Effect of nitrogen fixing bioinoculants resistant to inhibitors in castor bean (*Ricinus communis* L.) on the crop

Yadav Rashmi*, Pathak Dharam Veer and Kumar Rakesh
Department of Microbiology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, INDIA
*rashmi.yadav29@gmail.com

Abstract

In this study, effect of nitrogen fixing bioinoculants resistant to inhibitors in castor was studied on castor crop. Twenty nine free living diazotrophic bacteria were isolated from different rhizospheric soils of castor crop. On the basis of the ability of isolates to tolerate different concentrations of inhibitors and different plant growth promoting traits i.e. indole acetic acid (IAA) production, ammonia excretion, nitrate reductase (NR) activity and siderophore production under in vitro conditions, two isolates (CR5 and CR10) were selected and tested for growth promotion of castor crop under pot house conditions. Isolate CR5 was found to promote plant growth more efficiently.

So, for further studies on castor crop under field conditions, isolate CR5 was selected. Organic carbon increased slightly in most of the treatments while total nitrogen (N) and total phosphorus (P) contents of soil decreased gradually with advancement of the crop. However, an increase in total P content was observed initially after chemical fertilization. Highest diazotrophic viable count (6.57 log no. cfu/g soil) and seed yield (47.22 q/ha) were obtained with inoculation of isolate CR5 along with recommended dose of fertilizer (RDF) at harvest. Isolate CR5 showed 99% similarity with Klebsiella variicola strain A6128 on the basis of partial 16S rRNA gene sequencing. So, isolate CR5 is not only resistant to castor inhibitors but also promotes plant growth and yield significantly.

Keywords: Castor, Inhibitors, Yield, *Klebsiella variicola*.

Introduction

Castor (*Ricinus communis* L.) is a valuable oilseed crop and grown throughout the world because of its medicinal and industrial values.[19] It belongs to the family *Euphorbiaceae*.[12] It originated from the tropical belts of India and Africa, grown especially in arid and semi-arid regions where germination and plant growth may be affected by salinity stress intensively.[6]

It is grown worldwide on commercial scale, of which India, Brazil and China account for maximum production. Most of the global demand of castor oil is met by India as it is the largest producer of castor seeds.[16] Castor bean has been broadly acknowledged as a horticultural answer for all subtropical and tropical areas that address the requirement for crops with low input costs and simultaneously give customary cultivating a feasible income from current non beneficial terrains.[13]

Plant growth promoting rhizobacteria (PGPR) play an important role in promoting plant growth and development via diverse mechanisms by colonizing plant roots. Generally, these bacteria enhance plant growth directly by either providing nutrients (nitrogen, phosphorus and essential minerals)[3,33,35] and modulating plant hormone levels[14] or via indirect mechanisms as biocontrol agents.[11,17] Various researchers have studied the promotory effects of PGPR on growth and productivity of many plant species under normal and stressful conditions.[10,36,40]

Since, hazardous chemicals used as fertilizers and pesticides destabilize the agro-ecosystems, the application of beneficial rhizobacteria is a good alternative to enhance plant growth. The free living diazotrophic bacteria present in rhizosphere like *Azotobacter, Bacillus, Clostridium, Beijerinckia* and *Klebsiella* help in promotion of plant growth mainly by nitrogen fixation.[34] They can also be involved in production of hormones and plant protection by producing antibiotics and competing for resources[39]. Additionally, microorganisms induce systemic resistance and protect plants against pathogens.[31]

Many researchers have studied that application of biofertilizers to castor can enhance plant growth and yield by increasing soil fertility, availability of various nutrients to plants and saving N-fertilizers.[1,15] Patel et al[30] found that bio-fertilizers improve vegetative growth and productivity of castor when used in addition to organic fertilizers. Similarly, Kumar and Kanjana[22] found that the application of specific bacterial strains can accelerate the mineralization processes of organic matter in soil, which in turn enhances the vegetative growth and yields of castor by increasing nutrients availability to the plant.

Castor plant contains a number of toxic compounds like ricin, ricinine, *Ricinus communis* agglutinin etc. in different parts of the plant.[23] In addition, the plant also synthesizes a number of secondary metabolites like steroids, saponins, alkaloids, flavonoids, tannins, phenols, phytates, oxalates and glycosides.[18]

These groups of compounds are toxic for humans, plants, insects and microorganisms. Some of these inhibitors are quiet stable and can persist in soil for many years which not

only affect soil microorganisms but also the growth of plants grown in the same field later on. This also results in decline of soil fertility.[38,41]

However, there are reports on certain microorganisms which are resistant to castor inhibitors and effectively degrade them.[37,41] There are few reports of biofertilizers used to improve growth and yield of castor and no attempts have been made to use bioinoculants which can withstand its inhibitors. Therefore, the present investigation aims at application of nitrogen fixing bioinoculants resistant to inhibitors as biofertilizers for castor crop.

Material and Methods

Experimental site and investigation design: The research was conducted at CCS HAU RRS, Bawal (Haryana) under field conditions. *Azotobacter chroococcum* Mac-27 is a commercial inoculant used as reference strain. Six treatments were established using randomized block design in 18 plots: T1 (RDF), T2 (75% RDF), T3 (RDF + isolate CR5), T4 (75% RDF + isolate CR5), T5 (RDF + *Azotobacter chroococcum* Mac-27), T6 (75% RDF + *Azotobacter chroococcum* Mac-27). Each treatment had three replications. Plot size was 3 × 5 meters, row to row distance was 1 meter and plant to plant distance was 60 centimeters. Recommended doses of fertilizers for castor crop [$N_{80}P_{60}K_{30}S_{30}$ (Kg ha^{-1})] were applied as per the treatments. The seeds of castor var. DCH 177 were treated with fully grown (10^8 ml^{-1}) selected bacterial isolate CR5 and *A. chroococcum* Mac-27 and sown in field according to the treatments.

Determination of microbial count: Rhizospheric soil samples collected from castor grown field were used for microbiological analysis and diazotrophic bacterial count was determined at an interval of 30 days by dilution plate count method. The soil was serially diluted and hundred µl of each sample from various dilutions (10^{-3}, 10^{-4} and 10^{-5}) was spread over Jensen's nitrogen free medium plates. After incubating the plates for 3-5 days at 28±2ºC, colonies appeared were counted. The counts were calculated on per g soil basis using formula:

No. of cfu (colony forming units) x dilution factor/ volume taken (ml)

Analysis of soil chemical properties and seed yield: Organic carbon, total N and total P in soil were checked at 30 days interval by the methods of Kalembassa and Jenkinson,[21] Kjeldahl's methods[9] and John[20] respectively. Seed yield was determined after three pickings.

DNA extraction, PCR amplification and Partial 16S rRNA gene sequence analysis: In present study, the DNA of isolate CR5 was extracted by modified method as described by Ausubel et al.[2] Approximately 1500 bp DNA fragment was amplified from 16S rRNA gene as described by Lukow et al.[24] The partial sequence of 16S rRNA gene was obtained after purification from Chromous Biotech Pvt. Ltd., Bangalore and compared with the sequences already submitted in NCBI (National Centre for Biotechnology Information) database using BLAST programme. The sequence was submitted to GenBank for obtaining accession number. Phylogenetic tree of the isolate was prepared by using neighbour joining of BLAST programme.

Results

A total of 29 bacterial isolates were obtained from rhizospheric soils of castor from different locations. All the bacterial isolates were tested for resistance to inhibitors in castor and various plant growth promoting traits including IAA production, ammonia excretion, NR activity and siderophore production are *in vitro* conditions. Out of 29 soil isolates, isolates CR5 and CR10 were found to survive at highest concentration of inhibitors (7%). It shows high resistance of these isolates to inhibitors. Highest IAA production and ammonia excretion were also detected in isolate CR5 (13.99 µg ml^{-1} and 6.38 µg ml^{-1} respectively).

Highest NR activity was shown by isolate CR5 (150.92 µg nitrite ml^{-1}) followed by CR10 (128.01 µg nitrite ml^{-1}). Both isolates were positive for siderophore production (Table 1). On the basis of above characters, two isolates (CR5 and CR10) were selected for growth promotion of castor crop under pot house conditions.

Isolate CR5 was found to promote plant growth more efficiently through plant parameters including plant height (48.8 cm), root weight (fresh and dry) 17.0 g and 5.1 g respectively and shoot weight (fresh and dry) 6.0 g and 1.9 g respectively. Highest diazotrophic viable count was also observed in the rhizosphere of plants treated with isolate CR5 (6.74 log no. cfu/g soil). To see the full effect of isolate CR5 as biofertilizer on castor crop, a field experiment was set up. Basic physico-chemical properties of experimental field are detailed in table 2.

Table 1
Resistance to castor inhibitors and plant growth promoting traits of selected bacterial isolates

Isolate no.	% Inhibitors				NR activity (µg nitrite ml^{-1})	IAA production (µg ml^{-1})	Ammonia excretion (µg ml^{-1})	Siderophore Production
	1	3	5	7				
CR5	+++	+++	+++	++	150.92	13.99	6.38	+
CR10	+++	+++	+++	++	128.01	4.94	1.29	+

Table 2
Physicochemical properties of experimental field at the start of experiment

Parameter	Value / Type
Texture	Loamy sand
Electrical Conductivity	0.17 (dS/m)
Ph	8.34
Organic Carbon	0.20 (%)
Total N	348 (Kg/ha)
Total P	220 (Kg/ha)
Microbial biomass C	294 (Kg/ha)
Diazotrophic count	6.11 log no. cfu/g soil)

Table 3
Diazotrophic viable count in rhizosphere of castor under field conditions (log no. cfu/g soil)

Treatments	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS
T1	6.11	6.20	6.32	6.49	6.52	6.45	6.28	6.18	6.11
T2	6.04	6.08	6.23	6.45	6.46	6.38	6.18	6.08	5.95
T3	6.20	6.36	6.74	7.01	7.22	6.93	6.88	6.77	6.57
T4	6.20	6.32	6.59	6.90	6.94	6.81	6.77	6.54	6.46
T5	6.18	6.28	6.40	6.74	6.77	6.61	6.54	6.40	6.28
T6	6.15	6.23	6.34	6.57	6.58	6.36	6.32	6.26	6.18
Critical difference at 5%	N.S.	0.08	0.05	0.03	0.02	0.03	0.05	0.06	0.10

Survival and establishment of inoculated bacterial isolates: Studies on diazotrophic viable count on inoculation with isolate CR5 and *Azotobacter chroococcum* Mac-27 showed significant difference in all the treatments. The viable count in all the treatments increased upto 150 DAS.

Maximum count was observed in the rhizosphere of plants inoculated with isolate CR5 along with RDF (7.22 log no. cfu/g soil) followed by isolate CR5 along with 75% RDF (6.94 log no. cfu/g soil) at 150 DAS. However, a decrease in bacterial count was observed at 270 DAS in all the fertilization treatments (Table 3).

Soil chemical properties: Organic carbon, total nitrogen and total phosphorus were determined in soil at an interval of 30 days. No significant difference was observed in different treatments w.r.t. organic carbon. It was 0.21% in all treatments except the treatment having 75% RDF (0.20%) at 270 DAS (Table 4). Total N and total P contents of soil decreased in all the treatments irrespective of the inoculation of isolates with the advancement of the crop.

However, at 30 DAS an increase in total P content was observed. Highest total N was detected in the treatment having isolate CR5 along with RDF (343 Kg/ha) followed by the treatment having RDF and *A. chroococcum* Mac-27 along with RDF (342 Kg/ha) at 270 DAS (Table 5). Maximum total P was observed in treatment having isolate CR5 along with RDF (232 Kg/ha) followed by *A. chroococcum* Mac-27 along with RDF (231 Kg/ha) at 270 DAS (Table 6).

Effect of bacterial isolate on seed yield: The seed yield was determined after three pickings. Significant increase in seed yield was observed with inoculation of isolate CR5 as compared to their respective controls and reference strain *Azotobacter chroococcum* Mac-27. Maximum seed yield was obtained with inoculation of isolate CR5 along with RDF (47.22 q/ha) followed by isolate CR5 along with 75% RDF (45.92 q/ha).

The percent increase in seed yield on inoculation with isolate CR5, over respective controls was 11.16 and 8.75% at 75 and 100% RDF respectively. Moreover, when compared with reference strain *A. chroococcum* Mac-27, isolate CR5 showed percent increase of 4.84 and 7.12% at 75 and 100% RDF respectively (Fig. 1).

Identification of bacterial isolate on the basis of partial 16S rRNA gene sequencing: Over several years, 16S rRNA gene sequencing is being used as an important tool for identification of bacteria. In this study, isolates CR5 showed 99% similarity with *Klebsiella variicola* strain A6128 as indicated by 16S rRNA gene sequencing and comparison with the sequences already submitted in NCBI database using BLAST programme. Phylogenetic tree of the isolate was prepared by using neighbour joining of BLAST programme (Fig. 2). The GenBank accession number for isolate CR5 16S rRNA gene is MH398588.

Discussion

Castor is an important commercial plant with many uses, mainly the oil is important. However, it contains many

toxins which affect soil microbial community and other higher organisms. Biofertilizers which can withstand and may also degrade these inhibitors will not only help in maintaining soil fertility but also promote plant growth. Therefore, in the present study, attempts were made to evaluate bioinoculants which are resistant to inhibitors in castor for castor crop.

Table 4
Effect of different treatments on organic carbon (%)

Treatments	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS
T1	0.20	0.20	0.20	0.20	0.20	0.20	0.21	0.21	0.21
T2	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
T3	0.20	0.20	0.20	0.20	0.21	0.21	0.21	0.21	0.21
T4	0.20	0.20	0.20	0.20	0.20	0.21	0.21	0.21	0.21
T5	0.20	0.20	0.20	0.20	0.20	0.21	0.21	0.21	0.21
T6	0.20	0.20	0.20	0.20	0.21	0.21	0.21	0.21	0.21
Mean	0.20	0.20	0.20	0.20	0.20	0.21	0.21	0.21	0.21
Critical difference at 5%	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table 5
Effect of different treatments on total nitrogen (kg/ha)

Treatments	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS
T1	347	347	345	344	343	343	342	342	342
T2	346	345	343	342	340	339	339	339	339
T3	348	347	347	346	345	344	344	343	343
T4	347	346	344	342	341	341	340	340	340
T5	347	346	346	345	344	344	343	343	342
T6	346	345	344	343	341	341	341	340	340
Mean	346	346	344	343	342	342	341	341	341
CD at 5%	N.S.	N.S.	N.S.	2.15	3.22	2.71	2.71	2.77	2.54

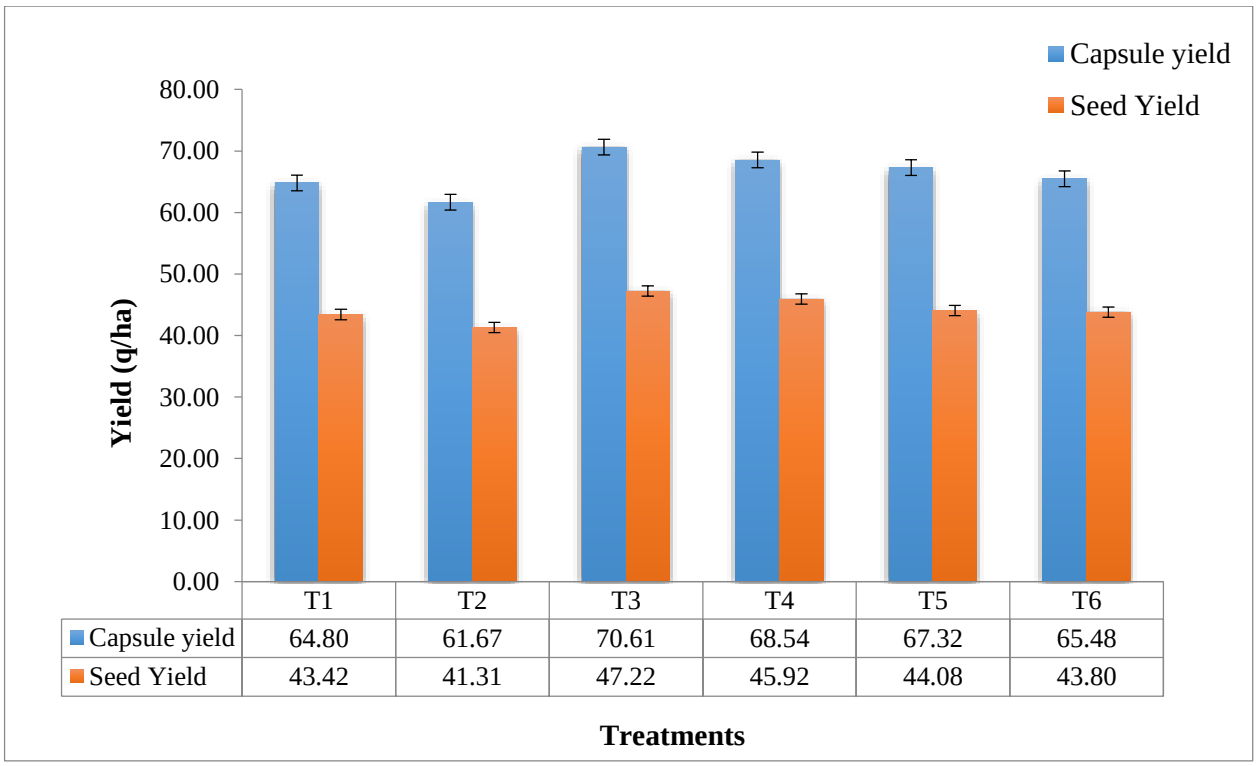

Figure 1: Effect of bacterial inoculants on capsule and seed yield of castor at harvesting

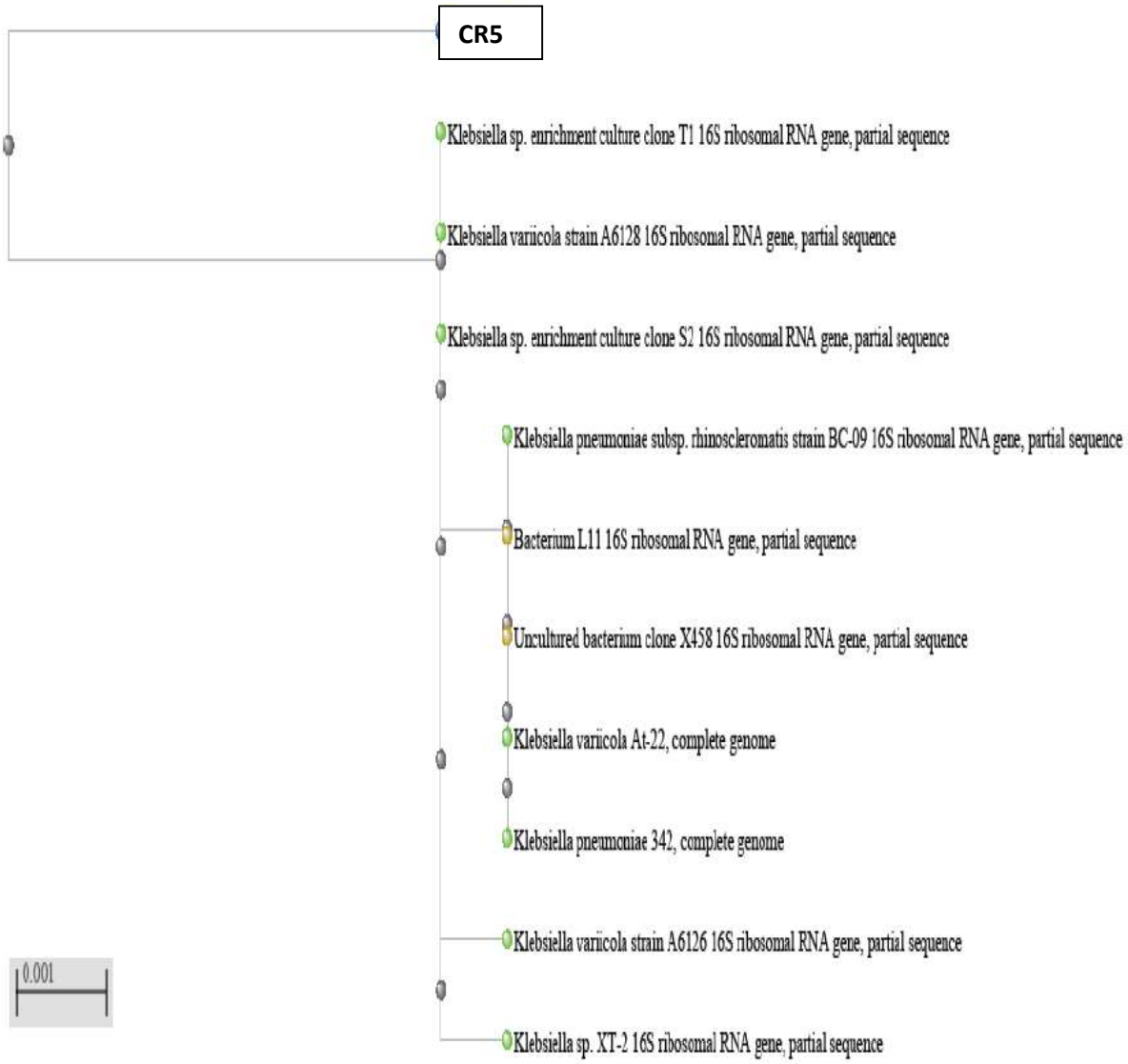

Figure 2: Phylogenetic Tree of bacterial isolate CR5

Table 6
Effect of different treatments on total phosphorus (kg/ha)

Treatments	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	210 DAS	240 DAS	270 DAS
T1	260	254	250	245	241	236	233	232	230
T2	256	251	246	242	238	234	231	228	227
T3	261	255	251	245	241	237	234	232	232
T4	257	252	246	243	237	234	231	229	227
T5	261	254	251	246	240	236	234	231	231
T6	258	253	245	241	235	232	230	229	228
Mean	258	253	248	243	238	234	232	230	229
CD at 5%	3.17	N.S.	3.17	3.18	3.17	2.85	2.71	N.S.	3.62

Out of 29 soil isolates, isolate CR5 and CR10 showed highest tolerance to inhibitors under *in vitro* conditions. In addition to this, these isolates also possess plant growth promoting traits like IAA production, ammonia excretion, NR activity and siderophore production. These are some of the important properties of rhizospheric bacteria contributing to plant growth. Further testing of selected isolates (CR5 and CR10) on the basis of above characters under pot house conditions proved that these isolates can survive under high concentration of inhibitors in castor rhizosphere and also promote plant growth efficiently.

However, isolate CR5 performed better and resulted in maximum increase in plant height, root weight (fresh and

dry) and shoot weight (fresh and dry). Highest count was also observed with the inoculation of isolate CR5. So, it was further studied under field conditions.

In this study, diazotrophic viable count in rhizosphere was determined in all the treatments at an interval of 30 days till 270 DAS. An increase in diazotrophic count was observed in all the treatments upto 150 DAS and later on a decrease in count was observed ranging from 5.95 - 6.57 log no. cfu/g soil at 270 DAS. The increase in population of rhizobacteria may be due to positive influence of the root exudates.[29] However, in later stages of plant growth, there is decrease in root exudation because the energy of the plant is channelized towards the seed and fruit formation.[25,26]

So, this may be the reason of decrease in the population of diazotrophic bacteria during later stages of plant growth as observed in this study. Another reason for decrease in diazotrophic population in later stages may be increase in concentration of castor inhibitors in soil, as microbes can tolerate these inhibitors only upto a certain limit.

A decline in native populations of rhizobium (*Bradyrhizobium* sp.) in 88 soil samples from 13 legume growing locations was reported by Venkateswarlu et al[38] when castor was cultivated in preceding year. Basinger et al[4] also observed negative effect of most toxic protein ricin form castor on microbial activity. Highest count was observed in the rhizosphere of plants treated with isolate CR5. This might be due to resistance of this strain to inhibitors of castor present in soil. Similarly, *Actinomycetes* concentrations as high as 30,000/g of soil have been identified in castor grown fields. Two bacterial genera, *Pseudomonas* and *Erwinia* were found to effectively degrade the toxic protein ricin in *in vitro* assays.[41]

Soil organic carbon is one of the most important factors that determines soil fertility. It also completes natural carbon cycle. It helps in promoting plant growth by providing nutrients and soil health by changing its biological and physical properties. In present investigation, increase in organic carbon content of soil was not significant. The amount of soil organic carbon is determined by many factors including soil nutrition and soil type, microbial activity, temperature, rainfall, farming methods and climate change.[5]

In this study, a gradual decline in total N content of soil was observed till 270 DAS. However, total P increased at 30 DAS after chemical fertilizer treatment and later on it also decreased gradually till 270 DAS.

Nitrogen and phosphorus are two major essential macronutrients required for plant growth, hence they are commonly added as fertilizers to enhance crop yield, as considerable part of soils is deficient in N and P. Thus, the use of PGPR including N_2-fixers and phosphate solubilizers as biofertilizers has become an area of interest in India to achieve maximum crop yields because nitrogenous and soluble phosphatic fertilizers cause environmental and economic problems.[7,8]

There was considerable increase in yield with the inoculation of isolate CR5 compared to its respective controls under field conditions. It showed percent increase of 11.16 and 8.75% at 75 and 100% RDF respectively when compared with uninoculated controls. Pishchik et al[32] also examined the effect of *Klebsiella mobilis* strains CIAM880 and CIAM853 on yield of potato when nitrogen fertilizer was added in low doses. They found that the yield of potato cultivars enhanced 1.2 to 1.4 fold as compared with uninoculated plants. Similarly, Mathukia et al[27] observed significant increase in seed and stalk yields in castor in two years by 14.7 and 14.6 % respectively over control when inoculated with *Pseudomonas striata.*

In addition, inoculation of phosphobacteria also resulted in better growth and yield parameters *viz.*, plant height, number of branches and spikes per plant, length of main spike, number of capsules per main spike and seed weight. The above findings can be explained on the basis of the fact that *Klebsiella variicola* strain A6128 established well in the rhizosphere of castor and resulted in increase in yield by providing various nutrients and other plant growth promoting substances.

16S rDNA has served as an important tool for the study of bacterial identification, classification and evolution because of a number of reasons. The function of 16S rRNA gene did not change over time, so the time (evolution) can be measured more accurately by random sequence changes in it. It is present as a multigene family in almost all bacteria. Moreover, the large size of 16S rRNA gene (1,500 bp) is well suited for informatics purposes. Finally, 16S rRNA gene can be sequenced completely by assembling the several amplified parts together. It provides species-specific signature sequences because of presence of highly conserved primer binding sites and hypervariable regions.[28] Isolate CR5 showed 99% similarity with *Klebsiella variicola* strain A6128.

Conclusion

The main goal of this work was to assess effect of a strain resistant to inhibitors of castor on yield of the crop. The selected isolate CR5 is the resistant strain to castor inhibitors which not only promotes plant growth and yield but also maintains soil health by maintaining soil microflora in castor grown fields. Therefore, from the above study, it was concluded that bacterial isolates from the rhizosphere of castor can withstand its inhibitors and promote plant growth and yield.

Acknowledgement

We thank Leelawati, L. K. Chugh and Anju Gaina for valuable comments on the study. We are also grateful to people of Regional Research Station (Bawal), Chaudhary

Charan Singh Haryana Agricultural University, Hisar, Haryana, India for their support during the course of study.

References

1. Aruna B., Bhadraiah B. and Pindi P.K., Effect of different AM fungi on biomass productivity of castor (*Ricinus communis)*, *Bioinfolet*, **12**, 176–179 **(2015)**

2. Ausubel F.M., Brent R., Kingston R.E., Moore D.D., Seidman J.G., Smith J.A., Struhl K., Albright L.M., Coen D.M., Varki A. and Chanda V.B., eds., Current Protocols in Molecular Biology, John Wiley, New York **(2001)**

3. Bashan Y. and Levanony H., Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture, *Can J Microbiol*, **36**, 591–608 **(1990)**

4. Basinger J., Zartman R., Zak J., Franscisco M.S., Green C. and Hopper N., Ricin influence on soil microbial respiration: Castor bean components and long-term effects, The ASA-CSSA-SSSA International Annual Meeting **(2005)**

5. Batjes N.H., Soil organic carbon stocks under native vegetation - revised estimates for use with the simple assessment option of the Carbon Benefits Project system, *Agric Ecosyst Environ*, **142**, 365-373 **(2011)**

6. Berman P.S.N. and Wiesman Z., Castor oil biodiesel and its blends as alternative fuel, *Biomass Bioenergy*, **35,** 2861-2866 **(2011)**

7. Bhardwaj D., Ansari M.W., Sahoo R.K. and Tuteja N., Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity, *Microb Cell Fact*, **13**, 66 **(2014)**

8. Brady N.C., Soil organic matter. The nature and properties of soils, 14th edn. Macmillan, New York, USA, 351-380 **(1990)**

9. Bremner J.M. and Mulvaney C.S., Nitrogen total. Methods of soil analysis, Part 2, Chemical and Microbiological Properties, eds., Page A.L., Miller R.H. and Keeney D.R., American Society of Agronomy, Madison, 643-698 **(1982)**

10. Colo J., Hajnal-Jafari T.I., Durić S., Stamenov D. and Hamidović S., Plant growth promotion rhizobacteria in onion production, *Pol J Microbiol*, **63**, 83-88 **(2014)**

11. Compant S., Duffy B., Nowak J., Clément C. and Ait Barka E., Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action and future prospects, *Appl Environ Microbiol*, **71**, 4951–4959 **(2005)**

12. Cooke T., Flora of the presidency of Bombay, Botanical survey of India, Calcutta, 627 **(1908)**

13. Gana A.K., Amosun A. and Alhaji B.B., Determination of number of manual hoe weeding for optimal yield of castor (Ricinus communis L., Euphorbiaceae) in Nigeria, *Glob J Bot Sci*, **2**, 21-25 **(2014)**

14. Glick B.R., Cheng Z., Czarny J. and Duan J., Promotion of plant growth by ACC deaminase-producing soil bacteria, *Eur J Plant Pathol*, **119**, 329–339 **(2007)**

15. Hussein M.M., ElHabbasha T.S.F. and Mekki B.B., Prospect of Bacterial Inoculants and Organic Fertilizers for Improving Growth, Productivity and Quality of Castor Bean (*Ricinus communis* L.) Plants in Newly Reclaimed Sandy Soils, *World J Agric Sci*, **9**, 421-428 **(2013)**

16. India, major producer of castor oil, https://www.thehindubusinessline.com/economy/agri-business/India-major-producer-of-castor-oil/article20116366.ece **(2011)**

17. Innerebner G., Knief C. and Vorholt J.A., Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system, *Appl Environ Microbiol*, **77**, 3202–3210 **(2011)**

18. Jena J. and Gupta A.K., *Ricinus communis* linn: A phytopharmacological review, *Int J Pharm Pharm Sci*, **4**, 25-29 **(2012)**

19. Jeyaseelan E.C. and Jashothan P.T.J., In vitro control of *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) by *Ricinus communis* L, *Asian Pac J Trop Biomed*, **2**, 717–721 **(2012)**

20. John M.K., Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid, *Soil Sci*, **109**, 214-220 **(1970)**

21. Kalembassa S.J. and Jenkinson D.S., A comparative study of titrimetric and gravimetric methods for determination of organic carbon in soil, *J Sci Food Agric*, **24**, 1089-1090 **(1973)**

22. Kumar N.S. and Kanjan D., Influence of integrated nutrient management practices on yield attributes, seed yield, oil yield and nutrient uptake of castor under irrigated conditions, *Indian J Agric Res*, **43**, 200-205 **(2009)**

23. Kumar O., Nashikkar A.B., Jayaraj R. and Vijayaraghavan R., Purification and biochemical characterisation of ricin from castor seeds, *Defence Sci J*, **54**, 345-351 **(2004)**

24. Lukow T., Dunfield P.F. and Liesack W., Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants, *FEMS Microbiol Ecol*, **32**, 241–247 **(2000)**

25. Martin J.K., Factors influencing the loss of organic carbon from wheat roots, *Soil Biol Biochem*, **9**, 1-7 **(1977)**

26. Martin J.K. and Kemp J.R., The measurement of C transfers within the rhizosphere of wheat grown in field plots, *Soil Biol*, **18**, 103-107 **(1986)**

27. Mathukia R.K., Shekh M.A. and Sagarka B.K., Irrigation and integrated nutrient management in castor (*Ricinus communis* L.), *Innovare J Agric Sci*, **2,** 3-4 **(2014)**

28. Mishra V., Kumar D., Srivastava V.K., Gautam A., Shukla S., Verma K.R. and Verma A.R., Identification of unknown bacteria by using biochemical tests and 16S RRNA sequencing from different soil samples, *Int J Adv Res Sci Eng*, **5**, 342-349 **(2016)**

29. Narula N., Remus R., Deubel A., Granse A., Dudeja S.S., Behl R.K. and Merbach W., Comparison of the effectively wheat roots

colonization by A. chroococcum and Panotea agglomeraus using serological techniques, *Plant Soil Environ*, **53**, 167-176 **(2007)**

30. Patel H.M., Bafna A.M. and Patel Z.N., Yield and quality of castor as affected by INM, *Gr Farming*, **1**, 263-265 **(2010)**

31. Pieterse C.M., Van der Does D., Zamioudis C., Leon-Reyes A. and Van Wees S.C., Hormonal modulation of plant immunity, *Annu Rev Cell Dev Biol*, **28**, 489-521 **(2012)**

32. Pishchik V.N., Chernyaeva I.I., Kozhemaykov A.P., Vorobyov N.I., Lazarev A.M. and Kozlov L.P., Effect of inoculation with nitrogen-fixing *Klebsiella* on potato yield. Nitrogen Fixation with Non-Legumes, eds., Malik K.A., Kluwer Academic Publishers, Britain, 223-235 **(1999)**

33. Richardson A.E., Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants, *Funct Plant Biol*, **28,** 897–906 **(2001)**

34. Saharan B.S. and Nehara V., Plant Growth Promoting Rhizobacteria: A Critical Review, *Life Sci Med Res*, **21**, 1-30 **(2011)**

35. Sharma A. and Johri B.N., Growth promoting influence of siderophore-producing Pseudomonas strains GRP3A and PRS9 in maize (*Zeamays* L.) under iron limiting conditions, *Microbiol Res*, **158**, 243–248 **(2003)**

36. Singh R.P., Jha P. and Jha P.N., The plant-growth-promoting bacterium Klebsiella sp. SBP-8 confers induced systemic tolerance in wheat (Triticum aestivum) under salt stress, *J Plant Physiol*, **184**, 57-67 **(2015)**

37. Ulanova R. and Kravchenko I., Lactic acid bacteria fermentation for detoxification of castor bean meal and processing of novel protein feeds supplement, *Int J Eng Sci Innov Technol*, **2**, 618-624 **(2013)**

38. Venkateswarlu B., Hari K. and Katyal J.C., Influence of soil and crop factors on the native rhizobial populations in soils under dryland farming, *Appl Soil Ecol*, **7**, 1-10 **(1997)**

39. Wu C.H., Bernard S., Andersen G. and Chen W., Developing microbe-plant interactions for applications in plant-growth promotion and disease control, production of useful compounds, remediation and carbon sequestration, *Microb Biotechnol*, **2**, 428–440 **(2009)**

40. Yadav H.K., Thomasi T. and Khajuria V., Effect of different levels of sulphur and biofertilizer on the yield of Indian mustard (Brassica juncea L.) and soil properties, *J Agric Phys*, **10**, 61-65 **(2010)**

41. Zartman R., Green C., Francisco M.S., Zak J., Jaynes W. and Boroda E., Mitigation of ricin contamination in soils: sorption and degradation, Texas Tech University, Lubbock, Texas **(2003)**.

(Received 20th April 2021, accepted 03rd July 2021)