Influence of Environmental Factors on Activities of Bacterial Population Associated With Rhizospheric Soil of Wheat Crop

Jupinder Kaur1* and S. K. Gosal1

1Department of Microbiology, Punjab Agricultural University, Ludhiana-141 004, India.

ABSTRACT

Aim: To study the effect of environmental factors on the activities of soil microbial population in the rhizospheric soil of wheat crop.

Study Design: An agroclimatic study was carried out to study the effect of environmental alterations on the activity of soil bacteria in a multifactor climate change experiment in which wheat crop was grown under field conditions and under temperature gradient tunnel maintained at different and higher temperature. Attempt was made to screen high CO2 and high temperature tolerant diazotrophic bacteria from wheat rhizosphere.

Place of Study: Department of Microbiology, PAU, Ludhiana.

Methodology: Nitrogen fixing bacteria were isolated on Jensen’s medium using serial dilution spread plate technique. The bacterial isolates were characterized biochemically using standard techniques as described in Bergey’s Manual of Determinative Bacteriology. The isolates were also assessed for their ability to produce indole acetic acid, siderophores production, ammonia excretion, qualitative phosphate solubilization and solubilization of phosphate by quantitative method.

Results: A total of 21 different nitrogen fixing bacteria were isolated from rhizospheric soil samples of wheat crop grown under field conditions and under temperature gradient tunnel. Out of 21 isolates, 8 isolates were able to grow upto 20% concentration of CO2 and 7 isolates showed growth upto 60°C. Tolerance to high CO2 and high temperature was observed to more in the bacteria isolated from the rhizospheric soil of wheat crop grown under temperature gradient tunnel.

*Corresponding author: E-mail: jupindercheema13@gmail.com;
Functional characterization of these isolates showed that the isolate WT5 had significantly higher IAA production (44.3 µg/ml) after 5 days of incubation in the medium supplemented with tryptophan. Among these 21 isolates, six were found to have P-solubilizing diazotrophic trait and five were found to show siderophore production on CAS agar plates. The amount of ammonia excretion was non-significant among the isolates and was in the range of 1.14-3.70 µg/ml. The isolate WF6 was found to be the best isolate in terms of the functional characteristics and tolerance to high CO$_2$ and temperature levels.

**Conclusion:** Results indicate that alterations in environmental factors may cause changes in activities of bacterial populations. These results illustrate the potential for complex community changes in terrestrial ecosystems under climate change scenarios that alter multiple factors simultaneously.

**Keywords:** Biochemical characterization; IAA production; nitrogen fixing bacteria; temperature and carbon dioxide tolerance; siderophore production.

1. INTRODUCTION

The microorganisms present in the soil are responsible for the cycling of carbon (C) and various other nutrients in the ecosystems. The activities of these bacteria are regulated by various biotic and abiotic factors such as, temperature, the quantity and quality of litter inputs, and moisture. Climatic changes will impact both abiotic and biotic drivers in ecosystems and the response of ecosystems to these changes. Feedbacks from ecosystem to the atmosphere may also be regulated by soil microbial communities [1]. Although microbial communities present in the soil regulate important ecosystem processes, but it is often unclear how the activities of microbial communities correlate with climatic perturbations and interact to effect ecosystem processes. Much of the ecosystem climate change research conducted till date has focused on macroscale responses to climatic change such as changes in plant growth, photosynthesis [2], plant community composition [3], and coarse scale soil processes [4], many of which may also indirectly interact to effect microbial processes. Studies that have addressed the role of microbial communities and processes have most often targeted gross parameters, such as microbial biomass, enzymatic activity, or basic microbial community profiles in response to single climate change factors [5].

Greenhouse gases naturally keep the earth warm by trapping heat in the atmosphere, thus increases in atmospheric concentration of these gases are predicted to shift the earth’s climate. The concentration of carbon dioxide (CO$_2$) in the atmosphere is increasing at an unprecedented rate, due to fossil fuel burning and land use changes. One of the pre-eminent manifestations of climate change is the increase in atmospheric CO$_2$ concentration which in turn leads to increased temperature (global warming). A predicted consequence of this rise in CO$_2$ concentration is the warmer temperature of the earth’s surface. Both CO$_2$ and temperature are the key variables of global climate and may cause significant changes in functional activities of bacteria present in the soil. Atmospheric CO$_2$ concentrations and warming can potentially have both direct and indirect impacts on soil bacterial communities. However, the direction and magnitude of these responses is uncertain [6]. The response of soil microbial communities to changes in atmospheric CO$_2$ concentrations can be positive or negative [7]. Further, climatic changes may increase or decrease the ratio of bacteria and fungi, as well as shift their community composition [8]. Increasing temperatures can increase in microbial activity, processing, and turnover, causing the bacterial community to shift in favor of representatives adapted to higher temperatures and faster growth rates [9], [10] isolated and characterized CO$_2$ tolerant bacteria. They found that microbes are capable of surviving stressful conditions created by the introduction of CO$_2$. In another study by [11], nitrogen fixing bacteria (rhizobia) were isolated and checked for their high temperature tolerance. Although the many single factor climate change studies described above have enabled a better understanding of how microbial communities may respond to any one factor, understanding how multiple climate change factors interact with each other to influence microbial community responses is poorly understood. For example, in a study conducted by [12], it was found that elevated atmospheric CO$_2$ and precipitation changes increase soil moisture in an ecosystem, but this increase may be counteracted by warming.
Similarly, warming may increase microbial activity in an ecosystem, but this increase may be eliminated if changes in precipitation lead to a drier soil condition. Such interactive effects of climate factors in a multifactorial context have been less commonly studied even in plant communities [2], and detailed studies are rarer still in soil microbial communities [13]. Clearly, understanding how bacterial populations will respond to these atmospheric and climate change drivers is important to make accurate predictions of how ecosystems may respond to future climate scenarios. The increased awareness of this global problem has led to increased pressure to minimize the impacts of elevated atmospheric concentrations of greenhouse gases and to screen microbial communities capable of tolerating higher concentrations of CO$_2$.

Wheat is one of the major crops cultivated in India and all over the world. It is the leading source of vegetable protein in human food, having higher protein content than soybeans or the other major cereals, maize (corn) or wheat. Because of its importance, effects of regional and global environmental changes on microbial flora of wheat need to be better understood. Keeping all these points in view, the research was carried to screen high temperature and CO$_2$ tolerant diazotrophic bacteria from rhizospheric soil of wheat. To address how multiple climate change drivers will interact to shape soil microbial communities present in wheat rhizosphere, a multifactor climatic change experiment in which wheat crop was grown under field conditions and under temperature gradient tunnel (TGT) maintained at different temperature was used. Attempt was made to screen high CO$_2$ and high temperature tolerant diazotrophic bacteria from wheat rhizosphere.

2. MATERIALS AND METHODS

2.1 Soil Sample Collection

Soil samples were collected from rhizospheric soil of wheat crop grown under field conditions and under TGT located at the research farm of PAU, Ludhiana. The dimensions of the TGT were 30 m length × 5 m width and the meteorological parameters at the various depths within the TGT and outside were monitored at hourly interval by automated weather station manufactured by Delta -T devices, UK. Temperature and moisture within the TGT were maintained using fans, exhaust fans and coolers. Wheat crop was raised within the TGT and in open by following the crop management practices recommended in the Package of Practices of PAU, Ludhiana. Soil temperature data recorded at different time intervals is represented in Table 1. Soil samples were collected at different time intervals from rhizosphere of wheat crop. Soil samples were collected by composite sampling method [14].

<table>
<thead>
<tr>
<th>Time interval (DAS)</th>
<th>Soil temperature (°C)</th>
<th>Moisture content (%)</th>
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<tbody>
<tr>
<td>0</td>
<td>F: 17.5</td>
<td>21.6</td>
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<tr>
<td></td>
<td>TGT: 20.0</td>
<td>25.2</td>
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<tr>
<td>60</td>
<td>F: 13.2</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>TGT: 17.2</td>
<td>16.4</td>
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<tr>
<td>120</td>
<td>F: 23.2</td>
<td>15.8</td>
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<tr>
<td></td>
<td>TGT: 28.5</td>
<td>17.9</td>
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<tr>
<td>Harvesting</td>
<td>F: 30.8</td>
<td>06.5</td>
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<tr>
<td></td>
<td>TGT: 34.7</td>
<td>08.7</td>
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</tbody>
</table>

F: field (open) and TGT: Temperature gradient tunnel

2.2 Isolation and Naming of Diazotrophic Bacteria

A total of 21 different diazotrophic bacterial isolates, 10 from rhizospheric soil samples of wheat grown under field conditions and 11 from rhizospheric soil samples of wheat grown under TGT were isolated. Isolation of rhizospheric bacteria was carried out on Jensen’s medium using serial dilution spread plate technique. Isolated colonies were picked up and purity of the isolates was ensured by streak plate method. Isolated colonies appearing at the tail end of the streak were picked up, subcultured and maintained on Jensen’s medium slants at 4°C for further studies [15]. The isolated diazotrophic bacteria were named according to the source of soil from which they were isolated. Ten diazotrophic bacteria which were isolated from rhizospheric soil of wheat grown under field conditions were named as WF1, WF2, WF3, WF4, WF5, WF6, WF7, WF8, WF9 and WF10. The 11 diazotrophic bacteria which were isolated from rhizospheric soil of wheat grown under TGT were named as WT1, WT2, WT3, WT4, WT5, WT6, WT7, WT8, WT9, WT10 and WT11.
2.3 Biochemical Characterization

The bacterial isolates were characterized biochemically for oxidase, catalase, citrate utilization, Methyl Red (MR) and Voges-Proskauer (VP), indole production and H₂S production using standard techniques as described in Bergey’s Manual of Determinative Bacteriology [16].

2.4 Functional Characterization

The isolates were assessed for their ability to produce indole acetic acid [17], siderophores production [18], ammonia excretion [19], Qualitative phosphate solubilization [20] and solubilization of phosphate by quantitative method [21].

2.5 Screening of High CO₂ Tolerant Diazotrophic Bacteria

Effect of various levels of CO₂ on the growth of diazotrophic bacterial isolates was studied using CO₂ incubator. Diazotrophic bacterial isolates were screened for CO₂ tolerance provided constant temperature. The isolates were inoculated in Jensen’s broth. Inoculated tubes were screened for growth at 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% of CO₂ levels at 28 ± 2°C of temperature [22].

2.6 Screening of High Temperature Tolerant Diazotrophic Bacteria

Effect of different temperatures on the growth of diazotrophic bacterial isolates was studied by growing the diazotrophic isolates in broth. The isolates were inoculated in Jensen’s broth. Inoculated tubes were incubated at 30, 33, 36, 39, 42, 45, 48, 51, 54, 57 and 60°C for 48 hrs and the temperature tolerance of diazotrophic bacterial isolates was observed [23].

3. RESULTS AND DISCUSSION

Atmospheric and climate change drivers may select for distinct soil bacterial communities, and these community changes may shape the way ecosystems function in the future. We used a multifactor climate change experiment to investigate how climatic change factors impact activities of soil bacterial communities. Using Biochemical and functional techniques, we targeted changes in the activity of the bacterial population isolated from rhizosphere of wheat crop. Isolated bacteria were also screened for their ability to tolerate high CO₂ and high temperature levels.

3.1 Biochemical Characterization

All the 21 isolates were characterized using standard biochemical methods as in the Bergey’s manual of systematic bacteriology. Biochemical characterization of all the isolates showed that all the isolates were catalase and citrate positive whereas negative for indole production and H₂S production test. Mostly the isolates were found positive for oxidase and MR test. Majority of the isolates found positive for MR test were from rhizospheric soil of wheat crop grown under field conditions whereas none of the isolates taken from rhizospheric soil of wheat crop grown under TGT were found to be positive for VP test (Table 2). [24] also characterized bacteria biochemically and found that majority of isolates were positive for oxidase and catalase, MR, citrate utilization and negative for indole and VP. [25] characterized bacteria using biochemical characterization and identified isolates as Bacillus sp. as the most dominant genera followed by Pseudomonas sp., Serratia sp., Flavobacterium sp., Micrococcus sp., Klebsiella sp., Azotobacter sp., Enterobacter sp., Xanthomonas sp., Staphylococcus sp. and Micrococcus sp. Pseudomonas striata and Enterobacter were the most important strains present in the rhizospheric soil of crops.

3.2 Functional Characterization

The different isolates were evaluated for functional characterization using IAA production, siderophore production, ammonia excretion and phosphate solubilization.

3.2.1 IAA production

IAA production in the bacteria isolated from rhizospheric soil of wheat crop grown under field conditions ranged from 7.20 to 15.55 µg/ml and 9.60 to 30.25 µg/ml in the medium without tryptophan and supplemented with tryptophan, respectively. Whereas IAA production in case of bacteria isolated from TGT was found to be in the range of 05.10 to 20.60 µg/ml and 11.70 to 36.00 µg/ml in the medium without tryptophan and supplemented with tryptophan, respectively. Maximum IAA
was produced by isolate WT5, isolated from rhizospheric soil of wheat crop grown under TGT in the medium supplemented with tryptophan (Table 3). Similar findings have been reported by [26]. Thus, ability to produce IAA among various bacterial isolates was observed to be more in the isolates isolated from rhizospheric soil of wheat crop grown under TGT. [27] reported that L-tryptophan was primary precursor for the formation of IAA in several microorganisms. Similarly in the present study, significantly more IAA production was observed in the medium with tryptophan than in the medium without tryptophan for all the isolates. The present results are in accordance with [28] who reported production of 10-35 μg/ml IAA in a medium supplemented with tryptophan. [29] also reported the production of 15-19 μg/ml IAA by various isolates. [30] observed that out of 72 isolates, 49 were able to produce IAA in presence of tryptophan. [31] found that IAA production by various isolates ranged from 15.70 to 36.33 μg/ml after 8 days of incubation.

### 3.2.2 Siderophore production

All the bacterial isolates were assessed for siderophore production on Chrome-azurol S (CAS) agar plates for 120 hours. Out of all the 21 diazotrophic bacterial isolates, 5 isolates produced a distinct orange coloured zone on the Chrome-azurol S agar plates, thus indicating the production of siderophores. The isolates WF2 and WF5 produced less than 1.0 cm zone on CAS agar whereas, 3 isolate (WF6, WT7 and WT8) produced more than 1.0 cm zone on CAS agar. Ability to produce siderophores was observed to be more in the isolates, isolated from rhizosphere of wheat crop grown under field conditions (Table 4). [32] reported the production of siderophore by CAS assay from all the seventeen strains of bacteria under study. Present results are in accordance with [33] who reported production of siderophore by 7 bacterial isolates using the CAS blue medium. [34] also evaluated the ability of Acinetobacterial isolates to produce siderophores qualitatively using CAS blue agar assay and observed the production of siderophore by 29 Acinetobacter isolates.

### Table 2. Biochemical characterization of diazotrophic bacteria isolated from rhizospheric soil of wheat

<table>
<thead>
<tr>
<th>Diazotrophic isolates</th>
<th>Oxidase</th>
<th>Catalase</th>
<th>Indole production</th>
<th>Methyl red</th>
<th>Voges proskauer</th>
<th>Citrate utilization</th>
<th>H₂S production</th>
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- Negative test; + Positive test
3.2.3 Ammonia excretion

The amount of ammonia excretion was in the range of 1.23-3.70 µg/ml in the bacterial isolates from wheat crop grown under field conditions whereas this range was found to be 1.14-3.50 µg/ml in the bacterial isolates isolated from rhizosphere of wheat crop grown under TGT. The maximum amount of ammonia (3.70 µg/ml) was excreted by isolate WF3 isolated from wheat crop grown under field conditions whereas, minimum amount of ammonia (1.14 µg/ml) was excreted by the isolate WT8 isolated from wheat grown under TGT (Fig. 1). All the isolates were able to excrete ammonia but there was non-significant difference in the amount of ammonia excreted by various isolates. Ammonium excretion of 260 µ Mol in *A. vinelandii* was reported by [35].

3.2.4 Phosphate solubilization

All the isolates were assessed qualitatively and quantitatively for phosphate solubilization. Various isolates were screened for their ability to form clear zone on Pikovskaya agar plates. Out of all the 21 diazotrophic bacterial isolates screened, 6 of the isolate were able to solubilize phosphate. Among these, 5 isolates were from rhizospheric soil of wheat grown under field conditions, one was from rhizospheric soil of wheat grown under temperature gradient tunnel. Out of these 6 phosphate solubilizing isolates, all isolates produced more than 1.0 cm zone.
Solubilization index of the diazotrophic bacterial isolates ranged from 0.1 cm to 1.3 cm at 1st day of incubation, 0.5 cm to 1.6 cm on 2nd day of incubation and 1.1 cm - 1.8 cm at 3rd day of incubation. Maximum solubilization index was observed for the isolate WF8 at 1st, 2nd as well as at 3rd day of incubation (Table 5). Among the 6 bacterial isolates, range of solubilized phosphorus was found to be 16.20 - 22.10 μg/ml but there was non-significant difference in the amount of P-solubilized by various isolates. After 9 days of incubation, the isolate WF8 (22.10 μg/ml) was found to be the most efficient phosphate solubilizer (Table 5). [36] postulated that good phosphate solubilizers produce halo around their colonies with diameters higher than 15 mm. Since, phosphate solubilization is a plasmid borne character so some strains loose their phosphate solubilizing capability after several cycles of inoculation. The present study finds support with the observations made by various researchers [24,37] who also screened phosphorous solubilizing bacteria from rhizospheric soil. [38] observed higher number of P solubilizing bacteria in rhizospheric soil as compared to non rhizospheric soil and these results are in agreement with present findings in which the soil samples were from rhizospheric region.

3.3 Temperature Tolerance of Isolated Diazotrophic Bacterial Isolates

Effect of variable temperatures on the growth of isolated diazotrophic bacteria was studied by growing the diazotrophic isolates in Jensen broth at 30, 33, 36, 39, 42, 45, 48, 51, 54, 57 and 60°C. All the 21 isolates were able to grow up to 48°C temperature. Further onwards, all the isolates were observed to have variable response to different temperatures. Seven isolates showed growth up to 60°C (Fig. 2). Ability of isolates to tolerate higher temperature was found to be more in the bacterial isolates isolated from rhizospheric soil of wheat crop grown under TGT. [39] also reported the ability of nitrogen fixing bacteria to grow even at 60°C same as in the present study the bacteria were able to grow up to 60°C. [23] reported the ability of bacterial isolates to grow at 50°C. Although in vitro temperature selection is not considered a promising approach for field applications [40], but high temperature tolerance can be useful for the purpose of isolating competitive PGPR in oscillating temperature fields. [41] reported that four isolates were able to grow at 4°C, whereas, one isolate was able to grow at 55°C and above. [42] observed that majority of isolates were able to grow properly at 20 to 28°C whereas, only two isolates were found to grow at 60°C. Similar results have been reported by [43] who found the survival of bacterial cultures at temperature ranging from 4 to 55°C. The ability of bacterial isolates to grow at variable temperature conditions might be due to their ability to survive and adapt under very low and high temperatures due to seasonal variations.

3.4 High Carbon Dioxide Concentration Tolerant Diazotrophic Bacteria

Effect of variable carbon dioxide concentrations on the growth of isolated diazotrophic bacteria was studied by growing the diazotrophic bacterial isolates in Jensen’s broth at 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% of CO₂. All the 21 isolates were able to grow upto 10% of CO₂ concentration.
Further onwards, all the isolates were observed to have variable response to different concentrations of CO₂. Eight isolates were observed to show growth up to 20% of CO₂ concentration (Fig. 2). Ability of isolates to tolerate higher CO₂ concentration was found to be more in the bacterial isolates isolated from rhizospheric soil of wheat crop grown under TGT.

4. CONCLUSION

Diverse bacteria were found to present in the rhizospheric soil of wheat crop. These bacteria were isolated from the soil samples taken from wheat crop grown under field conditions as well as from wheat crop grown under temperature gradient tunnel. The nitrogen-free media was used for the isolation of bacteria to ensure their nitrogen fixation activity. The isolated bacteria were characterized in this work based on their biochemical and functional properties. The bacteria isolated from wheat crop grown under temperature gradient tunnel showed higher tolerance to high CO₂ and high temperature levels. The isolated bacteria also showed plant growth promoting traits like production of indole acetic acid, phosphate solubilization, siderophore production etc. The isolate WF6 was found to be the best isolate in terms of functional characteristics as well as in terms of tolerance to high temperature and CO₂ levels. Our results demonstrate that environmental factors affect activities of soil bacteria. Our data highlight the need to know more about the physiology and ecology of the various uncultured, dominant, and responsive soil community members.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


23. Thanuja L, Ambika SR. Effect of bacterization of finger millet grains with...


