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# Suppression of common blight disease (*Xanthomonas axonopodis* pv. *phaseoli*) by a bacterial endophyte in soybean (*Glycine max*)*

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Soybean (*Xanthomonas axonopodis* pv. *phaseoli*) which causes bacterial common blight disease is an important pathogen in French bean and naturally infects the other legumes including soybean. This pathogen is widely distributed and causes substantial yield losses in beans (Vauterin *et al*. 1995). The increasing resistance to this pathogen and detrimental effect of chemicals on plants, soil and health force us to search an alternative for better disease management. *P. polymyxa* is a common soil bacterium found to be associated with plants with abilities to fix nitrogen, solubilize phosphorus, and produce antibiotics, auxin, chitinase and hydrolytic enzymes. The colonization of plant roots by *P. polymyxa* indicated that the bacteria colonized predominantly in root tip and form biofilms (Timmusk *et al*. 2005). Earlier work in our lab showed that soybean bacterial endophyte, *P. polymyxa* strain HKA 15 grown in nutrient broth at 48 h of incubation showed strong antibacterial activity against *X. axonopodis* pv. *phaseoli* strains M 5 and CP 1-1 (Mageshwaran *et al*. 2011). However, few attempts have been made on the effect of endophyte on suppression of pathogen *X. axonopodis* pv. *phaseoli* in soybean. With this background, the present investigation was aimed to study the effect of *P. polymyxa* HKA-15 on suppression of bacterial common blight disease in soybean.

The experiments were conducted during 2008–09. Soybean bacterial endophyte *P. polymyxa* HKA 15 was obtained from Division of Microbiology, IARI, New Delhi. Bacterial pathogen *X. axonopodis* pv. *phaseoli* strains M 5 and CP 1-1 were obtained from Division of Plant Pathology, IARI, New Delhi. The bacterial strains were grown in nutrient broth at 30°C and were maintained on Nutrient Agar (NA) slants at 4°C. The effect of *P. polymyxa* HKA-15 on soybean varieties was assessed under *in vitro* conditions. Fifty seeds of each soybean variety, viz NRC 12, and PK 262 were treated with log phase culture ($10^6$cfu/ml) of *P. polymyxa* HKA-15 for 10 min. Similarly uninoculated seeds immersed in sterile water for 10 min. served as control. The growth characteristics such as germination percentage (no. of seeds germinated/total no. of seeds) × 100, shoot length (mm) and root length (mm) were calculated. Vigour index was determined by multiplying seedling length and its germination percentage. Biomass in dry weight was determined by keeping the seedlings in hot air oven at 100°C for 24 hr.

The extraction of crude metabolite from *P. polymyxa* HKA 15 and test for its antibacterial activity against *X. axonopodis* pv. *phaseoli* strains M 5 and CP 1-1 was done as described earlier (Mageshwaran *et al*. 2011). *In vivo* biocontrol activity of *P. polymyxa* HKA 15 against bacterial common blight disease in soybean was carried out under poly house conditions. Surface sterilized seeds of soybean NRC 12 were submerged in double the volume of log phase inoculum of HKA 15 for 10 min. Uninoculated control seeds were submerged in sterile water. After incubation, excess inoculum was drained out and seeds were immediately sown. Pots were kept under controlled conditions where the temperature (30°C) and RH (80%) were maintained. Plants were irrigated with Jensen's Liquid Nutrient solution (N+) on alternate days up to 30 days. There were 10 treatments with five replications including a chemical control (Streptomycin sulphate@100 ppm). Germination count was taken at 10 days after sowing. Inoculation of pathogenic cultures was don7e by pin prick method at 30 days after sowing in which 2.5µl of bacterial pathogens was inoculated in damaged leaf surface. In each replication, 20 inoculations were made in 5 leaves (four inoculations/leaf). Hence 25 leaves were observed for the occurrence of disease symptoms in each treatment. To study the efficacy of biocontrol potential of crude extract (100 ppm) and HKA-15 cells ($10^6$ cfu/ml), 2.5 µl of these

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were inoculated in the area of pathogen treated surface. A total of 30 plants per treatment were screened for the appearance of disease symptoms in each treatment. The observations were recorded at 2 and 4 days after inoculation (DAI) and a numerical disease rating was assigned as follows. 0- Healthy leaf, 1- yellowish brown lesion with narrow margin, 2-dark brown lesion with broad margin, 3-dark brown lesion with half of the leaf surface, 4- damage to the whole surface. Mean disease rate (MDR) and per cent disease incidence (PDI) were calculated as per the following formulae

-Mean disease rate (MDR) = (a × 0) + (b × 1) + (c × 2) + (d × 3) + (e × 4)/(a + b + c + d + e) where a,b,c,d and e are the number of plants with the disease rating of 0,1,2,3 and 4 respectively. Per cent disease incidence (PDI) = (MDR × 100)/Max. grade. Data from the completely randomized design experiment on *in vivo* biocontrol activity were arc sine transformed prior to statistical analysis. Results were analyzed using one –way ANOVA (AGRES ANOVA package version 7.01; TNAU, India).

The growth parameters such as germination percentage (%), root length (mm) shoot length (mm), vigour index and biomass (g) were found higher in seeds inoculated with HKA 15 cells compared to uninoculated seeds of both soybean varieties NRC 12 and PK 262 (Table 1). Higher vigour index of 6640 and 6232 was observed in soybean varieties NRC 12 and PK 416 respectively when inoculated with HKA 15 cells compared to uninoculated control. Similar results were obtained when soybean seeds are treated with *Bacillus subtilis* cells and its metabolites showed higher seed germination percentage and growth parameters compared to control (Araujo *et al*. 2005).

The crude metabolite extracted from 48h old culture of *P. polymyxa* HKA 15 was tested for antibacterial activity against *X. axonopodis* pv. *phaseoli*. In agar diffusion assay, a clear zone of inhibition was observed around the well containing crude metabolite in NA plates spread with *X. axonopodis* pv. *phaseoli* strains CP 1-1 and M 5 (data not shown). However, there was no inhibition zone was observed around well containing methanol. The antagonism of metabolite produced by *Bacillus* against *Xanthomonas campestris* pv. *campestris* was reported (Monteiro *et al*. 2005). The results obtained in this experiment showed antagonism of *P. polymyxa* HKA 15 against *X. axonopodis* pv. *phaseoli* under *in vitro* conditions. In green house study, higher seed germination was recorded in treatments ($T_7$, $T_3$, $T_6$ and $T_4$) where seeds were treated with log phase culture ($10^6$cfu/ml) of *P. polymyxa* HKA 15 (Table 2). Two days after pathogen inoculation, the mean disease rate and percent disease incidence were maximum in negative control. MDR and PDI were recorded minimum in $T_7$, $T_8$, $T_3$ and $T_4$ along with positive chemical control. After four days of pathogen inoculation, maximum MDR (3.64, 2.96) and PDI (72.8, 60.8) were recorded in $T_1$ and $T_2$ respectively in which typical symptoms were noticed, i e brown lesion on entire leaves (Fig 1). Less MDR (1.24, 1.1) and PDI (24.8, 22) were recorded in $T_7$ and $T_8$, respectively where seeds and leaf were treated with HKA 15 cells. The screening and selection of the antagonistic bacteria for the control of *X. axonopodis* pv. *glycines* showed that strains of *Bacillus subtilis*, resistant to rifampicin at 20µg/ml have higher degree of antibiosis against the pathogen (Salerno and Sagardoy 2003). May *et al.* 1997 reported that under *in vivo* conditions, when mixtures of epiphytic bacterial isolate and pathogen inoculated at ratios >1 was able to prevent the formation of leaf spots in soybean. In our study, soybean seeds treated with HKA 15 cells followed by foliar treatment with cells showed minimum MDR and PDI compared to control at 4 days after inoculation of pathogen. However, application of crude extract on the pathogen inoculated leaves showed high MDR and PDI compared to treatment in which leaves treated with HKA 15 cells. The result may be due to lesser survivability of metabolite under *in vivo* conditions. The results from this study clearly indicated that the inoculation of *P. polymyxa* HKA 15 significantly reduced the incidence

Table 1 Plant growth promoting ability of *P. polymyxa* HKA 15 on soybean varieties NRC 12 and PK 262 in *in vitro*

| Variety | | Growth parameter | | | | |
|---|---|---|---|---|---|---|
| | | Seed germination (%) | Shoot length (mm) | Root length (mm) | Vigour index | Biomass (g) |
| NRC 12 | Inoculated | 83 | 61 | 29 | 6640 | 2.6 |
| | Un-inoculated | 54 | 45 | 23 | 3672 | 1.9 |
| PK 262 | Inoculated | 76 | 57 | 25 | 6232 | 2.1 |
| | Un-inoculated | 59 | 44 | 20 | 3776 | 1.4 |
| SED | | 1.63 | 1.29 | 0.66 | 8.16 | 0.13 |
| CD (*P* = 0.05%) | | 3.76 | 2.97 | 1.53 | 18.82 | 0.30 |


Fig. 1 Bacterial inoculation studies on leaf of soybean to evaluate control effect of HKA 15 against common blight

Table 2 *In vivo* biocontrol activity of *Paenibacillus polymyxa* HKA 15 against bacterial blight pathogen of soybean (NRC 12)

| Treatment | | 2 DAI | | 4 DAI | |
|---|---|---|---|---|---|
| | Seed germination (%) | MDR | PDI | MDR | PDI |
| T1 (*X. campestris* pv. CP 1-1 ) | 71.5(57.73)$^{f}$ | 1.44$^{h}$ | 28.8(6.89)$^{e}$ | 3.64$^{i}$ | 72.8(58.56)$^{i}$ |
| T2 (*X. campestris* pv. M 5) | 74.0(59.34)$^{e,f}$ | 1.32$^{g}$ | 26.4(6.59)$^{d}$ | 2.96$^{g}$ | 60.8(51.23)$^{h}$ |
| T3 (T1+ST@$10^{6}$/ml ) | 87.0(68.86)$^{a,b}$ | 1.16$^{d}$ | 23.2(6.18)$^{c}$ | 3.04$^{h}$ | 59.2(50.30)$^{g}$ |
| T4 (T2+ST@$10^{6}$/ml) | 85.5(67.61)$^{b,c}$ | 1.0$^{c}$ | 20.0(5.73)$^{b}$ | 2.28$^{f}$ | 45.6(42.47)$^{f}$ |
| T5 (T3+ CE@100ppm) | 79.0(62.72)$^{d,e}$ | 1.24$^{f}$ | 24.8(6.39)$^{c}$ | 1.92$^{d}$ | 38.4(38.29)$^{d}$ |
| T6(T4+ CE@100ppm) | 86.0(68.02)$^{a,b,c}$ | 1.20$^{e}$ | 24.0(6.28)$^{c}$ | 2.08$^{e}$ | 41.6(40.16)$^{e}$ |
| T7(T3+ CL @$10^{6}$/ml) | 90.0(71.56)$^{a}$ | 0.80$^{a}$ | 16.0(5.13)$^{a}$ | 1.24$^{b}$ | 24.8(29.86)$^{b}$ |
| T8(T4+ CL @$10^{6}$/ml) | 82.0(64.89)$^{c,d}$ | 0.88$^{b}$ | 17.6(5.38)$^{a}$ | 1.1$^{a}$ | 22.0(27.97)$^{a}$ |
| T9(T1+Streptomycin sulphate@100 ppm) | 69.0(56.16)$^{f}$ | 1.17$^{c}$ | 23.4(5.73)$^{c}$ | 1.24$^{b}$ | 24.8(29.86)$^{b}$ |
| T10(T2+Streptomycin sulphate @100 ppm) | 72.0(58.05)$^{f}$ | 1.0$^{d}$ | 20.0(6.20)$^{b}$ | 1.32$^{c}$ | 26.4(30.91)$^{c}$ |
| SED | 1.70 | 0.02 | 0.81 | 0.03 | 0.25 |
| CD ($P$ = 0.05) | 3.56 | 0.04 | 1.69 | 0.07 | 0.53 |

ST, Seed treatment; CL, culture; CE, crude extract; MDR, mean disease ratio; PDI, percent disease incidence; DAI, days after inoculation. Figures in parentheses are Arc sine transformed values. Treatment values followed by same alphabet do not differ significantly at $P$=0.05. Values are the means of two different experiments

of *X. axonopodis* pv. *phaseoli* which suggests the potential of *P. polymyxa* HKA 15 as a biocontrol agent for the control of bacterial common blight disease in soybean.

## SUMMARY

Soybean seeds inoculated with *Paenibacillus polymyxa* HKA 15, a bacterial endophyte of soybean showed higher germination percentage, root length, shoot length, vigour index and biomass compared to uninoculated seeds. The crude metabolite from *P. polymyxa* HKA 15 showed a clear zone of inhibition against strains (M 5 and CP 1-1) of bacterial common blight pathogen *X. axonopodis* pv. *phaseoli* in agar diffusion assay. Under poly house conditions, the effect of *P. polymyxa* HKA 15 on seed germination percentage and biocontrol activity against bacterial common blight disease was assessed. Soybean seeds treated with *P. polymyxa* HKA 15 showed significant increase in seed germination percentage compared to untreated seeds. Pin-prick inoculation of strains of *X. axonopodis* pv. *phaseoli*, strains, viz CP 1-1 and M 5 on soybean leaves treated with endophyte resulted in lowest mean disease ratio (MDR) of 0.80 and 0.88 at two DAI and 1.24 and 1.1 at four DAI and per cent disease incidence (PDI) of 16 and 17.6 at two DAI and 24.8 and 22 at four DAI respectively.

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