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Research Article

In vitro evaluation of rhizobacteria associated with diseased tomato plants for antagonistic activity against *Ralstonia solanacearum*, the causal agent of bacterial wilt

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Abstract

Bacterial wilt caused by the phytopathogen *Ralstonia solanacearum* is one of the most destructive diseases affecting the worldwide net production of tomato (*Solanum lycopersicum* L.). The present study aimed to isolate and determine the antagonistic effect of rhizobacteria associated with diseased tomato plants against the phytopathogen under *in vitro* conditions. A total of 32 bacterial strains were obtained from the rhizosphere of wilt-affected tomato plants and screened for their antagonistic activity using dual culture and agar well diffusion assays. Morphological, biochemical, and preliminary molecular characterizations were performed to identify the promising isolate. Among the isolates tested, a few exhibited significant inhibitory zones against *R. solanacearum*, indicating strong antagonistic potential. The isolate TRSAB1, later identified as *Stenotrophomonas pavanii,* showed potential *in vitro* growth inhibition of the phytopathogen as compared to the control. The result revealed that antagonist microbes associated with the rhizosphere soil of diseased tomato plants possess potential antagonist activities against the wilt pathogen and hence have prospects for exploration as a biocontrol agent. Further polyhouse and field evaluations are recommended to confirm their effectiveness and potential application in sustainable tomato disease management strategies.

Keywords: *Ralstonia solanacearum*, Bacterial wilt, Rhizobacteria, Antagonistic activity, Bio-control agents

Introduction

Bacterial wilts of tomato, potato, pepper, eggplant, etc. were among the first diseases that were proved to be caused by bacteria by the scientist Erwin Frink Smith (1988). Bacterial wilt occurs widely in tropical, subtropical, and some temperate regions of the world (Kelman, 1998) and is caused by a soil-borne vascular pathogen, *Ralstonia solanacearum* (Yabuuchi *et al.,* 1995). It has host plants of more than 450 plant species, both monocots and dicots, from 54 families (Smith *et al.,* 1988; Grimault *et al.,* 1994; Hayward, 1995; Swanson *et al.,* 2005). In India, it affects major economic crops of *Solanaceae* family, such as potato, tomato, eggplant, tobacco, pepper, etc. It also affects some other important crops like peanuts, bananas, and ginger. Because of the widespread geographic distribution, high survival properties, and unusual wide host range of the pathogen, control methods such as crop rotation, field sanitation, and use of resistant varieties provide limited success. Chemical control of this disease is usually ineffective, and soil fumigation has slight or no effect (Murakoshi & Takahashi, 1984; Nesmith & Jenkins, 1985). And also, no effective chemical product is commercially available for *Ralstonia* wilt. Disease resistance of a cultivar is usually not stable and/or durable (Hayward, 1991; Boucher *et al.,* 1992). Therefore, biological control is an alternative method to resolve some of these difficulties. This method also avoids environmental pollution. Several studies have been carried out to find bio-control agents to reduce bacterial wilt severity. Studies indicate that the rhizosphere soil of healthy plants is usually a good source for isolating PGPRs and Bio-control Agents (BCAs) (Guo *et al.,* 2004; Yang *et al.,* 2011). Since 1980, rhizobacteria have been investigated as a possible substitute to chemicals and are used to control a broad range of plant diseases either by producing antibiotics, siderophores, or competing for nutrients, predation, or induction of host resistance or by producing most recently discovered bio-surfactants (Défago *et al.,* 1990; Weller & Thomashow, 1994). Antibiotic production by microorganisms is considered a major event in the soil-borne disease suppression ability of rhizobacteria. A variety of antibiotic metabolites, including phenazine, carboxylic acid, pyoluterin, 2,4-diacetylphloroglucinol, oomycin, and cyanides, are produced by these microbes.

Thus, most sustainable and environmentally acceptable control methods for phyto-pathogens can be achieved using these bio-control agents due to the effort to reduce the use of agro-chemicals and their toxic residues in the environment and in food.

The objective of the present research work plan was to investigate the antagonistic potential of the indigenous rhizobacteria associated with the rhizosphere soil of diseased tomato crops against *R. solanacearum* under *in vitro* conditions.

Materials and methods

Sample collection

Rhizosphere soil samples from diseased tomato crops were collected from different sites of experimental fields of DIBER, Haldwani, Auli, and Pithoragarh, and also from other regions of Uttarakhand in January and March 2015. Soil samples and plants were tagged properly. Samples were immediately transferred to the laboratory aseptically and stored at 40 °C. A representative subsample of 10 grams of

soil from each soil sample was taken after following the conning and quartering procedure.

Isolation and multiplication of rhizobacteria

From each sub-sample of 10 grams of soil, 1 gram was taken, diluted in normal saline (0.85% NaCl), and serially diluted 10-fold, making dilutions 10^{-1} to 10^{-10}. From each dilution, 0.1 mL of the diluted samples was taken and spread over the Nutrient agar plates. For each dilution, triplicate plates were prepared with a control set. After incubation at 37 ℃ for 24-48 hours, single isolated colonies were picked and purified on fresh NA plates by the quadrant streak. Isolates were further subcultured twice. All the isolates were then stored in 30% glycerol at -80 ℃ and water stocks at room temperature until further investigation.

In vitro screening of potential antagonist

The different bacterial strains isolated from all the samples were screened *in vitro* for their antagonistic behavior towards the known strain of *Ralstonia solanacearum 00418* (obtained from ICAR-NAIMCCC). This test was performed by agar well diffusion method on Mueller-Hinton Agar plates (Balouiri *et al.,* 2016). The concentration of both the test organism and bacterial isolates was standardized to 1×10^8 CFU/mL. The volume for each bacterial isolate tested was 100 µL. Positive result showing isolates were further checked on different media such as SMSA Agar and King's medium B base agar plates for the presence/absence of a hollow zone of inhibition and the size of inhibition zones after incubation at 28 ℃ for 48 hours. Each strain was tested at least 5 times on each medium for confirmation.

Test for antibacterial activity

Antagonistic trait of the isolates was determined by the following three tests: HCN production test, ammonia production test, and siderophore production test.

HCN production test

Production of HCN was assessed on King's B medium containing 4.4 g/L of glycine. Freshly grown broth cultures were spread on the King's B medium containing glycine. A Whatman filter paper No. 1 soaked in 0.5% picric acid solution (in 2% sodium carbonate) was placed inside the lid of the plate. Plates were sealed with parafilm and incubated at 28±2 ℃ for 4 days. The development of orange to red colour indicates HCN production (Kumari *et al.,* 2017).

Ammonia production test

Bacterial isolates were tested for the production of ammonia in peptone water broth. Freshly grown cultures were inoculated in 10 ml peptone water and incubated for 48-72 hours at 37 ℃. After the incubation period, Nessler's reagent was added to each tube. The development of brown to yellow colour indicates the production of ammonia (Kumari *et al.,* 2017).

Siderophore production test

Production of siderophore was assessed on CAS agar plates (Louden *et al.,* 2011) following the protocol described by Schwyn and Neilands (Schwyn & Neilands, 1987).

Result and discussion

Isolation and multiplication of rhizobacteria

Five different soil samples have been collected from Haldwani, Pithoragarh, and Auli, Uttarakhand. From the samples, a total of 32 bacterial strains were isolated. Cultures were purified on Nutrient agar plates. Stored in 30% glycerol stocks at -80 ℃ (Figure 1).

In vitro screening of a potential antagonist

Out of 32 isolates, 8 of them, *viz.* TRSAB1, TSSIB1, TSSIIB1, TSSIIB7, TSSIIIB1, TSSVB6, ITRSB8, and PTRSB5 showed inhibition zones against the known strain of *R. solanacearum*. The isolate named TRSAB1 demonstrated the most promising *in vitro* antagonistic activity against *R. solanacearum* (NAIMCC-00418 strain). It showed the largest zone of inhibition having average zone size of 21 mm on the MHA plate (Figure 2 & Table 1). The most effective isolate (TRSAB1) exhibited the highest antagonistic activity, inhibiting radial growth of *R. solanacearum* by 35–40%, calculated as:

$$\text{Percentage inhibition} = [(C - T)/C] \times 100$$

where C = control radial growth, T = treatment radial growth

Test for antibacterial activity

Antibacterial activities such as HCN production test, Ammonia production test and test for Siderophore production are mention in Table 2.

HCN production test

All the isolates except TSSIIB7 were found to be negative for HCN production (Figure 3).

Ammonia production test

All the isolates were found to be negative for ammonia production (Figure 4).

Siderophore production test

The isolates TRSAB1 and TSSIIB1 are found to be positive for siderophore production test (Figure 5).

Identification of the isolates

16S rRNA sequencing of the isolate TRSAB1 was done at MTCC, Chandigarh, and it was identified as *S. pavanii* which belongs to the family Xanthomanadaceae, an

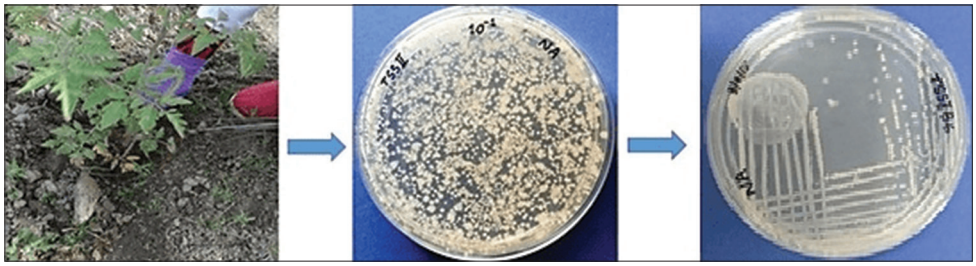

Figure 1: Isolation of rhizobacteria

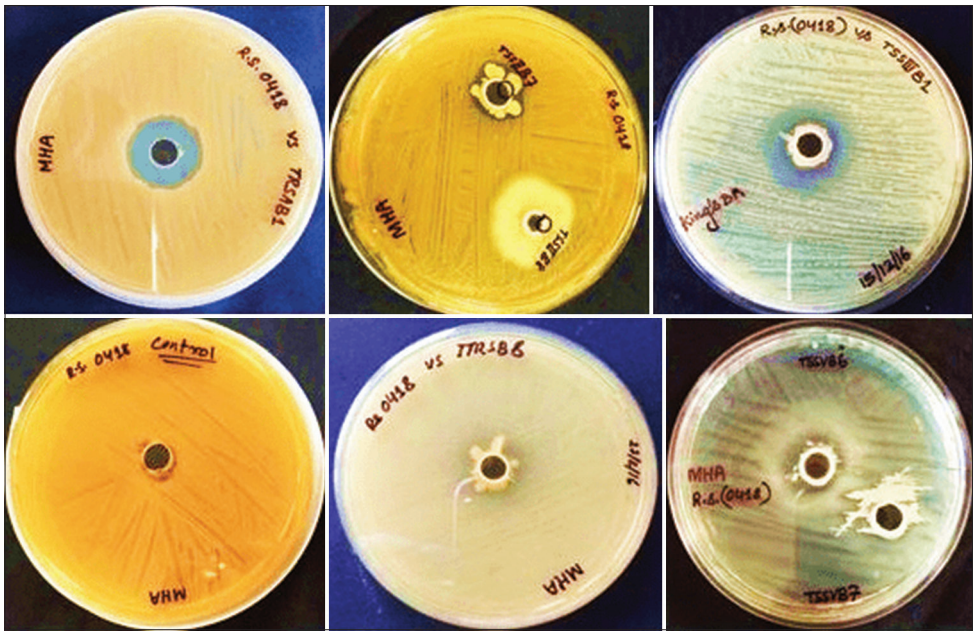

Figure 2: *In vitro* screening of potential antagonist

Table 1: *In vitro* antibiosis test in MHA plate

S. No.	Isolate code	*In vitro* antibiosis against *R. solanacearum*	
		On the MHA plate	On King's B A plate
1	TRSAB1	21 mm	24 mm
2	TSSIB1	20 mm	21 mm
3	TSSIIB1	12 mm	14 mm
4	TSSIIB7	14 mm	20 mm
5	TSSIIIB1	17 mm	17 mm
6	TSSVB6	17 mm	15 mm
7	ITRSB8	12 mm	11 mm
8	PTRSB5	13 mm	20 mm

Table 2: *In vitro* test for antibacterial activity

S. No.	Isolate code	HCN production test	Ammonia production test	Siderophore production test
1	TRSAB1	-	-	+
2	TSSIB1	-	-	-
3	TSSIIB1	-	-	+
4	TSSIIB7	+	-	-
5	TSSIIIB1	-	-	-
6	TSSVB6	-	-	-
7	ITRSB8	-	-	-
8	PTRSB5	-	-	-

endophytic nitrogen-fixing, rod-shaped, non-spore-forming, gram-negative bacterium, and was most closely related to *S. maltophilia*, *S. tumulicola*, and *S. chelatiphaga* (Ramos *et al.,* 2011) (Table 3).

16S rRNA gene sequences

GGCAGCACAGGAGAGCTTGCTCTCTGGGTGG
CGAGTGGCGGACGGGTGAGGAATACATCGGA
ATCTACTCTGTCGTGGGGGATAACGTAGGGA
AACTTTACGCTAATACCGCATACGACCTACGGGTG
AAAGCAGGGGACCTTCGGGCCTTGCGCGATTGAT
GAGCCGATGTCGGATTAGCTAGTTGGCGGG
GTAAAGGCCCACCAAGGGGAACAACCCGT
AGCTGGTCTGAAAGGATGAT CAGCCACACTGGAA
CTGAGACACGGTCCAGAACTCCTACGGGAGGCA
GCAGTGGGGAATATTTGGACAATGGGCGCAAG
CCTGATCCAGCCATACCGCGTGGGTGAAGAAG
GCCTTTCGGGTTTGTAAAGCCCTTTTTGTTTGGG
AAAGAAATCCAGCTGGCTAATACCCGGTTGGGA
TGACGGTACCCAAAGAATAAGCACCGGCTAAC
TTCGTGCCAGCAGCCGCGGTAATACGAAGGGT
GCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGT
GCGTAGGTGGTCGTTTAAGTCCGTTGTGAAAGCC
CTGGGCTCAACCTGGGAA CTGCAGTGGATACTGG
GCGACTAGAATGTGGTAGAGGGTAGCGGAATT
CCTGGTGTAGCAGTGAAATGCG TAGAGATCAGGA
GGA ACATCCATGGCGAAGGCAGCTACCTGGACC

Table 3: Strain identification

Hit taxon name	Hit strain name	Accession	% Similarity	Diff/Total nucleotides
Stenotrophomonas pavanii	DSM 25135(T)	LDJN01000038	99.23	11/1426
Pseudomonas beteli	ATCC 19861(T)	AB021406	99.08	13/1407
Pseudomonas geniculata	ATCC 19374(T)	AB021404	99.07	13/1404
Stenotrophomonas maltophilia	MTCC 434(T)	JALV01000036	99.02	14/1426
Pseudomonas hibiscicola	ATCC 19867(T)	AB021405	98.86	16/1406
CP001111_s	R551-3	CP001111	98.60	20/1426
Stenotrophomonas tumulicola	T5916-2-1b (T)	LC066089	98.04	28/1426
Stenotrophomonas chelatiphaga	DSM 21508(T)	LDJK01000058	97.97	29/1426

Figure 3: HCN production test

Figure 4: Ammonia production test

AACATTGACACTGAGGCACGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGGTAGTCCACGCC
CTAAACGATGCGAACTGGATGTTGGGTGCAA
TTTGGCACGCAGTATCGAAGCTAACGCGTTAAGT
TCGCCGCCTGGGGAGTACGGTCGCAAGACTGA
AACTCAAAGGAATTGACGGGGGCCCGCACAAGC
GGTGGAGTATGTGGTTTAATTCGATGCAACG
CGAAGAACCTTACCTGGCCTTGACATGTCGAGAA
CTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCG
AACACAGGTGCTGCATGGCTGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGCAACGA

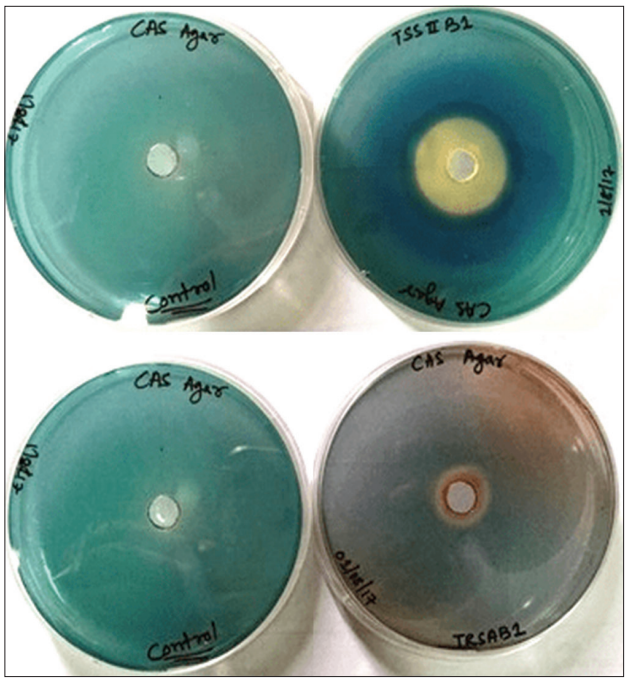

Figure 5: Siderophore production test

GCGCAACCCTTGTCCTTAGTTGCCAGCACGTAAT
GGTGGGAACTCTAAGGAGACCGCCGGTGACAA
ACCGGAGGAAGGTGGGGATGACGTCAAGTCATC
ATGGCCCTTACGGCCAGGGCTACACACGTACTA
CAATGGTAGGGACAGAGGGCTGCAAGCCGGCGA
CGGTAAGCCAATCCCAGAAACCCTATCTCAGTCC
GGATTGGAGTCTGCAACTCGACTCCATGAAGTCGG
AATCGCTAGTAATCGCAGATCAGCATTGCTGCG
GTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACACCATGGGAGTTTGTTGCACCAGAAG
CAGGTAGCTTAACCTTCGGGAGGGCGCTTGCC
ACGGTGTGGCCGATGACTGGGGTGAAGT

Conclusion

The rhizobacterium *S. pavanii*, isolated from the rhizosphere region of diseased tomato plants, has shown remarkable potential in suppressing bacterial wilt caused by *R. solanacearum*. The mechanism underlying this suppression appears to be multifaceted, involving the production of siderophores that chelate iron and limit pathogen growth, as well as the synthesis of biosurfactants that may enhance host resistance and disrupt pathogen colonization. These beneficial traits suggest that *S. pavanii* not only acts as an antagonist but also as a plant growth-promoting rhizobacterium (PGPR) capable of stimulating the plant's innate defense responses. Furthermore, the

findings provide valuable insights into the possibility of selectively enriching such beneficial rhizobacteria in the rhizosphere to promote the production of secondary bioactive compounds, including siderophores and antibiotics. Therefore, *S. pavanii* can be considered as a promising biological control agent for the eco-friendly and sustainable management of bacterial wilt in tomato, hence by reducing dependency on chemical pesticides and contributing to improved soil and plant health.

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Author contributions

Shraddha P. Mishra: Conceptualization, Methodology, Data curation, Investigation, Formal analysis, Writing – original draft. Anfal Arshi: Funding acquisition, Supervision, Writing – review & editing. Shardesh Kumar Chaurasia: Investigation, Formal analysis, Validation, Visualization. All authors approved the final manuscript.

References

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis, 6*(2), 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

Boucher, C. A., Gough, C. L., & Arlat, M. (1992). Molecular genetics of pathogenicity determinants of Pseudomonas solanacearum with special emphasis on hrp genes. *Annual Review of Phytopathology, 30*, 443-461. https://doi.org/10.1146/annurev.py.30.090192.002303

Défago, G., Haas, D., Berling, C. H., Burger, U., Keel, C., Voisard, C., Wirthner, P., & Wüthrich, B. (1990). Suppression of black root rot of tobacco and other root diseases by strains of *Pseudomonas fluorescens*: Potential applications and mechanisms. In D. Hornby (Ed.), *Biological control of soil-borne plant pathogens* (pp. 93-108). CAB International.

Grimault, V., Anais, G., & Prior, P. (1994). Distribution of *Pseudomonas solanacearum* in the stem tissues of tomato plants with different levels of resistance to bacterial wilt. *Plant Pathology, 43*(4), 663-668. https://doi.org/10.1111/j.1365-3059.1994.tb01604.x

Guo, J.-H., Qi, H.-Y., Guo, Y.-H., Ge, H.-L., Gong, L.-Y., Zhang, L.-X., & Sun, P.-H. (2004). Biocontrol of tomato wilt by plant growth-promoting rhizobacteria. *Biological Control, 29*(1), 66-72. https://doi.org/10.1016/S1049-9644(03)00124-5

Hayward, A. C. (1991). Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annual Review of Phytopathology, 29*, 65-87. https://doi.org/10.1146/annurev.py.29.090191.000433

Hayward, A. C. (1995). *Pseudomonas solanacearum*. In U. S. Singh, R. P. Singh & K. Kohmoto (Eds.), Pathogenesis and host specificity in plant disease: Histopathological, biochemical, genetic and molecular bases (Vol. 1, pp. 139-151). Elsevier.

Kelman, A. (1998). One hundred and one years of research on bacterial wilt. In P. Prior, C. Allen, J. Elphinstone (Eds.), *Bacterial wilt disease* (pp. 1-5). Springer. https://doi.org/10.1007/978-3-662-03592-4_1

Kumari, P., Khanna, V., & Kumar, P. (2017). Multifaceted rhizobacteria-mediated growth augmentation in chickpea. *Agricultural Research, 6*, 368-377. https://doi.org/10.1007/s40003-017-0275-5

Louden, B. C., Haarmann, D., & Lynne, A. M. (2011). Use of blue agar CAS assay for siderophore detection. *Journal of Microbiology & Biology Education, 12*(1), 51-53. https://doi.org/10.1128/jmbe.v12i1.249

Murakoshi, S., & Takahashi, M. (1984). Trials of some control of tomato wilt caused by *Pseudomonas solanacearum*. *Bulletin of the Kanagawa Horticultural Experiment Station, 31*, 50-56.

Nesmith, W. C., & Jenkins, S. F. (1985). Influence of antagonists and controlled matric potential on the survival of *Pseudomonas solanacearum* in four North Carolina soils. *Phytopathology, 75*, 1182-1187.

Ramos, P. L., Van Trappen, S., Thompson, F. L., Rocha, R. C. S., Barbosa, H. R., De Vos, P., & Moreira-Filho, C. A. (2011). Screening for endophytic nitrogen-fixing bacteria in Brazilian sugar cane varieties used in organic farming and description of *Stenotrophomonas pavanii* sp. nov. *International Journal of Systematic and Evolutionary Microbiology, 61*(4), 926-931. https://doi.org/10.1099/ijs.0.019372-0

Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry, 160*(1), 47-56. https://doi.org/10.1016/0003-2697(87)90612-9

Smith, I. M., Dunez, J., Phillips, D. H., Lelliott, R. A., & Archer, S. A. (1988). *European Handbook of Plant Diseases*. Blackwell Scientific Publications. https://doi.org/10.1002/9781444314199

Swanson, J. K., Yao, J., Tans-Kersten, J., & Allen, C. (2005). Behavior of *Ralstonia solanacearum* Race 3 Biovar 2 During Latent and Active Infection of Geranium. *Phytopathology, 95*(2), 136-143. https://doi.org/10.1094/PHYTO-95-0136

Weller, D. M., & Thomashow, L. S. (1994). Current challenges in introducing beneficial microorganisms into the rhizosphere. In F. O'Gara, D. N. Dowling & B. Boesten (Eds.), Molecular ecology of rhizosphere microorganisms: Biotechnology and the release of GMOs (pp. 1-18). VCH Verlagsgesellschaft.

Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H., & Nishiuchi, Y. (1995). Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. Nov. *Microbiology and Immunology, 39*(11), 897-904. https://doi.org/10.1111/j.1348-0421.1995.tb03275.x

Yang, M.-M., Mavrodi, D. V., Mavrodi, O. V., Bonsall, R. F., Parejko, J. A., Paulitz, T. C., Thomashow, L. S., Yang, H. T., Weller, D. M., & Guo, J.-H. (2011). Biological control of take-all by fluorescent *Pseudomonas* spp. from Chinese wheat fields. *Phytopathology, 101*(12), 1481-1491. https://doi.org/10.1094/PHYTO-04-11-0096