EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) AND FARMYARD MANURE (FYM) AMENDMENT ON GROWTH PARAMETERS AND ANTIOXIDANT LEVEL IN PADDY (ORYZA SATIVA L.) CROP UNDER SOIL SALINITY

SUMMARY OF

Thesis

SUBMITTED TO

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

LUCKNOW

FOR THE DEGREE OF

Doctor of Philosophy

IN

ENVIRONMENTAL MICROBIOLOGY

Submitted By

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(Enrolment No- 223/10)

Under the Supervision of

Dr. Jay Shankar Singh

DEPARTMENT OF ENVIRONMENTAL MICROBIOLOGY

(SCHOOL FOR ENVIRONMENTAL SCIENCES)

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

(A Central University, NAAC Accreditation 'A' Grade)

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SUMMARY

Soil salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on germination density, plant vigour and crop yield, limiting nutrient absorption and reducing the quality of the available water. For example, elevated salinity weakens plants due to the increase in osmotic pressure and the toxic effect of the salts (Munns and Tester, 2008; Paul and Lade, 2014). Saline soils show the following physical-hydric characteristics: low permeability, low hydraulic conductivity and aggregate instability (Freire, 2009). Salinity also affects photosynthesis mainly through a reduction in leaf area, chlorophyll content and stomatal conductance, and to a lesser extent through a decrease in photosystem II efficiency (Netondo et al., 2004; Barnawal et al., 2017). Salt stress as one of the most widespread abiotic constraints in food production may also result in the negative ecological, social and or economic outcomes. Agricultural crops drastically affected in high salt concentration. High salt concentration lower down crop production and affect soil physiochemical and ecological balance of the ecosystem. Successful remediation of salt degraded areas for crop production, based on sustainable management practices evolving efficient, low cost, easily adaptable methods, is the challenge.

Rice (*Oryza sativa* L.) is a vital agent for nutritional security and according to report of International Rice Research Institute (IRRI)-Philippines, 90% of global production of rice is consumed by the Asian population. Food and Agriculture Organisation (FAO)-United Nations, data shows (*http://faostat3.fao.org*), after sugar cane the next three first crops in terms of production (million tonnes) in the world are the cereal maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.). In 2011, global production of rice was almost 723 MT. Seeds utilizes different prompts from the earth to terminate dormancy and where and when to set up another plant. Primary signals utilized by seeds for sprout as part of world compromise light, nutrients, suitable heats, water substance, and likely different signs. Undisputedly plants are flexible in environment with the possibility to build up a plenty of morphological patterns relying on growth conditions to which they are exposed. This morphological flexibility has empowered plants to colonize almost every edge of globe and to survive in the harshest conditions. Rice exhibits wide adaptability to different environments, which makes it the most widespread crop in the world. It can grow in saline condition, drought conditions or in shallow water (up to 50 cm of water), and in a wide range of latitudes and up to 3000 m altitude. For this reason, it is considered a strategic crop for food security in the world by the FAO. However methodologies have been utilized aiming at enhancing multiple stress resilience in early decades but inadmissible results occurs. Escalating salinity fields imposed severe osmotic damages on paddy plant and declined grain productivity. Applications of agrochemicals in rejuvenation of saline soils drastically deteriorate soil

physicochemical and biological properties. Thus, it is now necessary to these Global concerns somewhat mitigated through upgraded sustainable options.

The emerging environmental issues such as soil pollution, land degradation, loss of soil fertility or soil microbial diversity, or rise of average global temperatures, among many others, are adversely impacting the various ecosystems (Singh and Gupta, 2018). The changes in plant and animal communities are relatively slow, but alterations in soil microbial community compositions and their ecological functioning happen much more rapidly, but mostly stay ignored. The soil microbial communities are the key responders to any environmental change, but details on what exactly happens to microbial community composition and their functional role as a response to variations in soil parameters changes to most of the cases are still quite unclear. Microbes, a tiny living with unmatchable capacity offer an innovative and feasible option in agriculture and got position of decent bioengineers for engineered tainted agro-ecosystems (Singh, 2015). Beneficial microbes associated with plants rhizosphere are known to stimulate plant growth and enhance plant resistance to biotic (diseases) and abiotic (salinity, drought, pollutions, etc.) stresses. Roots are the vital part of plant can taste which supplements are available in soil and answer is easily shown in plant health. They can also taste and integrate signalling by means of different chemicals substances that are delivered in their distinctive organs, as well as by microorganisms, plants, and animals in their environments (Cheynier et al., 2013; Fonseca et al., 2014). Root exudate varies with plant genotypes, recruit microbial partners towards and along roots (Patel et al., 2015). Rhizosphere microbial counts go beyond to 1×10^{11} microbial cells per gram root (Egamberdieva et al., 2008) in eutrophic while it has decline up to 10^4 under stressed terrestrial ecosystems. Microbes under rhizosphere can trustily modulate root exudate patterns (Patel et al., 2015), improves rhizospheric architecture (Vimal et al., 2017) and induce systemic resistance to consequent pathogen attack (Glick, 2014).

Plant-growth-promoting-rhizobacteria (PGPR), a key component of soil micro-biota, could play vital roles in the maintenance of plant fitness and soil health under stressed environments. The PGPR got special promotions among soil microbes during last few decades due to unmatchable capability. After successful tuning with the plant roots rhizobacteria utilizes carbohydrates, amino acids, organic acids and exercises with different plant growth promontory traits (Choudhury et al., 2014). PGPR boost plant vigor by means of different mechanisms, altering root architecture (Grobelak et al., 2015), initiate phytohormone levels (Glick, 2012), pathogen reduction (Singh et al., 2013), stress tolerance (Vimal et al., 2018a) and comprehension these unpredictable cross-kingdom interactions inspires us into root formative science and bacterial signaling (Singh et al., 2011; 2015; 2018a, b). The PGPRs are proficient in modulating the root system architecture which is a critical factor of productivity (Singh 2015). The capability of PGPR to influence plant development and root processes was excellently addressed by Hatesami and Maheshwari, 2018. By contrast, the mechanisms by which PGPRs up-regulate cell division, and improve the equilibrium between proliferation and differentiation in the primary root and lateral root initiation sites, remain largely unidentified (Veloccia et al., 2016). These adjustments are built up by changing plant endogenous signalling pathways. The PGPR got tremendous attention in to taking care of soil and plant health due to environmental calamities (Vimal et al., 2016; 2017; 2018a, b). The PGPR provides great promise in sustainable future soil fertility and crop productivity managements. The PGPR communications with their host plant have knocked the minds of researcher for advancement in PGPRs based microbial technology.

The application of organic manure as a soil conditioner in combination with suitable salt tolerant PGPR strains could improve the soil-plant-microbe interaction and may enhance the crop yield under stressed soil conditions. Countless number of microbes as algae, mycorrhizal fungi, PGPR processes in different nutrient cycling. Thus amendments of organic manures in stressed soils as bio-fertilizer, compost, and vermin-composts offers a blameless bio-agent in rejuvenation of degraded ecosystem. The PGPR in association with organic amendments like FYM may significantly reduces the amount of energy demanding inputs, such as chemical fertilizers and can contribute to mitigation and adaptation for paddy plant under saline soils. Investigations are needed in this area that how and how much of the PGPR with FYM amendments facilitates plant growth, enhanced paddy crop production and regenerate soil fertility. Thus, the present doctoral research hypothesizes that the addition of salt tolerant PGPR inoculant + FYM (as organic amendment) to paddy soil could be a novel and cost-effective tool to enhance soil fertility and alleviate the salinity stresses of paddy crop plants. The present study was conducted to investigate the efficacy of salt tolerant PGPR strain + FYM on paddy plant growth productivity with following 3 objectives.

(i) To analyze the impact of PGPR and farmyard manure (FYM) applications on soil physico-chemical properties.

(ii) To examine the impact of PGPR and FYM applications on plant growth parameters and antioxidant level in paddy crop in saline soil.

(iii) To assess the correlation between the PGPR and FYM application and plant growth parameters and yields.

Note: For more clarity it is important to mention here that whole experimental work of present study broadly may be divided into two parts.

Part I	**Part II (3 objectives)**
Isolation and characterization of salt tolerant plant growth promoting rhizobacteria from saline soils (Chapter 3)	Application of isolated salt tolerant most efficient PGPR strain *C. albidum* SRV4 in combination with FYM in field conditions (Chapters 4, 5, 6 and 7)

Part I

Twenty nine promising halo-tolerant PGPRs were isolated and screened for their PGP traits from the rhizosphere of naturally growing plants of saline soils located at Sandila region of Hardoi, Uttar Pradesh, India. Out of 29 isolates, one most efficient salt-tolerant isolate having potential PGP attributes, was selected for further study. Based on 16S rRNA gene sequencing and BLASTn analysis, the isolate was identified as *Curtobacterium albidum* SRV4 strain. The SRV4 expressed positive attribute for nitrogen (N_2) fixation, exopolysaccharide production (EPS), hydrogen cyanide (HCN), Indole-3-acetic acid (IAA), and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. The higher doses of NaCl negatively affected paddy plant physiology and growth parameters. Paddy plants in pot experiment treated with SRV4 showed significant differences ($P < 0.001$) in improvement in plant growth parameters, photosynthetic pigment efficiency, membrane stabilization index and proline content. A significant variation ($P < 0.001$) in enhancement in antioxidative enzymatic activities catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and ascorbate peroxidase (APX) and K^+ uptake in paddy plants was noted due to *C. albidum* SRV4 inoculation. The *C. albidum* SRV4 has been found as effective microbial agent to improve photosynthetic efficiency, modulation of osmolytes and antioxidative enzymes due to efficient PGP attributes, development of induced systemic tolerance and alleviating salt stress in paddy plants. The sequence data of *C. albidum* SRV4 has been deposited to the GenBank nucleotide sequence database with the accession number KX 81071.

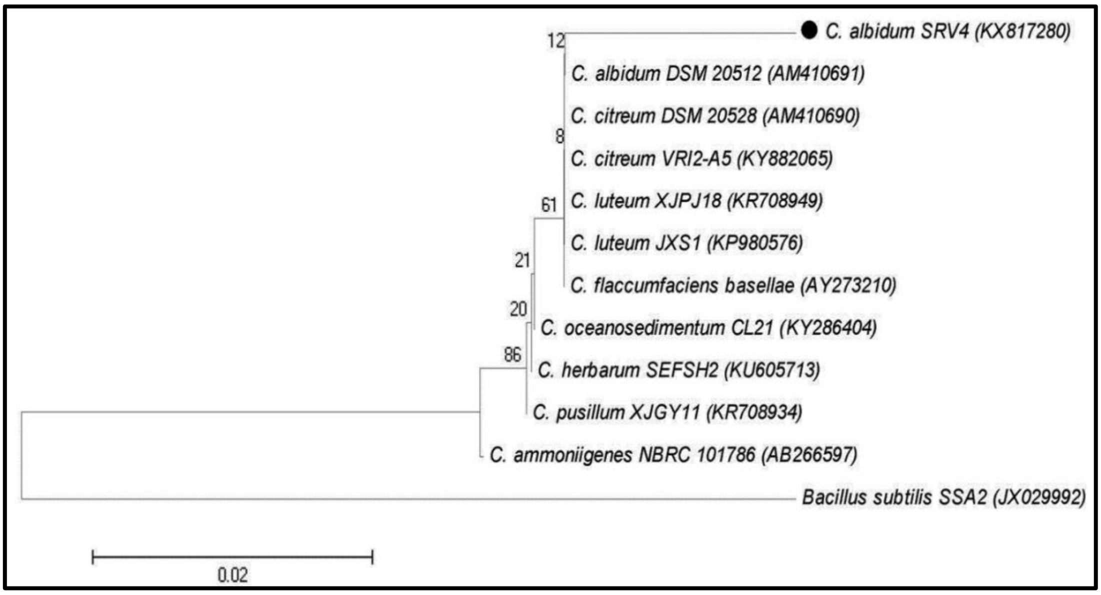

Figure 1. Phylogenetic relationship of isolated rhizobacterial strain SRV4 with other closely related bacterial strains based on 16s r-RNA gene sequence available from National Center for Biotechnology Information (NCBI) database library. The above isolated and identified salt tolerant PGPR *C. albidum* SRV4 strain was used as microbial inoculant in combination with FYM for further paddy crop field experiment

Part II

Experimental Field Design and Paddy Crop Cultivation

The field experiment was conducted for two successive paddy crop cycles in the year 2015 and 2016, at Field Experimental Station, Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India. For both the study years, 2015 and 2016, four treatments used in this experiment were: (a) Control, (b) *C. albidum* SRV4 strain, (c) FYM (50 t ha^{-1}) and (d) FYM (50 t ha^{-1}) + *C. albidum* SRV4 strain. Therefore, total 12 experimental plots (each having 3m×2m dimensions) were established in completely randomized block design (CRBD) with each treatment having three replications.

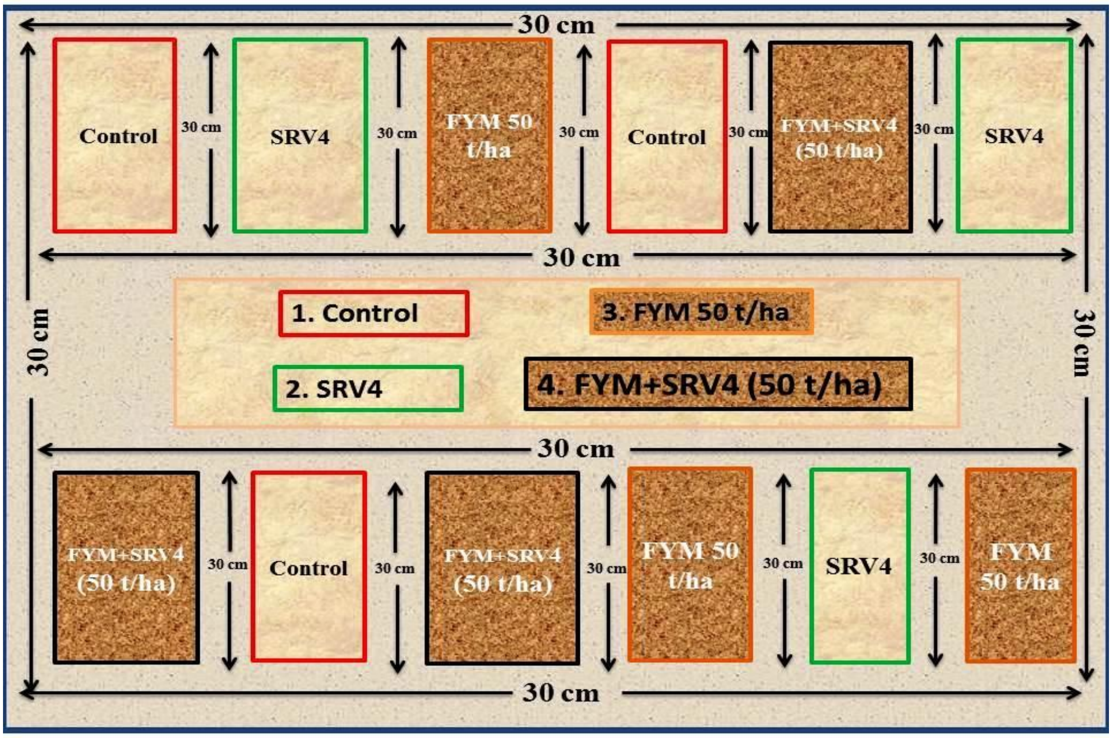

Figure 2. Experimental plots for the cultivation of paddy crop during 2015 and 2016

For present investigation, the selected experimental crop was paddy (*Oryza sativa* L.). The rice variety namely HUR 3-4 (Hindu-University-Rice 3-4) was obtained from Department of Genetics and Plant Breeding, Institute of Agriculture Sciences, Banaras Hindu University (South campus), Mirzapur, Uttar Pradesh. The rice seeds sterilization, inoculum preparation and application to paddy field were done as described in Chapter (3). The nursery of rice cultivar was prepared in month of June during both the years 2015 and 2016.

Five paddy hills having equal height was transplanted in month for both the years 2015 and 2016 at 20cm×20cm dimensions in each plot. A total of 600 paddy hills were transplanted in each plot. The SRV4 inocula were sprayed on paddy plant parts on panicle initiation stage at the rate of 2 litres $plot^{-1}$. The matured paddy crop was harvested during the month of November in both years 2015 and 2016.

Objective 1: To Analyze the Impact of PGPR and Farmyard Manure (FYM) Applications on Soil Physico-Chemical Properties

The results of different FYM+SRV4 amendments on soils physico-chemical showed significant ($P < 0.001$) variations due to treatments. The application of FYM significantly reduces the pH and EC, BD and TSS level of paddy soils in both the study years 2015 and 2016. However, improvement in Organic-C, available-N, available-P and available-K was observed in FYM+SRV4 treated plots during both the crop cultivation years. Based on the results, this study suggests that application of FYM in combination with PGPR *C. albidum* SRV4 had significant impact on soil conditions due to beneficial activities of plant growth promoting inoculant and under salinity stress.

Objective 2: To Examine the Impact of PGPR and FYM Applications on Plant Growth Parameters and Antioxidant Level in Paddy Crop in Saline Soil

Paddy plant growth variables (plant height, tiller number, panicle number) and rice grain yields was noted maximum in SRV4+FYM amended plots compared to other treatments. ANOVA showed significant variations in paddy plant growth variables ($P < 0.001$) and rice grain yields (P= < 0.01 to < 0.001) due to treatments in both the years 2015 and 2016. Two-way ANOVA for the pooled data of paddy plant growth variables and rice grain yield across different treatments and years showed that impact of treatment was only significant ($P < 0.001$), while impact of years and treatment × year interaction was not significant. Across different treatments maximum paddy plant height in both the years 2015 (135±6.77 cm) and 2016 (139±7.68 cm) was noted in FYM+SRV4 amended plots compared to other treatments. The maximum tiller numbers per paddy plant were appears in FYM+SRV4 amended plots in both crop cultivation years. Similarly the panicle numbers were recorded maximum in FYM+SRV4 amended plots in 2015 and 2016. For both the years, also the weight of 1000 rice grain was found maximum in FYM+SRV4 amended plots compared to other treatments. The maximum rice grain production was found in FYM+SRV4 amended plots for 2015 (5.04±0.53 t ha^{-1}) and 2016 (5.85±0.57 t ha^{-1}). It is suggested that the efficient salt tolerant PGPR SRV4 strain isolated from saline soils with FYM amendments (50 t ha^{-1}) nourishes paddy degraded soils, enhances availability of nutrients, modulates plant antioxidants and improves plant health and ultimately the paddy crop yield.

Antioxidant enzyme activities (CAT, SOD, POX and APX) were found lowest in SRV4+FYM amended plots and highest in control plots. ANOVA showed significant variations (P= <0.01 to <0.001) in antioxidant enzyme activities in both the years 2015 and 2016 during paddy crop cycles. When the data of antioxidant enzyme activities were polled across different treatments and years, two-way ANOVA showed significant differences ($P<0.001$) only for treatments, while impact of years and treatment × year interaction was not significant. In the present experiments, an enhanced antioxidative enzymes production in paddy plants of control plots, possibly could be to neutralize the adverse impact of higher salinity stress and is in conformity with the results of other investigations.

Objective 3: To Assess the Correlation between the PGPR and FYM Application and Plant Growth Parameters and Yields

To find out the relationship between, soil physico-chemical properties, paddy crop growth parameters (plant height, tiller number, panicle number and rice grain yields) and antioxidant enzyme activities (CAT, SOD, POX and APX), across different treatments and years, pooled data were subjected to regression analysis. A negative correlation between soil physico-chemical properties and paddy plant growth parameters was observed across different treatments and years. The results of present study showed that input of FYM and SRV4 strain, alone or in combination, resulted in significant reduction in soil pH, EC, TSS and BD of paddy soils, while organic-C available-N, -P, -K (Table 5.1) and paddy plant growth parameters (plant height, tiller and panicle numbers and rice grain yields) was enhanced significantly. This probably could be the reason for a negative relationship between soil physico-chemical properties and paddy plant growth parameters in this study.

The results of correlation analysis showed a negative relationship between soil paddy plant growth parameters and antioxidative enzymes activities when data were pooled across different treatments and years. The results of present study showed that input of FYM and SRV4 strain, alone or in combination, resulted in significant increase in paddy plant growth parameters (plant height, tiller and panicle numbers and rice grain yields), while antioxidant enzymatic activities (CAT, SOD, POX and APX) was reduced significantly. In this study, an increase paddy plant growth parameters and decrease in antioxidant enzymatic activities due to FYM and SRV4 treatments could be the reason for a negative relationship between soil paddy plant growth parameters and antioxidant levels.

Conclusions

The aim of the present investigation was to isolate and identify the efficient PGPR strains from the saline soils that could be evaluated for improve the growth and yield of paddy crop under salinity stress. The seeds inoculated with SRV4 strain increased paddy plant growth, yields compared to the non-inoculated controls. The paddy yield components were enhanced even more, when rhizobacteria treated seedlings was transplanted in FYM amended plots. Significant variation confines fertility rejuvenation, crop production and plant antioxidant capacity. In general a negative correlation between crop growth parameters and antioxidant enzyme activities were observed. The negative correlations between plant growth parameters and antioxidant enzymes levels in the present investigation indicated that inoculation of salt tolerant *C. albidum* SRV4 to soil reduces the salinity stress in paddy plants. These results suggest that higher antioxidative enzyme requirement by paddy plant at higher salinity levels, could play as important role to protect plants against oxidative injury. Based on the above results, this study suggests that inoculation of *C. albidum* SRV4 had significant impact on paddy growth due to their plant growth promoting attribute under salinity stress. With reference to the combined effect of microbial inoculants and their survival under salinity stress and their competition with other indigenous microbial flora, it would be more effective and viable to apply the locally well adapted salt tolerant native microbial agents like *C. albidum* SRV4 to ensure the best results on paddy crop productivity under salinity stress. This field experiments possibly validate SRV4 strain potentiality in stress management and therefore, in association with FYM amendment it can play a vital role in restoration of soil fertility and considered as potential bio-tool to promote sustainable paddy agricultural management in salinity affected area of Lucknow and other similar regions.

Although, the ecologically sustainable paddy agricultural practices are essential to contribute significantly for food security, and efficient agriculturally important microbes (PGPR inoculants) are playing very crucial role in high paddy and others crop production due to their immense plant growth promoting attributes. Nowadays, bio-formulations (microbes or their metabolites) use in combination with organic amendments has been considered as promising means for crop sustainability in stress soil conditions. Inoculation of agriculturally beneficial microbes along with their metabolites in agriculture fields, may be a potential option, proving more eco-friendly with multidimensional roles and consequently, demonstrating the new opportunity to develop the microbial-formulations for safe and sustainable agriculture. These sustainable crop productions are possible because of their better adaptability and survival under stresses, and ultimately that result in attenuating the chemicalization (pesticides/fertilizers use) in agriculture sector. However, the eco-friendly viable microbial-formulations progress at commercial level and constraints associated with the techniques are not yet explored in detail. It should be always kept in mind that the unpredictable biotic and abiotic edaphic factors that determine the survival and potential of the microbial inoculants responses and functioning after their delivery in the field conditions. As most plant-inoculants interactions after addition in the field conditions are as yet unexplored and high-throughput sophisticated molecular tools/techniques are limited, so we only can predict that plant-microbes inoculants could be a general strategy in benefits of soil fertility. Therefore, there is need to assess critically such non-target special effects of microbial inoculants at broad level, and to validate such consequences before their delivery in the natural field conditions.