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ORIGINAL ARTICLE

Chryseobacterium nankingense sp. nov. WR21 effectively suppresses *Ralstonia solanacearum* growth via intensive root exudates competition

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Abstract Previous studies demonstrated that the *Chryseobacterium* sp. WR21 could effectively control the bacterial wilt disease caused by *Ralstonia solanacearum* through effective root colonization. The strain WR21 exhibited a low level of DNA homology with *Chryseobacterium* strains DSM 15235^T (24.1%), DSM 17724^T (24.8%), and DSM 18014^T (10.4%), suggesting that WR21 may represent a novel species, for which the name *Chryseobacterium nankingense* sp. nov. is proposed. The in vitro competition experiments with strain WR21 indicated it significantly inhibited growth of the pathogen in co-culture with six of nine tested nutrients (e.g. root exudates) that could be utilized by strain WR21 and *R. solanacearum*. Similar trends were observed in co-culturing experiments using tissue exudates of tomato. A positive relationship ($r = 0.785$) was noticed between the differences in the average growth rate of both strains and the disease suppression effects. In conclusion, *Chryseobacterium nankingense* sp. nov. WR21 exhibits antagonism through nutrient competition that might be used for achieving biocontrol of *Ralstonia solanacearum* induced wilts.

Keywords *Ralstonia solanacearum* · Bacterial wilt · Resource competition · Root exudates · Biocontrol

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Introduction

Ralstonia solanacearum, the causal agent of bacterial wilt disease, inflicting increasing economic losses over time, has emerged as a constant threat to tomato production in Southern China (Hayward 1991; Xue et al. 2009). Rhizosphere has been a predominant source of a wide variety of biocontrol agents both cultivable as well as that of metagenomics origin (Riaz et al. 2008; Dessaux et al. 2016). The application of plant growth-promoting rhizobacteria (PGPRs) to control soil-borne diseases has been widely recognized as an environment friendly alternative to the use of chemicals (Kheirandish and Harighi 2015; Ling et al. 2010; Tan et al. 2013). Mutualism, commensalism, neutralism, competition, amensalism, parasitism, predation, and various combinations thereof have been demonstrated as effective processes of microbial physiology that help PGPRs achieving effective biocontrol against soil-borne pathogens (Francis et al. 2010; Pérez-García et al. 2011). Microorganisms that are better adapted to utilize root exudates in a particular environment under a given set of conditions compete better for these nutrients thereby efficiently colonizing the roots and the rhizosphere (Dessaux et al. 2016). Theoretically, biocontrol agents that colonize the rhizosphere for long periods can effectively utilize such root exudates, thereby providing them an advantage in competition. Soil carbon concentration is one of the key factors that limits microbial growth in soil (Helal and Sauerbeck 1986). Carbon release in the rhizosphere through root exudates affects the chemical, physical and biological characteristics of the rhizosphere to differ from those of the bulk soil. The magnitude of these changes in soil properties is largely determined by the amount and type of carbon released from the roots in addition to the intrinsic soil characteristics (Haichar et al. 2014; Jones et al. 2004). Hence, it was observed that 75% of the soil bacteria that suppressed the tomato bacterial wilt disease could in fact efficiently utilize pectic exudates (the primary exudates of tomato plants) (Shiomi et al. 1999). These bacteria were identified as β-*Proteobacteria* and were the primary reason for the suppression of *R. solanacearum*. Similarly, Malic acid, an organic acid, is a root exudate of *Arabidopsis thaliana* that can successfully induce the colonization by *Bacillus subtilis* FB17 (Rudrappa and Bais 2008).

Recently, effective tomato root colonizing bacteria could also be isolated from the rhizosphere of *Ralstonia*-wilted plants where *R. solanacearum* was present in higher density (greater than 10^8 cfu g^{-1} dry soil) (Huang et al. 2013). It is supposed that, if PGPRs successfully survive in a pathogen-prevalent environment, they must have developed particular survival strategies, which would help them under the stress exerted by the pathogens. Consequently, the *Chryseobacterium* sp. strain WR21 was found to be prevalent in the rhizosphere soil of diseased tomato plants (Huang et al. 2013). Further studies clearly demonstrated that the *Chryseobacterium* sp. could not only successfully colonize the tomato rhizosphere but also reduced the density of *R. solanacearum*, and hence the incidence of bacterial wilt disease (Huang et al. 2013). However, the mechanism by which *Chryseobacterium* sp. strain WR21 successfully colonized the tomato rhizosphere and competed against the *R. solanacearum* remained unclear.

To address this question, root exudate utilization efficacy was hypothesized to be the determining factor for *Chryseobacterium* sp. WR21, for both root colonization and competitive exclusion of the pathogens. To examine this hypothesis, the ability of *Chryseobacterium* sp. strain WR21 and the pathogen to utilize 48 chemical nutrients, previously used to mimic tomato root exudates (Wei et al. 2015), was compared in this study. Previous studies suggested that the strain WR21 was closely related to *Chryseobacterium daecheongense* (HQ220102), nevertheless, only exhibiting an identity of 97% based on 16S rRNA gene sequence clustering (Huang et al. 2013). Therefore, in the present study, DNA–DNA hybridization and other chemotaxonomic analyses were performed to further identify the taxonomic status of *Chryseobacterium* sp. strain WR21. On the basis of the physiological, chemotaxonomic, and phylogenetic data, it has been proposed that the strain WR21 represents a novel species of the genus *Chryseobacterium*.

Materials and methods

Strains and culture conditions

The *Chryseobacterium* sp. strain WR21 (GenBank accession: JF700397) was maintained on Luria–

Bertani agar plates at 30 °C (LB, peptone 10 g l^{-1}, yeast 5 g l^{-1} and NaCl 10 g l^{-1}) (Huang et al. 2013). *Ralstonia solanacearum* QL-Rs1115 strain (GenBank accession: GU390462) tagged with the pYC12-mCherry plasmid was used as a model bacterial pathogen (Wei et al. 2011; Tan et al. 2016). *R. solanacearum* QL-Rs1115 was maintained on Casein Peptone Glucose agar plate (CPG) supplemented with 0.005% triphenyl tetrazolium chloride (m/v) for two days at 30 °C. The bacterial strains were provided by the Laboratory of Rhizosphere Micro-ecology, Nanjing Agricultural University, China.

Phenotypic, physiological, and biochemical characterization

The Gram reaction was determined by the conventional Gram-staining method (Smibert and Krieg 1994). Cell morphology was observed under a Zeiss light microscope at ×1000 magnification after three-day growth at 28 °C on Tryptose Soya Agar plate (TSA). Catalase and oxidase activity was tested as described by McCarthy and Cross (1984). The optimum pH range (pH 4.0–10.0 at intervals of 0.5 pH unit) for growth was determined in TSB that was buffered with either citrate/phosphate or Tris/hydrochloride buffers. Tolerance to NaCl was tested in TSB containing 0–7% NaCl (w/v) at 0.5% increments. Growth on TSA was tested under various temperature conditions (5, 10, 20, 25, 28, 30, 37, 40, 42, and 45 °C). Additional biochemical tests were performed to assess the carbon source utilization pattern by the Biolog GN2 and the API ZYM, API 20E, and API 20NE (BioMérieux Co., Ltd., Marcy-l′Etoile, France) microtest systems according to the methods outlined by the manufacturer. The DNA base composition analyses (mol% G+C) was conducted by the Identification Service of the Deutsche Sammlung von Mikroorganismen and Zellkulturen (DSMZ), Braunschweig, Germany. DNA–DNA hybridization was performed to determine the genomic relatedness as described by Ley et al. (1970), with modifications as described by Huss et al. (1983), using a Cary model 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-Thermostatted 6 × 6 multi-cell changer and a temperature controller equipped with an in situ temperature probe (Varian Co., Ltd., USA).

The 16S rRNA gene sequence was identified by BLAST search analysis on EzTaxon-e server and the identification of phylogenetic neighbors and calculation of pairwise 16S rRNA gene sequence similarity were achieved (Kim et al. 2012). The phylogenetic analysis was performed using the software package MEGA version 6.0 after multiple alignments of the sequence data with CLUSTAL X (Tamura et al. 2013). Distances were calculated using distance options according to Kimura's two-parameter model, and clustering was performed with Neighbor-Joining. Confidence values for the branches of phylogenetic trees were determined by using bootstrap analyses (based on 1000 re-sampling).

Resource utilization and competition pattern of *Chryseobacterium* sp. WR21 and *R. solanacearum* in laboratory microcosms

Firstly, the resource consumption pattern of each bacterial strain was determined individually in single-species monocultures on 48 compounds representative of amino acids, organic acids, and sugars found in tomato root exudates (supplementary Table S1). Briefly, overnight cultures were adjusted to an optical density of 0.1 (OD_{600}) and were grown in microtiter plates in 150 µl OS minimal medium (Schnider-Keel et al. 2000) supplemented with each chemical compound with the final concentration of 10 mmol l^{-1}. After 48-h growth at 25 °C with agitation, the optical density was recorded using a SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, CA, USA). Wells with an $OD_{600} > 0.05$ were scored as positive for growth on a given substrate.

Depending upon the above results, nine nutrients, which could be used by both *Chryseobacterium* sp. strain WR21 and the pathogen *R. solanacearum,* were selected for the nutrient competition test. The *Chryseobacterium* sp. strain WR21 and *R. solanacearum* were first grown alone in a liquid nutrient medium (NA, glucose 10.0 g l^{-1}, peptone 5.0 g l^{-1}, yeast extract 0.5 g l^{-1}, beef extract 3.0 g l^{-1}, pH 7.0) on a shaker at 170 rpm and 30 °C for 12 h. Afterwards, the densities of *Chryseobacterium* sp. strain WR21 and *R. solanacearum* were adjusted to 10^7 cells ml^{-1} in 0.85% NaCl solution. Suspensions of monocultures or a 1:1 mixture (volume) of *Chryseobacterium* sp. strain WR21 and *R. solanacearum* were inoculated into nine different nutrients environments, each of which comprised OS minimal medium supplemented with one kind of nutrient. The final concentration of each

nutrient was 10 mmol l^{-1} and the initial density (OD_{600}) of the inoculum was 0.05. The cultures were grown in microtiter plate (200 µl $well^{-1}$) at 30 °C with agitation. Bacterial growth was recorded every 2 h based on the optical density (OD_{600}), and pathogen growth was recorded based on the red fluorescence signal (excitation: 587 nm, emission: 610 nm) for 48 h.

Resource competition pattern of *Chryseobacterium* sp. WR21 and *R. solanacearum* in tissue exudates of tomato

The root exudates collected according to Kamilova et al. (2005), were sterilized by filtering through 0.22-µm membrane for further use. The inoculant suspensions of *Chryseobacterium* sp. strain WR21 and the pathogen *R. solanacearum* were adjusted to 10^7 -cells ml^{-1} in 0.85% NaCl solution. Suspensions of monocultures or a 1:1 mixture (volume) of *Chryseobacterium* sp. WR21 and *R. solanacearum* were inoculated into 96-well microtiter plate with 200 µl root exudates in each cell. The initial density (OD_{600}) of the inoculum was 0.05, and the cultures were grown at 30 °C with agitation for 72 h. Bacterial and pathogen growth was determined using the above explained method.

Data analysis

All experiments were performed at least in triplicate. Differences among treatments were assessed with one-way ANOVA or t-tests at the end of each assay and means were subjected to Duncan's multiple range tests at $P < 0.05$ using SPSS BASE ver.16.0 statistical software (SPSS Inc., Chicago, USA). Regression analysis was performed using Sigma-plot 12.0. Illustrations and tables were also completed by Sigma-plot 12.0.

Results

Identification of *Chryseobacterium* sp. strain WR21

The *Chryseobacterium* sp. strain WR21 was Gram-negative, strictly aerobic, non-spore-forming, nonmotile, rod-shaped, 0.5–0.8 µm wide, and 1.5–2.0 µm long. The colonies of *Chryseobacterium* sp. WR21 appeared circular, shiny, and smooth with entire margins, and produced a yellow non-diffusible flexirubin-type pigment.

The growth of *Chryseobacterium* sp. strain WR21 occurred at 15–40 °C, pH 5.0–8.0, and 0–4.0% NaCl (w/v), being optimal at 25–30 °C, pH 7.0–7.5, and 0.5% NaCl (w/v). The strain WR21 showed positive catalase, oxidase, and indole production activity. H_2S production, nitrate reduction, urease, arginine dihydrolase, and β-galactosidase activities were negative. Starch hydrolysis was positive while aesculin, casein, and gelatin hydrolyses were negative. *Chryseobacterium* sp. strain WR21 did not utilize glucose, mannose, arabinose, maltose, glyconate, and sodium acetate as sole carbon source. The strain WR21 produced acid from glucose and maltose. The G+C content of *Chryseobacterium* sp. strain WR21 was 36.6 mol% (Table 1).

The fatty acid profiling depicted that the major detected fatty acids (>5% of the total fatty acids) were iso-$C_{15:0}$ (38.1%), iso-$C_{17:1}\omega9c$ (18.8%), 17:0 iso-$C_{17:0}$3-OH (17.1%), and the summed feature 3 ($C_{16:1}\omega7c$/15 iso 2-OH, 10.5%) (Table 2). DNA–DNA hybridization was performed between strain WR21 and *Chryseobacterium daecheongense* DSM 15235^T, *Chryseobacterium wanjuense* DSM 17724^T, and *Chryseobacterium gambrini* DSM 18014^T (Table 3). Low levels of DNA homology were found between *Chryseobacterium* sp. strains WR21 and DSM 15235^T (24.1%), DSM 17724^T (24.8%), or DSM 18014^T (10.4 %) (Table 3). The *Chryseobacterium* sp. strain WR21 (ID 12-199) was distinct from DSM 15235^T (ID 12-200), DSM 17724^T (ID 12-201), and DSM 18014^T (ID 12-202) based on the recommended threshold value of 70% DNA homology for the definition of a bacterial species by the *ad hoc* committee (Friginal et al. 2014) (Table 3).

The 16S rRNA sequence of *Chryseobacterium* sp. strain WR21 was a continuous stretch of 1418 base pairs. Pairwise similarity calculations indicated that the closest relative of *Chryseobacterium* sp. strain WR21 was *C. daecheongense* (98.0%), *C. wanjuense* (97.5%), and *C. gambrini* (97.3%). The phylogenetic reconstruction, based on Neighbor-Joining analyses, indicated that *Chryseobacterium* sp. strain WR21 occupied a distinct branch from its closest relatives within the genus *Chryseobacterium* (Fig. 1).

Table 1 Characteristics that differentiate *Chryseobacterium* sp. strain WR21 from phylogenetically related type species *Chryseobacterium daecheongense* DSM 15235^T, *Chryseobacterium wanjuense* DSM 17724^T, and *Chryseobacterium gambrini* DSM 18014^T

Characteristic	*Chryseobacterium* species			
	WR21	DSM 15235^T	DSM 17724^T	DSM 18014^T
Growth at				
5 °C	−	+	+	+
42 °C	−	−	+	+
Nitrate reduction	−	+	−	−
Indole production	+	−	−	−
Utilization of				
Glucose	−	+	+	+
L-Arabinose	−	+	−	+
D-mannose	−	+	+	+
Maltose	−	+	+	+
Glyconate	−	+	−	−
Acetate	−	+	+	+
Acid production from				
Glucose	+	−	+	+
Maltose	+	−	−	−
Hydrolysis of				
Starch	+	+	+	−
Gelatin	−	+	+	+
DNA G+C content (mol%)	36.6 ± 0.2a	36.6 ± 0.1a	37.8 ± 0.2a	37.8 ± 0.3a

All strains tested positive for the presence of flexirubin-type pigments and for the hydrolysis of aesculin and casein. +, Positive; −, negative. Values after the sign "±" represent the SD between replicates. Different letters among strains indicate significant differences ($P < 0.05$)

Table 2 Cellular fatty acid profiles of *Chryseobacterium* sp. strain WR21 and related type species *Chryseobacterium daecheongense* DSM 15235^T, *Chryseobacterium wanjuense* DSM 17724^T, and *Chryseobacterium gambrini* DSM 18014^T

Cellular fatty acid	*Chryseobacterium* species			
	WR21	DSM 15235^T	DSM 17724^T	DSM 18014^T
$C_{16:0}$	1.7 ± 0.1	tr	tr	tr
$C_{17:0}$2-OH	tr	nd	nd	3.0 ± 0.5
iso-$C_{15:0}$	38.1 ± 0.5ab	51.2 ± 0.8a	40.0 ± 0.6a	29.4 ± 0.5b
iso-$C_{15:0}$3-OH	2.8 ± 0.1a	2.0 ± 0.2b	3.7 ± 0.2a	2.3 ± 0.1ab
iso-$C_{17:1}\omega 9c$	18.8 ± 0.2ab	7.6 ± 0.2b	11.7 ± 0.1ab	25.6 ± 0.2a
iso-$C_{17:0}$	1.8 ± 0.1a	3.0 ± 0.2a	2.9 ± 0.1a	tr
iso-$C_{17:0}$3-OH	17.1 ± 0.3ab	15.7 ± 0.4ab	21.9 ± 0.2a	14.0 ± 0.2b
anteiso-$C_{15:0}$	tr	tr	tr	5.9 ± 0.3
Unknown ECL 13.565	3.9 ± 0.1	tr	2.9 ± 0.2	tr
Unknown ECL 16.582	1.4 ± 0.1	nd	nd	nd
Summed feature 3[a]	10.5 ± 0.2ab	10.3 ± 0.1b	11.0 ± 0.2ab	11.2 ± 0.2a

Values are the percentage of total fatty acids. Fatty acids that account for less than 1% of the total fatty acids in all strains studied are not shown. Values after the sign "±" represent the SD between replicates. Different letters among strains indicate significant differences ($P < 0.05$)

ECL equivalent chain length (i.e. the identity of the fatty acids is unknown), *tr* Trace (less than 1%), *nd* not detected

[a] Summed feature 3 contains iso $C_{15:0}$ 2-OH and/or C16:1ω11c

Table 3 DNA–DNA similarity (in 2 × SSC at 65 °C) of *Chryseobacterium* sp. strain WR21 and related type species *Chryseobacterium daecheongense* DSM 15235^T, *Chryseobacterium wanjuense* DSM 17724^T, and *Chryseobacterium gambrini* DSM 18014^T

	DNA–DNA similarity
Chryseobacterium daecheongense DSM 15235^T	24.1 (25.5)
Chryseobacterium wanjuense DSM 17724^T	24.8 (16.8)
Chryseobacterium gambrini DSM 18014^T	10.4 (10.5)

Values in parentheses are results of measurements in duplicate

On the basis of the physiological, chemotaxonomic, and phylogenetic data, strain WR21 represents a novel species within the genus *Chryseobacterium*. Here, we named *Chryseobacterium* sp. strain WR21 as *Chryseobacterium nankingense* sp. nov., as it was isolated from the rhizosphere of a tomato field near Nanjing (Huang et al. 2013).

Competition for nutrients between *Chryseobacterium nankingense* strain WR21 and *Ralstonia solanacearum*

Forty-eight nutrients were tested in this study. Twenty of them could be utilized by *C. nankingense* strain WR21 and 23 by the pathogen *R. solanacearum* (RS). Among these nutrients, nine were utilized by both *C. nankingense* WR21 and RS (Table 4).

Chryseobacterium nankingense WR21 exhibited different suppression effects when grown in the presence of different nutrients. Suppression effects greater than 40% were observed with six nutrients: L-Asparagine (Asp), L-Glutamine (Gln), L-Histidine (His), L-Leucine (Leu), Myo-Inositol (Ino), and 2-Oxoglutarate (Oxo). The strongest effect was

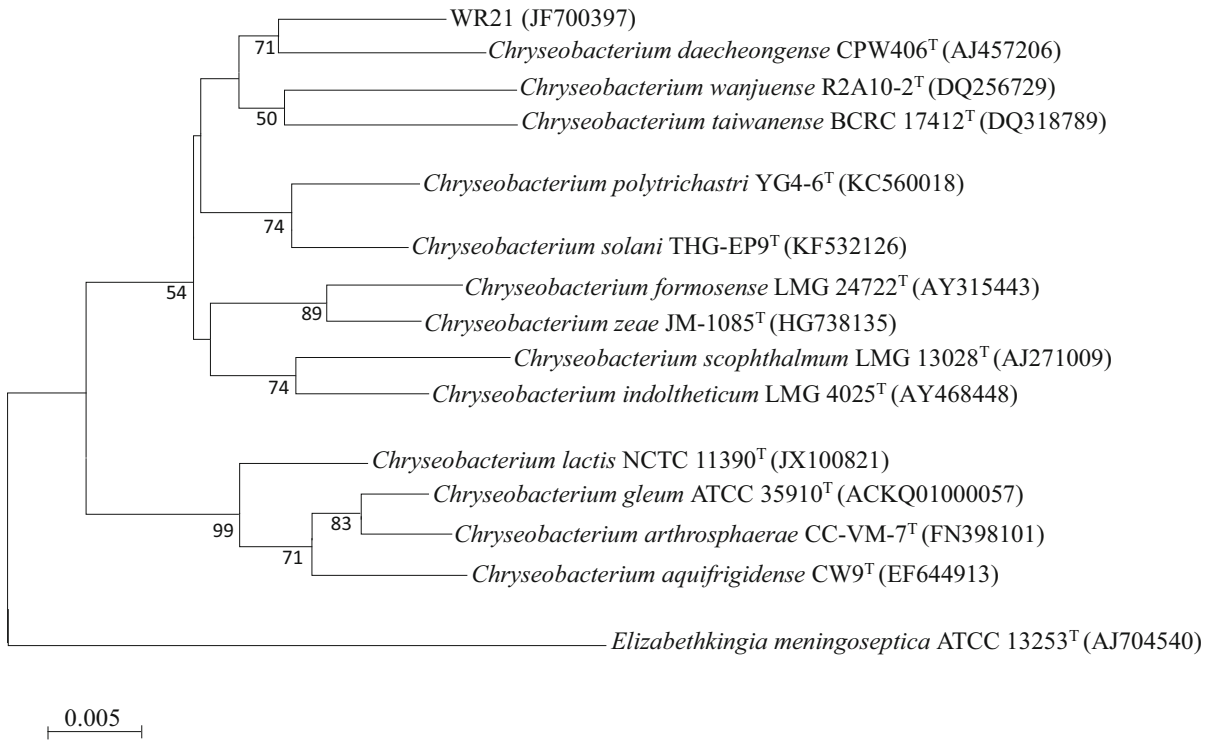

Fig. 1 Dendrogram of strain WR21 among species of the genus *Chryseobacterium*. It was obtained by using 16S rRNA gene sequences and distance matrix (Neighbor-Joining) analysis, species of some genera within the family *Flavobacteriaceae* were used to define the root. *Numbers* at branching points refer to bootstrap values (1000 re-samplings, only values greater than 50% are shown). *Bar* indicates two substitutions per 100 nucleotide positions

observed with Asp (57.6%), which was significantly higher than other nutrients excluding Gln ($F = 197.1$, $df = 8, 18$, $P < 0.001$). *C. nankingense* WR21 exhibited lower than 30% suppression (29.2, 19.0, and 12.4%, respectively) with remaining three nutrients Glucose (Glu), L-Proline (Pro), and L-Threonine (Thr) (Fig. 2a). *C. nankingense* WR21 exhibited a higher average growth rate (i.e. OD_{600} h^{-1}) compared to *R. solanacearum* with seven out of nine common nutrients (Asp, Glu, Gln, His, Leu, Ino, and Oxo) and presented a lower average growth rate than *R. solanacearum* with two nutrients (Pro and Thr). The highest and lowest growth rates were both exhibited by *C. nankingense* WR21 with Gln and Pro, respectively, and the highest one was significantly higher than all others excluding Ino ($F = 96.8$, $df = 8, 18$, $P < 0.001$) (Fig. 2b). We analyzed the linear relationship between the difference of the average growth rates between *C. nankingense* WR21 and RS, the D-value was calculated from the average growth rates of *C. nankingense* WR21 to RS with the unit of $\triangle OD_{600}$ h^{-1}, and the suppressive effects. The results showed a positive relationship ($r = 0.785$, $P < 0.05$) (Fig. 2c).

Competition for tomato root exudates between *Chryseobacterium nankingense* strain WR21 and *Ralstonia solanacearum*

Chryseobacterium nankingense WR21 exhibited a higher growth rate than *R. solanacearum* in root tissue exudates of tomato in monoculture conditions, and the OD_{600} of *C. nankingense* WR21 remained higher than that of *R. solanacearum* throughout the experiment. At the end of the experiment, the OD_{600} of *C. nankingense* WR21 was 0.521, whereas the OD_{600} of *R. solanacearum* was 0.464 (Fig. 3a). Compared to the monoculture conditions, the growth of *R. solanacearum* was significantly inhibited in co-culture experiments ($P < 0.05$) (Fig. 3b). The fluorescence of *R. solanacearum* ranged from 1217.9 down to 634.5 at the end of the experiment (72 h after cultivation), and the suppression effect was calculated as 47.9%.

Table 4 Different nutrients utilized by *Chryseobacterium* sp. strain WR21 and the pathogen *Ralstonia solanacearum* tagged with the pYC12-mCherry plasmid (RS).

Nutrients	Utilization by strains		Nutrients	Utilization by strains	
	WR21	RS		WR21	RS
Acetic acid	−	+	Myo-Inositol	+	+
L-Alanine	−	+	2-Oxoglutarate	+	+
β-Alanine	−	+	L-Phenyalanine	+	−
L-Arginine	+	−	L-Proline	+	+
L-Asparagine	+	+	Pyruvic acid	−	+
Citric acid	−	+	Succinic acid	−	+
Citrulline	−	+	Sucrose	−	+
Ethanolamine	−	−	Tartaric acid	−	+
Fructose	−	+	L-Threonine	+	+
Galacturonic acid	−	+	L-Tryptophan	+	−
Glucose	+	+	L-Valine	+	−
L-Glutamine	+	+	Maltose	+	−
L-Histidine	+	+	L-Arabinose	+	−
Isoleucine	+	−	D-Galactose	−	+
L-Lysine	+	−	D-Mannose	+	−
L-Leucine	+	+	D-Ribose	+	−
Malic acid	−	+	D-Mannitol	−	+
L-Methionine	−	−	Inosine	+	−

"+" indicates positive utilization of nutrients, while "−" indicates negative activity

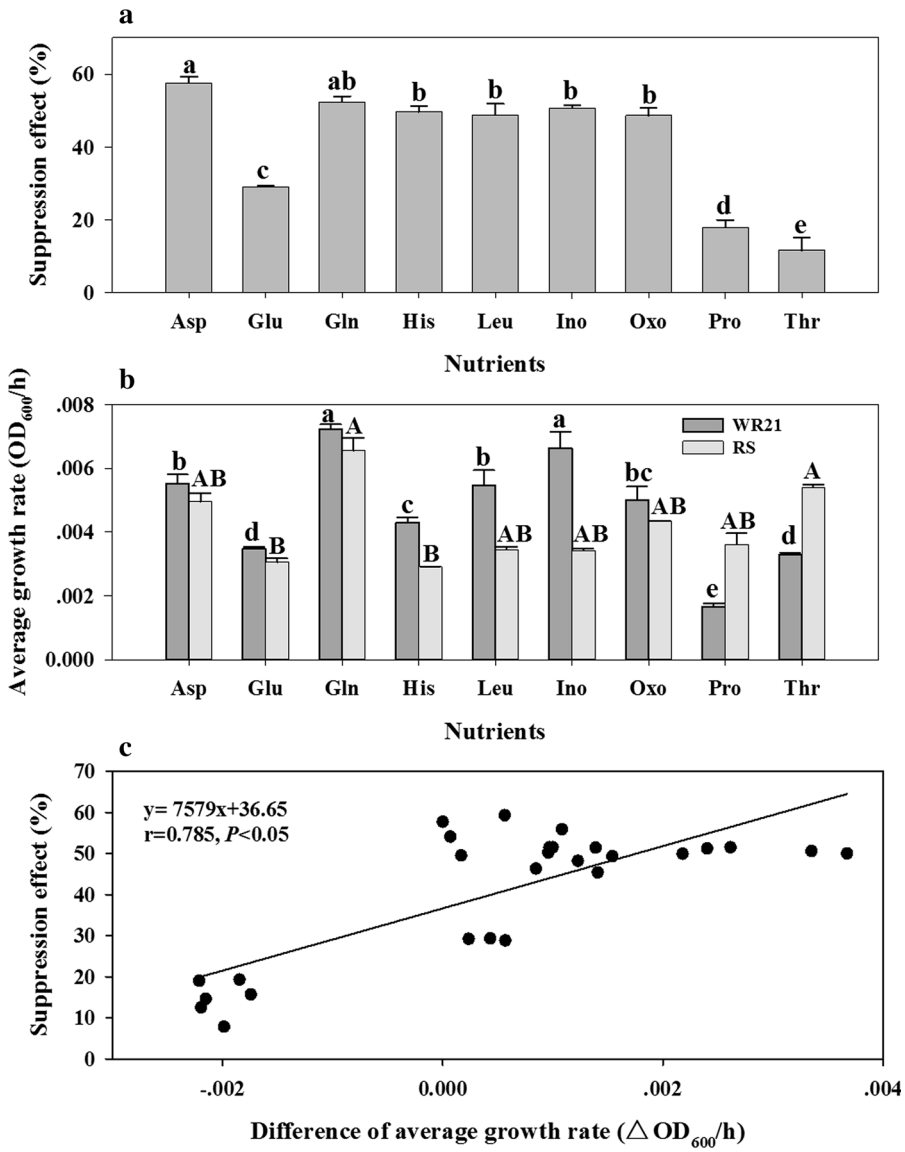

Fig. 2 Suppression effect (**a**) and average growth rate (**b**) of *Chryseobacterium nankingense* strain WR21 and/or the pathogen *Ralstonia solanacearum* tagged with the pYC12-mCherry plasmid (RS) in medium containing nutrients utilized by both of them and linear relationship between the suppression effect and the difference in the average growth rate (D-value) of the two strains (**c**). *Error bars* represent the SD between replicates. *Different lower-case letters* (or *upper-case letters*) on the *error bars* indicate significant differences ($P < 0.05$) among the growth rate of strain WR21 (or RS) in different nutrients. The line added in Fig. 2c was computed by the linear relationship of D-value calculated from average growth rates of WR21 to RS and the suppression effects

Discussion

The genus *Chryseobacterium* was first proposed in 1994 and comprised 63 species Vandamme et al. (1994). (Vandamme et al. 1994; Euzéby 1997). However, the novel taxa are continuously emerging within this group, some of which are pathogenic to humans and animals as well (Bernardet et al. 2005). Strains belonging to this genus are distributed among a wide variety of habitats, such as plants, soil, sewage, freshwater, marine sediments, clinical environments, and the food processing industry. The importance of

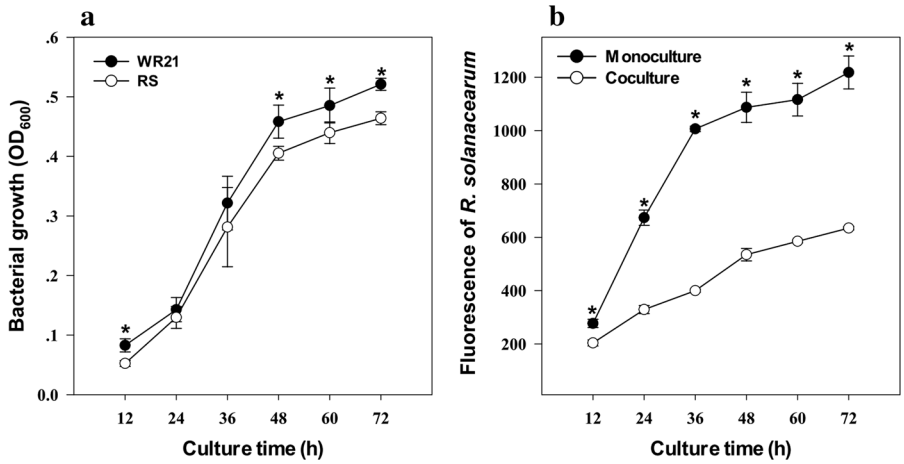

Fig. 3 Dynamic growth of *Chryseobacterium nankingense* strain WR21 and *Ralstonia solanacearum* in tissue exudates at 12, 24, 36, 48, 60, and 72 h after cultivation. **a** *C. nankingense* strain WR21 and *Ralstonia solanacearum* grew alone. The growth ability was demonstrated as OD_{600}. **b** The dynamic fluorescence change indicated the growth of *Ralstonia solanacearum* tagged with the pYC12-mCherry plasmid grew alone or co-cultured with *C. nankingense* strain WR21. *Error bars* represent the SD between replicates. *Asterisk* on the *error bar* indicates that there was a significant treatment effect at each evaluation time point, based on *t* tests ($P < 0.05$)

Chryseobacterium can be recognized from the recent studies demonstrating that the genus could serve as a novel source of bioactive compounds, such as proteases that help them to survive under specific environments (Wang et al. 2008). In addition, secondary metabolites confer a selective and competitive advantage to the bacteria that produce them against other microorganisms, further contributing to the rhizo-competence and root colonization abilities of the competent biocontrol agents (BCAs) (Compant et al. 2010; Lugtenberg and Kamilova 2009).

Chryseobacterium nankingense WR21 was able to compete effectively with *R. solanacearum* for unique compounds and root tissue exudates, which provide a partial explanation for the underlying mechanism for the colonization ability exhibited by *C. nankingense* WR21 (Huang et al. 2013). Although, rhizosphere environment is lucrative, it is pertinent to note that the concentrations and variety of nutrients in the rhizosphere soil are much greater than those in the bulk soils. Also, their composition change with respect to plant age, physiology and interactions with the environment. Hence, for greater adaptability and performance, the BCAs must compete for efficient nutrient and niche acquisition in order to survive in the rhizosphere (Haichar et al. 2014; Ziegler et al. 2013). Once installed, these BCAs outcompete the pathogens and hence the disease incidence is decreased. In this study, among the tested nutrients, four amino acids (Asp, Gln, His, and Leu; Fig. 2a) strongly supported the suppression of the *R. solanacearum* growth by *C. nankingense* WR21. Positive contribution of higher amounts of amino acids in exudates in enhancing bacterial chemotaxis towards roots has been reported, such as the chemotaxis of *Pseudomonas fluorescens* towards the root exudates in solarized soil (Weger et al. 1987; Gamliel and Katan 1992). The specific nutrient utilization by proficient colonizers might distinguish them from less efficient colonizers (Oksinska et al. 2011).

In conclusion, we found a novel biocontrol agent *Chryseobacterium nankingense* sp. nov. WR21 that could effectively compete for nutrients (especially certain amino acids) with *R. solanacearum*. This effect might aid *C. nankingense* WR21 in its successful colonization of the tomato rhizosphere, where it could suppress bacterial wilt disease caused by *R. solanacearum*.

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