Rhizobium isolates antagonistic activity against *Fusarium* sp. FT-04 cause of *Fusarium* wilt of Tomato

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ABSTRACT

Seventy one *Rhizobium* strains isolated from root nodules of legume plants; *Vigna sinensis* L., *Dalbergia oliveri* G., *Mimosa pudica* L., *Pterocarpus indicus* W., *Sesbania grandiflora* D., *Cassia bakeriana* C. and *Butea monosperma* L. Characterization of colonies *Rhizobium* isolates were colonized on yeast manitol congo red agar, there were white or colorless, translucent, elevated and mucilaginous. After staining, there were rod shaped, gram negative, 0.3-0.5 µm of cell and non endospore forming. *Rhizobium* strains were produced PHB granules. All strain showed growth in 3 days and truned the yeast extract mannitol agar media containing bromthymol blue to yellow color showing that all were fast grower and acid producers. Their colonies were large (2-4 mm in diameter). The antagonist activity of *Rhizobium* isolates were tested by dual culture, 27 isolates inhibited the mycelium growth of *Fusarium* sp. FT-04. Isolated PWI18 (36% growth inhibition) was the most effective *in vitro*. These results indicated that rhizabial could be element in Fusarium wilt disease.

INTRODUCTION

Rhizobium are present in root nodules as pleomorphic or bacteroid forms which are normally involved in fixing atmospheric nitrogen into combined form (ammonia) utilizable by the host plant. Cell rods shape in 0.5-0.9x1.2-3.0 µm and usually contain granules of poly-β-hydroxybutyrate (PHB). Cells stain gram negative. Colonies are circular, convex, semitranslucent, raised and mucilaginous, usually 2-4 mm in diameter within 3-5 days on yeast mannitol mineral salt agar (Holt at al., 1994). *Rhizobium* can live either in the soil or within the root nodules of host legume plants (Shahzad et al., 2012). Various strains of *Rhizobium* have been described as effective biological control agents antagonistic to many fungal plant pathogens. *Rhizobium* sp. significantly reduced wilt and root rot on common bean and chickpea caused by *Fusarium* sp. (Arfaoui et al., 2006)

Fusarium spp. are soil borne fungi that specifically attack many agricultural crops causing wilt or foot and root rots that result in significant yield losses (Hassan et at., 1996). *Rhizobium* were isolated and found to effectively control various soil borne plant pathogenic fungi under green house and field conditions (People et al., 1995). The aim of the present work was to characterize and select *Rhizobium* isolates with antagonistic activity against Fusarium wilt of tomato caused by *Fusarium* sp. FT-04. Isolates were purified from legume plants species and their effects on fungal growth by applying dual culture technique.

EXPERIMENT

Microorganism

The isolates of *Rhizobium* were isolated from root nodule of different legume plants species, which were collected from different locations in Udon Thani province, Thailand as listed in Table 1. The collected nodules were surface-sterilized with 95% ethanol and 3% H_2O_2 and washed thoroughly with distilled water. *Rhizobium* strains were obtained by streaking the crushed root nodules on yeast extract mannitol congo red agar,YMCA (10g/L manitol, 0.5g/L K_2HPO_4, 0.2g/L $MgSO_4 \cdot 7H_2O$, 0.1g/L NaCl, 4g/L $CaCO_3$,0.4g/L Yeast extract, 0.25% congo red, 15g/L agar, pH 7.0) agar plates and incubated at 37±2°C. After 5 days of incubation, *Rhizobium* colonies were obtained. The white, translucent, elevated and mucilaginous colonies were piked up and transferred to YMA slant for further characterization (Malisorn & Prasarn, 2014).

Fungal culture (*Fusarium* sp. FT-04) was obtained from the culture collection of the microbiology laboratory of Programe in Biology, Faculty of Science, Udon Thani Rajabhat University, Udon Thani province, Thailand.

Table 1 Sources of *Rhizobium* isolates.

Isolates	Origin	Number of isolates
MPU	*Mimosa pudica* L.	17
CCR	*Cassia bakeriana* C.	8
VES	*Vigna sinensis* L.	18
DOL	*Dalbergia oliveri* G.	17
PWI	*Pterocarpus indicus* W.	19
BKU	*Butea monosperma* L.	0
SDE	*Sesbania grandiflora* D.	4
	Total	83

Morphology Characteristic

The morphological characterized *Rhizobium* isolates. Colonies are circular, convex, semitransluscent, raised, and musilaginous usually 2-4 mm in diameter within 3-5 days and incubated at 37±2°C. Acid production on yeast extract mannitol bromtylmol blue agar (YMBA) was observed. Similarly using Gram staining technique (Cappucino & Sherma, 2001) pink or red colored gram negative rods in 0.5-0.9x1.2-3.0 µm. and Sudan black B staining (Kumari & Dhingra, 2013) presented PHB granules were observed respectively.

Effect of Rhizobium isolates in dual culture

In *vitro* antagonism tests were performed on potato dextrose agar (PDA) in 9-cm Petri plates by applying a dual culture technique (Arfaoui et al., 2006). Rhizobium isolates were streaked across the centre of the plate. One disce 5 mm in diameter cut from the edge of a 5 days-old culture of *Fusarium* sp. FT-04 were placed at each side of the antagonist. The distance between the two microorganisms was 2.5 cm. Plates were incubated at 37±2^{o}C for one week. Percent growth inhibition of *Fusarium* sp. FT-04 after 7 days was calculated by the formula of Whipps (1987): (R1-R2)/R1*100 where R1 is the fungal diameter growth (measured in cm) in the tested antagonist and R2 is the fungal diameter growth (measured in cm) in the control non- antagonist. Growth inhibition was measured on percentage.

ANALYSIS

Eighty three strains of bacteria were isolated from root nodules of different legume plants species *Vigna sinensis* L., *Dalbergia oliveri* G., *Mimosa pudica* L., *Pterocarpus indicus* W., *Sesbania grandiflora* D., *Cassia bakeriana* C. and *Butea monosperma* L. in Udon Thani province, Thailand as listed in Table 1. After identification by morphology of genus *Rhizobium*. We found seventy one strains belonging to *Rhizobium*. The character of 71 strains of *Rhizobium* were obtained on YMBA after incubation at 37±2^{o}C. The colonies were having circular, convex, semitrasluscent, raised and mucilaginous. Typical colonies had a dimeter of 2-4 mm. All strains of *Rhizobium* isolated were showing the production of acid during growth. General microscopic view of them showed rod cell, gram negative and presented PHB granules.

Among the 71 *Rhizobium* isolated tested in dual culture, 27 inhibited the growth of *Fusarium* sp. FT-04 in the range 18-36 % growth inhibition. Isolated PWI18 (36% growth inhibition) was the most effective *in vitro* as Table 2.

Table 2. Effect of *Rhizobium* isolates on in vitro and % growth inhibition of *Fusarium* sp. FT-04 in dual culture.

Rhizobium isolates	% growth inhibition
PWI02, PWI04	18
PWI11	23
PWI19	24
PWI12	25
PWI15	26
PWI13, PWI14	27
PWI06	28
PWI01	29
MPU12, MPU14 , DOL02 , VSE08 , VSE09 , VSE18 , PWI05 , PWI16 , PWI17 , DOL02	30
DOL13 , PWI07	31
PWI03 ,DOL03	32
PWI09	33
PWI08	34
PWI18	36

CONCLUSION

In the present study, strains of *Rhizobium* were isolated from root nodules of legume plants species. Legume are herbaceous woody plants that produce seeds in pods; examples of legumes include peas, beans, alfalfa, vetches and cloves (Shahzad et al., 2012). The strains of *Rhizobium* were isolated from different legumes plants such as common bean (*Phaseolus vulgaris* L.), clover (*Trifolium repens* var. *repens*), chickpea (*Cicer arietinum* L.), Lentil (*Lens culinaris* Medik.), cowpea (*Vigna unguiculata* L.) and red bean (*Phaseolus vulgaris* L.) (Küçük, 2013).

All strain showed growth in 3 days and truned the yeast extract mannitol agar media containing bromthymol blue to yellow color showing that all were fast grower and acid producers. Their colonies were large (2-4 mm in diameter) mucilaginous, circular, convex with rised and semitransluscent (Holt et al., 1994).

Microscopic examination revealed that the isolates were pink and/or red shaped and gram negative in nature. *Rhizobium* strains were produced PHB granules (Holt et al., 1994; Kumari & Dhingra, 2013).

Rhizobium strains showed different interactions with the tested fungi. In this study, some *Rhizobium* strains inhibited the growth of *Fusarium* sp. FT-04, so they may have the potential to act as biocontrol agents. Similary to *Fusarium oxysporum* strain F4 was inhibited by *Rhizobium cicero* N28 (Küçük, 2013). Most *Rhizobium* isolates were able to form inhibition zones in vitro Reduction of fungal growth *in vitro* by several rhizobia

and formation of inhibition zones were presumably due to the metabolites released by the bacteria into the culture medium. These metabolite may include antibiotics and/or cell wall degrading enzymes (Hmissi et al., 2011).

Our results therefore suggest that the *Rhizobium* isolates could be used effectively as biocontrol agents of Fusarium wilt of tomato.

REFERENCES

Arfaoui, A., Sifi, B., Boudabous, A., El Hadrami, I., and Chérif, M., Identification of *Rhizobium* isolates possessing antagonistic activity *against Fusarium oxysporum* F.SP. *ciceris*, the causal agent of Fusarium wilt of chickpea, *Journal of Plant Pathology*, vol. 88, no. 1, pp. 67-75, 2006.

Cappuccino, J. G., and Sherman, N., *Microbiology a laboratory manual (6th)*, Pearson Education, USA, 2001.

Hassan Dar, G. H., Zargar, G. M., and Being, G. M., Biocontrol of Fusarium root rot in the common bean (*Phaseolus vulgaris* L.) by using symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*, *Microb. Ecology*, vol. 37, pp. 74-80, 1996.

Hmissi, I., Gargouri, S., and Sifi, B., Attempt pf wheat protection against *Fusarium culmorum* using *Rhizobium* isolates, *Tunisian Journal of Plant Protection*, vol. 6, no. 2, pp. 75-86, 2011.

Holt, G. J., Krieg, R. N., Sneath, H. A. P., Stalry, T. J., and William, T.S., *Bergey's manual of determinative bacteriology 9th ed*, Williams & Wilkins, USA, 1994.

Küçük, Ç., In vitro antagonism of *Rhizobium* stranis isolated from various legumes, *Journal of Applied Biologal Science*, vol. 7, no. 1, pp. 24-30, 2013.

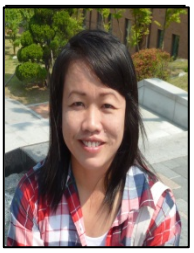
Kumari, P., and Dhingra, K. H., Isolation and characterization oh PHB producing micro-organisms isolated from root nodules of legominous plants, *The Bioscan*, vol. 8, no. 1, pp. 109-113, 2013.

Malisorn, K., and Prasarn, C., Isolation and characterization of *Rhizobium* spp. from root legume plants species, *Khon Kaen Agr. J*, vol. 24, suppl. 4, pp. 157-160, 2014.

People, M. B., Ladha, J. K., Alamed, M., and Schroder, E. C., In vitro evaluation of bacteria for the biological control of *Macrophomina phaseolina*, *World journal of Microbiology and Biotechnology*, vol. 11, pp. 183-185, 1995.

Shahzad, F., Shafee, M., Abbas, F., Babar, S., Tariq, M. M., and Ahmed, Z., Isolation and biochemical characterization of *Rhizobium meliloti* from root nodules of Alfalfa (*Medica sativa*), *The journal of animal & plant Science*, vol. 22, no. 2, pp. 522-524, 2012.

Whipps, J. M., Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi, *New Phytologist*, vol. 107, pp. 127-142, 1987.



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