

Criterion – 3

Research, Innovations and Extension

NAAC- SSR (2nd Cycle)



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3.3.1(1)

Biofertilizer

(NPK Microbial Consortium)

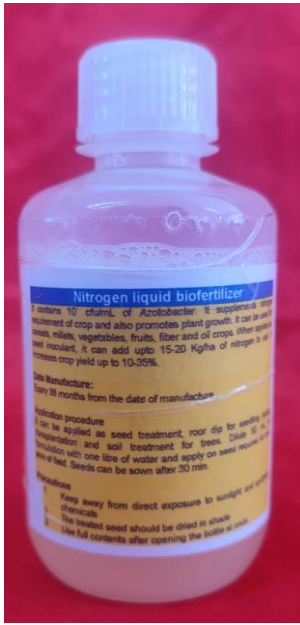
and Biopesticide



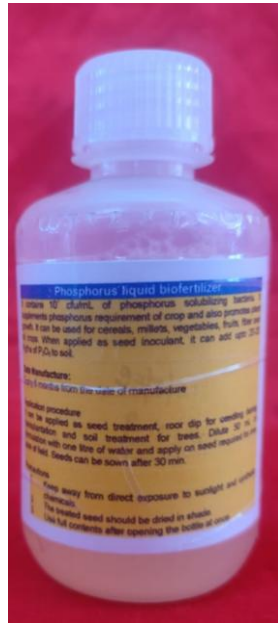
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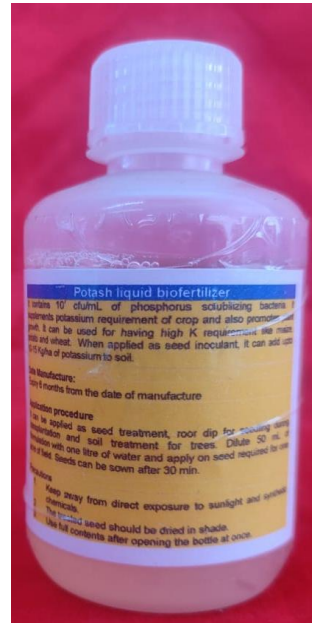
Bio-fertilizers i.e. NPK (Nitrogen, Phosphorus and Potassium) microbial consortium



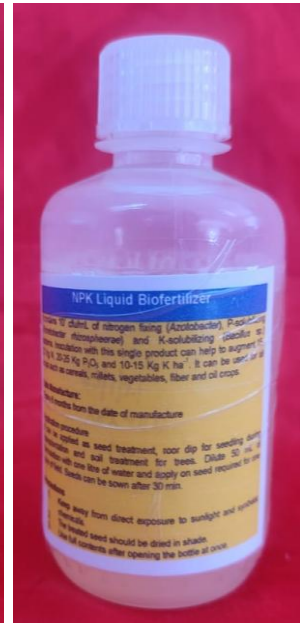
**Nitrogen
biofertilizer**



**Phosphorus
biofertilizer**



**Potassium
biofertilizer**



**NPK Microbial
Consortium**

Applications of developed microbial consortium



**NPK Microbial
Consortium**



**NPK Chemical
fertilizer**



Un-inoculated



Endophytic Microbes from Diverse Wheat Genotypes and Their Potential Biotechnological Applications in Plant Growth Promotion and Nutrient Uptake

Kusam Lata Rana¹ · Divjot Kour¹ · Tanvir Kaur¹ · Imran Sheikh¹ · Ajar Nath Yadav¹ · Vinod Kumar² · Archna Suman³ · Harcharan Singh Dhaliwal¹

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Abstract Endophytic microbes residing inside the tissues of plants play a significant role to enhance the growth and health of plants by different plant growth-promoting mechanisms. In the present investigation, N₂-fixing endophytic bacteria were isolated and characterized by plant growth. A total of one hundred fifty-nine endophytic bacteria were isolated from surface-sterilized roots and stem of different genotypes of wheat growing in the Divine Valley of Baru Sahib, Himachal Pradesh. The isolated bacterial endophytes were screened in vitro for plant growth-promoting attributes. Out of one hundred fifty-nine, thirteen endophytic bacteria were selected based on multifarious plant growth-promoting attributes. Among plant growth-promoting activities, hydrogen cyanide producers (19%)

were higher when compared to siderophores producers (16%) and P-solubilizers (16%), ammonia producers (14%), K-solubilizers (14%), IAA producers (12%), Zn-solubilizers (5%), N₂-fixers (2%) and biocontrol (2%). One of the isolates EU-B2RT.R1 demonstrated that a significant level of nitrogenase activity, P-solubilization and IAA production was identified as *Acinetobacter guillouiae* EU-B2RT.R1 based on 16S rRNA gene sequencing and BLAST analysis. *Acinetobacter guillouiae* EU-B2RT.R1, exhibiting multifarious beneficial traits, is further evaluated for plant growth promotion of wheat cultivar PBW 343+Lr24+GPC in pot experiment under greenhouse conditions. The *Acinetobacter guillouiae* EU-B2RT.R1 with multifarious plant growth-promoting activity has emerged as one of the efficient biofertilizers that need to be explored for sustainable agriculture.

Kusam Lata Rana and Divjot Kour contributed equally to the present work.

Significance Statement The significance of present work is the isolation of nitrogen-fixing endophytic bacterium isolated from wheat, having huge potential role in plant growth promotion under in vitro condition and in future can be developed as nitrogen biofertilizers.

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SHORT COMMUNICATION

First report on *Rahnella* sp. strain EU-A3SNfb, a plant growth promoting endophytic bacterium from wild wheat relative *Aegilops kotschy*

Rajeshwari Negi¹ · Tanvir Kaur¹ · Rubee Devi¹ · Divjot Kour² · Imran Sheikh³ · Vikrant Tyagi³ · Ajar Nath Yadav¹

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Abstract Endophytic biology is an emerging field due to their wide range of application in various sectors including agriculture, pharmaceutical, medical, and industries. The endophytic phytomicrobiomes residing inside the tissue of the plant have gained the momentum for plant growth promotion (PGP), crop production and protection along with sustaining the environmental health. Considering the importance of sustainability, the present study aimed to characterize and evaluate the plant growth promoting endophytic microbes with nitrogen fixing ability. A total of 52 putative endophytic bacteria were isolated from wild wheat relative *Aegilops kotschy* growing in the Himalayan regions. All the isolates were screened for nitrogen-fixing attributes, and the selected nitrogen fixing bacteria were screened for

other PGP attributes including solubilizing of phosphorus, potassium and zinc; production of siderophores, ammonia and hydrogen cyanide. Efficient microbe having capability to fix nitrogen was identified as *Rahnella* sp. strain EU-A3SNfb using 16S rRNA gene sequencing and BLASTn analysis. The selected bacterial strain was evaluated for growth of *Aegilops kotschy*, and results showed the significant improvement in the physiological parameters, chlorophyll, total soluble sugar, phenolics and flavonoid content as compared to recommended dose of chemical fertilizer and untreated control. This efficient bacterial strain EU-A3SNfb could be used as bioinoculants for cereal crops growing in hilly region for agricultural sustainability.

Keywords *Aegilops kotschy* · Endophyte · Plant growth promotion · *Rahnella* sp. · Sustainability

Rajeshwari Negi and Tanvir Kaur contributed equally to the present work.

Significance statement: Present era is more inclined towards the research on endophytic microbes and development of bioinoculant for sustainable agriculture. The present investigation has reported bacterial strains which could be used as biofertilizers for plant growth, enhanced crop yield and crop protection for agricultural sustainability.

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Nitrogen is the most abundant element of the atmosphere and essential macronutrient required by the plants in the large quantity. It is limiting factor for the plants despite its abundance as it could not be utilized because of its strong trivalent bond. Nitrogen requirement of the plants is fulfilled by the utilization of urea, the chemical fertilizer. Urea use in the field has shown the significant enhancement in the crop yield, but it also resulted in many environmental problems such as soil fertility and diversity loss. The alternative of urea to supply nitrogen to the plants is the reduction in the element into the ammonia, which could be achieved by the microbes through the process biological nitrogen fixation. Endophytic bacteria, residing in plants are one such organism which gained momentum for fixing the atmospheric nitrogen. It has been found that endophytic nitrogen fixing bacteria contributes up to 47% of N to the plants. Bacterial endophytes are the sustainable approach



Alleviation of Drought Stress and Plant Growth Promotion by *Pseudomonas libanensis* EU-LWNA-33, a Drought-Adaptive Phosphorus-Solubilizing Bacterium

Divjot Kour¹ · Kusam Lata Rana¹ · Imran Sheikh¹ · Vinod Kumar² · Ajar Nath Yadav¹ · Harcharan Singh Dhaliwal¹ · Anil Kumar Saxena³

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Abstract Drought is a very common abiotic stress worldwide in arid and semiarid areas. It decreases the growth and yield of the crops. Due to drought stress, there is insufficient intake of the nutrients, the low rate of photosynthesis and limited supply of water in plants. Inoculating crops with plant growth-promoting rhizobacteria (PGPR) mitigates the deleterious effects of stress by promoting beneficial effects such as helping them in the acquisition of less available nutrients, increasing the levels of plant growth regulators, improving the physiological health of the plants. In the present study, drought-tolerant phosphorus-solubilizing rhizobacteria with multifunctional plant growth-promoting attributes were isolated from different cereal crops grown in the Divine Valley of Baru Sahib, Himachal Pradesh, using a different nutrient

combination. A total of 86 bacteria were isolated from different growth media. All 86 could tolerate 5% PEG, and 19, 6 and 6 isolates could tolerate 6%, 7% and 8% PEG-8000, respectively. Among 86, 48 drought-adapted and P-solubilizing strains were selected and screened for diverse PGP attributes such as solubilization of potassium and zinc, production of siderophores, hydrogen cyanide, ammonia and 1-aminocyclopropane-1-carboxylate deaminase. The efficient drought-adaptive P-solubilizing strain was used for seed germination and plant growth-promoting ability in different pot assays under laboratory and greenhouse conditions at different water regimes. The strain EU-LWNA-33 positively influencing the growth parameters and physiological parameters was identified using 16 S rRNA gene sequencing as *Pseudomonas libanensis*. To our knowledge, this is the first report for *P. libanensis* EU-LWNA-33 to solubilize a considerable amount of P under the water-deficient conditions. The use of stress-adaptive and P-solubilizing PGPR provides significant promise to overcome the challenges of sustainable agriculture in stressed environmental conditions.

Divjot Kour and Kusam Lata Rana contributed equally in the present manuscript

Significance Statement Drought-tolerant microbes as biofertilizers play an important role in the survival and growth of plants during stress. Further, these bioinoculants are eco-friendly to replace chemical fertilizers.

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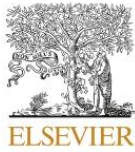
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Phosphorus biofertilizer published paper

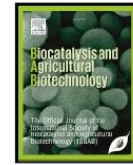
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Microbe-mediated alleviation of drought stress and acquisition of phosphorus in great millet (*Sorghum bicolor* L.) by drought-adaptive and phosphorus-solubilizing microbes

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ABSTRACT

Among the extreme habitats, drought is most harsh abiotic stress affecting growth, development and productivity of crops. Plants also face limitations of certain nutrients such as phosphorus required for different physiological and metabolic activities. Stress-adaptive phosphorus-solubilizing microbes in rhizospheric soil can help plants to combat water scarcity and overcome the problem of phosphorus unavailability to plant systems. The present investigation deals with the isolation of drought stress adaptive and P-solubilizing microbes from rhizospheric soil of different cereals and pseudocereals and their role in mitigation of drought stress in great millet. A total of 193 rhizospheric microbes were isolated and screened for their capability to solubilize phosphorus under drought stress. Twenty isolates exhibited P-solubilizing attribute under drought stress, which were further screened for plant growth promoting (PGP) traits such as solubilization of zinc and potassium; production of Fe-chelating compounds, indole acetic acid, hydrogen cyanide and ammonia. On basis of multi-functional PGP traits, two efficient and potential microbes were evaluated for PGP in great millet *in vitro* under green house with different water regimes. The isolates were found to be efficient in terms of enhancing accumulation of different osmolytes such as glycine betaine, proline, sugars, increased chlorophyll content, and decreasing lipid peroxidation. The isolates were identified by 16S/18S rRNA gene sequencing as *Streptomyces laurentii* EU-LWT₃-69 and *Penicillium* sp. strain EU-DSF-10. To best of our knowledge *Streptomyces laurentii* has been reported first time as PGP and drought adaptive bacterium. PGP drought-adaptive phosphorus solubilizers could be used as bioinoculants for crops under water scarcity ecosystems.

1. Introduction

Climate is changing day by day and plants face a variety of stresses by the changing environment. Among diverse environmental stresses, drought is one of the most destructive stresses which negatively influence the crop plants (Hasanuzzaman and Fujita, 2012). Climate limits the growth of the plants as well the production of the food crops (Keyvan, 2010). Additional problems arise due to the limitation of the nutrients in particular less concentration of available phosphorus (Sharma et al., 2013) and to meet phosphorus demands of the

plants, modern agricultural systems have to depend on chemical fertilizers. The use of the chemical fertilizers has no doubt enhanced the yield but at the same time has also left undesirable effects on the ecosystem (Kumar and Shastri, 2017).

Biotechnology offers various eco-friendly approaches to alleviate such problems by the use of plant growth promoting microbiomes (PGPMs) as biofertilizers and to achieve agricultural and environmental sustainability. These PGPMs can also alleviate the adverse effects of such problems by different mechanisms including the fixation of the biological nitrogen; acquisition of phosphorus (P), potassium (K),

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Amelioration of drought stress in Foxtail millet (*Setaria italica* L.) by P-solubilizing drought-tolerant microbes with multifarious plant growth promoting attributes

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Abstract

Drought is the most limiting factors affecting plant development. It severely affects the crops and leads to serious reductions in yield. There are certain nutrients which also act as limiting factor for plants such as phosphorus and nitrogen. Under the conditions of nutrient limitations, growth is greatly reduced. Plant associated microbiome are gaining attention as they help the host (plant) to combat stress conditions. In the present study, stress-adaptive and phosphorus-solubilizing microbes were isolated from rhizosphere of different crops such as wheat, maize, foxtail millet, and finger millet growing in NW Indian Himalayas. A total of 70 microbes were isolated using different defined and selective growth media. The isolated microbes were screened for plant growth promoting (PGP) ability of phosphate solubilization using three different insoluble phosphorus (P) substrates (apatite, tricalcium phosphate and rock phosphate) under the drought stress conditions (5–8% PEG-8000). Among isolated microbes 27 isolates exhibited P-solubilizing attribute under the water deficient conditions. The two efficient drought-adaptive and P-solubilizing isolates were identified as *Acinetobacter calcoaceticus* EU- LRNA-72 and *Penicillium* sp. EU-FTF-6, respectively, by 16S rRNA and 18S rRNA gene sequencing. The isolates EU- LRNA-72 and EU-FTF-6 were evaluated for plant growth promoting (PGP) traits and mitigation of drought stress in foxtail millet under the controlled and natural conditions. The isolates *A. calcoaceticus* EU- LRNA-72 and *Penicillium* sp. EU-FTF-6 efficiently mitigated the adverse effects of drought in foxtail millet by enhancing the accumulation of glycine betaine, proline, sugars, and decreasing lipid peroxidation. The drought tolerant P-solubilizing microbes could be useful for plant growth promotion and mitigation of drought stress for crops growing under the water deficient conditions.

Keywords Agricultural sustainability · Drought · Foxtail millet · PGPMs · Phosphorus solubilization

Introduction

Drought is a very complex phenomenon which affects different parts of the world and causes negative impacts on social, economic and environmental aspects. Global warming and extreme dry conditions are ultimately going to hinder food productions (Niu et al. 2018). Drought stress causes devastating effects on different growth stages of plants. All the physiological processes are completely disturbed during drought due to disruption of membrane structure, enzyme activities, and ultra structural cellular components (Hasanuz-zaman et al. 2018). Drought, thus, is one of the most critical expression of environmental stress which affects plant's biochemistry and physiology. On the other hand, phosphorus (P), essential macronutrient for metabolic activities, is the major growth limiting nutrient. It is often required in

Divjot Kour and Kusam Lata Rana contributed equally to present work.

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Phosphorus solubilizing stress tolerant rhizobacteria for growth promotion of wheat (*Triticum aestivum* L.)

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Abstract

Phosphorus is needed by plants for their growth and development as well as different metabolic activities simultaneously being the major yield limiting plant nutrient. The application of the phosphatic fertilizers is the only alternative to prevent the phosphorus deficiency in the plants but the over use of the fertilizers depletes the soil fertility and pollutes the environment. In the present research, 190 bacteria were purified from the rhizosphere of wheat collected from the Keylong region. All the 190 isolates were screened for P-solubilization attribute. Ten selected P-solubilizing strains including EU-KLD-02, EU-KLD-12, EU-KLD-13, EU-KLD-17, EU-KLD-19, EU-KLD-20, EU-KLD-23, EU-KLD-55, EU-KLD-70 and EU-KLD-76 were then further characterized for other attributes of plant growth promotion and tolerance to different abiotic stress. The selected strains were quantified for phosphorus under normal and conditions of abiotic stress. The efficient strains were identified as *Serratia marcescens* EU-KLD-2, *Pseudomonas azotoformans* EU-KLD-13, *Staphylococcus aureus* EU-KLD-23 and *Pseudomonas* sp. EU-KLD-70. All the ten isolates with multiple PGP attributes were used as bioinoculants in green house experiment on wheat crop. The isolates efficiently improved the studied physiological and growth parameters of wheat. P-solubilizers as biofertilizers are potential candidates to reduce the utilization of chemical fertilizers as well as are a better alternative for environmental sustainability. The present investigation deals with the isolation of P-solubilizing bacteria from rhizospheric soil of wheat, screening for PGP attributes, abiotic stress tolerance and their role in plant growth promotion of wheat,

Keywords Abiotic stress · Bioinoculants · Plant growth promotion · P-solubilization · Wheat

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Introduction

In India, agriculture has become a considerable private sector and in future this will also become lifeline of Indian economy. In percentage of the population of world, the stipulation of food will increase. The rate of increase in the global population has been estimated to reach 9.6 billion by 2050 (United Nations 2013). Current agricultural practices are not sustainable either economically or environmentally. Agricultural production extremely increased after green revolution by the use of chemical fertilizers and pesticides. The excessive use of chemicals has led to deleterious effect on the environment and the insects developed resistance against these usual and ordinary pesticides. The awareness among the people about the sustainable agricultural practices has increased. The scientific community and researchers are also aware of sustainable management practices. The primary importance of sustainable agriculture is



First Report on Novel Psychrotrophic Phosphorus-Solubilizing *Ochrobactrum thiophenivorans* EU-KL94 from Keylong Region in Great Himalayas and Their Role in Plant Growth Promotion of Oats (*Avena sativa* L.)

Divjot Kour¹ · Ajar Nath Yadav^{2,3}

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Abstract

Cold stress leads to the disruption of the cellular homeostasis in plants and generation of reactive oxygen species (ROS) and productivity losses. In the present study, 94 psychrotrophic phosphorus-solubilizing bacteria with multiple plant growth-promoting (PGP) capabilities were isolated from rhizosphere of wheat. The most efficient strain EU-KL94 showing highest amount of solubilized phosphorus and maximum number of PGP attributes was identified using 16S rRNA sequencing as *Ochrobactrum thiophenivorans*. *Ochrobactrum thiophenivorans* EU-KL94 along with recommended doses of the chemical fertilizers as controls were used for alleviation of cold stress in oats. The strain improved the root and shoot length, dry and fresh weight, proline, glycine betaine, chlorophyll content as well as the superoxide dismutase (SOD) and glutathione reductase (GR) activities of oats under cold stress conditions. *Ochrobactrum thiophenivorans* with all promising plant growth activities under cold stress could be used as an environmental friendly strategy for mitigation of low temperature stress. To the best of our knowledge, *Ochrobactrum thiophenivorans* has been reported for the first time as P-solubilizer and as bioinoculants in oats for cold stress mitigation.

Introduction

Global demands for food, fodder and fuel have increased due to increasing population and there is critical need to fulfill these demands by increasing the productivity in a sustainable way or utilizing the lands for cultivation which were not suitable for growing crops such as the regions of high altitude. These regions suffer from certain limitations such as salinity, drought, frost and cold being imposed on them by the nature [1]. Among diverse abiotic stresses, cold

temperature is challenging for agriculture. Low-temperature stress is a serious problem globally as it negatively impacts the crop productivity [2].

The impact of the cold stress on the plants greatly depends on the degree of severity and the time for which crop is exposed. The seedling stage is known to be the most sensitive to the chilling conditions. The plants affected by the chilling stress show diverse symptoms such as increased production of stress ethylene, occurrence of surface lesion, tissue break down and leakage of plant metabolites [3]. The unsaturation of the fatty acids leads to reduction of the membrane fluidity, lipid to protein composition and ratios in cell membrane [4]. Coldness reduces the uptake of the water by the roots which lead to dehydration [5]. Cold stress at reproductive stage delays heading and causes pollen sterility which in turn is responsible for grain yield reduction [6, 7].

The undesirable effects of the cold stress can be alleviated by the use of the cold stress adaptive and plant growth-promoting PGP microbes colonizing the rhizospheric region. The consideration of the beneficial microbial diversity is important to shift toward the sustainable approaches. In hilly regions and cold conditions, psychrotrophic microbes

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Mitigation of low temperature stress and plant growth promotion in barley (*Hordeum vulgare* L.) by inoculation of psychrotrophic P-solubilizing *Serratia nematodiphila* EU-PW75

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Abstract

Agriculture is one of the exposed sectors to abiotic stress conditions. There are diverse abiotic stress in which low temperature is an important abiotic stress limiting plant growth and development. The low temperature (0–20 °C) adaptive microbial communities are of particular interest because of their adaptability to prevailing conditions. Phosphorus is an important growth nutrient for the plants. Low temperature and phosphorus limitations act as the stress factors for the plants. Psychrotrophic microbiomes with P-solubilizing ability are gaining attention because of their amazing role in enhancing the availability of P and growth under low temperatures. In the present study, different crops with their rhizospheric soil from Poonch region of Jammu and Kashmir were collected for isolation of potential plant growth promoting psychrotrophic bacteria. A total of 65 isolates were obtained on different growth media. Among 65 isolates, 29 exhibited P-solubilizing capability. On basis of P-solubilizing ability and multiple PGP attributes, bacterial strain EU-PW75 was identified on basis of 16S rRNA gene sequencing as *Serratia nematodiphila*. The strain EU-PW75 was used for plant growth promotion of barley under conditions of low temperature where it efficiently improved the growth and physiological parameters of barley. The low temperature adaptive microbes with PGP activities could be useful for cold stress alleviation and plant growth promotion of crops growing under the low temperature.

Keywords Barley · Low temperature · PGP microbes · Plant growth promotion · P-solubilization · Psychrotrophs

Introduction

Eighty percent of the earth's surface is estimated to be exposed to 15 °C or below at different times of the year (Kawahara 2002). Cold environment is harsh owing to major factor being low temperature along with some minor factors such as UV rays, low availability of nutrients and water, freeze–thaw cycles, and osmotic pressures (Rafiq et al. 2019). Microbial communities flourishing under extreme

cold conditions are found abundantly over the earth's surface such as deep sea depths, polar and temperate regions (Subramanian et al. 2011). Cold adaptive microbiomes undergo physiological adaptations to thrive in such cold environment and have been extensively studied from past few years by diverse culture-dependent and culture-independent techniques (Yadav et al. 2018b). Diverse novel microbial genera have been reported from cold habitats; *Psychrobacter* from coral, *Pocillopora cydonia* (Zachariah et al. 2016), *Massilia* from ice core (Shen et al. 2015), *Sphingomonas* from alpine glacier cryoconite (Zhang et al. 2011) and *Cryobacterium* from glacial soil (Reddy et al. 2010). Cold adaptive fungi including *Akernaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Geomyces*, *Lecanicillium*, *Monodictya*, *Mucor*, *Penicillium*, and *Rhizopus* have been also reported from Antarctica (Kostadinova et al. 2009).

Cold adaptive microbiomes are emerging as rich sources of several valuable tools for wide applications and development of novel technologies. In recent times, these microbiomes are gaining interest and are being focused in

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Potassium biofertilizer published paper



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Plant growth promotion of barley (*Hordeum vulgare* L.) by potassium solubilizing bacteria with multifarious plant growth promoting attributes

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Abstract

Potassium (K) is the foremost macronutrients for growth of plant, soil health and fertility. The huge application of NPK chemical fertilizers negatively impacts the economy and is a threat to environmental sustainability. The rapid depletion of K mineral in soil is due to the application of agrochemicals agricultural fields for the production of crops in India. In present investigation, K-solubilizing microbes (KSM) were isolated and enumerated from cereal crops growing in Sirmour Himachal Pradesh. A total 125 bacteria were isolated and screened for K- solubilization on Aleksandrov agar plates and found that 31 bacterial strains exhibited K-solubilization. These 31 K-solubilizing strains of bacteria were additionally screened for other plant growth promoting (PGP) potential including solubilization of minerals, production of siderophores, ammonia, hydrogen cyanide and indole acetic acids. The performance of an efficient K-solubilizer was evaluated for plant growth promoting ability in pot assay under *in vitro* conditions. The strain EU-LWNA-25 positively influenced shoot length, fresh weight, carotenoids and total sugar content than the full dose, half dose and control. The strain enhancing physiological and growth parameters was identified by BLASTn analysis as *Pseudomonas gessardii* EU-LWNA-25. K-solubilizing plant growth promoting bacteria could be suitable bioinoculants for Rabi seasonal crops and overcomes the challenges of sustainable agriculture in K-deficient soil.

Keywords

Biodiversity, Bio-inoculants, PGP attributes, Potassium solubilization, Sustainable agriculture

Introduction

Potassium (K), the 7th most abundant, is one of the vital nutrients which is requisite in large quantity for functioning of cells by all living organisms. It plays a major role in activation and production of enzymes that are involved in regulating the photosynthesis rate i.e. adenosine triphosphate and transportation of water and nutrients throughout the plant. The deficient supply of potassium in plants depressed the translocation of amino acids, calcium (Ca), magnesium (Mg), nitrates, and phosphates. In soil, the nutrient is present in different insoluble forms, majorly fixed forms which cannot be absorbed by plant systems (1, 2). To fulfill the plant nutrient requirement, potassium fertilizer is being used on a huge level around the globe. The use of fertilizer also has a harmful impact on the environment and it is necessary to have a substitute indigenous source of potassium that produces crops in



First Report on Rhizospheric Silicate Mineral Weathering Bacteria from Indian Himalayas and Their Roles for Plant Growth Promotion of Tomato (*Solanum Lycopersium L.*)

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Abstract Potassium silicate rock is one of the main sources of soil potassium (K), which is the essential macronutrients for plant growth. Potassium is absorbed in the form of K⁺ ion by plants. The amount of K⁺ is a limited factor, thus excessive quantity of K fertilizer is being applied in agricultural field worldwide. The excessive K fertilizers application has a substantial negative impact on the environmental sustainability and economy. Silicate mineral weathering bacteria are one of the better substitutes of K fertilizer, which can weather the rock of potassium silicate and releases K⁺. In present study, silicate-mineral weathering bacteria (SMWB) from rhizospheric samples of cereal and pseudocereal crops were isolated and evaluated for the growth promotion of widely cultivable tomato. A total 154 rhizospheric bacteria were isolated and screened for potassium solubilizing attributes by using Aleksandrov agar media amended with K-alumino silicate and mica as insoluble source of potassium. Isolated bacterial strains were screened and found 56 bacterial strain exhibited silicate mineral weathering attribute. Among 56 bacterial strains, *Bacillus albus* EU-AN-25 was

found to be the most efficient SMWB. The performances of these selected silicate mineral weathering isolates were found to promote plant growth higher than the recommended dose of chemical fertilizers and untreated control. The silicate-mineral weathering bacterium was found to have more efficient and could be employed as an agricultural tool for crops grown in high-altitude, low-temperature alpine, and sub-alpine regions.

Keywords Bioinoculant · Plant growth · Potassium solubilization · Sustainable agriculture

Soil contains various mineral elements, among which potassium (K) is the third most essential nutrient. This macronutrient plays a vital role in the metabolic and physiological processes of the plant life. Plant growth, photosynthesis, rate of the assimilation, sugar accumulation, growth of root, devotement of seed, plant yield, fiber quality and overall development are the major role played the K mineral in the plant life [1]. Moreover, potassium helps in enhancing the stress tolerance and efficiency of water use. Biotic stress tolerance is also improved by the potassium mineral [2]. In soil, potassium is the most abundant macro-mineral, and it is present in the number of appreciable mineral ores in the soil system that constitutes nearly 2.5% of the lithosphere. This mineral is present in the three diverse forms in the soil, i.e., insoluble form (80–90%), interlayer K of non-expanded clay minerals such as lattice K and illite in K feldspars (1–10%) and non-exchangeable pool [3]. Among these three forms of the K plant absorb mineral as cation which is lesser in the amount [4]. Thus, to fulfill the required amount of K mineral in plants K fertilizer was introduced in the beginning of the green revolution [5]. The extensive and long-term utilization of agrochemicals is the known to serious environmental

Kartika Shukla, Rajeshwari Negi and Tanvir Kaur contributed equally to the present work.

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Research article

Microbial consortium with nitrogen fixing and mineral solubilizing attributes for growth of barley (*Hordeum vulgare* L.)[☆]Tanvir Kaur^a, Rubee Devi^a, Sunil Kumar^a, Imran Sheikh^b, Divjot Kour^c, Ajar Nath Yadav^{a,*}^a Microbial Biotechnology Laboratory, Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, 173101, India^b Department of Genetics, Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, 173101, India^c Department of Microbiology, Akal College of Basic Sciences, Eternal University, Baru Sahib, India

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ABSTRACT

Bioinoculants provide better opportunity for ecological farming practices to improve the plant growth and enhanced crop productivity. Different types of bioinoculants containing single microbial culture and multiple microbial strains in single formulation could be used for agricultural sustainability. The different efficient microbial strain in single formulation as a consortium is an emerging trend in the present era. The present study deals with the isolation of nitrogen fixing, phosphorus and potassium solubilizing microbes from rhizospheric soil and root's internal tissues of different cereal/pseudocereal crops and their application as a microbial consortium for the growth of cereal crops. A total of 152 rhizospheric and endophytic bacteria were isolated and screened for the plant growth promoting (PGP) traits of nitrogen fixation, solubilization of phosphorus, and potassium. Among all the isolates, nine were found to fix nitrogen, fifteen and eleven exhibited phosphorus and potassium solubilization activity, respectively. Three selected efficient bacterial strains were identified using 16S rRNA gene sequencing as *Erwinia* sp. EU-B2SNL1 (N-fixer), *Chryseobacterium arthrosphaerae* EU-LWNA-37 (P-solubilizer), and *Pseudomonas gessardii* EU-MRK-19 (K-solubilizer). The inoculation of these three bacterial strains on barley crop as single inoculum and as microbial consortium enhanced the growth and physiological parameters including root/shoot length and biomass, chlorophyll, carotenoids, phenolics, flavonoids and soluble sugar content in comparison with untreated control. The microbial consortium was found to be more effective as compared to single inoculum. The microbial consortium of nitrogen fixing and mineral solubilizing microbes could be used as bio-fertilizer for plant growth and soil health.

1. Introduction

Macronutrients, nitrogen (N), phosphorus (P) and potassium (K) are the basic need for plant growth which play several significant roles in the entire life of the plants. These nutrients perform beneficial activities in the metabolism of plant and protect them from various abiotic and biotic factors exerted from outer environment. Macronutrients help in increasing the quality and quantity of crop grain (Tripathi et al., 2014). The fulfillment of nutrient requirements until the middle of the twentieth century was relied on organic manures, but the beginning of green revolution, the chemical fertilizers are used for high production and its consumption drastically increased with every progressed year worldwide. In India, during the 1950–1951, the consumption of N fertilizer

(urea), P fertilizer (di-ammonium phosphate) and K fertilizer (potash) was 0.06, 0.01 million ton (Mt) and almost nil, respectively and by 2000–2001 the consumption of N, P, K fertilizer hiked to 10.8 Mt, 1.8 Mt and 0.81 Mt, respectively and which resulted an increment of crop the yield by 190 folds (Pathak et al., 2010). According to FAOSTAT 2021 report 27 billion tons of NPK fertilizer is being yearly used.

In earlier times, i.e. from 1950–1991 the use of the NPK chemical fertilizers increased the annual per capita food availability to 208 kg but after a decade it declined to 192 kg. The reason behind the stagnation rate of food availability was due to fertilizers harmful impact such as N fertilizer leads to acid deposition, nitrate leaching into ground water, eutrophication, loss of biodiversity and production of greenhouse gas that contributes to ozone depletion (Powelson 1993). Whereas, P fertilizer

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Plant growth promotion of pearl millet (*Pennisetum glaucum* L.) by novel bacterial consortium with multifunctional attributes

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Abstract

Intensive agricultural practices with chemical fertilizers are becoming the reason of environmental deterioration. To feed the ever increasing worldwide population with sustainability goals is a one major challenge and biostimulants developed from the beneficial soil and plant microbiome is a better approach for farming practices to increase the crop productivity. In agricultural fields various categories of biostimulants are utilized that contains one microbial culture and multiple strains of microbes in single formulation as microbial consortium. The mixture of microbial species in a formulation is an emerging technology in the present era because of its multiple benefits for plant growth and plant protection for agro-environment sustainability. The present study deals with the isolation of rhizospheric and endophytic bacteria from different cereal and pseudocereal crops and development of a single inoculum as well as consortium for the cereal crops growth. A total of 147 bacteria (rhizospheric and endophytic) were sorted out and were screened for plant growth promoting attributes of nitrogen fixation, phosphorus and potassium solubilization. Among all the bacterial isolates, three potential strains EU-PEN-6, EU-PRP-12 and EU-PRK-4 exhibiting N-fixing, P and K-solubilizing attributes were identified using 16S rRNA gene sequencing as *Pseudomonas extremorientalis*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*, respectively. In best of our knowledge, the present investigation has firstly reported *P. extremorientalis*, *B. subtilis* and *B. amyloliquefaciens* associated with the endophytic region of wheat and rhizosphere of pearl millet. The strains inoculation on pearl millet as single culture and as bacterial consortium improved the parameters like length and biomass of root/shoot, chlorophyll, carotenoids, total soluble sugar content, phenolics, and flavonoids over untreated control. The bacterial consortium was found to have more potential over single culture inoculation. A bacterial consortium could be used as bioinoculants for cereal crops growing in hilly regions.

Keywords Agricultural sustainability · Biostimulants · Bacterial Consortium · Pearl millet · Plant growth promotion

Abbreviations

BLAST	Basic Local Alignment Search Tool
CFU	Colony forming unit
gDNA	Genomic DNA
K	Potassium

LSD	Least significant difference
N	Nitrogen
NA	Nutrient agar
NCBI	National Center for Biotechnology Information
OD	Optical density
P	Phosphorus
PGP	Plant growth promoting

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Introduction

Cereals crops are the staple food for the worldwide population and they cultivated in more than 734.32 million ha of area. Globally, 2980.2 million tonnes of cereal crops



Synergistic Effect of Endophytic and Rhizospheric Microbes for Plant Growth Promotion of Foxtail Millet (*Setaria italica* L.)

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Divjot Kour² · Ajar Nath Yadav¹

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Abstract Microbial bioformulations in agriculture is vastly known and accepted for the sustainable agriculture and most of the bioformulation applied in the agricultural fields are developed from a single strain which have displayed inconsistent results. Microbial consortium is one new and promising bioformulation that could be used for growth and development of the crops. In the present investigation, isolation of plant (cereal and pseudocereal)-associated rhizospheric and endophytic microbes exhibiting nitrogen fixation, phosphorus and potassium solubilization capability was carried out. Afterward, the microbial mixture was developed for the growth promotion of foxtail millet crop. A total of 163 bacteria were sorted out and they were screened for nitrogen fixation, P- and K-solubilization attributes. Potent NPK strains were identified as *Stenotrophomonas rhizophila* EU-FEN-32 (N-fixer), *Pseudomonas marginalis* EU-FRP-16 (P-solubilizer) and *Bacillus* sp. strain EU-FRK-38 (K-solubilizer) using 16S rRNA gene sequencing. These strains inoculation on foxtail millet as microbial consortium improved the growth and physiological parameters in comparison with respect to chemical fertilizer and untreated control. The compatible mixture of microbes was found to have more potential and it could be used as bioinoculants for cereal crops growing in hilly regions.

Keywords Bioformulations · Foxtail millet · Microbial consortium · Sustainable agriculture

Globally, agricultural practices are not uniform, but the utilization of agrochemicals is common. Total 890 man-made agrochemicals are known which plays crucial role in enhancing the crop yield. The utilization of agrochemicals on the other hand leaves the hazardous effect on the environment and humans (mostly farmers). Formulation based on the microbes (bacteria, and fungi) are the finest substitute of agrochemical which sustains the agriculture as well as environment. Microbe's exhibits diverse indirect and indirect plant growth promoting (PGP) attributes, such as fixation and nutrients (nitrogen, phosphorus, potassium, zinc, and selenium) solubilization; production of growth hormones, antibiotics, nutrient chelator (siderophores) which benefits in the plant growth development [1]. Beneficial microbes exhibiting multifarious PGP traits as bioinoculants could be used as singly or as consortium (mixture of two or more microbes). The majority of available bioformulations developed ever since are derived from single species. Particularly from the last decade, microbial bioformulations were also developed containing two or more microbial species as combining the microbes gives combined benefits. Thus, the conglomeration of beneficial microbes is an efficient bioinoculants over the single microbe containing bioformulations [2].

The rhizospheric soil and samples of different cereal crops (maize, wheat) and pseudocereal crops (millets and amaranth) were collected from the green slopes of the Shivaliks, Himachal Pradesh. The samples were placed in the sterilized plastic bags and stored at 4 °C until the isolation of endophytic and rhizospheric bacteria. The isolation of cultural endophytic and rhizospheric bacteria were performed using serial dilution plating method on different

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Mutualistic Effect of Macronutrients Availing Microbes on the Plant Growth Promotion of Finger Millet (*Eleusine coracana* L.)

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Abstract

Globally, man-made agrochemicals plays crucial role in plant growth promotion and boost crop yield. The agrochemicals overuse leaves the detrimental damage on the environment and humans. Biostimulants developed from single or multiple microbes (archaea, bacteria, and fungi) could be the appropriate alternative of agrochemical which sustains the agriculture as well as environment. In the present investigation, 93 beneficial bacteria associated with rhizospheric and endophytic region were isolated using diverse growth media. The isolated bacteria were screened for macronutrients availing traits including dinitrogen fixation, phosphorus and potassium solubilization. The bacterial consortium was developed using selected bacteria with multifunctional attributes and evaluated for the growth promotion of finger millet crop. Three potent NPK strains were identified as *Erwinia rhapontici* EU-FMEN-9 (N-fixer), *Paenibacillus tylopili* EU-FMRP-14 (P-solubilizer) and *Serratia marcescens* EU-FMRK-41 (K-solubilizer) using 16S rRNA gene sequencing and BLAST analysis. The developed bacterial consortium inoculation on finger millet resulted in the improvement of growth and physiological parameters with respect to chemical fertilizer and control. The compatible mixture of bacteria was found to have more ability to increase the growth of finger millet and it might be utilized as biostimulants for nutri-cereal crops growing in hilly regions.

Introduction

The cereals of the future, millets are increasingly gaining attention because of their health benefits across the globe. Apart from health benefits millets are sustainable crop alternatives to other crops with respect to water scarcity. The small seeded nutri-cereals comprises of seven different types of millets such as pearl millet, foxtail millet, finger millet, barnyard millet, kodo millet, proso millet, and little millet [1]. Finger millet (ragi) is a minor millet cereal which is solely consumed by the humans and it provides different health benefits due to high content of dietary fibre, phenolic compounds, and calcium. This nutri cereal is also antitumorogenic, atherosclerogenic and antidiabetic, along with

antimicrobial and antioxidant properties [2]. Finger millet is widely practiced across the Asia and different types of agrochemicals mainly consisting of macronutrients are being applied to the increase productivity. The utilization of agrochemicals such as urea, diammonium phosphate and muriate of potash benefits the optimal growth and productivity of crop but on the other hand these chemicals utilization for extended period could cause severe damage to the environment, humans and microflora of soil. In environment, conventional agrochemicals could cause contamination in groundwater, eutrophication, degradation of the soil quality and pollution in air. Moreover, the chemical fertilizers use could also reduce the profit margin of the growers as nutrients utilization efficiency is limited. The less or no use of chemical fertilizer is not possible as growing population demand of the food couldn't be fulfilled [3].

As an alternative, biostimulants developed from microbes could be utilized for the growth and production of the crops. Microbial-based biofertilizers is one of the safe and finest substitutes of agrochemical fertilizers which also promote the agricultural and environmental sustainability. The use of biostimulants offers multiple benefits such as quality grain production, soil fertility improvement and plant protection from abiotic and biotic factors. The production and

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Potential applications of mineral solubilizing rhizospheric and nitrogen fixing endophytic bacteria as microbial consortium for the growth promotion of chilli (*Capsicum annum* L.)

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Abstract

Over the decades, chemical fertilizers are indiscriminately used in agriculture to increase the crop production, but unfortunately it is considered as a non-sustainable approach due to adverse impact on environment and human health. Bioinoculants provide a better opportunity for ecological farming practices to enhance crop productivity. Different types of bio-inoculants containing single and multiple strains as consortium could be used for crop production soil health. In the present study, efficient microbial consortium consisting mineral solubilizing, and nitrogen fixing microbes were evaluated for growth and physiological parameters of chilli. Rhizospheric and endophytic bacteria were isolated from different cereal crop (foxtail millet, finger millet, maize, oats and, wheat) growing in Himachal Pradesh. All the 145 isolates were primarily screened for minerals solubilization (phosphorus and potassium) and nitrogen fixation attributes. The selected P-solubilizer, K-solubilizer, and N-fixer strains were further screened for solubilization of Zn and Se; production of siderophores, ammonia, indole acetic acid, and hydrogen cyanide. The potential P-solubilizer, K-solubilizer and N-fixer bacterial strains were identified on the basis of 16S rRNA gene sequencing as *Erwinia persicina* EU-A3SK3, *Halomonas aquamarina* EU-B2RNL2, and *Pseudomonas extremorientalis* EU-B1RTR1, respectively. The inoculation of these bacterial strains on chilli as microbial consortium resulted in the increased growth (shoot/root biomass and length; number of leaves, branches and fruits per plant) and physiological parameters (content of chlorophyll, total soluble sugar, phenolics and flavonoids) of the treated plants over the individual strain inoculated plants and untreated control. These potential strains of mineral solubilizers and nitrogen fixer as biofertilizer could be utilized for the horticulture crops growing in hilly region for sustainable agriculture.

Keywords Chilli · Consortium · Microbiomes · Plant growth promotion · Sustainable agriculture

Abbreviations

PGP Plant growth promoting
OD Optical densities

mL Milliliter
ARA Acetylene reduction assay
CFUs Colony-forming units
LSD Least significant differences
CD Critical difference
IAA Indole-3-acetic acid

Rubee Devi and Tanvir Kaur contributed equally to the present work.

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Introduction

Sustainable agriculture aims to establish a healthy and natural environment along with producing the food. Until middle of the twentieth century, organic manure was used for the fulfillment of nutritional requirements, but with the beginning of green revolution, the use of chemical fertilizers has increased drastically with each passing year across the world. Over the long period of time, chemical fertilizers



Indigenous plant growth-promoting rhizospheric and endophytic bacteria as liquid bioinoculants for growth of sweet pepper (*Capsicum annuum* L.)

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Abstract

Agrochemicals provide vital nutrients for plant growth to enhance crops yield, but they can pose major agro-environmental issues. Bioinoculants have attracted more and more attention due to their cost effective-eco-friendly and pollution-free characteristics. The aim of this study was to determine whether using a variety of bioinoculants that include both individual and group members could reduce the need for chemical fertilizer. In the modern era, individual and multiple strain formulation as bioinoculants and bacterial consortium is need of agricultural sustainability. A total 132 bacteria were sorted out from soil and internal tissues of the plant and screened for PGP characteristics including nitrogen fixer, phosphorus, and potassium solubilization. Among 132 bacteria, 13 were found to fix nitrogen, 17 and 14 bacteria were able to solubilize phosphorus, and potassium respectively. Efficient bacterial isolates were identified using 16S rRNA gene sequencing as *Bacillus thuringiensis* EU-CRP-15 (P-solubilizer), *Bacillus horikoshii* EU-CRK-18 (K-solubilizer), and *Pseudomonas trivialis* EU-CEN-2 (N-fixers). Inoculation of individual and consortium bioinoculants had a favorable effect on seed sprouting with the increase concentrations of inoculum. These three compatible and individual bacterial strains inoculated on sweet pepper enriched the growth and physiological characteristic of plant (plant length, root length, fresh weight, and biomass of the plant), and (chlorophyll, carotenoids, flavonoids, phenolics, and total soluble sugar content) over chemical fertilizers and untreated control plant. The plant growth promoting bacteria viz; N₂-fixer as well as P and K solubilizers can be utilized as bioinoculants for the growth promotion of plants and increasing soil fertility.

Keywords Agriculture sustainability · Bioinoculants · Bacteria consortium · K-solubilizer · N₂-fixer · P solubilizer

Abbreviations

PGP microbes	Plant growth promoting microbes
PGPR	Plant growth promoting rhizobacteria
CFU	Colony Forming Unit

Introduction

Sustaining food is a significant challenge for human beings due to increase in human population (Godfray et al. 2010). To meet the high food demand for the growing population, agricultural production must be increased significantly. At the time of green revolution, there was an increase in food production per unit area due to indiscriminate utilize of agrochemicals and pesticides for better food, and fiber production to maintain high yields, conventional agriculture relies heavily on chemical inputs, such as fertilizers. Still, sources of the fertilizer are becoming increasingly scarce, and the use of excessive amounts of fertilizers and pesticides adversely affects soil, plant and human, quality of groundwater, making these agricultural practices unsustainable in the long period (Pérez et al. 2003). The researchers suggest using beneficial plant microbes as bio-fertilizers in place of agrochemical to maintain crop productivity. Bioinoculants is an

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Research article

Assessment of nitrogen-fixing endophytic and mineral solubilizing rhizospheric bacteria as multifunctional microbial consortium for growth promotion of wheat and wild wheat relative *Aegilops kotschy*[☆]



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Plant growth promotion

ABSTRACT

Microbes play crucial functions in maintaining the health and growth of the plants directly or indirectly by supplying nutrients. These microbes could be used as biofertilizers for the enhancement of soil health and growth of crops. In preset investigation, potential microbes from endophytic and rhizospheric region of *Aegilops kotschy* growing in green slopes of Shivaliks, Himachal Pradesh were sorted out and screened for plant growth promoting attributes including phosphorus and potassium solubilization. The potential bacterial strains were identified through 16S rRNA gene sequencing and developed as microbial consortium for the plant growth of wheat and wild wheat relative *Aegilops kotschy*. A total 125 isolates of bacteria were sorted out and among them 36 were found as P-solubilizers and 19 showed K-solubilization attribute and two highly potential bacterial strain were identified as *Bacillus tropicus* EU-ARP-44 (P-solubilizer; $270.5 \pm 0.00 \text{ mg L}^{-1}$) and *B. megaterium* EU-ARK-23 (K-Solubilizer; $51.3 \pm 1.7 \text{ mg mL}^{-1}$). The microbial consortium of *Rahnella* sp. strain EU-A3SNfb (N-fixers; MN294545), *B. tropicus* EU-ARP-44 (P-solubilizer) and *B. megaterium* EU-ARK-23 (K-solubilizer) evaluation in *Aegilops kotschy* and wheat crop resulted in the enhancement of growth as well as physiological parameter including shoot/root length, fresh/dry weight and chlorophyll, carotenoid, total soluble sugar content, phenolic and flavonoid content as compared to un-inoculated control. Microbial consortium consisting of potential plant growth promoting (PGP) bacterial strains could be used as biofertilizer and bioinoculants in cereals crop growing in hilly region.

1. Introduction

Cereals derivative 'cereals' are a type of grasses that are consumed all over the globe as it provide much more energy than any other crop type. They are rich sources of minerals, carbohydrates, vitamins, fats, proteins and oils. Worldwide different types of cereals are grown such as rice, millet, wheat, sorghum, barley, rye and maize (Muhammad et al., 2013). Wheat (*Triticum aestivum*) is one of the oldest and important cultivable staple crop of the globe as it forms cohesive dough that could be made into noodles, soup, pasta and other food products. The appropriate production of wheat requires a huge amount of macronutrients including phosphorus (P), nitrogen (N) and potassium (K) which are the most essential requirement for the production of wheat. The nutrient NPK

helps in the uptake and assimilation of other nutrients, protein biosynthesis, biomass production and yield. The amount of available form of nutrients (NPK) is fewer in soil and insufficient supply may leads to reduced wheat yield. To fulfill the nutrient requirement, externally NPK fertilizers were being added throughout the world which helps in increasing the wheat yield. The external fertilizers added usually developed through the chemical processes and they are known to leave a deleterious effect on the environment, humans, animals, and soil macro and microflora.

Beneficial microbes associated with plants could be used in agricultural farms as an alternative of chemical fertilizers. Plant associated microflora plays significant role in improving soil fertility, plant health and growth (Jyolsna et al., 2021; Yadav et al., 2021). Apart from plant

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Co-inoculation of nitrogen fixing and potassium solubilizing *Acinetobacter* sp. for growth promotion of onion (*Allium cepa*)

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Abstract

The use of plant growth promoting microbes (PGP) microbes in agriculture has increased due to negative impact of the chemical fertilizers. PGP microbes enhance the plant growth and productivity by diverse mechanisms such as the biological nitrogen fixation; solubilization of phosphorus, potassium and zinc; production of siderophores, plant growth regulators, hydrolytic enzymes, and various phytohormones. PGP microbes as bio-inoculants have been used as sources to improve plant nutrients in sustainable agriculture. The present study was designed to investigate the impact of single as well as the co-inoculation of a nitrogen fixer (*Acinetobacter guillouiae* EU-B2RT.R1) and a potassium solubilizer (*Acinetobacter calcoaceticus* EU- LRNA-72) in combination with chemical fertilizers for growth promotion of onion. Each strain used as bio-inoculants positively impacted shoot length, root length, biomass, phenolic, flavanoids, total soluble sugars and chlorophyll content of onion. Thus, nitrogen fixing and potassium solubilizing bacteria could be a promising and potential alternative for utilizing as source of fertilizers to horticultural system and to maintain greater nutrients availability in soil for agricultural sustainability.

Keywords Biofertilizers · Consortium · K-solubilizers · Nitrogen fixer · Onion

Abbreviations

PGP Microbes	Plant growth promoting microbes
PGPR	Plant growth promoting rhizobacteria
VAM	Vesicular Arbuscular Mycorrhiza

Introduction

Modern agricultural approaches and crop production focuses on maintaining the fertility of the soil, protecting the environment, controlling the pests simultaneously targeting the

reasonable productivity. In this context, the application of the chemical fertilizers as well as the pesticides has been the major approach. However, the long-term use of agrochemicals has been harmful for the environment. Excessive use of chemicals has resulted in the deterioration of the fertile soil, generation of highly resistant pests, as well as raised public concerns about the environment and health (Samayoa et al. 2020).

In recent decades, keeping in view the harmful side of the chemical fertilizers, governments and growers are targeting to reduce their use in agricultural as well as the horticultural systems. Growers are aiming to meet the demands of the population by producing healthy food. The increase in the use of the bioresources has been the major target of the horticultural sector. In this regard, the use of the plant growth promoting (PGP) microbes has become vital part. PGP microbes can be isolated from phyllospheric and rhizospheric region or internal tissue as endophytes. These microbiomes also inhabit bulk soil. PGP microbes have been in study and research from the beginning of the twentieth century and their mode of action at the physiological level is now well understood (Ruzzi and Aroca 2015).

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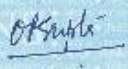
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<p>प्रमाणित किया जाता है कि पेटेटी को उपरोक्त आवेदन में बयां प्रकटित INSECTICIDAL FORMULATION OF NOVEL STRAIN OF BACILLUS THURINGENSIS AK 47. नामक आविष्कार के लिए, पेटेंट अधिनियम, 1970 के उपबंधों के अनुसार आज तारीख 20th day of August 2014 से बीस वर्ष की अवधि के लिए पेटेंट अनुवक्त किया गया है।</p> <p>It is hereby certified that a patent has been granted to the patentee for an invention entitled INSECTICIDAL FORMULATION OF NOVEL STRAIN OF BACILLUS THURINGENSIS AK 47. as disclosed in the above mentioned application for the term of 20 years from the 20th day of August 2014 in accordance with the provisions of the Patents Act, 1970.</p>			
			
अनुदान की तारीख : 06/07/2020 Date of Grant :	पेटेंट नियंत्रक Controller of Patent		
<p>टिप्पणी - इस पेटेंट के नवीकरण के लिए फीस, यदि इसे बनाए रखा जाना है, 20th day of August 2016 को और उसके पश्चात प्रत्येक वर्ष में उन्नीस दिन होगी। Note. - The fees for renewal of this patent, if it is to be maintained will fall / has fallen due on 20th day of August 2016 and on the same day in every year thereafter.</p>			

REVIEW ARTICLE

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Endosymbiotic microbes from entomopathogenic nematode (EPNs) and their applications as biocontrol agents for agro-environmental sustainability

Preety Tomar¹, Neelam Thakur^{1*} and Ajar Nath Yadav²

Abstract

Background: The biological diversity on planet earth is declining day by day, due to different factors such as excessive applications of pesticides. The utilization of chemical pesticides affected environment as well as microorganisms. The awareness among the peoples towards the hazards by the residual toxicity of chemical pesticides should be developed for agro-environmental sustainability.

Main body: Entomopathogenic nematodes (EPNs) are the bacto-helminth parasites which show classical mutualism with the genera *Xenorhabdus* and *Photorhabdus*. The nematodes along with its endosymbiotic bacteria have a bio-control potential which could be used to reduce chemical pesticides. Applications of bioagents have been reported and resulted in considerable reduction in pathogens. Furthermore, these bioagents are biodegradable, eco-friendly and easy to apply for protection of crops against diverse pathogenic organism. The nematode-bacterium complexes are effective against huge range of bacteria, fungi, nematodes and insects that are harmful to the crops. Along with biocontrol potential, the endosymbionts produce diverse secondary metabolic compounds, exoenzymes and toxic compounds that show antibiotic, antimycotic, nematicidal, miticidal and anticancerous properties.

Conclusion: The present review deals with the diversity of endosymbiotic microbes from EPNs and their role in bio-control for the agro-environmental sustainability.

Keywords: Agricultural sustainability, Biocontrol, Diversity, Entomopathogenic nematode, *Photorhabdus*, *Xenorhabdus*

Background

Entomopathogenic nematodes (EPNs) are microscopic roundworms that belong to the families Heterorhabdidae and Steinernematidae of phylum Nematoda. EPNs are beneficial nematodes that exhibit a holoparasitic mode of survival (Bhat et al. 2020). The EPNs have been reported to survive in most of environmental conditions

except psychrophilic conditions of Antarctica (Hominick 2002). The EPNs from genus *Steinernema* and *Heterorhabditis* were considered deadly fatal for a number of agricultural insects (Liu et al. 2020). Globally, 17 species of genus *Heterorhabditis* and 100 species of genus *Steinernema* have been reported that are found to be lethal for insect pests (Bhat et al. 2020). These nematodes showed mutualistic associations with endosymbiotic bacterial species that live inside the nematode. A major role has been played by these bacterial endosymbionts in nutritional physiology (Feldhaar 2011). The endosymbionts *Xenorhabdus* and *Photorhabdus* reside in symbiotic

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RESEARCH

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Synergistic effect of entomopathogens against *Spodoptera litura* (Fabricius) under laboratory and greenhouse conditions

Neelam Thakur¹, Preety Tomar¹, Sakshi Sharma¹, Simranjeet Kaur¹, Sushma Sharma², Ajar Nath Yadav³ and Abd El-Latif Hesham^{4*}

Abstract

Background: Entomopathogens such as nematodes, bacteria and fungi are well recognized for their biocontrol potential. This study was carried out to examine the insecticidal properties of the *Heterorhabditis bacteriophora* Poinar, *Beauveria bassiana* Balsamo-Crivelli, *Bacillus thuringiensis* Berliner, individually and in combination against 3rd instar larvae of *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera) under controlled laboratory and greenhouse conditions at Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh.

Results: The results demonstrated that the combined applications of the tested entomopathogens resulted in 100% insect mortality under the laboratory conditions. Among the individual concentrations, applications of 200 IJs/ml were noticed highly virulent with (98%) mortality, followed by *B. thuringiensis* (96%) and then by *B. bassiana* (92%). However, single treatments were also evaluated that further showed a highest mortality in the target pest by *H. bacteriophora*, followed by *B. thuringiensis*. Among the combined treatments by *H. bacteriophora* plus *B. thuringiensis* (200 IJs + 1×10^{12} CFU/cm²) more effective caused (100%) mortality were noticed in the laboratory and (28%) under the greenhouse conditions than *H. bacteriophora* plus *B. bassiana* (200 IJs + 1×10^{10} conidia/cm²) that caused (100%) mortality and (34%) damage under both, laboratory and greenhouse conditions.

Conclusion: Laboratory bioassay and greenhouse evaluation tests demonstrated that the combined sprayed treatments showed reliable and fast synergism. This study could be recommended to the farmers to control the pest.

Keywords: *Bacillus thuringiensis*, *Beauveria bassiana*, Bio-efficacy, *Heterorhabditis bacteriophora*, *Spodoptera litura*

Background

Spodoptera litura (Fabricius, 1755) (Lepidoptera: Noctuidae), known as tobacco caterpillar, beet armyworm, lesser armyworm, small mottled willow, cutworm and pigweed caterpillar, is the most serious insect pest in the countries like Japan, China, India, Pakistan (Ghaffar et al. 2002) and South Asia (Qin et al. 2004). It is a very destructive and polyphagous insect pest that causes damage to various crops such as potato, cotton, capsicum, tomato, soybean,

okra, clover and onion (Saleem et al. 2016). The larvae feed on leaves of the cultivated plants that lead to complete defoliation in the early stage causing severe crop damage in India (Firake and Behere 2020). Commercially important vegetable Capsicum (*Capsicum annum* Linnaeus) (Solanales: Solanaceae) grown worldwide is highly infested by *S. litura* (Baikar and Naik 2016). Constant use of pesticides leads to environmental contamination and pesticide residues in all foodstuffs all over the world (WHO 2017). This leads to develop safer, novel, biodegradable biopesticides as insecticidal alternatives (Chaudhary et al. 2017).

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RESEARCH ARTICLE

Indigenous entomopathogenic nematode as biocontrol agents for insect pest management in hilly regions

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Abstract

The present investigation emphasized on the development and use of entomopathogenic nematodes (EPNs) as a bio-insecticide. The success in controlling insect pests in the soil environments increased the production and use of the adapted indigenous EPNs species for insect management in the fields. EPNs as biocontrol agents were capable for high virulence, easy for application, safe for non-target animals and eco-friendly in nature. These nematodes have ubiquitous nature. These occur in low population in their natural habitat which was mass multiplied in the laboratory. In the present investigation, 5 concentrations (30, 60, 90, 120 and 150 IJs) of *Heterorhabditis bacteriophora* strain S₁₅ were applied against the 3rd and 4th instar larvae of four major agricultural insect pests, namely *Helicoverpa armigera* (Hubner), *Spodoptera litura* (Fabricus), *Agrotis segetum* (Denis and Schiff-ermüller) and *Mythimna separata* (Walker) under laboratory conditions at different time exposure (24, 48, 72 and 96 h). It was observed that the 3rd and 4th larval instars of all 4 insects (*H. armigera*, *S. litura*, *A. segetum* and *M. separata*) were highly susceptible for the pathogenesis caused by *H. bacteriophora* strain S₁₅. Amongst all insects, both the larval instars of *M. separata* are highly susceptible for EPNs infection with highest 96% and 98% mortality in highest dose @150 IJs. In 3rd instar larvae of other insects such as *H. armigera*, *S. litura* and *A. segetum* larval mortality ranges from 84%, 92% and 94% respectively. Among 4th instar larvae of *H. armigera*, *S. litura* and *A. segetum* the pathogenicity varies from 88%, 94% and 96%, respectively. The recorded median lethal concentration (LC₅₀) in 3rd instar larvae of *H. armigera*, *S. litura*, *A. segetum* and *M. separata* varies from 36.15, 30.05, 30.97 and 23.81 IJs/larva. Similarly in 4th instar larvae of *H. armigera*, *S. litura*, *A. segetum* and *M. separata*, LC₅₀ ranged from 31.41, 28.64, 26.92 and 20.64 IJs/larva respectively. Statistically significant variations were observed in the data recorded on the mortality, in all the treatments. EPNs are the best weapon to overcome insect resistance problems and must be employed to manage insect population.

Keywords

Agrotis segetum, Biocontrol, *Helicoverpa armigera*, *Mythimna separata*, *Spodoptera litura*

Introduction


Over the next few decades, the world's population predicted to be reached about 10 billion (1). Most of the people in India depend upon agriculture to fulfil their every day needs. The main concern of the agricultural industry is

RESEARCH

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Eco-friendly management of *Spodoptera litura* (Lepidoptera: Noctuidae) in tomato under polyhouse and field conditions using *Heterorhabditis bacteriophora* Poinar, their associated bacteria (*Photorhabdus luminescens*), and *Bacillus thuringiensis* var. *kurstaki*

Neelam Thakur¹, Preety Tomar^{1*} , Jaspreet Kaur¹, Simranjeet Kaur¹, Anuja Sharma¹, Samiksha Jhamta¹, Ajar Nath Yadav², Harcharan Singh Dhaliwal², Rajesh Thakur³ and Seema Thakur³

Abstract

Background Insect pests cause substantial agriculture losses annually, and their regulation through chemical pesticides instigates a prolonged detrimental impact on the environment and human health. The upsurge in difficulties such as pesticide residue, soil degradation and pest resistance acted as the pacesetter for research on biological control with a prime focus on entomopathogens. To aid in knowing the biocontrol potential of these creatures, the present work deals with the applications of entomopathogenic nematode *Heterorhabditis bacteriophora* EUPT-SD, and entomopathogenic bacteria (EPBs) *Photorhabdus luminescens* and *Bacillus thuringiensis* var. *kurstaki* against the 4th larval instar of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in the laboratory experiment, polyhouse as well as field condition.

Results The results demonstrated that EPB, *P. luminescens*, caused the highest 100% larval mortality at the highest concentration of 5×10^2 CFU/ml after 96 h of infection, followed by *B. thuringiensis* var. *kurstaki* which resulted in 98% at a concentration of 5×10^2 CFU/ml and 92% mortality at the treatment with *H. bacteriophora* EUPT-SD (140IU/ml) under laboratory bioassay study. In the polyhouse and field evaluation tests, again the *P. luminescens* was recorded as the most effective, followed by *B. thuringiensis* and *H. bacteriophora*, respectively. It was found that the treated plots experienced lesser damage when compared to non-treated plots.

Conclusion Applications of these pathogens are nature friendly and are a proficient alternative to synthetic chemical insecticides. It is suggested from the present investigation that the use of *P. luminescens* was the best biocontrol agent to overcome the local pest problems of this region because it is safe for animals, humans, non-target insect pests, plants, as well as for the environment.

Keywords *Spodoptera litura*, Bioassay, Entomopathogens, Management, Tomato

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RESEARCH

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Biocidal potential of indigenous isolates of Entomopathogenic Nematodes (EPNs) against tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

Preeti Tomar and Neelam Thakur*

Abstract

Background: Entomopathogenic nematodes (EPNs) have been regarded as the most convenient strategy for insect pest management. The native strains of EPNs: *Heterorhabditis bacteriophora* EUPT-SD, *H. bacteriophora* EUPT-R, *H. bacteriophora* EUPT-KN, *H. bacteriophora* EUPT-K and *H. bacteriophora* EUPT-H isolated from mid-Himalayan region of Himachal Pradesh were tested in laboratory for their multiplication and virulence against 3rd and 4th larval instars of the tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), the serious polyphagous pest affecting a wide range of agricultural crops worldwide.

Results: All the EPN strains were effective against 3rd and 4th larval instars of *S. litura*. Insect mortality reached 90–96% after 96 h at nematode concentrations of 150 infective juveniles (IJs)/ml. The insect mortality was also recorded at low concentrations of IJs, but the most exposure period was required. High virulence was shown by *H. bacteriophora* EUPT-SD 96 and 94%, followed by *H. bacteriophora* EUPT-R 92 and 90%, *H. bacteriophora* EUPT-KN 92 and 90%, *H. bacteriophora* EUPT-K 92 and 90% and *H. bacteriophora* EUPT-H 92 and 90%, respectively, against 3rd and 4th larval instars in terms of reproductive potential and killing. All the insects were alive in the absolute control.

Conclusion: Utilization of EPNs for the management of *S. litura* may be the best method to overcome the insect resistance problems and to manage the population of this insect pest. It may be an effective method and may be a partial substitute of synthetic insecticides, thus minimizing the excessive use of synthetic chemicals. The results demonstrated the potential of indigenous EPNs isolates against *S. litura*, but before further recommendation, multiplication field trials need to be conducted to confirm their efficacy at farm level.

Keywords: Entomopathogenic Nematodes, *Heterorhabditis bacteriophora*, *Spodoptera litura*, Virulence, Reproductive potential

Background

The tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), is a serious insect pest that feeds upon a wide variety of crops having very high fecundity with elevated reproduction rates and

migratory behaviour. Young larvae feed on leaves, and 4th and 5th larval instars cause maximum damage by completely skeletonized the leaves, which declined the photosynthesis ability of infected plants (Yadav et al. 2017). In Himachal Pradesh, the control strategies applied to manage the population of these insect pests are generally chemicals based which are hazardous to living beings and costly. Applications of chemical-based synthetic insecticides are the solution only for shorter duration that ultimately resulted in negative impacts over

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