International Journal of Agricultural Technology 2023 Vol. 19(2):833-848
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Straw microorganisms with combined cellulolytic and chitinolytic activities drive decomposition of rice straw and antagonism against *Rhizoctonia solani* causing rice sheath blight

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Xuan, D. T., Rosling, A., Alström, S., Khuong, N. Q., Dung, T. V., Trinh, N. T. D. and Högberg, N. (2023). Straw microorganisms with combined cellulolytic and chitinolytic activities drive decomposition of rice straw and antagonism against *Rhizoctonia solani* causing rice sheath blight. International Journal of Agricultural Technology 19(2):833-848.

Abstract Decomposition of cellulose and chitin substrates plays an important role in ecosystem functions. The relationships between the microbial diversity and the ecosystem functions of decomposition and antagonistic processes were examined using rice straw. A microcosm was designed for investigating complexity in microbial interactions of 16 different microorganisms belonging to 6 different functional groups and were designated as generalists or specialists depending on the number of hydrolases produced. Multiple regression analysis showed that increasing number of isolates, the presence of three fungal and one bacterial isolate and two fungal isolates degraded significantly to the decomposition process of rice straw. Straw was decomposed by one fungus and one bacterium, three fungal and two bacterial isolates and the 16-isolates mixture were significantly inhibited growth of the phytopathogenic *Rhizoctonia solani* causing rice sheath blight on the decomposed straw.The growth of *R. solani* was reduced significantly by 62% - 93% compared to the non-inoculated controls. There was a negative correlation between rice straw weight loss and the growth of *R. solani.* It is suggested that the specific isolates may play an important role in ecosystem functions of decomposition and antagonistic processes.

Keywords: Rice straw weight loss; Antagonism; Functional group; Cellulolytic; Chitinolytic micro-organisms

Introduction

In nature, cellulose and chitin are the most abundant organic components (Boer *et al.*, 2005; Li, 2006). Their degradation is an important feature in the global recycling of carbon (C) and nitrogen (N) and is primarily a microbial

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process (Gooday, 1990). Bacteria and fungi are the key agents involved in the degradation of organic material because they are among the few organism groups in soils that produce enzymes capable of degrading recalcitrant compounds (Romani *et al*., 2006) and convert these polymeric molecules into smaller ones that ultimately can be assimilated (Chróst, 1991). After addition of cellulose to an agricultural soil, an initial phase featuring predominantly its bacterial decomposition has been recognized, followed by a stage dominated by fungal decomposition (Minerdi *et al*., 2001). The degradation rate of chitin in soil appears to be similar to that of cellulose. In fungi, chitinase(s) have a physiological as well as a nutritional role in relation to their mycoparasitic activity. Although bacteria do not contain chitin as a native cell component, numerous bacteria are able to hydrolyze chitin during the interaction with other organisms containing chitin in their cell walls (Wohl and Mc Arthur, 2001).

Diversity represents the range of significantly different kinds of organisms and their relative abundance in natural habitats. Biodiversity and C-cycling have been focused to do research in recent decades because of environmental changes induced due to various anthropogenic activities which are likely to continue (Nielsen *et al.,* 2011). Researchers have examined how microbial communities varying in species composition and diversity influenced the decomposition process of plant residues, and thus released of plant available nutrients. Some studies have been shown that microbial diversity enhances the decomposition rate while contradicting reports have found to be no effect of the microbial diversity in decomposition rate or declined in decomposition rate, or the presence of specific members of the microbial community rather than changes in the diversity per se, and determined the decomposition rate (Murray and Woodward, 2003; Heemsbergen *et al*., 2004; Hättenschwiler *et al*., 2005; Nielsen *et al*., 2011; Yang *et al*., 2016). The nature of these different types of interactions, inhibitory, neutral or facilitative might be related to the degree to which species involved differently in their impact on soil processes (Heemsbergen *et al*., 2004). The effect of bacterial co-inoculation with white rot fungi, *Heterobasidion annosum*, *Resinicium bicolor*, and *Hypholoma fasciculare* on spruce wood blocks, the degradation of the wood tended to be consistently higher in co-inoculated blocks than in the control blocks which inoculated with fungus alone, even though wood decay could not directly be described to the bacteria itself (Murray and Woodward, 2003). Wohl *et al.* (2004) found in a controlled environment that greater species richness of cellulolytic bacteria supported a greater number of individuals and subsequently higher rates of total cellulose degradation than fewer one.

In paddy fields, pathogens can thrive in presence or absence of a plant host. Among them sheath blight caused by *Rhizoctonia solani* is considered an

economically important soil-borne fungal pathogen because of its ability to cause both pre- and post-damping off in many crops including rice (Mendes *et al.*, 2011). *R. solani* can survive in the field as a saprophyte or in a dormant form as sclerotia. Crop residues are colonized by the pathogen may play an important role in epidemics due to sheath blight in intensive rice production systems (Cu *et al*., 1996). The primary infection of *R. solani* on rice seedlings is very important for the later spread and development of this disease (Cuong *et al.*, 2010). Early removal of *R. solani* colonized plant residues from the field reduces sheath blight and makes epidemics less severe at later stages of plant growth. Levels of maturity of composts have been found to affect their disease suppressive activity. Nelson *et al*. (1983) found that substrates amended with fresh compost hardwood bark are conducive to damping-off caused by *R. solani*, while amendment with mature bark compost was consistently suppressive to *R. solani*. Chung *et al.* (1988) found a positive relationship between disease suppressiveness and the degree of decomposition.

The aims of this study were to investigate the effects of microbial and functional diversity on rice straw decomposition under aerobic conditions and to what extent microbial decomposition of straw is linked to antagonistic ability of the decomposed straw.

Materials and methods

Straw-degrading microorganisms

Ten bacterial and 6 fungal isolates were selected from previously isolated rice-stubble material and representing different cellulolytic and chitinolytic activities as described by Xuan (2012), Xuan *et al*. (2016). The nifH gene was identified (Zehr and Turner, 2001) for two isolates which out of the the ten bacterial isolates, and were designated as potential diazotrophs in this study. The isolates were identified by the partial sequence of the 16S rRNA (Xuan *et al*., 2012) and ITS regions (Kennedy *et al*., 2005) respectively. All sequences were deposited in GenBank (Table 1).

The isolates were categorized into 6 different functional groups based on their ability to produce different level of cellulase(s) and chitinase(s) enzymes as 1= strong cellulolytic (S cellu) fungi, 2= weak cellulolytic and moderate chitinolytic (W cellu + M chit) fungi, 3= strong cellulolytic (S cellu) bacteria, 4= strong cellulolytic and strong chitionlytic (S cellu + S chit) bacteria, 5= weak cellulolytic and strong chitinolytic (W cellu + S chit) bacteria, and 6= weak cellulolytic and nitrogen-fixing (W cellu + N-fixing) bacteria (Table 1). In our study isolates with cellulase production were designated as specific

isolates while those with two functional traits were designated as highly specific isolates.

Microcosm and experimental design

Table 1. Functional and taxonimic characterization of ten bacterial and 6 fungal isolates included in this study

Isolate ID	Best match in Genbank[a]	Percentage of similarity[b] (%)	Activity[c]		N fixing gene[d]	Functional group[e]	Category S/G[f]	Accession number[g]
			Cellulolytic	Chitinolytic				
Fungi								
Sar	*Sarocladium oryzae*	100	+++	-	-	1	S	JQ582425
Dot	*Massariosphaeriatyphicola*	89	+++	-	-	1	S	JQ582427
Gib	*Fusarium* sp.	100	+++	-	-	1	S	JQ582429
Sta	*Stachybotrys bisbyi*	99	+	++	-	2	G	JQ582426
Rhi	*Rhizomucor variabilis*	99	+	++	-	2	G	JQ582428
Den	*Dendryphiella* sp. BR354	94	+	++	-	2	G	JQ582430
Bacteria								
Ba1	*Bacillus* sp.	97	+++	-	-	3	S	JQ438848
Ba2	*Bacillus pumilus*	100	+++	-	-	3	S	JQ438851
Pan	*Pantoea dispersa*	100	+++	-	-	3	S	JQ438850
Fle	*Chitinophaga pinensis*	98	+++	+++	-	4	G	JQ438849
Bur	*Burkholderia gladioli*	99	+++	+++	-	4	G	JQ438853
Se1	*Serratia marcescens*	99	+	+++	-	5	G	JQ438852
Se2	*Serratia marcescens*	99	+	+++	-	5	G	JQ438846
Ba3	*Bacillus pumilus*	98	+	+++	-	5	G	JQ438845
Rhiz	*Rhizobium* sp.	98	+	-	Y	6	G	JQ438844
Her	*Herbaspirillum* sp.	99	+	-	Y	6	G	JQ438847

[a] Based on ITS region and 16S rRNA gene sequencing for fungal and bacterial isolates, respectively, [b] Percentage similarity to closest relative in GenBank, [c] Functional characterization of cellulolytic and chitinolytic activity. +++ strong activity; ++ moderate activity; + weak activity; - no activity, [d] N_2- fixing gene in bacteria identified by the nested PCR protocol. Y means presence of this gene,[e] Defined from c and d, [f] S means specialist having one trait; G means generalist having two traits

A mixture of rice straw was collected from the two fields after rice harvest which dried at 60 ℃ and then chopped into approximately 5 cm pieces. Five different treatments were set up with either single isolate inoculation or inoculation with different mixtures of isolates and functional groups: 1) 16 sets with 1 isolate (1iso:1f), 2), 6 sets with 2 isolates from one functional group, (2 iso:1f), 3) 13 sets with 2 isolates from 2 functional groups (2 iso:2f), 4) 4 sets with 6 isolates from 6 functional groups (6 iso:6f), and 5) one set with all 16 isolates from 6 functional groups (16 iso:6f) (Table 2). Isolates representing the specific functional groups were randomly selected for mixed treatments. All the isolates were multiplied on 1.5% TSBA and Hagem broth (HB; 0.5 g L^{-1} NH_4NO_3, 0.5 g L^{-1} KH_2PO_4, 0.5 g L^{-1} $MgCl_2$. $7H_2O$, 5 g L^{-1} malt extract, Merck and distilled water) for bacteria and fungi, respectively. Fresh inocula were prepared by suspending fresh cultures of bacteria in tryptic soy broth (10 g L^{-1}, Merck and distilled water) and careful blending fresh fungal mats in 1% HB.

Table 2. The microcosm study of five treatments including single straw associated isolates and different mixtures of straw associated isolates representing different functional groups and categories

Treatments	**1 iso[a]:1f[b]**	**2 iso:1f**	**2 iso:2f**		**6 iso:6f**	**16 iso:6f**
	Sar Dot Gib	Gib+Sar	Gib+Den Sar+*Her*	Gib+*Bur*	Gib+Den+*Pan*+ *Bur*+*Se2*+*Rhiz*	Gib+Dot+ Sar +Den+ +Rhi+Sta+*Ba1*+*Ba2*+ *Pan*+ *Fle*+ *Bur*+*Ba3*+ *Se1*+*Se2*+*Rhiz*+ *Her*
	Sta Rhi Den	Den+Rhi	Dot+*Ba2*	Den+*Ba3*	Sar+Rhi+ *Ba2*+ *Fle* +*Se1*+*Her*	
	Ba1 *Ba2*	*Ba1*+*Pan*	Rhi+*Ba2*	*Ba1*+*Her*	Dot + Sta + *Ba1*+ *Fle* + *Se2*+ *Her*	
	Pan *Fle* *Bur*	*Fle*+*Bur*	*Ba1*+*Bur*	Sta+*Fle*	Dot + Rhi + *Pan*+ *Bur* + *Ba3*+ *Rhiz*	
	Se1 *Se2* *Ba3*	*Ba3*+*Se1*	*Ba2*+*Se1*	Dot+*Se1*		
	Rhiz *Her*	*Rhiz*+ *Her*	*Bur*+*Her*	Sta+*Rhiz*		
Number of sets	16	6	13		4	1

Regular style represents fungal isolates and italic style represents bacterial isolates.

Microcosms used for the experiment consisted of an autoclavable polyethylene bag (50 cm x 31 cm) containing 15 g dry-weight straw and distilled water adjusted to give 60% moisture in order to make the environment suitable for microbial growth. The bags were closed with a cotton plug and autoclaved twice for one hour each. The microbial suspensions were diluted to approximately 10^6 CFU mL^{-1} and injected aseptically into the microcosms (5 mL/microcosm) using a sterile syringe. The injection hole was sealed aseptically. For the mixed inoculations, an equal amount of each isolate was added to make a total of 5 mL/microcosm before inoculation.

The control microcosms were prepared in the same manner but injected with microbe-free nutrient broths. All treatments were performed in 3 replicates. Microcosms were spaciously placed in a growth room and incubated at 30°C for 6 weeks (with light/ dark regime of 12/12 hours). The moisture content of the rice straw in the microcosms was adjusted to 60% based on the weight of the bag during the whole experimental period by injecting SDW aseptically whenever necessary.

After six weeks, the straw was harvested and divided into two equal portions from each microcosm. The first portion of the decomposed straw was homogenized and 2 grams of decomposed straw of each replicate were transferred to sterilized falcon tube 50ml and freeze-dried before DNA extraction. The remained decomposed straws of these sets was used to calculate the percentage of straw weight loss (SWL) during the incubation experiment by drying them overnight in an oven at 105 ℃. The second sets of decomposed straws were transferred into petri dishes to study their antagonistic potential against *R. solani*.

DNA extraction, PCR amplification and pyrosequencing of the decomposed straw from all treatments

All decomposed straw freeze-dried sample sets were crushed by using Robot coupe (France) and homogenized before DNA extraction. Five milligrams of crushed straw were used for DNA extraction using 1ml extraction buffer (3% CTAB, 2.6M NaCl, 0.15M Tris- HCl, 2mM EDTA, and pH 8). The mixture of crushed straw was incubated at 65°C for 1 hour. After centrifugation, the supernatant was further extracted with chloroform (1:1), precipitated by 1.5 volume of cold isopropanol, the pellet was washed with cold ethanol 70% and dissolved in 50 μL miliQ water. DNA samples were purified using the JetQuick DNA DNA purification kit (Genomed GmbH, Lohne, Germany). The DNA samples were used for pyrosequencing of both bacterial and fungal treatments, see details below.

For the bacteria-treated samples, the primer pair 341F (CCTAYGGGRBGCASCAG)-tags/806R (GGACTACNNGGGTATCTAAT) targeted the V3 and V4 regions of the 16S rRNA (Sundberg *et al.,* 2013). PCR amplification was conducted in a 2720 Thermal Cycler (Life Technologies) in 50 μL reaction mixture of 5 ng μL^{-1} of DNA, 200 nM of each nucleotides, 1.5 mM $MgCl_2$, 0.2 μM of each primers, 0.025U μL^{-1} polymerase (DreamTaq Green, Thermo Scientific, Waltham, MA) in buffer. The PCR reaction was conducted with 5 min at 95 ℃; 30 cycles of (1min at 95 ℃; 1 min at 55 ℃; 2 min at 72 ℃); 15 min at 72 ℃.

For fungi-treated samples, the primer pair ITS7(GTGAATCATCGAATCTTTG)/ITS4(TCCTCCGCTTATTGATATGC)-tags targeted the ITS regions of the fungi (Ihrmark *et al.,* 2011). PCR amplification was conducted in a 2720 Thermal Cycler (Life Technologies) in 50 μL reaction mixture of 5ng μL^{-1} of DNA, 200nM of each nucleotides, 2.75 mM $MgCl_2$, 0.3 μM of ITS4-tags, 0.5 μM of ITS7, 0.025U μL^{-1} polymerase (DreamTaq Green, Thermo Scientific, Waltham, MA) in buffer. The PCR reaction was conducted with 5 min at 94 ℃; 35 cycles of (30 s at 94 ℃; 30 s at 56 ℃; 30 s at 72 ℃); 7 min at 72 ℃.

All PCR products of either bacterial or fungal samples were purified using the AMPure kit (Beckman Coulter, Brea, CA). Concentrations of the samples were quantified using a Qubit® 2.0 Fluorometer (Invitrogen, USA), and PCR products were mixed in equimolar proportion into a pooled sample, freeze-dried and used for 454-sequencing after addition of sequencing adaptors by ligation. Adaptor ligation and sequencing was performed by LGC Genomics GmbH (Berlin, Germany) on a GLFLX Titanium system (Roche, Basel, Switzerland).

The 16S rRNA and ITS pyrosequencing were processed using the RDP pyrosequencing pipeline (http://pyro.cme.msu.edu/). After quality trimming following the defined parameters on the RDP pipeline, the different samples were retrieved as FASTA files based on the tag added into the samples. The FASTA file was completed with sequence(s) of single isolate(s) inoculated in the straw. They were aligned together and the alignment file was further clustered to determine whether inoculated isolates still were present after 6 weeks of the experiment. The fungal ITS data was also processed using the SCATA pipeline (http://scata.mykopat.slu.se/) which gave congruent results with RDP.

Effects of decomposed straw on pathogen growth

The second portion of the decomposed straw from the 16 single isolate sets (1iso:1f), the set of mix of 16 isolates (16 iso:6f) and the non-inoculated

control were inoculated with *R. solani* TG-05. The pathogen was originally isolated from paddy soil in the Tien Giang province, Vietnam (Cuong *et al.*, 2010). A mycelia plug (6 mm diameter) from a 3-day-old *R. solani* culture was inoculated aseptically on the surface of the decomposed straw (10 g per plate) in Petri dishes (84 mm diameter). The three replicates from the microcosm were used as three separate replicates in this sub-study. The inoculated plates were incubated at 25 ℃ for 4 weeks with 12 hours light/ 12 hours dark intervals. The moisture of the straw was maintained at about 60% during the entire experimental period. The growth of *R. solani* was measured as the diameter of mycelial growth at the end of the experiment.

Statistical analyses

To achieve normal distribution, the percentage of SWL was arcsine square root transformed and the diameter of *R. solani* growth was transformed as ranked in ascending order of the raw values. One-way ANOVA was used to test the differences in SWL among treatments, between bacteria, fungi, bacterial-fungal co-inoculations and non-inoculated controls, and to compare the growth of *R. solani* on straw decomposed by single and mix inoculants (1iso:1f, 16 iso:6f and control). The effect of number of isolates, functional groups, and all single isolates was evaluated by multiple regressions using a general linear model. A least significant difference (LSD) test was used for pairwise comparisons of the growth of *R. solani* in sets within the 2 iso:2f treatment for straw decomposition and sets of 1 iso:1f, 16 iso:6f in comparison to the non-inoculated control. The correlation between SWL and the growth of *R. solani*, as well as the relationship between the number of inoculated microorganisms and their survival, was estimated by a linear regression model.
All statistical analyses were performed in the software package STATISTICA (data analysis software system, version 10. www. statsoff.com. StatSoft, Inc.2011).

Results

An overall significant effect of inoculations was observed 6 weeks after incubation on SWL ($P<0.05$) compared to that in the non-inoculated control. The SWLs ranged on average from 11% in the 1iso:1f treatment to 20% for the mixed treatment (16 iso:6f) with the highest number of isolates. The means of SWL in treatments containing more than two functional groups were also significantly different from the control (Figure 1). The inoculated fungi and bacteria generally survived over the course of the experiment but some were

extinct or could not be detected with the chosen sequencing-method. Survival data for each treatment is given in Figure 1b.

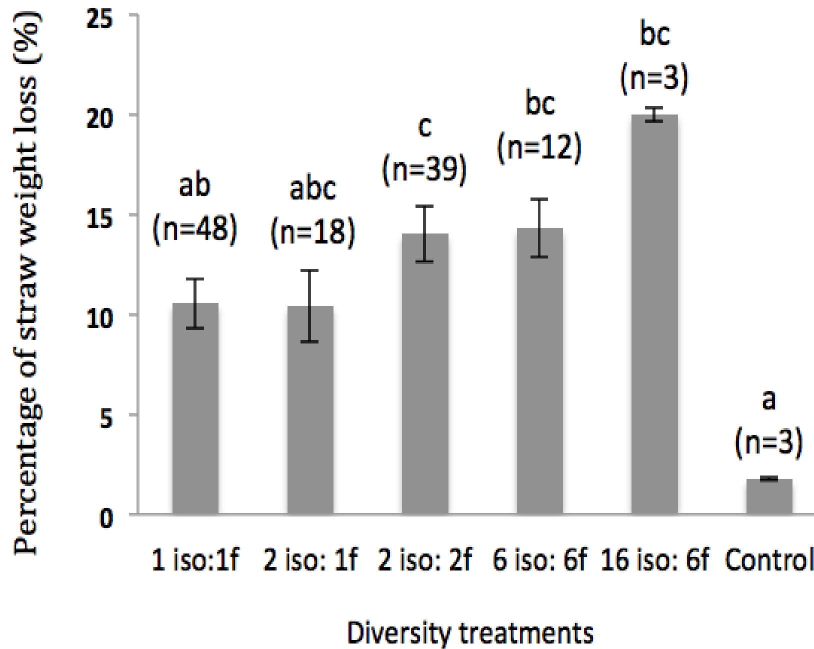

Figure 1. Percentage of straw weight loss of the survival of straw microorganisms after 6 weeks of incubation of 5 treatments of 16 different straw associated fungi and bacteria inoculated singly or in mixture with different functional groups (values are mean ± SE, n shows replicates of the treatment); iso means isolate and f means functional group. Same letters donates no significant difference at P >0.05

The multiple regression analyses showed that the five fungi identified as *Stachybotrys bisbyi* (Sta), *Fusarium* sp. (Gib), *Sarocladium oryzae* (Sar), *Dendryphiella* sp. BR354 (Den) and *Rhizomucor variabilis* (Rhi), and one bacterium *Burkholderia gladioli* (Bur) as well as the increasing number of isolates in the mixtures contributed significantly to the SWL value (P<0.001). The Sta designated as a generalist proved to be the strongest decomposer (F= 46.38, P<0.001). The second most important factor was an increasing number of isolates (F=31.99, P<0.001), (Table 3). The fungal high specific isolates; Sta, Rhi and Den contributed more effectively to SWL than the fungal specific isolates, Sar and Gib (Table 3).

Table 3. SWL caused by different single isolates and an increasing number of isolates from multiple regression analyses that explained the general diversity effect on rice straw decomposition

Factors	Category	d.f.[c]	MS	F
Intercept		1	4553.2	98.9***
Sta	G[a]	1	2135.4	46.4***
Increasing number of isolates	G+S	1	1472.1	32.0***
Rhi	G	1	1288.9	28.0 ***
Den	G	1	597.2	13.0 ***
Sar	S[b]	1	546.1	11.9***
Gib	S	1	501.8	10.9**
Bur	G	1	448.1	9.7**
Error		115	46.0	

Regular style represents fungal isolates and italic style represents bacterial isolates; [a] G means generalist. [b] S means specialist. [c] d.f. means degree of freedom. *P< 0.05; **P < 0.01; ***P< 0.001

Standard error (SE) of the SWL within treatments decreased with the increasing number of isolates and functional groups (y = - 0.27x + 2.12; R^2= 0.4). The SWL in the microcosms inoculated with either fungal isolates only or those with a mixture of bacterial and fungal isolates was significantly higher than in microcosms inoculated with bacteria only and the non-inoculated controls (P<0.001) (Figure 2a). It found a positive correlation (y=0.62x - 0.01, R^2= 0.76 (p<0.001) between the number of inoculated microorganisms and the number of surviving microorganisms were detected by means of pyrosequencing at the end of the experiment.

The growth of *R. solani* was significantly reduced as a whole on the decomposed straw (P= 0.007) compared to its growth on the straw from the non-inoculated control sets when observed four-week after inoculations, (Figure 2b). The growth of *R. solani* on the straw decomposed by the specialist fungus Gib and the bacterium *Pantoea dispersa (Pan)*, the high specific isolated fungi: Den, Rhi, Sta and bacteria: *Bur, Ba3* as well as by the mix inoculant of 16 isolates was significantly reduced from 62% to 93% depending on the treatments compared to the control sets (P<0.05) (Figure 2b). In addition, there was a negative correlation between the SWL and the growth of *R. solani* (R^2= 0.38, P<0.001).

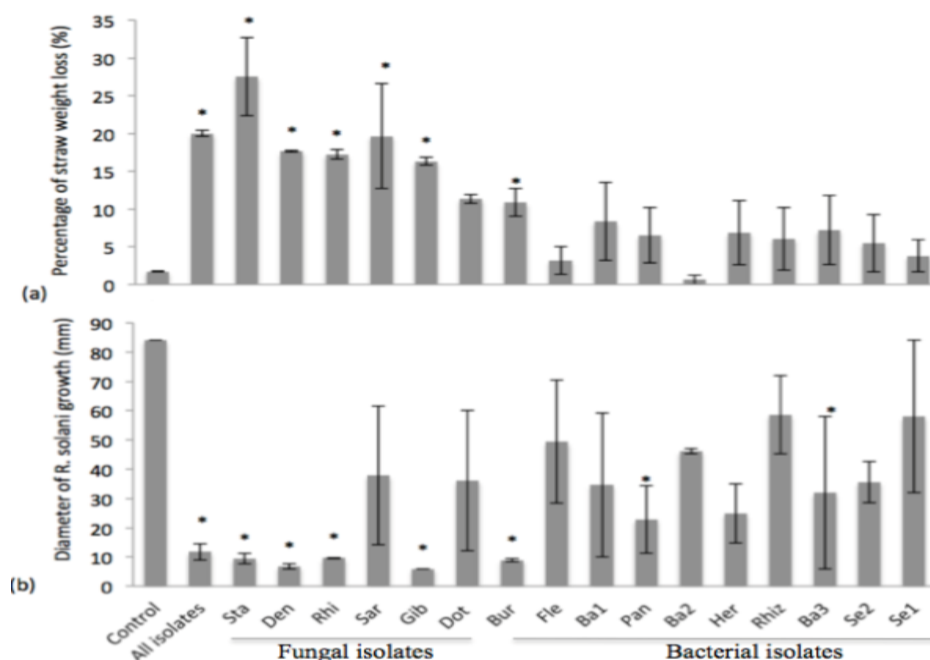

Figure 2. Effects of the 16 single isolate sets and the 16-isolate-mixture set on (a) the SWL and (b) on the growth of *R. solani* (values are mean ± SE, n= 3). *P< 0.05 compared to the control set

Discussion

Our results demonstrated that an increasing number of isolates seems to increase in the percentage of straw weigh loss decomposition rate. Similar patterns were reported in other earlier studies reviewed by Nielsen *et al*. (2011). The increasing in decomposition may be explained by a complementary interaction of non-overlapping nutrient resources (Naeem *et al*., 1999) or inclusion of the effective isolates in high diversity treatments. *In vitro* study, Wohl *et al*. (2004) found facilitative effects on cellulolytic bacterial species utilizing cellulose as a sole carbon source. It is observed in the study that both diversity and single isolate effects on the effective straw decomposition and the SWL which caused by the increased number of isolates was weaker compared to the single inoculation with *Stachybotrys* sp. Schimel (1995), Brussaard (1997) and Groffman and Bohlen (1999) suggested that the species–specific composition of microorganisms affects cellulose decomposition. This is also evident in our study where the inoculation with the strong straw-decomposer *Stachybotrys* sp. exhibited weak cellulolytic and moderate chitinolytic activity in a mixture with other isolates which had a significant impact on the

decomposition process. Another possible explanation is that both complementary and competitive interactions occur simultaneously. The reduced variation in decomposition rates with increased diversity that agreed well with the theoretical predictions, as well as examples from other systems, that species richness may be important for the stability of ecosystem functions, including aspects of C cycling (Yachi and Loreau, 1999; Loreau *et al.,* 2001).

It is reported to be the first time to include the number of isolates and the functional diversity of both bacteria and fungi to their effects on organic matter decomposition. Our results are in line with those reported by Schneider *et al*. (2010) who investigated the proteome of one fungus and one bacterium involved in beech leaf litter *(Fagus sylvatica)* decomposition. The authors found that the fungus played a key role as a decomposer and the bacterium was a cheater which took advantage of the decomposing activity of the fungus. In this study, the fungi proved to be the most important group in rice straw decomposition and mix-isolate treatments. It is possible that some bacteria are able to utilize fungal-derived substrates. This assumption is consistent with the results of Romani *et al*. (2006) who found that enzymatic activities related to cellulose decomposition increased in treatments with fungi and a mixture of fungi and bacteria compared to those in treatments with bacteria alone. The authors studied the effects of the natural bacterial community and 5 fungal isolates obtained from an aquatic habitat on the decomposition of *Phragmites* leaves.

Our study found that microcosms inoculated with those denoted as fungal generalists performed best in terms of rice straw decomposition compared to those denoted as specific isolates. The bacterial high specific isolates, *Burkholderia* (*Bur*) was the only among bacterial isolates that significantly contributed to the rice decomposition in the microcosms. This observation is interesting its functional traits which are similar to the most effective generalist fungi. It has been postulated that a competitive exclusion favors specialists with competitive advantage over the generalists (Gause, 1932; Wohl *et al*., 2004). Our finding contradicted this postulate in both high specific isolates and specific isolates were presented among the best straw decomposers. The additive chitinolytic trait of specific isolates may give them a competitive advantage against fungi in the microcosm community although the trait may not directly be important for the straw decomposition.

Our results found a negative correlation between SWL and mycelial growth of *R. solani* with practical implications for rice crop health. It provided a link to previous observations through reduced pathogen outbreaks resulting from compost amendment in agriculture (Noble and Conventry, 2005). Our finding also found that an additional perspective of studying species with multi-

functional mechanisms influencing the ecosystem services such as nutrient recycling and antagonistic processes. For the cellulose decomposition process, most studies have focused only on the cellulolytic activity of the organisms (Nielsen *et al.,* 2011). However, the activities of chitinase(s) production and nitrogen-fixation, other than cellulolytic activity may play a role when microorganisms interact with each other to utilize the cellulose substrate. Our results found that the most effective isolates are combined traits as a vital for future understanding the relationship between the functional diversity and the ecosystem services such as microbe-mediated disease suppressiveness in composts. Rice straw decomposed with five high specific isolates with combined traits, the mix of 16-isolates with different levels of the two traits as well as the specific isolates 1 fungus and 1 bacterium with one trait only (cellulolytic activity), inhibited the growth of the phytopathogen *R. solani*. It could be explained by their effective utilization of chitinase(s) for encountering *R. solani* mycelia. Morissette *et al.* (2006) found that the expression of the endochitinase coding gene *sechi44* produced by *Stachybotrys elegans* was increased in the presence of *R. solani* cell wall. Benyagoub *et al.* (1994) reported that *S. elegans* had a strong ability to degrade the cell wall of *R. solani*. Our isolate *Stachybotrys* sp. (Sta) was shown to be effective in both straw degradation and antagonism of *R. solani* suggesting it to be a good future candidate for biological control agent.

Inhibition of *R. solani* may also be explained by mycotoxins produced by the inoculant fungal isolates. It is likely for the fungus *Fusarium* sp. (Gib) which is documented to produce antimicrobial substances with an effect against fungi (Praveena and Padmini, 2011), and possibly against *R. solani*. *Fusarium* sp. in our study did not produce chitinase(s), but it was still able to inhibit the growth of *R. solani*. It is possible that the inhibition of *R. solani* growth by *Fusarium* sp. is due to depletion of energy sources and/or the production secondary metabolites (Nelson *et al*., 1983). Surprisingly, among 10 bacterial isolates from the rice straw tested in this study, only the bacterium *Burkholderia* sp. (*Bur*) significantly degraded straw and inhibited the plant pathogen. *Burkholderia* sp. has previously been documented for its potential as a beneficial agent in agriculture (Anandham *et al*., 2009). Cuong *et al*. (2011) reported that *Burkholderia* sp. isolated from rice fields in Vietnam colonized *R. solani* hyphae and strongly inhibited its growth in both dual culture and water-surface microcosm assays. It is concluded that our results contribute to the understanding of competitive exclusion, ecosystem stability, and the relative roles of fungi and bacteria in straw degradation as well as in natural suppression of soil borne fungal pathogens under aerobic conditions.

Acknowledgements

This study was funded by The Ministry of Educational Training (MOET), Vietnam, The Swedish Institute (SI), Vetenskapsrådet and The Swedish university of Agricultural Sciences, Uppsala, Sweden.

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(Received: 26 May 2022, accepted: 30 December 2022)