ISSN: 1394-7990
Malaysian Journal of Soil Science Vol. 14: 95-99 (2010) Malaysian Society of Soil Science

Green Bean (*Vigna radiata*) Seedling Growth Inhibition by *Chromobacterium violaceum* under In-vitro Condition

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ABSTRACT

Chromobacterium violaceum is a pathogenic soil bacterium producing violacein and hydrogen cyanide both of which is controlled by quorum sensing with the same signal molecule homoserine lactone (C6-HSL). A study was carried out to determine if quorum sensing was a factor that was required for inhibiting the growth of green bean (*Vigna radiata*) seedling. The results showed that *C. violaceum* which reached quorum level inhibited the growth of green bean seedling as much as 86.5% for the shoot length and 92.1% for the root length. However inhibition was reduced to 37.5% for the shoot and 17.5% for root if the quorum level of *C. violaceum* was not reached under an aseptic environment. Furthermore sterilised inoculant (killed) which had not reached quorum level would not affect the growth of green bean seedlings. These results indicate that quorum sensing in *C. violaceum* is a factor that determines its inhibitory effect on seedling growth.

Keywords: *Chromobacterium violaceum*, quorum sensing, seedling growth, *Vigna radiata* inhibition

INTRODUCTION

Chromobacterium violaceum is a Gram-negative facultative anaerobic bacterium pathogenic to mammals and human. It inhabits soil and water and is widely found in the tropical and subtropical regions of the world (McGowan and Steinberg 1995). It produces an antibiotic - violacein, a purple pigment that gives *C. violaceum* its characteristic violet colour (Forbes 2002). It also produces hydrogen cyanide (HCN) (Sneath 1956) and both substances are controlled by quorum sensing. Quorum sensing is a cell-to-cell communication system used by bacteria to control gene expression by signal molecules (Miller and Bassler 2001). The signal use by *C. violaceum* to control the violacein and HCN is homoserine lactone (C6-HSL) (Srivastava and Gera 2006). Hydrogen cyanide was reported to have a negative effect on root metabolism and inhibit plant growth (Lambers 1980; Schippers *et al.* 1990). Other cyanogenic bacteria such as *Pseudomonas fluorescens* are also reported to inhibit the growth of beans by cyanide production (Alstrom *et al.* 1989).

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The tropical climate in Malaysia offers a very conductive environment for the growth of *C. violaceum* and it is believed to be widely distributed locally in agriculture and non-agriculture soils. It was reported that Malaysia has the highest human infection of *C. violaceum* in Southeast Asia (Anupop 2008). No study has been reported on the effect of *C. violaceum* on crop growth in Malaysia although cyanide production by rhizobacteria is considered a possible mechanism of plant growth inhibition. This study was carried out to determine if quorum sensing in indigenous isolates of *C. violaceum* is a determining factor in plant growth inhibition.

MATERIALS AND METHODS

Seeds of green bean were surface sterilized by shaking in 1% sodium hypochlorite for 4 minutes followed by 70% ethanol for 2 minutes and rinsed in sterilized distilled water for one minute. The seeds were pre-germinated for 24 hours on wet cotton in a Petri dish prior to its transfer into a 50 mL test-tube. The tubes were filled with sterilized vermiculite to the 15 mL level of the tube. A single colony of *C. violaceum* from the stock culture was transferred into LB broth (Luria and Burrous 1955) media tubes A and B and grown to log phase by 16 hours. Tube A was *C. violaceum* inoculant which did not reach quorum level whereas Tube B was incubated for three days at room temperature until quorum level was reached. Quorum level is the condition where the signal molecule C6-HSL reaches a certain level to activate the genes expression for production of violacein and cyanide (Srivastava and Gera 2006). The quorum level was reached as indicated by the presence of violacein that turns the media into purple colour (*Fig.1)*. In Tube A, no purple colour was observed indicating that quorum level was not reached. A 0.5 ml inoculant from Tube A and Tube B was used to inoculate the seedlings. As a non-inoculated control, the inoculant was replaced with distilled water and the same volume of *Azospirillum brasilense* Sp7 inoculant was used as a comparison. Five tubes were used for each treatment as replicates. The average shoot and root length was measured after seven days of growth and percentage increase or decrease in growth was compared against treatment using distilled water only.

RESULTS AND DISCUSSION

Results in Table 1 show the average shoot and root length obtained after seven days of growth and the percent increase (+) or decrease (-) in terms of shoot and root length. The results clearly show that *C. violaceum* is inhibitory to the growth of green bean seedling. The greatest reduction in shoot and root length (86.5% and 92.1%, respectively) was obtained from seedlings treated with *C. violaceum* that had reached quorum level indicating that cyanide is produced and inhibits the growth of shoot and root. Even if *C. violaceum* that has reached quorum was killed by sterilisation, the cyanide that has been produced during its growth is still active and inhibits the green bean seedlings and reduces shoot growth by 87.5% for shoot and 92.1% for root. Treatment with *C. violaceum* that did not reach quorum level still inhibits the growth of both shoot and root.

The reduction was recorded at 37.5% for shoot and 17.5% for root. However for sterilized non-quorum *C. violaceum*, the effect was negligible compared to the control. This was because the dead bacteria were not able to reach quorum level to produce cyanide while no other inhibitory compounds were produced during the treatment. Seedlings treated with *A. brasilense* Sp7, a plant-growth promoting rhizobacteria, showed promotion of growth by increasing shoot length by 13.5% and root length by 20.6%.

TABLE 1
Effect of *C.violaceum* on root and shoot length of green bean (*V. radiata*) after seven days of growth

Treatments	Average shoot length (cm)	Reduction / increase (%)	Average root length (cm)	Reduction/ increase (%)
Sterilised distilled water (Control)	10.4 cm	0 %	6.3 cm	0 %
A. brasilense Sp7	11.8 cm	+13.5 %	7.6 cm	+20.6 %
C. violaceum non- quorum level (Tube A)	6.5 cm	-37.5 %	5.2 cm	-17.5 %
C. violaceum quorum level (Tube B)	1.4 cm	-86.5 %	0.5 cm	-92.1 %
C. violaceum Non quorum level (Tube A) - Sterilised	10.8 cm	+3.8 %	6.2 cm	-1.6 %
C. violaceum quorum level (Tube B) - Sterilised	1.3 cm	-87.5 %	0.5 cm	-92.1 %

The results showed clearly the inhibitory effect of *C. violaceum* on green bean seedlings. The effects were most pronounced when the plants were inoculated with *C. violaceum* that has reached quorum level whereas the effect is less pronounced if the inoculants has not reached quorum level. One of the characteristic of *C. violaceum* is production of an antibiotic, violacein and also cyanide when it reaches quorum level (Forbes 2002). Cyanide has been implicated as a possible agent of plant growth inhibition (Alstrom and Burns 1989) and has been shown to have a negative effect on the growth of velvet leaf (*Abutilon theoprasii*) and corn (*Zea mays*) (Adam and Zdor 2001). In 2001, Kremer and Souissi confirmed that HCN produced by cyanogenic rhizobacteria is the major inhibitory compound on grass. These results confirm the possible inhibitory effect of *C. violaceum* similar on other HCN-producing pseudomonads as reported by Schippers *et al.* (1990). The results also indicate that inhibitory effect is negligible if the quorum level has not been reached supporting the idea that quorum level is needed for the production of cyanide which will inhibit the growth of green bean. As long as quorum level is not breached, cyanide will not be produced and plant growth will not be inhibited.

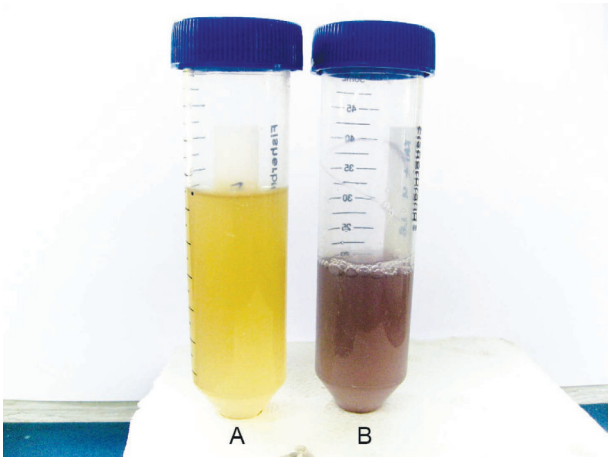

Fig. 1: C.violaceum culture reaching quorum level (B) and non quorum level (A). Culture that reached quorum level can be differentiated based on the purple colour of the media

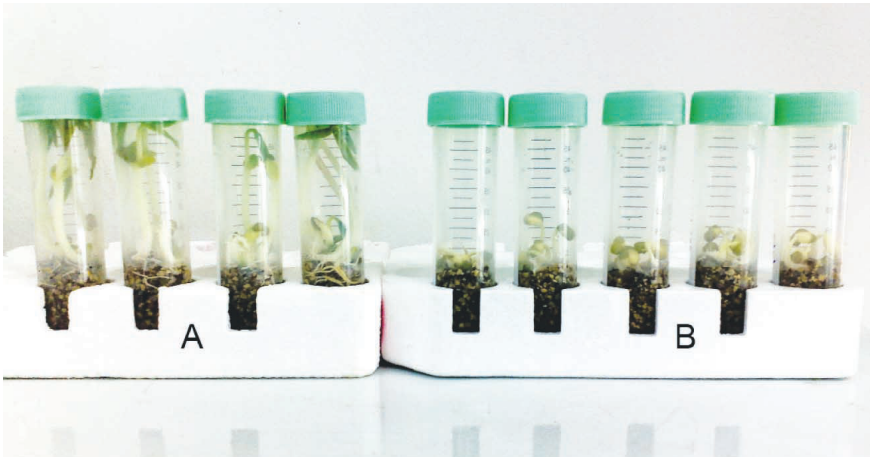

Fig. 2: Effect of non-quorum level C.violaceum (A) and quorum level C.violaceum (B) inoculations on seedling growth of green bean (V. radiata)

Although the results demonstrate the inhibitory effect of *C. violaceum* when quorum level is reached, it is believed that such a quorum level will be difficult to reach under a natural environment. Thus it is expected that the effect of *C. violaceum* in agriculture soils would be minimal. However, a detailed study needs to be carried out to confirm this hypothesis.

CONCLUSION

The quorum level of *C. violaceum* is the determining factor that inhibits shoot and root growth under in-vitro conditions.

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