field aimed at their preservation [4] as the transposition which represents an important source of genetic variability can be induced [5] [11]. In this manner, bacteria try to find their adaptation through intra-strains variability as the benefits of heterogeneity among a cell population enhances the persistence of bacteria [1].

4. CONCLUSIONS

The magnetic treatment significantly improves the bacterial population with shorter generation time under different stress like Nacl, pH and temperature. This increased population of Bacillus sp. will increase the nitrogen fixing efficiency thus leading to greater yield. The enzyme activities and metabolites (IAA, ACC and Siderophore Production) were also improved under in the influence of magnetic treatment. The magnetic fields affect the cells whichever of two ways. The major is through the cell wall and would contain the expression and production of proteins and metabolites, such as enzymes, the second is affecting the cells intracellular and engaged the affect within the cell. The applicable magnetic field is applied for a time period and an intensity, which is based on the

recipient of the field, the medium and the desired result. Experiments in this study showed 1700 μT magnetic field range quit good for further research on Bacillussp.

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