ISSN: 1394-7990
Malaysian Journal of Soil Science Vol. 25 : 59-65 (2021) Malaysian Society of Soil Science

Effect of *Chromobacterium violaceum* on Plant Growth-Promoting Rhizobacteria (PGPR) under *In-vitro* Conditions

Loke W.K. and Saud H.M.

Department of Agriculture Technology,
Faculty of Agriculture Universiti Putra Malaysia 43400 UPM Serdang,
Selangor Darul Ehsan, Malaysia

ABSTRACT

Chromobacterium violaceum is a pathogenic soil bacterium that produces violacein and several types of antibiotics which are active against amoebae, trypanosomes, and Gram-positive and Gram-negative bacteria. The production of antibiotics is controlled by a quorum sensing system with a signal molecule called homoserine lactone (C6-HSL). In both methods (interaction and non-interaction), *C. violaceum* which reached quorum level produced antibiotics and killed all the selected PGPR (*Azospirillum brasilense* Sp7, *Rhizobium* UPMR1102 and *Bacillus sphaericus* UPMB10) but did not kill the selected PGPR in concentration below their quorum level. This study indicates that quorum sensing is involved in the effect of *C. violaceum* on selected PGPR and has the potential to threaten the use of PGPR in agriculture.

Keyword: *Chromobacterium violaceum*, Plant Growth-Promoting Rhizobacteria (PGPR), quorum sensing.

INTRODUCTION

Chromobacterium violaceum is a Gram-negative facultative anaerobic pathogenic bacterium to mammals (David *et al*. 2012; Hammerschmitt *et al*. 2017; Donny *et al.* 2018) and humankind (Shao *et al*. 2002; Kothari *et al*. 2017). 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, and which is active against most microbes including protozoa, fungi, bacteria and virus (Forbes *et al*. 2002; Duran *et al*. 2007; Duran *et al.* 2016). Other antibiotics produced from *C. violaceum* are aerocyanidine which is active against Gram-positive organisms, aerocavin which is active against Gram-positive and Gram-negative organisms, 3,6-dihydroxyindoxazene and Factor Y-T0678H (6-hydroxy-3-oxo-1,2-benzisoxazolin), both of which are active against Gram-negative bacteria and also several types of antibiotics which are active against amoebae and trypanosomes (Nelson and Carlos 2001). The production of all the antibiotics is controlled by a quorum sensing system (McClean *et al.* 1997; Lee *et al*. 2013).

*Corresponding author : E-mail: *halimi@upm.edu.my*

Quorum sensing is a cell-to-cell communication system used by bacteria to control gene expression by signal molecules where the bacteria are able to produce an antibiotic after reaching a certain level of cell-population density or quorum level (Miller and Bassler 2001; Lowery *et al.* 2008). In this system, *C. violaceum* accumulates a certain level of signal molecules to form a signal-receptor complex binding to activate transcription for antibiotics production. No production occurs if accumulation is below the quorum level (Sun *et al.* 2004; Stauff and Bassler 2011). The signal used by *C. violaceum* to control the production of antibiotics is homoserine lactone (C6-HSL) (McClean *et al*. 1997; Srivastava and Gera 2006).

The tropical climate in Malaysia offers a very conductive environment for the growth of *C. violaceum* and is believed that it is 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 (Jitmuang 2008). The widely distributed *C. violaceum* in soil is directly in contact with other beneficial microbes like plant growth-promoting rhizobacteria (PGPR).

Plant growth-promoting rhizobacteria (PGPR) were first described by Kloepper and Schroth (1987); the use of soil bacteria may be highly advantageous to plants by colonising roots and following inoculation of the seeds to enhance plant growth. In recent years, many PGPR have been isolated from soil and each isolated PGPR was found to contain one or more functions to improve plant growth (Zahir *et al.* 2004; Smith *et al*. 2015). It gives direct and indirect benefits that enhance the plant growth by improving the plant metabolites (Backer *et al.* 2018). However, the interaction and effect between *C. violaceum* and PGPR remains unknown. The aim of this work was to determine the effect of *C. violaceum* on selected PGPR.

MATERIALS AND METHODS

Two tests were conducted to investigate the effect of *C. violaceum* on selected PGPR. In test 1, there was an interaction between *C. violaceum* and PGPR during the culturing process while in test 2, no interaction was found between both bacteria until *C. violaceum* reached the final concentration. These tests are able to show whether the interaction between both bacteria has an effect on the quorum sensing mechanism and the production of antibiotics.

Test 1 - Interaction

The selected PGPR used in this experiment were *Bacillus sphaericus* UPMB10 (Gram-positive), *Rhizobium* UPMR1102 (Gram-negative) and *Azospirillum brasilense* Sp7 (Gram-negative). The inoculating loop was sterilised and used to pick up a single colony of *C. violaceum* and selected PGPR with each combination into 6 tubes (A1, A2, A3 and B1, B2, B3) containing LB broth. In tubes A1, A2 and A3, *C. violaceum* was cultured at 30°C with shaking for 16 h before it reached quorum level; in tubes B1, B2 and B3, *C. violaceum* was left to incubate for a further 3 days until it reached quorum level and the solutions had turned purple in colour. Ten-fold serial dilutions were made by transferring 1.0 ml of solution from

tubes A and B of each combination to the new tubes to achieve a final dilution of $1:10^2$ in the final tubes. One ml was transferred from the tubes to petri plates of LB agar and spread onto the surface of agar using an alcohol-flamed glass rod. The Petri plates were incubated at 30°C for 3 days.

Test 2 – Non Interaction

A single colony of *C. violaceum* was transferred into the LB broth media test tubes (C1, C2, C3 and D1, D2, D3). C1, C2 and C3 test tubes were cultured at 30°C by shaking for 16 h before the concentrations of the bacteria in both tubes reached quorum level; in test tubes D1, D2 and D3, the colony was cultured at 30°C by shaking for 3 days until quorum level was achieved and the solution turned purple in colour. Tetracycline antibiotic was added into both the test tubes to kill the *C. violaceum* and the concentration was maintained at non-quorum level in test tubes C1, C2 and C3 while it was maintained at quorum level in test tubes D1, D2 and D3.

Single colonies of *Azospirillum brasilense* Sp7, *Rhizobium* UPMR1102 and *Rhizobium* UPMR1013 containing a cosmid vector pLAFR1 (Vanbleu *et al.* 2004) that is resistant to Tetracycline antibiotic (Figure 1), and 2 ml fresh LB broth were transferred into different test tubes (C1, C2, C3 and D1, D2, D3) containing *C. violaceum* at quorum level and non-quorum level concentration and cultured at 30°C by shaking for 16 h. A ten-fold serial dilution was made by transferring 1.0 ml of solution from tubes C1, C2, C3 and D1, D2, D3 to new tubes to achieve a final dilution of $1:10^2$ in the final tubes. A sterilised inoculating loop was used to streak the 1.0 ml solutions from the test tubes on the selective media agar surface of petri plates which contained the Tetracycline antibiotic. The petri plates were incubated at 30°C for 3 days.

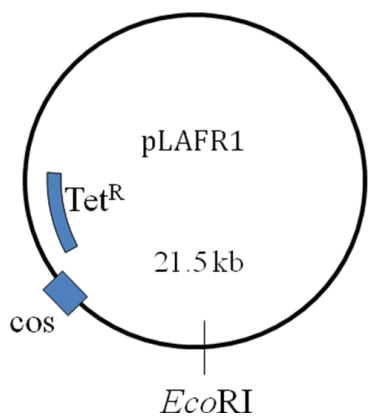

Figure 1. Cosmid pLAFR1

RESULTS AND DISCUSSION

In test 1, there was contact between *C. violaceum* and PGPR before *C. violaceum* reached quorum level. The interaction between both bacteria may affect growth rate and signal molecule production. This may have been the cause for some of the test tubes requiring more than 3 days to reach quorum level. From the results, petri plates with the content of test tubes B1, B2, B3 showed only the *C. violaceum* growing on the agar surface while the petri plates with the content of test tubes A1, A2, A3 showed the selected PGPR *Bacillus sphaericus* UPMB10, *Rhizobium* UPMR1102 and *Azospirillum brasilense* Sp7 growing together with *C. violaceum* (Figure 2). These results show that *C. violaceum* in B1, B2 and B3 will only produce antibiotics after reaching quorum level and having killed the selected PGPR in the test tubes.

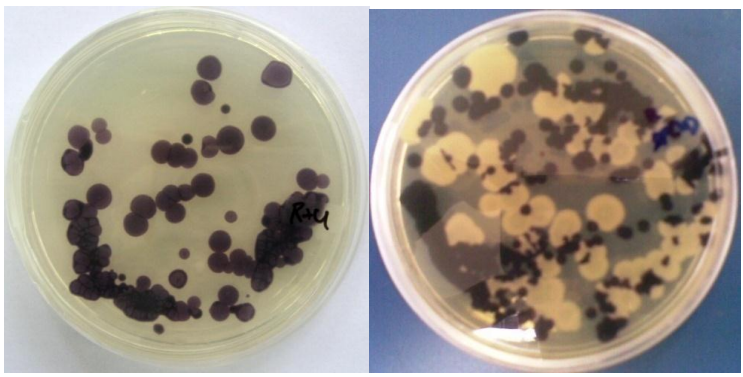

Figure 2. Effect of C. violaceum on PGPR from tube B (left) and tube A (right)

In test 2, there was no contact between *C. violaceum* with PGPR before *C. violaceum* reached quorum level and was killed by the antibiotic. The concentration of *C. violaceum* from both test tubes C and D was controlled by Tetracycline antibiotic before coming into contact with PGPR. The petri plates with the content of test tubes C1, C2, C3 showed that *Azospirillum brasilense* Sp7, *Rhizobium* UPMR1102 and *Rhizobium* UPMR1013 contained cosmid pLAFR1 that is resistant to Tetracycline antibiotic growing on the agar surface while the petri plates with the content of test tubes D1, D2, D3 showed that no bacteria can be cultured. The inhibited *C. violaceum* in quorum level produced antibiotics and kill all the selected PGPR in tubes D1, D2 and D3 though there was no interaction.

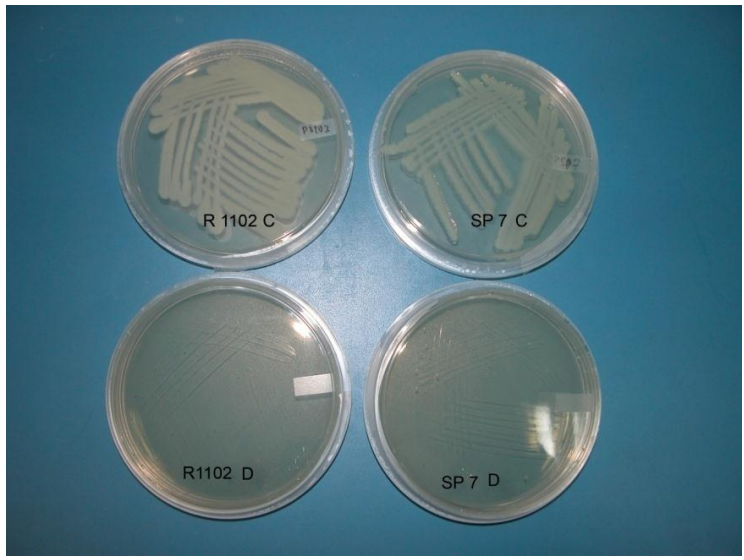

Figure 3. Effect of C. violaceum on PGPR from tubes C and D

CONCLUSION

From both these tests on the effect of *C. violaceum* on selected PGPR, it is concluded that *C. violaceum* will only produce antibiotics or kill the beneficial microbes after reaching quorum level; moreover, it will not have any impact if the concentration is below their quorum level. Although in natural conditions, it is very difficult to achieve a quorum level, the existence of *C. violaceum* in our local soils and their effect cannot be underestimated. The tropical climate in Malaysia offers a very conductive environment for the growth of *C. violaceum* and it has the potential to serve as a threat to beneficial bacteria in our agricultural areas.

REFERENCES

Backer, R., J.S. Rokem, G. Ilangumaran, J. Lamont, D. Praslickova, E. Ricci, S. Subramanian and D.L Smith. 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science* 9(1473): 1-17.

David, X. L., J.D. Peter and B.P. Gail. 2012. *Chromobacterium violaceum* infections in 13 non-human primates. *J Med. Primatol*. 41(2): 107–114.

Duran, N., G.Z. Justo, C.V. Ferreira, P.S. Melo, L. Cordi and D. Martins. 2007. Violacein: properties and biological activities. *Biotechnol. Appl. Biochem.* 48: 127–133.

Duran, N., G.Z. Justo, M. Duran, M. Brocchi, L. Cordi, L. Tasic, G.R. Castro and G. Nakazato. 2016. Advances in *Chromobacterium violaceum* and properties of violacein - its main secondary metabolite: a review. *Biotechnol. Adv*. 34: 1030–1045.

Donny, Y., A.J. Faez Firdaus, A.M. Azman Shah, N.A. Simaa, A.T. Tuba Thabitah, R.M Mariani, A.A.R. Firdaus and T. Rahmat. 2018. *Chromobacterium violaceum* infection in two black-handed gibbons: a veterinary case report. *Malaysian Journal of Veterinary Research* 9(1): 103-109.

Forbes, B. A., F.S. Daniel and S.W. Alice. 2002. Diagnostic Microbiology (11th Ed.). Mosby. St. Louis, Missouri, pp 423-434.

Jitmuang, A. 2008. Human *Chromobacterium violaceum* infection in Southeast Asia: case reports and literature review. *Southeast Asian Journal of Tropical Medicine and Public Health* 39(3): 452-460.

Kloepper, J. W. and M.N. Schroth. (1978). Plant growth-promoting rhizobacteria on radishes. Proceedings of the 4th International Conference on Plant Pathogenic Bacteria. Angers, France: *Station de Pathologie Végétale et Phytobactériologie* 2: 879–882.

Kothari, V., S. Sharma and D. Padia. 2017. Recent research advances on *Chromobacterium violaceum. Asian Pacific Journal of Tropical Medicine,* 10(8): 744–752.

Lee, J., J. Wu, Y.Y. Deng, J. Wang, C. Wang, J.H. Wang, C. Chang, Y. Dong, P. Williams and L.H. Zhang. 2013. A cell-cell communication signal integrates quorum sensing and stress response. *Nat. Chem. Biol*. 9: 339-343.

Lowery, C. A., T.J. Dickerson and K.D. Janda. 2008. Interspecies and interkingdom communication mediated by bacterial quorum sensing. *Chem. Soc. Rev*. 37: 1337–1346.

Hammerschmitt, M. E., V.M. Rolim, G.G. Snel, F.M. Siqueira, D. Driemeier and S.P. Pavarini. 2017. *Chromobacterium violaceum* infection in a horse. *Journal of Comparative Pathology*. 156(4): 334-338.

McClean, K. H., M.K. Winson, L. Fish, A. Taylor, S.R. Chhabra, M. Camara, M. Daykin, J.H. Lamb, S. Swift, B.W. Bycroft, G.S. Stewart and P. Williams. 1997. Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acyl homoserine lactones. *Microbiology* 143: 3703–3711.

McGowan, Jr J. E. and J.P. Steinberg. 1995. Other gram negative bacilli. *Principles and Practice of Infectious Diseases* (4th ed) ed. G.J. Mandell, J.E. Bennett and R. Dolin, pp 2106-2115. New York, NY: Churchill Livingstone.

Miller, M. B. and B.L. Bassler. 2001. Quorum sensing in bacteria. *Annual Review of Microbiology* 55: 165-199.

Nelson, D. and F.M. Carlos. 2001. *Chromobacterium violaceum*: A Review of Pharmacological and Industrial Perspectives. *Critical Reviews in Microbiology* 27(3): 201-222.

Shao, P. L., P.R. Hsueh, Y.C. Hang, C.Y. Lu, P.Y. Lee, C.Y. Lee and L.M. Huang. 2002. *Chromobacterium violaceum* infection in children: a case of fatal septicaemia with nasopharyngeal abscess and literature review. *Pediatr Infect Dis. J*. 21: 707–9.

Smith, D. L., D. Praslickova and G. Ilangumaran. 2015. Inter-organismal signaling and management of the phytomicrobiome. *Front. Plant Sci*. 6: 722.

Srivastava, S. and C. Gera. 2006. Quorum-sensing: the phenomenon of microbial communication. *Current Science* 90: 10-15.

Stauff, D. L. and B.L. Bassler. 2011. Quorum sensing in *Chromobacterium violaceum*: DNA recognition and gene regulation by the CviR receptor. *J. Bacteriol.* 193(15): 3871-3878.

Sun, J., R. Daniel, I. Wagner-Dobler and A.P. Zeng. 2004. Is autoinducer-2 a universal signal for interspecies communication: A comparative genomic and phylogenetic analysis of the synthesis and signal transduction pathways. *BMC Evol. Biol*. 4: 36–46.

Vanbleu, E., K. Marchal and J. Vanderleyden. 2004. Genetic and physical map of the pLAFR1 vector. *DNA Sequence* 15(3): 225-227.

Zahir, A. Z., M. Arshad, W.T. Frankenberger. 2004. Plant growth promotingrhizo bacteria: applications and perspectives in agriculture. *Adv. Agron*. 81: 97–168.

.