Advances in Bioresearch
Adv. Biores., Vol 9 (4) July 2018: 43-47
©2018 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.4.4347

Advances in Bioresearch

ORIGINAL ARTICLE

Growth promotion and biocontrol efficacy of Cauliflower by Plant Growth Promoting bacterial consortia

W. Jothy[1] and Srividya Shivakumar[*1]
1. Department of Microbiology,
Jain University, 18/3, 9th Main, Jayanagar, 3rd Block,
Bangalore, Karnataka, 560011, India
* Corresponding author E-mail: sk.srividya@jainuniversity.ac.in

ABSTRACT
Plant Growth Promoting Rhizobacteria (PGPR) associated with plant roots play a major role in the growth of the plants by providing them with growth promoting hormones, solubilizing phosphates, producing siderophores, secreting various hydrolysing enzymes etc. Though a single bacterium may not be multifarious in terms of its growth promoting activities, it can still be made available to the plants by a bacterial consortia with multiple activities as they could exhibit a synergistic effect on plant growth. The present study aims at testing the efficacy of a bacterial consortia on the growth parameters of Cauliflower plants and their biocontrol potentials. Three compatible bacterial isolates Bacillus subtilis J20A, *Bacillus subtilis* J20B and *Pseudomonas aeruginosa* J22P *were tested for their biocontrol activity against six different phytopathogens. Isolate* J20A *was more potent followed by* J22P *and* J20B. *All the isolates showed promising plant growth promoting traits. Seed germination potential in consortium treated plants were found to be 21% more than the untreated control plants. There was a threefold increase in vigour index in a consortium treated plants (1361) than the control plants (441). Similarly consortium treated plants showed two fold (7cm) and three fold (6cm) increase in terms of shoot and root length compared with the control plants (3cm and 2 cm respectively). Fresh and dry weight of shoot and root also showed a significant increase in consortium treated plants than the control plants. This is a clear indicative of using bacterial consortia as an efficient biofertilizer which can exhibit plant growth promoting and biocontrol activities.*
Key words: PGPR, consortium, seed germination, vigour index

Received 12.03.2018 Revised 11.04.2018 Accepted 28.06.2018

How to cite this article
W. Jothy and Srividya Shivakumar. Growth promotion and biocontrol efficacy of Cauliflower by Plant Growth Promoting bacterial consortia. Adv. Biores., Vol 9 [4] July 2018.31-36.

INTRODICTION

Sustainable Agriculture has become more popular in recent years as an excellent alternative measure to Industrial Agriculture since there is a considerable reduction in the use of harmful chemicals, soil erosion, nutrient depletion etc., of the fertile land [1]. In the development of sustainable crop production system. Plant Growth Promoting Rhizobacteria play a vital role [2]. PGPR show a direct or indirect effect on plant growth. It increases the growth of the plant by providing hormones, hydrolysing enzymes, chelating agents, etc., [3,4]. Application of PGPR increases crop productivity and improves soil fertility [5]. It can also improve disease resistance in plants by synthesising antimicrobial compounds against pathogens [6,7]. Cauliflower (*Brassica oleracea*) belongs to the family Cruciferaceae. The edible portion is known as 'curd'. In India two seasonal varieties are cultivated and they are early season and late season crop. Cauliflower is well known for reducing cholesterol and improving immune system. It is also rich in calcium and minerals. Many diseases like damping off, downy mildew, leaf spot, powdery mildew, etc., affects cauliflower and cause huge economic loss. Many bacteria are used as potential biocontrol agents which can inhibit the growth of the phytopathogens. The present study aims at testing the efficacy of bacterial consortium in improving the growth characteristics of cauliflower and their biocontrol potency.

MATERIALS AND METHODS

Three compatible bacterial isolates *Bacillus subtilis* J20A (GenBank Accession Number: MG601747.1), *Bacillus subtilis* J20B (GenBank Accession Number: MG602478.1) and *Pseudomonas aeruginosa* J22P (GenBank Accession Number: MG738317.1) were used for this study and were maintained in nutrient agar slants. Antagonistic potentials of these isolates were tested using dual-culture plate assay [8] against six fungal phytopathogens *(Cylindrocladium camelliae* MTCC2097*, Fusarium moniliforme* MTCC2088, *Sclerotinia sclerotiarum* MTCC8785, *Fusarium oxysporum* MTCC2087, *Rhizoctonia solani* MTCC4634*, Thielaviopsis basicola* MTCC1467*)* obtained from Microbial Type Culture Collection, IMTECH, Chandigarh which were maintained in PDA slants. The percentage of fungal growth inhibition was calculated with the formula,

$$\frac{C-T}{C} \times 100$$

Where, C=growth in control and T= growth in test. The plant growth promoting attributes of the above three isolates were analysed. The parameters analysed were siderophore production [9], PO_4 solubilization [10,11], Biosurfactant production [12], HCN production [13], ammonia production [14], Indole Acetic Acid production [15]. Analysis of hydrolytic enzymes like Protease [16], β-1,3-Glucanase [17], Chitinase [18], Lipase [19], Cellulase [20] were also carried out. The bacterial isolates were grown in nutrient broth overnight and the cell number was adjusted to $3X10^8$ CFU ml^{-1} with spectrophotometer. Equal volume of all the three isolates were mixed together to make a bacterial consortia. The cauliflower seeds were procured from Indian Institute of Horticultural Research (IIHR) Bangalore, Karnataka, India. Seeds were surface sterilized with 0.1% $HgCl_2$ and washed twice in sterile distilled water. Seeds were then dried and seed bacterization was carried out. Sterile seeds were soaked in the bacterial consortium with 1% Carboxymethyl Cellulose (CMC) and left undisturbed overnight for seed coating. CMC acts as a binding agent. After seed coating, the bacterial suspension was drained out and the seeds were dried under sterile air. In a protray sterile soil was taken and 5 seeds per pots were planted and the entire experiment was carried out in triplicates. The trays were kept in a greenhouse with a temperature of 22±2°C. and with a photoperiod of 16 h light and 8 h dark. The growth parameters were analysed after 15 days of planting. Irrigation was done periodically to avoid drying of the soil and plants. Seed germination potential, vigour index, shoot and root length, fresh and dry weight of the shoots and roots were analysed. All the data were subjected to one way Analysis Of Variance (ANNOVA) and the means were compared with Duncan's Multiple Range Test (DMRT) ($P<0.05$) using IBM SPSS software version 20.

RESULT AND DISCUSSION

Antagonistic assay of the bacterial isolates showed varied percentage of inhibition of phytopathogens. Isolate J20A showed highest percentage of inhibition against four of the five phytopathogens tested. *F.oxysporum* was highly sensitive (80%) to isolate J20A. *S. sclerotiarum* showed 57% of inhibition to isolate J20A but isolate J22P was very effective in inhibiting *S. sclerotiarum* which was 65% which shows that the isolates J20A and J22P together could show a cumulative inhibitory effect on *S. sclerotiarum*. Research done by Ruiz *et al.,* showed 60% inhibition of pathogen using *Bacillus subtilis* [21]. Sid *et al*., also showed a similar result with *Bacillus subtilis* in inhibiting *Phytophthora* and *Rhizoctonia* [22]. Cazorla proved the antagonistic activity of *Bacillus* though the secretion of hydrolysing enzymes [23]. Study carried out by Killani *et al.,* proved the antagonistic ability of *Bacillus subtilis* against fungal phyto pathogens *Fusarium verticilloides*, *F. equiseti, F. solani, F. oxysporum,* and *Rhizoctonia solani* [24]. Isolate J22P was the next potent antagonist which inhibited three pathogens more effectively. It also showed significant inhibition of the other remaining pathogens too indicating its effectiveness as a potential biocontrol agent. (Table 1).

Table 1: Antagonistic potentials of the bacterial isolates against phytopathogens

Isolate No.	% Inhibition					
	C.camelliae	*F. moniliforme*	*S. sclerotiorum*	*F. oxysporum*	*R. solani*	*T. basicola*
J20A	68.3±1.5^a	65.3±1.5^a	57±2.6^{ab}	80±2^a	77.6±2.5^a	67.6±2.5^a
J20B	54±3.6^c	46.0±5.5^b	55±4.5^b	55±1^c	67.3±2.5^b	58.3±2.8^b
J22P	60±2.0^b	68.3±1.5^a	65±5.0^a	76±1.7^b	75.3±0.5^a	55±5^b

*values are mean of three replicates ± standard deviation. Means within a column with the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($p<0.05$).

Table 2: Plant growth promoting characteristics of the bacterial isolates

PGPR traits / Isolates	J20A	J20B	J22P
Siderophore production	-	+	+++
PO_4 solubilisation	-	-	+++
Biosurfactant production	-	-	+++
HCN production	-	-	+++
Ammonia production	++	-	+++
IAA	+++	++	+
Protease	+++	+++	+
β-1,3-Glucanase	++	+++	-
Chitinase	+++	+++	-
Lipase	+++	+++	+
Cellulase	+++	+++	-

-, Negative, +, Positive, +, small halos < 0.7cm, ++, medium halos >0.7cm, +++, large halos >1.3cm

Table 3: Effect of PGPR on the growth parameters of cauliflower plants

Treatments	Seed germination (%)	Vigour Index	Mean shoot length (cm)	Mean root length (cm)	Fresh weight ($gplant^{-1}$)		Dry weight ($gplant^{-1}$)	
					Shoot	Root	Shoot	Root
J20A	68.0±2.6 d	478.3±21 f	3±0.05 e	3±0.11 g	0.7±0.15 f	0.03±0.00 g	0.008±0.002 d	0.0002±0.0001 d
J22P	76.6±2.0 bc	605±16 e	4±0.15 d	3±0.15 f	1.4±0.05 e	0.06±0.00 fg	0.02±0.01 d	0.0006±0.0002 d
J20B	78.6±0.5 bc	710±21 d	4±0.05 c	4±0.20 e	1.8±0.05 d	0.1±0.01 f	0.10±0.01 c	0.002±0.001 d
J20A + J22P	79.3±5.1 bc	816±39 c	5±0.15 b	5±0.05 c	2.2±0.15 c	0.4±0.01 c	0.12±0.01 c	0.033±0.025 b
J22P + J20B	82.3±2.0 b	889±36 b	5±0.11 b	5±0.05 b	2.6±0.20 b	0.5±0.02 b	0.18±0.01 b	0.066±0.020 a
J20A + J20B	77.3±3.7 bc	732±46 d	4±0.05 c	4±0.11 d	2.1±0.11 c	0.2±0.03 e	0.11±0.03 c	0.007±0.0006 cd
J20A + J22P + J20B	95.0±3.0 a	1361±39 a	7±0.20 a	6±0.20 a	4.4±0.25 a	0.9±0.03 a	0.23±0.02 a	0.026±0.015 bc
Control	74.0±5.2 c	441±33 f	3±0.20 f	2±0.05 h	1.7±0.11 d	0.2±0.03 d	0.01±0.00 d	0.0002±0.0001 d

Data represent the mean ± standard deviation of triplicates. Means were compared with DMRT. Means within the column in each parameter with the same letters are not significantly different by ONE WAY ANOVA ($p<0.05$)

There are many evidences [18] which showed the presence of many PGPR traits in *Pseudomonas* with an excellent antagonistic activity against many phytopathogens. Though isolate J20B was not an effective antagonistic strain, it is still used in this study because it possesses good PGPR properties. It showed the presence of not only the siderophores but also different types of highly effective hydrolysing enzymes. Isolate J22P exhibited a significant level of biosurfactant production, HCN production and phosphate solubilisation while these properties were not found in isolates J20A and J20B. Presence of best antagonistic properties, chelating agents like siderophores, defence mechanism through HCN production, hydrolysing enzymes and PO_4 solubilisation to make the nutrients available to the plants, presence of chitinase to hydrolyse the fungal cell wall and increased level of growth hormones makes the above bacterial isolates the best consortia with all the beneficial characteristics which can be made available for the growth of the plants (Table 2). Similar results were obtained by earlier researches done by Sasirekha e*t al*.,[18], Glick [25] and Dey [26]. When this consortia was applied to cauliflower seeds and growth parameters were analysed, the seed germination percentage showed 21% increase in consortium treated plants than the control plants. Plants treated with J22P + J20B showed 82.3% of seed germination while the consortium with all the three isolates showed 95% of seed germination proving the synergistic effect of these microbes. Vigour Index was found to be increased twofold (1361) than the control plants (441) indicating the increased potency of the microbial consortia on the growth of the plants. Shoot length and root length of the consortium treated plants were measured to be 7 cm and 6 cm, respectively, which was twofold and threefold more than the control plants which showed only 3 cm and 2 , respectively. Consortium treated plants showed a drastic increase in fresh and dry weight of shoots and roots. Fresh and dry weight of shoot of the consortia treated plants was found to be 90 % and 7.3 % more than the control plants. Similar result was observed in terms of dry weight of shoots and roots (Table 3). Evidence of plant growth enhancement was also proved by many other studies. Research done by Sasirekha *et*

al.,[18] showed the presence of many PGPR trait in *Pseudomonas* and their effect on plant growth was well recorded. The are many other supporting evidences as well. Findings of the research done by Ertan [27] showed the positive effect of PGPR on the growth parameters of cucumber. Similar study was also done by Melek [28] on cauliflower and it is promising result in the field of sustainable agriculture. Influence of varying levels of P and N fertilizers on the efficacy of PGPR was analysed by Manoj *et al.,* using cauliflower plant as the host system and the results were more favourable on the growth of the plant [29]. Results of Vibha *et al.,* [30]; Adesemoye *et al.,* [31]; Cakmakci *et al.,*[32] and Garcia *et al.,* [33] also supports the use of plant growth promoting rhizobacteria in green house as well as on the growth of transplants.

CONCLUSION

The present research work focussed on developing a bacterial consortia with efficient plant growth promoting characteristics and wide range of biocontrol activities. It was proved that the PGPR traits though were present in different isolates, showed a cumulative growth enhancement when applied on cauliflower seeds proving their efficacy as a microbial consortia. Further the isolates inhibited more than one phytopathogens which is indicative of their wide spectrum activity. This findings paves a way to use bacterial consortia as a potential plant growth promoting biofertilizer with broad spectrum biocontrol activity without affecting the environment and a milestone in the field of sustainable agriculture.

ACKNOWLEDGEMENTS

The authors acknowledge Jain University for providing necessary facilities to carry out this research work. We are thankful to Indian Institute of Horticultural Research (IIHR) Bangalore, Karnataka, India for providing cauliflower seeds.

CONFLICT OF INTEREST

The authors have declared that no competing interest exists.

REFERENCES

1. Sarah, V., Julia, L., Nicolas, J. & Jens, N. (2015). What is sustainable Agriculture? A systematic Review. Sustainability. 7:7833-7865.
2. Zahid, M., Abbasi, M.K., Hameed, S. & Rahim, N. (2015). Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zea mays* L.). Front Microbiol., 6:207.
3. Kloepper, J., Reddy, M., Rodríguez-Kabana, R., Kenney, D., Kokalis-Burelle, N., Martinez-Ochoa, N. & Vavrina, C. (2004). Application for rhizobacteria in transplant production and yield enhancement. Acta. Hortic., 631: 217-230.
4. Saharan, B.S. & Nehra, V. (2011). Plant growth promoting rhizobacteria: a critical review. Life Sci. Med. Res., 1:1–30.
5. Nehra, V. & Choudhary, M. (2015). A review on plant growth promoting Rhizobacteria acting as bioinoculants and their biological approach towards the production of sustainable agriculture. J. Appl. Nat. Sci., 7(1):540–556.
6. Singh, A.K. (2004). Effect of nitrogen and phosphorus on growth and curd yield of cauliflower var. Snowball-16 under cold arid region of ladakh. Haryana Journal of Horticulture Science. 33(1):127-129.
7. Mayak, S., Tirosh, T. & Glick, B.R. (2004a). Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol. Biochem., 42:565-572.
8. Idris, H.A., Labuschagne, N. & Korsten, L. (2007). Screening rhizobacteria for biological control of *Fusarium* root and crown rot of sorghum in Ethiopia. Biol. Control*.,* 40: 97-106.
9. Schwyn, B. & Neilands, J.B. (1987). Universal chemical assay for the detection and determination of siderophores. Anal. Biochem., 160: 47-56.
10. Pikovskaya, R. E. (1948). Mobilization of phosphorous in soil in connection with vital activity of some microbial species. Microbiologia. 17: 362-370.
11. Richardson, A.E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust. J. Plant Physiol*.,* 28: 897-906.
12. Carrillo, P., Mardaraz, C., Pitta-Alvarez, S. & Giulietti, A. (1996). Isolation and selection of biosurfactant-producing bacteria. World. J. Microb. Biot., 12: 82–84.
13. Lorck, H. (1948). Production of hydrocyanic acid by bacteria. Physiol. Plant. 1: 142–146.
14. Cappuccino, J.C. & Sherman, N. (1992). In: Microbiology: A Laboratory Manual, Third ed. Benjamin/cummings Pub. Co., New York pp.125–179.
15. Brick, J.M., Bostock, R.M. & Silverstone, S.E. (1991). Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Appl. Environ. Microbiol., 57: 535-538.
16. Chaiharn, M., Chunhaleuchanon, S., Kozo, A. & Lumyong, S. (2008). Screening of rhizobacteria for their plant growth promoting activities. KM I TL Sci. Tech. J., 8: 18-23.

17. Teather, R. M. & Wood, P. J. (1982). Use of congo red-polysacharide interaction in enumeration and characterization of cellulolytic bacteria from bovine Rumen. Appl. Environ. Microbiol., 43: 777-780.
18. Sasirekha, B., Srividya, S. & Sullia, S.B. (2012). Identification of multi-trait PGPR isolates and evaluation of their potential as biocontrol agents. Acta. Biologica. Indica. 1:61-67.
19. Bornscheuer, U.T., Bessler, C., Srinivas, R. & Krishna., S.H. (2002). Optimizing lipases and other enzymes for efficient application. Trends. Biotechnol., 20: 433–437.
20. Miller, G. L. (1959). Use of Dinitrosalicyclic Acid Reagent for Determination of Reducing Sugar. Anal. Chem., 31: 426-428.
21. Ruiz, S.E., Cristobal, A.J., Reyes, R.A., Tun, S.J., Garcia, R.A. & Pacheco, A.J. (2014). *In vitro* antagonistic activity of *Bacillus subtilis* strains isolated from soils of the Yucatan Peninsula against *Macrophomina phaseolina* and *Meloidogyne incognita,* Phyton, Int. J. Exp. Bot., 83: 45-47.
22. Sid, A.A., Ezziyyani, M.S., Perez, C. & Candela, M.E. (2003). Effect of chitin on biological control activity of *Bacillus* spp. and *Trichoderma harzianum* against root rot disease in pepper (*Capsicum annuum*) plants. Eur. J. Plant Pathol., 109: 633-637.
23. Cazorla, F.M., Romero, D., Perez, G.A., Lugtenberg, B.J.J., De Vicente, A. & Bloemberg, G. (2007). Isolation and Characterization of Antagonistic *Bacillus subtilis* Strains from the Avocado Rhizoplane Displaying Biocontrol Activity. J. Appl. Microbiol., 103: 1950–1959.
24. Killani, A.S., Abaidoo, R.C., Akintokun. A.K. & Abiala, M.A. (2011). Antagonistic Effect of Indigenous *Bacillus subtilis* on Root-/Soilborne Fungal Pathogens of Cowpea. Researcher. 3: 11-18.
25. Glick, B.R. (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol., 41: 109-117.
26. Dey, R., Pal, K.K., Bhatt, D.M. & Chauhan, S.M. (2004). Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol. Res., 159: 371-394.
27. Ertan, Y., Melek, E., Atilla, D. & Kenan, K. (2015). Plant growth promoting Rhizobacteria improved seedling growth and quality of cucumber (*Cucumis sativus* L.). In: Conference on Chemical, Food and environment engineering, (ICCFEE'15), 11-12.
28. Melek, E., Metin, T., Ertan, Y., Adem, G., Recep K. & Atilla, D. (2014). Effect of plant growth promoting Rhizobacteria on growth, nutrient, Organic acid, amino acid and hormone Content of cauliflower (*Brassica oleracea* L. Var. *Botrytis*) transplants. Acta. Sci. Pol. hortorum cultus., 13(6): 71-85.
29. Manoj, K., Rajesh, K., Thakur, B.S. & Spehia, R.S. (2011). Effect of plant growth-promoting rhizobacteria at varying Levels of N and P fertilizers on growth and yield of Cauliflower in mid hills of Himachal Pradesh, J. Farm Sci., 1(1): 19-26.
30. Vibha, N., Baljeet, S. & Madhu, C. (2016). Evaluation of *Brevibacillus brevis* as a potential plant growth promoting Rhizobacteria for cotton (*Gossypium hirsutum*) crop, SpringerPlus.5:948.
31. Adesemoye, A.O., Obini, M. & Ugoji, E.O. (2008). Comparison of plant growth promotion with *Pseudomonas aeruginosa* and *Bacillus subtilis* in three vegetables. Braz. J. Microbiol., 39: 423–426.
32. Cakmakci, R., Donmez, F., Aydın, A. & Sahin, F. (2006). Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. Soil. Biol. Biochem., 38: 1482–1487.
33. Garcia, L.J.A., Probanza, A., Ramos, B. & Manero, F.J.G. (2003). Effects of three plant growth promoting rhizobacteria on the growth of transplants of tomato and pepper in two different sterilized and nonsterilized peats. Arch. Agro. Soil Sci., 49: 119–127.