ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

EXPLORING THE ABIOTIC AND BIOTIC STRESS TOLERANCE POTENTIAL OF RHIZOBACTERA ISOLATED FROM CYAMOPSIS

Ritika Jain*, Meenu Saraf

Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India

**Corresponding author: smile_gudiya03@yahoo.co.in*

ABSTRACT

Agriculture plays a vital role for any economy primarily for developing and under developed economies. Increasing abiotic as well as biotic stresses adversely affects crop productivity across the world. Microorganisms inhabiting the Rhizospheric region of plant soil are known to play an important role in alleviating these stresses, thus enhancing crop productivity and yield. The present study was carried out to isolate the Rhizospheric bacteria from Cyamopsis showing potential to tolerate abiotic and biotic stresses. To carry out this, bacteria were isolated from Rhizospheric soil of Cyamopsis which were collected from different regions of Gujarat. These isolates were screened for tolerance to different abiotic stresses such as temperature, pH, salt and drought. Highly abiotic stress tolerant isolates were further tested for biotic stress against pathogenic bacteria and fungi. Among the 80 bacterial isolates, best grown 30 cultures were tested for different abiotic stress. Four cultures i.e. MN40, KM1, KM6 and AK17 showing high tolerance to abiotic stresses were further investigated for biotic stress tolerance. Selected cultures were tested for their antagonistic activity against pathogenic fungi viz., *Macrophomina phaseolina, Fusarium oxysporium, Sclerotinum rolfissii and Trichoderma spp.* Furthermore, antimicrobial activities of all 4 selected bacterial strains were tested against different test organisms viz., Gram negative bacteria (*Salmonella typhi*) and Gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus*). Amongst the 4 selected bacterial strains, KM6 shows highest antagonistic activity.

Keywords: Abiotic Stress, Biotic Stress, Cyamopsis, Antagonism.

1. INTRODUCTION

Abiotic and biotic stresses have a negative impact on crop growth and agricultural productivity worldwide. Rain fed agro-systems such as those in India are adversely affected by abiotic tresses such as high temperature, salinity and drought. An estimated 20% of total cultivated and 33% of irrigated agricultural lands are affected by salt stresses [1] and by 2050, drought and salinity are expected to affect more than 50% of arable land [2, 3]. Pathogens such as fungi, bacteria, virus, nematodes etc. also affect plant health and pose a threat to agricultural sustainability worldwide. Interestingly there is interplay between abiotic and biotic stresses and abiotic stresses such as high temperature and drought also influence the spread of pathogens and insects [4-6]. Often a range of abiotic and biotic stresses act simultaneously on plants and thus it becomes imperative to ameliorate the effects of these stresses to improve agricultural productivity.

Microorganisms inhabiting the Rhizospheric/endorhizospheric region of plants are known to promote plant growth, nutrient management and disease control through various direct and indirect mechanisms [7-9]. Certain bacteria ameliorate abiotic and/or biotic stressors contributing in enhanced plant growth and productivity [10]. Plant-growth-promoting rhizobacteria (PGPR) induces physical and chemical changes in plants that results in enhanced tolerance to abiotic stress [11]. Their interactions with the plants enhance the capability of plants to fight against abiotic stresses by evoking various kinds of local and systemic responses that improve metabolic capability of the plants [12]. Certain bacteria like Pseudomonas produces exopolysaccharides (EPS) under stress conditions, which protects them from drought conditions stress by enhancing water retention capability and regulating the diffusion of carbon sources in microbial environment [7,8]. Some PGPR strains produce cytokinin and antioxidants resulting in accumulation of abscisic acid (ABA) and degradation of reactive oxygen species. These antioxidant enzymes provides with oxidative stress tolerance [13]. Certain

PGPR are known to act as biological control agents either by producing antagonistic substances that inhibits the development of phytopathogens or by inducing resistance to pathogens [14]. As bio-control agents, PGPR act in myriad ways, such as by decreasing the level of ethylene in plants [15], production of auxin phytohormone [16] etc. In the present study we aim to isolate rhizobacteria which were able to tolerate both abiotic and biotic stresses. Rhizobacteria were tested for abiotic stresses such as drought, salinity, temperature and pH. They were also tested for antagonism against pathogenic bacteria and Fungi. Such rhizobacteria will be helpful for efficient management of abiotic and biotic stresses in crop production.

2. MATERIAL AND METHODS

2.1. Isolation of Bacteria

The bacterial strains were isolated from the Rhizospheric soil of Cyamopsis (cluster beans) collected from different regions of Gujarat. 5 gram of soil samples was diluted with 95 ml of sterile distilled water, dispersed equally by shaking at 150 rpm for 30 minute at 28°C and further serial dilution was done up to 10^7 fold. Aliquots (100µl) of the diluted samples were spread onto different media plates like Nutrient Agar, Kings B, Okons, Yeast Extract Mannitol Agar (YEMA), Ashby's and incubated at 28°C for 24 hrs to study the diversity of bacteria in soil samples. After diversity study the best grown cultures were picked and streaked on freshly prepared Nutrient agar (NA) plates. The bacterial cultures were store at 4°C for further study.

2.2. Screening of isolates for Abiotic stress tolerance

Isolates were screened for their ability to tolerate different abiotic stresses (high temperature (55°C), salinity (20% NaCl concentration), drought (-0.73 MPa osmotic pressure) and pH (4-12) using Nutrient broth (NB). Growth of all the isolates was recorded using spectrophotometer at 600 nm with uninoculated medium as blank. Bacterial isolates were considered stress tolerant if OD of ≥ 0.1 was recorded.

2.2.1. pH Tolerance

The pH of the culture medium was adjusted to 4, 6, 7, 8, 10 and 12 using sterile buffers. Nutrient broth with different pH was prepared and inoculated with 1% of overnight raised bacterial cultures. After incubating at 28°C under shaking condition at 120 rpm for 24 hrs, growth was measured at 600 nm.

2.2.2. Drought Tolerance

Nutrient broth with different water potentials (- 0.05, - 0.15, -0.30, -0.49, -0.73 MPa) was prepared by adding appropriate concentrations of polyethylene glycol (PEG 6000) and was inoculated with 1% of overnight raised bacterial cultures in NB. After incubation at 28°C under shaking condition (120 rpm) for 24 h, growth was estimated by measuring the optical density at 600 nm using a spectrophotometer.

2.2.3. Temperature tolerance

10ml of Nutrient broth was dispensed into 30ml capacity screw cap tubes and autoclaved. Already active bacterial suspension (0.1ml) was poured into these media containing autoclaved screw cap tubes & incubated at 20°C, 30°C, 40°C, 50°C and 55°C. After culture were allowed to grow for 24 hrs, their absorbance was measured at 600 nm.

2.2.4. Salinity Tolerance

Nutrient broth with different salinity was prepared by adding NaCl of different concentration (1%-20%) and was inoculated with 1% of overnight raised cultures in NB. After incubation at 28°C under shaking condition (120rpm) for 24 hrs, growth was estimated by measuring the optical density at 600nm.

2.3. Antagonistic activity of the selected strain

2.3.1. Antifungal activity

The fungal strains used in this study were *Macrophominia phaseolina, Fusarium oxysporium, Sclerotinum rolfissii,* and *Trichoderma spp.* These fungal pathogens were first grown in Petri plates containing Potato dextrose agar medium (PDA) and incubated at 28°C for 5 days. 1 cm^2 fungal plug from the previously active fungal cultures was inoculated in the centre of a plate with PDA and a loopful culture (24hrs old) of bacterial strain was inoculated at a distance of 2.5 cm from the pathogen. The plates were then incubated at 28°C for 72h & check antifungal activity after every 24hrs. Uninoculated plates were used as control and colony growth inhibition (%) was calculated using below formula:

$$I=\{(C-T)/C\}x\ 100$$

where I is the % of inhibition, C is the colony growth of pathogen in control and T is the colony growth of pathogen in test culture.

2.3.2. Antibacterial activity

Agar diffusion method was used to evaluate antibacterial activity of selected bacterial strains. Bacterial strains to be

tested for antibacterial activity were grow in Nutrient Broth (NB) medium and incubated at 30°C for 24 hrs. Nutrient Agar (NA) medium petriplates were prepared to check antimicrobial activity of selected isolates. 100µl of cell suspension of target strains i.e *Salmonella typhi, Staphylococcus aureus, Bacillus subtilis* and *Micrococcus luteus*, cultured for 24 hrs were spread on the plates. Then well of 5mm diameter was formed in the NA plates with a sterile cork borer and these well were filled with cell free supernatant of bacterial isolates. These plates were incubated for 24 hrs at 30°C.

3. RESULTS AND DISCUSSION

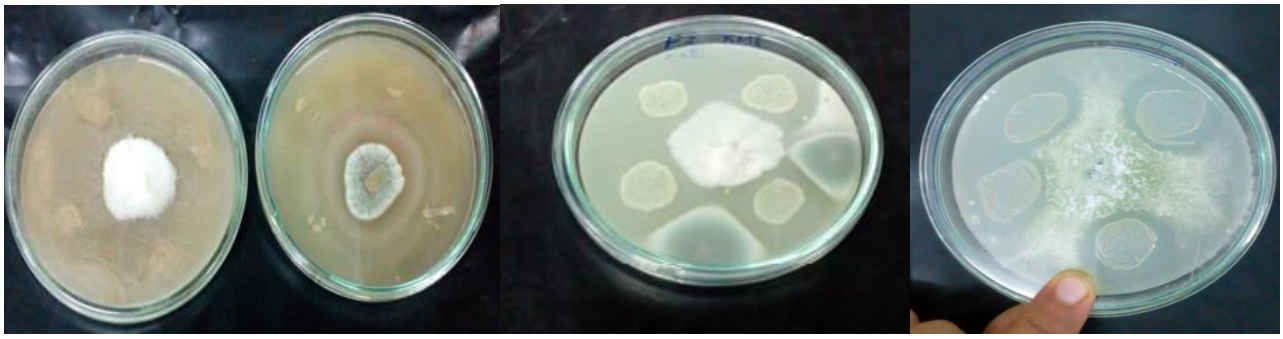
3.1. Isolation of bacteria

A total of 80 bacterial strains were isolated from the Rhizospheric soil sample collected from different regions of Gujarat. Out of the 80 strains, fast growing and morphologically different 30 colonies were selected for further abiotic stress study.

3.2. Stress tolerance of the selected bacterial isolates

Out of 30 isolates, three (MN5, MN7, KM 1) could tolerate NaCl concentration up to 20% while four isolates (MN40, KM6, AK17 & AK4) could grow in up to 18% NaCl concentration. Damodaran et al. [17] found two Bacillus spp. showing NaCl tolerance and having PGPR traits. Johri et al. [18] isolated the phosphate solubilizing bacteria that were salinity tolerant and survived at 5% NaCl concentration. Kannika and Maneewan et al [19] found that when tomato plants were inoculated with their isolated bacterial strains under different NaCl concentration there was a significant increase in plant morphological parameters especially at 30-90 mM NaCl concentration. Tank and Saraf [20] reported that strains Pseudomonas fluorescens and P. aeruginosa survived at 6% NaCl concentration and have positive effect on tomato plant growth. Results shows that isolates (MN40, AK17, KM1) can tolerate temperature ranging from 20°C to 55°C while four isolates (MN23, MN27, MN38, KM6) could tolerate temperature up to 50°C. Similarly Manasa et al. [21] found that their two bacterial strains Rhizobium and Pseudomonas fluorescenes were able to tolerate temperature up to 45°C and showing other activities like phosphate solubilization, nitrogen fixation and IAA production. This study shows that four isolates (AK17, KM1, KM6, KM11) were able to tolerate osmotic pressure ranging from -0.05 to -0.73Mpa. Sandhya et al. [22] found that their three Bacillus spp. have ability to tolerate different matrix potential and the production of EPS increased with increasing water potential. Six isolates (MN5, MN7, MN23, AK17, KM1, KM17) showed growth at pH range from to 4-12. The ability of isolates to tolerate these different abiotic stresses is summarized in Table 1.

Four isolates showing high potential to tolerate different abiotic stresses (MN40, AK17, KM1, KM6) were further tested for their Antifungal and Antibacterial activity. Antifungal activity result reveals that isolate MN40 has antifungal activity against *Sclerotium rolfsii* only. The isolate KM6 has antifungal activity against all the four pathogenic fungi i.e *Fusarium oxysporium, Macrophomina phaseolina, Sclerotium rolfsii and Trichoderma spp.* and highest antifungal activity against *Fusarium oxysporium* (Fig. 1). Similarly antibacterial activity result shows that isolate MN40 can resist the growth of only 1 pathogenic bacterial strain i.e *Staphylococcus aureus* while isolate KM6 hinders the growth of all four pathogenic bacterial strains i.e *Bacillus subtilis, Micrococcus luteus, Staphlococcus aureus, Salmonellla typhi* (Fig. 2).



(a) (b) (c) (d)

Fig. 1: Showing Antagonistic Activity of KM6 bacterial strain against (a) *F.oxysporium* (b) *M.phaseolina* (c) *S. rolfissii* (d) *Trichoderma spp.*

KM6 shows highest antibacterial activity against *S.aureus* strain. Kumar et al. [23] reported that out of their 40 isolates, 18 isolates which were identified as *P. fluorescens* were showing strong antifungal activity against *Fusarium oxysporium* and *Rhizoctonia bataticola* by producing antifungal metabolites. Sadfi et al. [24] found that fungus *F.roseum* was unable to grow in the presence of *B. cereus X16*. Yoshida et al. [25] reported the inhibitory effect of *B. amyloliquifaciens* against anthracnose disease of mulberry leaves. Arora et al. [26] isolated to strains of *Rhizobium meliloti* which produced siderophore and have antagonistic activity against *Macrophomina phaseolina*. Antagonistic activities of bacterial isolates were summarized in Table 2 and 3.

Table 1: *In Vitro* stress tolerance ability of the isolates

Isolates	NaCl Concentration (%)	Temperature (°C)	Drought (Mpa)	pH range
MN2	1-12	20-40	0.05-0.30	4-7
MN5	1-20	20-30	0.05-0.49	4-12
MN7	1-20	20-30	-	4-12
MN11	1-10	30	-	4-8
MN13	1-7	20-40	0.05-0.30	4-7
MN17	1-12	20	0.05-0.15	4-8
MN18	1-4	20	-	7
MN21	1-12	20-40	0.05-0.49	7
MN23	1-4	20-50	0.05-0.49	4-12
MN27	1-8	20-50	0.05-0.15	4-7
MN36	1-16	30-40	-	7-8
MN38	1-14	20-50	-	4-8
MN40	1-18	20-55	0.05-0.49	4-10
AK1	1-4	20-30	-	4-8
AK3	1-14	20	-	7-8
AK4	1-18	20	0.05-0.30	7
AK5	1-4	20-30	0.05-0.30	4-7
AK9	1-8	40	0.05-0.49	4-8
AK12	1-16	30-40	-	7-8
AK17	1-18	20-55	0.05-0.73	4-12
KM1	1-20	20-55	0.05-0.73	4-12
KM6	1-18	20-50	0.05-0.73	6
KM7	1-6	20-40	0.05-0.49	4-8
KM9	1-14	20	-	7
KM11	1-12	20-30	0.05-0.73	7
KM15	1-3	30-40	0.05-0.30	4-8
KM16	1-4	20-40	0.05-0.49	4-12
KM17	1-3	20-30	0.05-0.15	4-7
KM19	1-10	30	-	7
KM20	1-7	30-40	0.05-0.30	4-8

Table 2: Antagonistic activity of bacterial isolates against fungal pathogens

Isolates	Growth Inhibition (%)			
	A	B	C	D
MN40	0.0	0.0	25.6	0.0
KM1	35	33.04	46.9	0.0
KM6	82.6	80.9	66.2	40.3
AK17	0.0	52.2	37.4	0.0

A=*F.oxysporium*, B=*M.phaseolina.*, C=*S. rolfissii*, D= *Trichoderma spp*

Table 3: Antimicrobial activity of bacterial isolates against test organism

Isolates	Zone of Inhibition (mm)			
	A	B	C	D
MN40	0	0	10	5
KM1	8	8	16	0
KM6	18	10	21	11
AK17	10	6	15	7

A=*Bacillus subtilis*, B=*M.luteus*, C=*S.aureus*, D=*S.typhi*

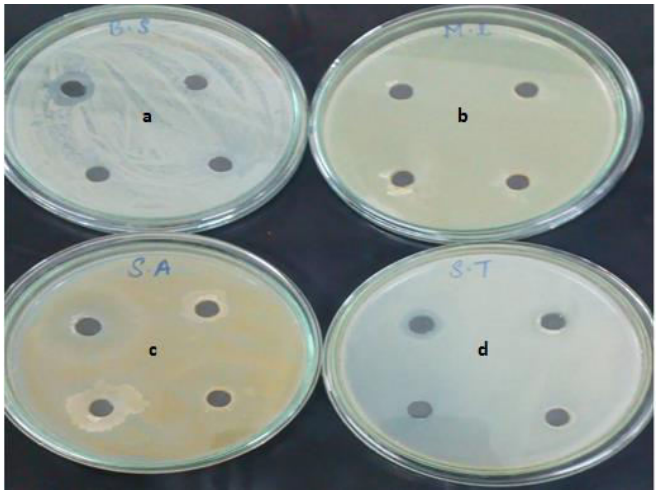


Fig. 2: Showing antibacterial activity of selected strains against (a) *Bacillus subtilis* (b) *M.luteus* (c) *S.aureus* (d) *S.typhi*

4. CONCLUSION

By this study we were able to isolate bacteria which exhibit strong tolerance to different types of abiotic and biotic stresses. These isolates possess good antifungal and antibacterial properties. These isolates can be used as bio inoculants for those areas which suffer from abiotic stresses. These microbial inoculants can help in fight against plant diseases caused by pathogenic bacteria and fungi. The isolate KM6 shows good antagonistic activity against pathogenic bacteria and fungi which causes great loss to crops.

5. ACKNOWLEGMENTS

Authors are thankful to Department of Microbiology and Biotechnology, Gujarat University for providing required facilities to carry out this work. This research was financially supported by Department of Science and Technology (DST) New Delhi under Women Scientist (WOS-A) scheme.

Conflicts of Interest

We declare that there are no conflicts of interest.

6. REFERENCES

1. Tanji KK. *Agricultural salinity assessment and management*, 1990; pp.71-92.
2. Jamil A, Riaz S, Ashraf M, Foolad MR. *Critical Reviews in Plant Sciences*, 2011; **30**:435-458.
3. Jarvis A, Lane A, Hijmans RJ. *Agriculture, Ecosystems & Environment*, 2008; **126**:13-23.
4. Coakley SM, Scherm H, Chakraborty S. *Annual Review of Phytopathology*, 1999; **37**:399-426.
5. Seherm H, Coakley SM. *Australasian Plant Pathology*, 2003; **32**:157-165.
6. Duveiller E, Singh RP, Nicol JM. *Euphytica,* 2007; **157**:417-430.
7. Vardharajula S, Ali SZ, Grover M, Kishore N, Venkateswarlu B. *Journal of Oilseed Research*, 2009; **26**:600-601.
8. Vardharajula S, Ali SZ, Grover M, Reddy G, Venkateswarlu B. *Biology and Fertility of Soil*, 2009; **46**:17-26.
9. Saxena AK, Lata Shende R, Pandey AK. *Basic research applications of mycorrhizae. I K International Pvt Ltd, New Delhi,* 2005; pp 453-474.
10. Singh RP, Jha PN. *Frontier in Microbiology*, 2017; **8**:1945.
11. Yang J, Kloepper JW, Ryu CM. *Trends in Plant Science*, 2009; **14**:1-4
12. Nguyen D, Rieu I, Mariani C, Van Dam NM. *Plant Molecular Biology*, 2016; **91**:727-740.
13. Stajner D, Kevresan S, Gasic O, Mimica-Dukic N, Zongli H. *Biologia Plantarum*, 1997; **39(3)**:441-445.
14. Glick BR. *Canadian Journal of Microbiology,* 1995; **41**:109-117.
15. Glick BR, Cheng Z, Czarny J, Duan J. *New perspectives and approaches in plant growth-promoting Rhizobacteria research*, 2007; pp 329-339.
16. Patten CL, Glick BR. *Applied and Environmental Microbiology,* 2002; **68**:3795-3801
17. Damodaran T, Sah V, Rai RB, Sharma DK, Mishra V, Jha SK, Kannan R. *African Journal of Microbiology Research*, 2013; **7**: 5082-5089.
18. Johri JK, Surange S, Nautiyal CS. *Current Microbiology*, 1999; **39(2)**:89-93.
19. Chookietwattana K, Maneewan K. *Soil & Environment*, 2012; **31**:30-36.
20. Tank N, Saraf M. *Journal of Plant Interactions*, 2010; **5(1)**:51-58.
21. Manasa K, Subhash R, Triveni S. *Journal of Pharmacognosy and Phytochemistry,* 2017; **6(5)**:764-767.
22. Grover M, Ali SZ, Sandhya V, Rasul A, Venkateswarlu B. *World Journal of Microbiology and Biotechnology,* 2011; **27**:1231-1240.
23. Kumar NR, Arasu VT, Gunasekaran P. *Current Science*, 2002; **82**:1463-466.
24. Sadfi N, Chérif M, Fliss I, Boudabbous A, Antoun H. *Journal of Plant Pathology*, 2001; **83**:101-118.
25. Yoshida S, Hiradate S, Tsukamoto T, Hatakeda K, Shirata A. *Phytopathology*, 2001; **91**:181-187.
26. Arora NK, Kang SC, Maheshweri DK. *Current Science,* 2001; **81**:673-677