(RESEARCH ARTICLE)

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A new method for controlling rice blast (*Magnaporthe grisea*) using effective microbes as biocontrol agents

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Abstract

Rice blast caused by *Magnaporthe grisea* is a noteworthy ailment influencing rice developed zone. Use of biocontrol agents (BCA) seen as an option in contrast to the utilization of synthetic fungicide since biocontrol is an environmentally friendly plant disease controlling approach. The goal of this exploration is planning to screen natural control agents for controlling *M. grisea*, and considering biocontrol is a natural un-hurtful plant disease controlling original thought. 639 bacterial isolates were screened from various parts of rice tissues, of which potential biocontrol scores were distinguished by their capacities in antagonism inhibition and secreting extracellular hydrolytic protein. Biocontrol tests against *M. grisea* were done on 30 bacterial isolates with potential biocontrol limit after pre-choice through ARDRA and novel investigation on strains with high assessment scores. 9 BCAs with clear biocontrol activity (over 50%) in greenhouse tests were obtained. *Bacillus cereus* H-L out 6 and *Bacillus subtilis* D-S in 61 performed well in greenhouse tests. In general, correlation coefficient is 0.80 between analysis points of 35 tried BCA strains and correlation coefficient between antagonism test and biocontrol efficacy demonstrate 0.76 against *M.grisea*.

Biocontrol efficacies results in greenhouse tests indicated positive correlation with analysis points, suggesting that the BCAs screening and method developed is adaptable for screening and controlling *M.grisea.*

Keywords: Biocontrol agents; Biocontrol efficacy; Extracellular metabolites; *Magnaporthe grisea*

1 Introduction

To a great extent plant, diseases and abiotic stresses have hampered worldwide rural profitability. Utilization of BCAS or microorganisms opposing to plant pathogens is an option and a maintainable method for plant insurance. Fruitful organic control in view of plant related adversaries not just requires superior information of the intricate direction of diseases concealment by opponents in light of biotic and abiotic factors, yet in addition requires a learning of the elements and organization of plant-related bacterial networks and what triggers plant colonization [1]. Moreover, screening of BCAs has principally centered on isolating organic control nitrogen-settling microorganisms [1].

Overseeing rice blast requires cautiousness and watchful incorporation of numerous procedures and methods learned in all parts of the world by singular rice maker. A solitary oversight can result in crop failure from this vital plant disease [2].

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This disease is a method that shows the reality, trickiness, and life span of some plant ailments. Rice blast has been generally examined all through the world. Numerous specialists have viewed it as a method ailment for the investigation of hereditary qualities, the study of disease transmission, atomic pathology of host parasite cooperation's and science [3], which has prompted scan for new BCAs from different sources, for agribusiness trim assurance. Utilization of BCAS or microorganisms hostile to plant pathogens is a feasible method for plant assurance. Effective natural control in view of plant related opponents not just requires superior information of the mind boggling direction of diseases concealment by rivals because of biotic and abiotic factors, yet in addition requires a learning of the elements and synthesis of plant-related bacterial networks and what triggers plant colonization [4]. In any case, research of screening proficient natural control specialists could be utilized to restrain the contagious pathogen (blast). In this investigation, an original thought for controlling rice blast (Magnaporthe grisea) using effective bacteria as biocontrol agents were examine.

BCAs new method based on antagonism inhibition ability, activities of extracellular hydrolytic enzymes and the banding designs gotten by amplified ribosomal DNA restriction analysis and (ARDRA), section sequencing were examine. Greenhouse tests are direct to test their practical effects in controlling M. grisea and the correlation is analyze between antagonism plus biocontrol efficacies and the assessment points plus biocontrol efficacies.

2 Material and methods

2.1 *M. grisea* culture

M. grisea strain *GUY11* was utilized in this investigation. Strain squares were kept up on strew decoction and corn (SDC) media at 25 $^{\circ}$C for five days oblivious taken after by 2 long periods of constant enlightenment under glaring light [22]. Biocontrol strains were refined on Luria-Bertani (LB) agar at 25°C for 2 days. Rice cultivar Lac23, utilized in this test is widely planted in Suakoko, Liberia. Plastic cups disinfected at 120 $^{\circ}$C for 30 min loaded up with rich humus soil from CARI Suakoko, Bong County, Liberia, were sown with rice seeds for three back to back days in greenhouse test. The greenhouse was disease and insect-free maintained at 25-40 $^{\circ}$C with relative humidity of 80% and 12 h/12 h day/night photoperiod (800-μmol photons/m^2/s of light provided amid the daytime).

2.2 Isolation of microbe's strains

Rice tests were gathered from Central Agricultural Research Institute (CARI), Suakoko Bong County in Liberia and bacterial strains were segregated from rice tissues of stems, endorhiza, rhizosphere, endosphere and phyllosphere separately (repeat in 3 tests). Three grams of fresh weight (FW) from rice tissues were separated into parts set into a cleaned Erlenmeyer flask and suspended in 27 mL of a clean 0.85% NaCl solution to isolate microbe strains. The suspension was incubated at 25 $^{\circ}$C with shaking at 180 rpm for 30 min and afterward settled 3 min; the resulting supernatant was serially diluted, plated on R2A medium plate (for soil tests) or Luria-Bertani (LB) agar (for tissue tests), and incubated at 28 $^{\circ}$C for 48 h to acquire cultures (form, color and texture) containing 50-500 CFU. Colonies with various morphologies from every microenvironment were transferred to LB agar, purified, and then stored at – 70 $^{\circ}$C in LB stock containing 40% glycerol as portrayed by [18].

2.3 Screen for antagonism towards *M. grisea*

WA medium (5 g peptone, 10 g glucose, 3 g beef extract, 5 g NaCl in 1 L medium) plate was partitioned into four equivalent segments by two vertical lines. Single colony of microbe strains were picked with sterile toothpick and cross-inoculated 3 cm from the center point of the plate along one vertical line antagonistic and left one plate without immunization of microorganisms as a control. *M. grisea* hyphae block (4 mm diameter circle taken on the edge of the culture) was put at the center point of a plate, inoculated at 28 ^{0}C for 72 h. The in vitro antagonistic activity was reviewed with 0, 1, 2, or 3 in view of the diameter (in mm) of the semicircular hyaline zones after 48 h: index (0) show no side effects; index (1), 1–5 mm; index (2), 5.1–10 mm; index (3), >10 mm.

2.4 Detection of activities of Extracellular hydrolytic enzymes and Siderophores

Microbe isolates were tested for in vitro activities of their extracellular hydrolytic compounds (cellulase, chitinase, glucanase, protease and siderophores), which were shown by distinct semicircular hyaline zones around microbe colonies on particular agar media. Cellulase action was resolved as described by [20], chitinase movement was tested in minimal medium [5], and [24] identified glucanase action. Skim milk agar (50 mL of sanitized skim milk blended at 55 ^{0}C with 50 mL of 1/5 WA medium containing 2 % agar) was utilized for the identification of protease action, which was shown by casein debasement [6]. Siderophore's appearance was resolved as beforehand [22].

2.5 ARDRA fingerprint investigation and Identification of microbes isolates

For ARDRA investigation, 89 microbes DNA was prepared utilizing the Mini BEST Bacterial Genomic DNA Extraction pack (TaKaRa Biotechnology Co., Ltd). The partial nucleotide sequence of the amplified 16S rDNA was resolved utilizing the accompanying primers: L1494-1514 (turn around) 5'- CTA CGG (AG) TA CCT TGT TAC GAC-3', U 8-27 (forward) 5'- AGA GTT TGA TC (AC) TGG CTC AG-3' in a computerized DNA sequencer (Yang et al., 2008). Amplification was performed with a Pettier Thermal Cycler PTC-200 (Bio-Rad, Watertown, MA, USA) utilizing an underlying denaturation step at 94 ^{0}C for 5 min, and in this manner 35 cycles of denaturation at 94 ^{0}C for 1 min, annealing at 56 ^{0}C for 2 min and expansion at 72 ^{0}C for 2 min, followed by a final extension at 72 ^{0}C for 10 min. The PCR products (10 µl) were processed for 2.5 h utilizing the restriction enzymes AluI and MspI. The restriction fragments were isolated on a mix gel (1.5% agarose + 2.25% Synergel) running in 1.0 × TBE buffer at 80 V for roughly 5 h, and stained with ethidium bromide, and captured under UV transillumination. We repeated the trial three times to confirm the reproducibility of the outcomes.

2.6 Greenhouse test

In Greenhouse, selected microbe isolates were developed in LB independently at 28 ^{0}C with 280 r/min for 24 h delicate shaking. At that point, microbe cells were pelleted by centrifugation, washed and suspended in a clean 0.85% NaCl arrangement, and acclimated to 5 × 10^3 CFU/mL with water for utilize. Thirty isolates were researched for their biocontrol efficacies in Greenhouse, each including 30 microbe treatments and 2 control treatments. Every treatment comprised of three replicates of 24 rice plants for each replicate.

Mycelia plugs of the fungus were develop on WA medium at 25 ^{0}C for three days and after that inoculated on straw decoction corn (SDC) medium, incubated at 28 ^{0}C for 3 days, and exchanged to a dark chamber for 5 days. We harvested the fungus conidia utilizing 15 ml sterile refined water containing 0.5% gelatin and used to inoculate the plants. The suspension was sifted through four layers of sterile cheesecloth, and kept in a flask 4 ^{0}C. We gauged conidia fixation utilizing a hemocytometer and adjusted to 10^3 conidia/ml, before spraying. Inoculation was done at night by spraying the *M.grisea* spore suspension (at 15 ml/ per replicate) onto entire plants. After Inoculation, the plants were well kept oblivious chamber and secured with dark plastic sheets for 24 h, in order to stimulate infection. From that point, we exposed the plants simultaneously to 12 h of light and 12 h of dark for up to seven days. We recorded and statistically analyzed disease index at 20 days after Inoculation. Relative disease index and biocontrol efficacy were calculated with the following formula, rice plant were lesions for the disease index (Di) utilizing a method of (0-3) described, as follows: index (0) show no side effects; index (1) average shoot injuries with circular shapes 1-5 mm long and generally half of the leaf zone; index (2) typical blast lesions 5.1– 10 mm and normally 75% of the leaf area, and index (3) >10 mm establishing all leaves dead respectively, [22].

We calculated relative disease index and biocontrol efficacy as follows:

Relative disease index (%) = [∑ (The number of diseased plants in each index × the number of index) / (Total number of plants investigated × the highest disease index)] × 100%

Biocontrol efficacy (%) = [(Relative disease severity of Control 1-Disease severity of bacterial treatment) / Disease index of Control 1] × 100.

2.7 Statistical investigation

The investigation was performed utilizing Pearson correlation applied to densitometry curves formed by the fingerprints [17]. We performed Clustering examination utilizing the unweighted pair grouping method based on arithmetic averages (UPGMA) to decide the population structure of the isolates. After deleting the isolates with same ARDRA unique mark and a similar appraisal of potential biological control qualities, the chose microbe strains were recognized by sequencing their16s rRNA quality, and the sequences were compared, utilizing the basic local alignment search apparatus (BLAST), with the reference successions in the Nucleotide Sequence Database of NCBI (National Center for Biotechnology Information).

Data on relative disease index and biocontrol efficacy were liable to examination of variance (ANOVA) utilizing the factual programming Data Processing System (DPS variant 7.05) to decide the distinctions among the treatments. The means of treatments demonstrating significant differences were separated at 5% level of significance utilizing the Fisher's least significant difference (LSD) test so as to decide the best treatment. Microsoft Excel 2010 (Microsoft Corporation) was utilized to figure the conventional correlation coefficients of the biocontrol efficacy of isolates with their assessed biocontrol potential points basing on activity of extracellular hydrolytic enzymes in vitro, production of siderophores and antagonism capacity.

3   Results

3.1   Detection of strains with potential Biocontrol efficacy against *M.grisea*

Rice samples were accumulated in two distinctive rice development periods (tilling and heading periods) a farm filed of Central Agricultural Research Institute (CARI) Suakoko, Bong County, Liberia, of which bacterial population density was detected and presented in (Table 1).

Table 1 Concentration per sample obtained from different substrates under rice blast affected and non-affected conditions and Proportion of bacterial isolates against *M. grisea* with activities of cell degrading enzymes

Strain source	Time/period	Bacteria concentration (CFU/g)	Number of bacterial isolates	Number of isolates with antagonism ability and metabolite enzyme ability					
				Antagonism points	Chitinases	Cellulases	Proteases	Glucanase	Siderophores
Interior stem	Tiller stage	6.5×10^6	50	20	2	8	3	5	2
	Booting stage	6.4×10^5	70	39	1	18	4	10	4
	Ripe stage	6.0×10^5	35	12	0	6	1	3	1
Surface stem	Tiller stage	6.2×10^4	24	5	0	3	0	1	1
	Booting stage	6.5×10^5	26	10	1	4	0	2	3
	Ripe stage	5.5×10^5	20	6	0	3	1	2	0
Phyllosphere	Tiller stage	3.5×10^3	30	4	0	2	0	0	2
	Booting stage	3.0×10^4	45	11	1	5	1	2	2
	Ripe stage	6.2×10^6	28	3	1	1	0	1	0
Rhizophere	Tiller stage	6.0×10^3	19	8	0	3	1	3	1
	Booting stage	3.0×10^7	10	5	0	3	1	1	0
	Ripe stage	4.2×10^3	6	3	0	1	1	0	1
Endorhiza	Tiller stage	4.4×10^5	60	11	0	6	1	2	2
	Booting stage	6.5×10^6	75	18	2	8	2	3	3
	Ripe stage	6.4×10^6	25	5	0	2	0	1	2
Endosphere	Tiller stage	4.4×10^5	30	12	2	5	1	2	2
	Booting stage	6.2×10^5	39	15	3	7	1	2	2
	Ripe stage	4.2×10^5	22	3	0	1	0	1	1
Soil	Tiller stage	3.2×10^5	10	3	0	1	0	1	1
	Booting stage	4.3×10^5	15	4	0	2	1	1	0
	Ripe stage	5.5×10^6	13	6	2	1	1	1	1

Dual culture assay against *M.grisea*, (B) Bacteria concentrations represent the total bacteria concentration isolated in tissue of rice samples. In vitro activities of extracellular metabolites (protease, cellulase, chitinase, glucanase, and siderophores) of bacterial isolates were indicated by hyaline zones formed on specific plates. The percentage of the isolates producing a specific extracellular metabolite from a microenvironment = (The number of the isolates producing a specific extracellular metabolite from a microenvironment / the total number of isolates from that microenvironment) × 100%.

639 strains from plates with countable microbe isolates were isolated from various parts of rice tissue (Oryza sativa), including root, surface and inside of stems, endorhiza, phyllosphere, soil, and rhizosphere. For the most part, the measure of microbe isolates from surface plant tissues were higher than those from inside (Table 1).We estimated, the antagonistic ability and activities of extracellular hydrolytic chemicals (cellulase, chitinase, glucanase, protease and siderophores) and assessed on these microbe isolates with said (Table 1).

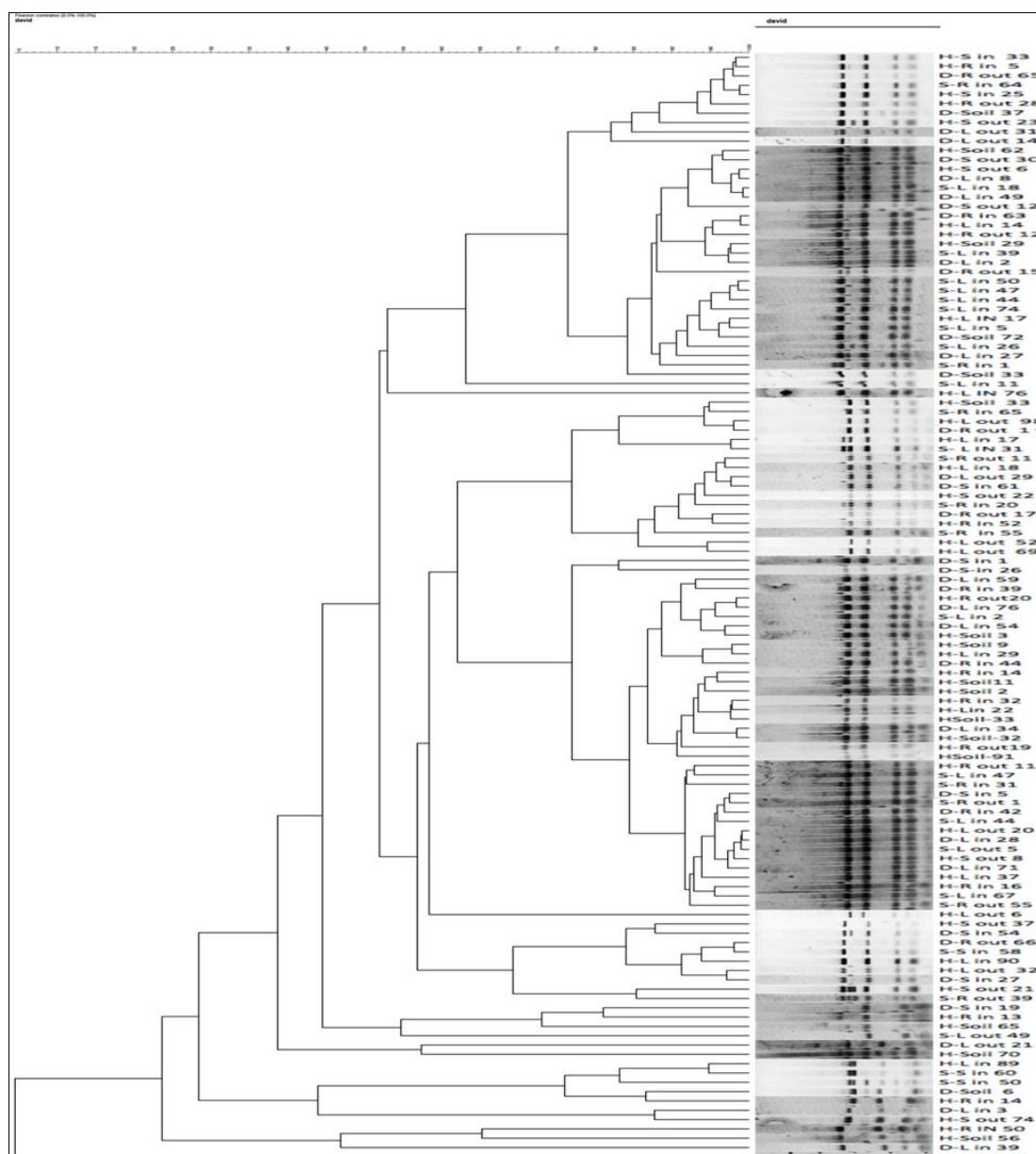## 3.2 ARDRA examination and assessing points of strains with potential biocontrol efficacy

Figure 1 The dendrogram was constructed using GelCompar®II version 4.5 (Applied MathsBVBA). The analysis was performed using Pearson correlation applied to the densitometric curves reference by (9), followed by clustering analysis using the unweighted pair-rouping method based on arithmetic averages (UPGMA).

One hundred and twenty-two microbe isolates (with no under 2 assessing points) were chosen; and ARDRA fingerprint analysis was done to remove microbe isolates with same species as indicated by the 100% similarity of banding patterns obtained by ARDRA analysis (Figure 1). We at that point analyzed a subset of isolates by their assessing points and thirty microbe isolates with most noteworthy assessing points were taken from each cluster, for greenhouse test.

ARDRA fingerprint analysis and Identification of bacterial isolates

3.3 Biocontrol efficacy test against *M. grisea* under Greenhouse condition

Greenhouse experiment with 30 selected microbes isolates referencing 9 isolates with obvious biocontrol efficacy higher than 50%, (Table 2) are acquired, which are (D-R out 65, D-L in 8, H-L out 6, H-Soil 33, H-S out 20, H-Soil 62, H-R in 14, S-S in 58, and D-S in 61). Two strains are screened from rhizosphere (D-R-out 65, and H-R in 14), two are from phyllosphere (D-L in 8, and H-L out 6), two strains from soil (H-Soil 33 and H-Soil 62) and three strains from stem of rice plant (H-S out 20, S-S in 58 and D-S in 61).

3.4 Correlation investigation between biocontrol efficacy and assessment points

We obtained nine strains with significant biocontrol efficacy (over 50%) in greenhouse test. We used Pearson relationship examination to research the relationship between the assessment points and their biocontrol efficacy in light of those insights (Table 2). The relationship between assessment points and greenhouse test results is 0.80 (Fig. 3A), coefficient between antagonism test and greenhouse test results is 0.76 (Fig. 3B). However, *Pantoea ananatis* H-L out 6 and *Bacillus cereus* D-S in 61 showed significant outcomes in greenhouse. *Bacillus cereus* H-L out 6 and *Bacillus subtilis* D-S in 61 evidently decrease *M.grisea* disease index in greenhouse (Table. 2).

Table 2 Assessments of 30 bacterial strains with potential cell degrading enzymes activities and their disease index/biocontrol efficacy in controlling *M. grisea* caused by GUY11

Treatments	Identify results	Similaraty %	Atangonism	Protease	Cellulase	Chitinase	Glucanas	Siderophores	Assessment points	Disease Index (%)	Biological control Efficacy/ (%)
H-L out 6	*Bacillus cereus*	99	2	1	3	1	3	2	12	16.67±5.56qr	76.50%
D-S in 61	*Bacillus cereus*	99	2	1	1	2	2	2	10	18.52±3.21qr	71.00%
H-Soil 62	*Bacillus subtilis*	99	2	1	2	0	1	0	6	33.21±4.97op	63.00%
H-Soil 33	*Bacillus subtilis*	99	1	0	3	0	1	1	6	35.19±8.49nop	68.50%
D-L in 8	*Bacillus cereus*	99	2	1	2	0	0	1	6	36.11±2.78mnop	60.25%
H-R in 5	*Bacillus subtilis*	99	2	0	0	0	0	0	2	45.19±7.14ghijklmn	22.00%
H-R in 14	*Bacillus cereus*	98	2	0	3	0	0	0	5	49.81±3.16fghijkl	52.75%
D-R out 65	*Bacillus subtilis*	99	2	0	1	0	1	1	5	53.7±6.42defghij	57.50%
S-L in 26	*Bacillus cereus*	99	2	0	0	0	0	0	2	54.32±13.4defghi	26.67%
H-S out 20	*Bacillus cereus*	99	1	1	1	1	1	1	6	53.02±9.92efghijk	58.42%
S-S in 58	*Bacillus subtilis*	99	1	0	2	1	0	1	5	54.07±6.42defghi	57.00%
S-L in 47	*Enterobacter*	99	1	0	1	0	1	0	3	46.11±10.2ghijklmn	34.17%
H-S out 8	*Bacillus subtilis*	99	1	0	0	0	1	1	3	44.07±5.01hijklmno	34.50%

D-S in 54	*Enterobacter*	98	1	0	0	1	1	1	4	47.04±0.32ghijklm	48.50%
D-S in 19	*Bacillus subtilis*	99	1	0	0	0	1	1	3	50.99±7.86fghijkl	31.17%
H-R in 50	*Bacillus cereus*	99	1	1	0	1	1	0	4	41.67±7.35klmno	47.75%
D-L in 3	*Bacillus subtilis*	99	1	0	1	0	0	0	2	43.09±4.69ijklmno	21.83%
H-Soil 11	*Bacillus cereus*	96	1	0	0	0	1	0	2	41.23±2.04lmnop	24.33%
S-L in 11	*Bacillus subtilis*	99	1	0	0	0	1	0	2	58.77±7.46cdef	20.67%
D-S in 26	*Bacillus subtilis*	99	1	0	0	1	1	0	3	50.68±3.26fghijkl	31.58%
D-R in 63	*Bacillus cereus*	99	1	0	0	2	0	0	3	54.94±2.47defgh	35.83%
S-R out 1	*Bacillus cereus*	99	0	0	0	1	1	0	2	52.96±9.71efghijk	28.50%
H-Soil 9	*Enterobacter*	99	0	0	1	0	0	0	1	64.81±8.49cdef	12.50%
H-L in 18	*Bacillus cereus*	99	0	0	0	0	1	1	2	56.67±4.01cdefg	23.50%
H-L in 17	*Bacillus cereus*	94	1	0	0	0	1	0	2	54.32±13.4defghi	26.67%
S-L in 50	*Bacillus subtilis*	85	1	1	0	0	0	0	2	51.23±4.66fghijkl	20.83%
D-L in 2	*Bacillus cereus*	98	0	0	0	0	0	1	1	63.7±7.56bcde	17.50%
S-R in 64	*Bacillus cereus*	99	0	0	0	1	0	0	1	65.12±6.17bcd	12.08%
H-L in 14	*Bacillus cereus*	99	1	0	0	0	1	0	2	56.51±2.2cdefg	23.71%
H-Soil 9	*Bacillus subtilis*	92	1	1	0	0	0	0	1	53.02±9.92efghijk	11.25%

Closest Genbank library strain, using nucleotide blasting of the 16S rDNA sequence in NCBI (National Center of Biotechnology Information. (B) The percentage means the similarity between our BCAs and known bacterial strain with Data in NCBI. (C) Hydrolytic enzyme activities and metabolite production were demonstrated by plate assay (+, hydrolysis represents a point; –, no hydrolysis represents zero point). (D) The values are Mean ± Std. Deviation, followed by the same letter within a column are not significantly different as determined by the LSD test (P < 0.05).

4 Discussion

A few investigations have reported detailed potential biocontrol agents against *M. grisea* fungus. In this work, BCAs were isolated from contaminated/non-tainted (root, surface and inside of stems, endorhiza, phyllosphere, soil, and rhizosphere) rice plant with a specific end goal to enhance the assortments of BCAs. Moreover, to accomplish effective biocontrol is to locate an appropriate wellspring of potential BCAs and analyze the vivo and in vitro of the crop. Previous, research on rice antagonistic microbe has mainly focused on isolating biocontrol nitrogen-fixing microbe [2] and plant development advancing microorganisms [4] (Fig. 2A and B). Actually, our examination center around a method technique for controlling rice blast (*Magnaporthe grisea*) utilizing effective microscopic organisms as biocontrol agents, had demonstrated antagonistic activity, which, recommend that antagonistic activity against *M. grisea*, and activities of extracellular hydrolytic enzymes, were indispensable to the biocontrol efficacy of these BCAs.

Typical blast symptoms on rice leaves (21 days after inoculation) and biocontrol strains antagonistic activities against *M.grisea caused by GUY11*
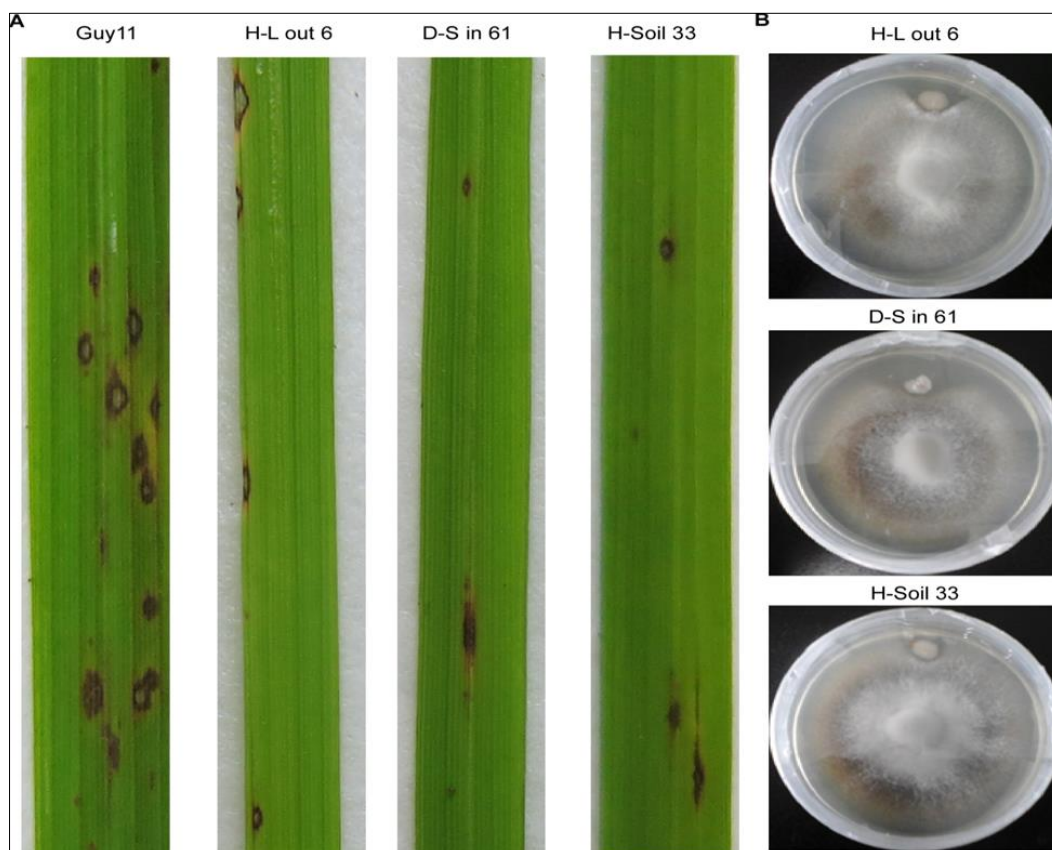

Figure 2 (A) Pathogenicity test on rice leaf plants and conidia suspension (5×10^4 spores/ml) sprayed on rice leaves. Diseased leaf was photographed at 5 day after inoculation. (B) Biocontrol strains inoculated on WA medium and cultured at 28°C for 7 days

The greater part of these microbe isolates with antagonist activities were screened from rice in vitro tissue; the *in vitro* test was highly encouraging because the bacterial antagonism ability and metabolite enzyme ability significantly reduced blast disease. Mechanism activity of biocontrol agents are generally classified as activities of substances competition, parasitism, and antibiosis [3] (Table 1). In past investigation, it was accounted for that few microbe isolates could suppress the development of pathogenic growths *M.grisea* [23].

In this investigation, the component of restraint of microbe isolates against *M. grisea* apparently caused by the movement of substance created by *Bacillus* delivers an assortment of anti-infection agents that are effective against fungi such as, [22] reported that antibiotic could restrain the development of pathogenic fungi. Fengycin creates by *Bacillus subtilis* had inhibitory action against *M. grisea* [23]. Furthermore, a few types of *Bacillus*, such as *B. cereus II.14* indicated chitinolytic action [1]. In this way, suggesting our method strategy for controlling rice blast (*Magnaporthe grisea*) for screening biocontrol agents in similar investigations.

ARDRA classifies analysis of BCAs and method procedure, helped us get distinctive hereditary foundation and lessen outstanding task at hand by abstaining from utilizing BCAs of same species in our examination. In some applicable research, order strategies based on genetic background were not been concerned well [7], which brought about duplication of same species and time-consuming.

The accomplishment of biocontrol may rely upon reasonable strategy and survival of the microbial agents. Microbes as biocontrol agents have advantages over fungal and are largely effective when applied as a preventive application to suppress the disease. In accordance with this examination, we have beforehand assessed a few strains of microbes isolated from rice plant for their antagonistic ability against *M. grisea* [22] (Table 2) and we gained nine BCAs with over 50%, which showed ideal biocontrol capacity. Moreover, the strategy of getting BCAs from various habitats of

infected/non-infected plant tissues may enhance the likelihood of isolating helpful BCAs with essential attributes from natural habitat.

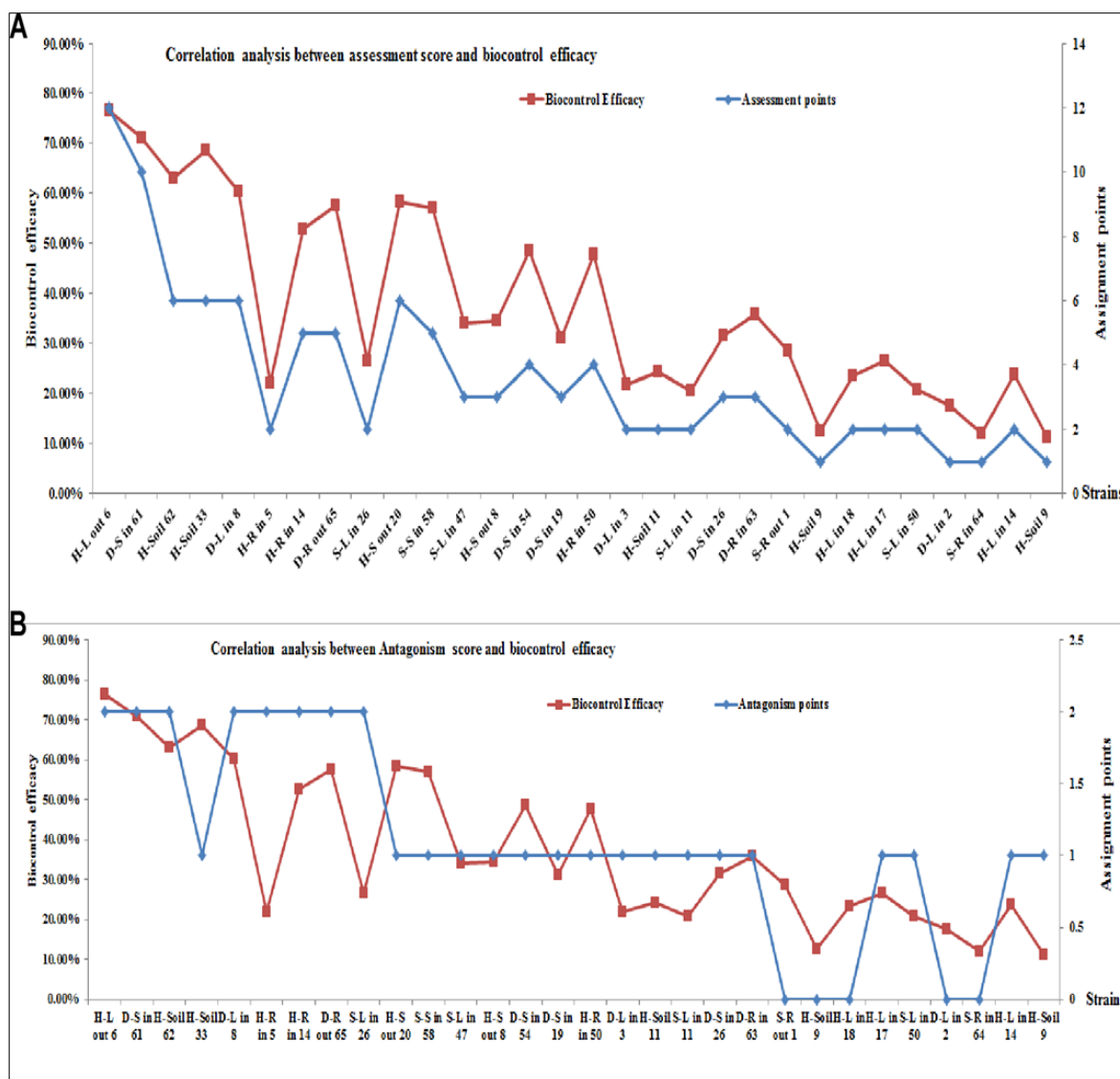Correlation Analysis between Biocontrol Efficacy and Assessment Scores

Figure 3 (A) Correlation analysis between antagonism and biocontrol efficacy to *M. grisea* is coefficient 0.76. (B) Correlation analysis between assessment and biocontrol efficacy to *M. grise* in greenhouse test is 0.70. (C) The dark red square rhombus lines representing bacterial biocontrol efficacy uses the ordinate on the left, while the blue diamond rhombus lines representing the assessment of antagonism and hydrolytic enzymes activities on the right ordinate

Besides, those nine BCAs with over 50% showed significant correlation in greenhouse with strains' assessment point yet some dissimilarity of performance in greenhouse showing that BCA strains may exhibit distinctive biocontrol capacity against maladies caused by fungal pathogen in a similar plant. Biocontrol efficacies of nine BCAs were higher in greenhouse, likely because of various colonization sites and ecological temperature [28] (Table 2). Then again, couple of different characters of bacterial strains, for example, motility and nutrient competing ability, are perhaps adjusted by colonizing condition too [8]. For secluding compelling biocontrol microscopic organisms in various conditions, soil composes and atmospheres are likewise considered. Like most other fungal pathogens, conidia of *M. grisea* assume a central role in the disease cycle. The infection process is initiated with attachment and conidia germination on the plant surface and appressoria formation from the end of the germ tubes, [23] (Fig 2A and B). H-L out 6 and D-S in 61significantly increased the growth of the rice in relation to the control, of which they significantly elevated the heights of rice plants compared with control plants (Fig. 3A). This demonstrated that these antagonistic bacteria promoted the

growth of the rice plants; by limiting, the effects of *M. grisea* on the plant (Fig. 3B). Additionally research will be done under field condition to translate the steadiness of the biocontrol agents recognized in this examination.

To the best of our insight, this investigation is the first to report a gathering of a viable microbes, to be specific *Bacillus cereus* (H-L out 60) and *Bacillus subtilis* (D-S in 61) strain as BCAs against *M.grisea* in light of procedure set up in our institution (CARI), which showed great possibility because of high correlation coefficient (Fig. 4A and B). *B. cereus* (H-L out 60) and *B. subtilis* (D-S in 61) demonstrate evident biocontrol against *M.grisea* in greenhouse (Table 2). We hypnotize it might credit to the better adaptability of strains (H-L out 60 and D-S in 61) in variety environment, as various colonizing sites even one rice plant might face complete disparate environmental condition.

5   Conclusion

In this investigation, greenhouse test results demonstrated significant relationship with strains' appraisal point (0.80), and correlation coefficient between antagonism and biocontrol efficacy of greenhouse test indicate 0.76 which implies the method for determination of biocontrol agents is suitable for the pathogen that cause *M.grisea* in rice.

Compliance with ethical standards

Acknowledgments

Disclosure of conflict of interest

Authors declare no conflict of interests.

References

[1]   Ali S, Shah M. Human action recognition in videos using kinematic features and multiple instance learning. IEEE transactions on pattern analysis and machine intelligence. 2010; 32: 288-303.

[2]   Balsanelli E, Tadra-Sfeir MZ, Faoro H, Pankievicz V, Baura VA, Pedrosa FO, Souza EM, Dixon R, Monteiro RA. Molecular adaptations of Herbaspirillum seropedicae during colonization of the maize rhizosphere. Environ. Microbiol. 2016; 18(8): 2343-2356.

[3]   Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EV, Schulze-Lefert P. Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant Biol. 2013; 64: 807-838.

[4]   Corcione CE, Freuli F, Maffezzoli A. The aspect ratio of epoxy matrix nanocomposites reinforced with graphene stacks. Polym. Eng. Sci. 2013; 53(3): 531-539.

[5]   Cretoiu MS, Korthals GW, Visser JH, van Elsas JD. Chitin amendment increases soil suppressiveness toward plant pathogens and modulates the actinobacterial and oxalobacteraceal communities in an experimental agricultural field. Appl. Environ. Microbiol. 2013; 79(17): 5291-5301.

[6]   Durante-Mangoni E, Signoriello G, Andini R, Mattei A, De Cristoforo M, Murino P, Bassetti M, Malacarne P, Petrosillo N, Galdieri N, Mocavero P. Colistin and rifampicin compared with colistin alone for the treatment of serious infections due to extensively drug-resistant Acinetobacter baumannii: a multicenter, randomized clinical trial. Clinical infectious diseases. 2013; 57(3): 349-358.

[7]   Er MK, Tunaz H, Ceyda ÜC, Bariş C, Işikber AA. Occurrence of entomopathogenic fungi on insect pests of stored wheat and maize in Central and South Anatolia in Turkey. Turk. J. Entomol. 2016; 40: 3.

[8]   LeRoux M, Peterson SB, Mougous JD. Bacterial danger sensing. J. Mol. Biol. 2015; 427(23): 3744-3753.

[9]   Rademaker J, De Bruijn F. Characterization and classification of microbes by rep-PCR genomic fingerprinting and computer assisted pattern analysis. DNA markers: protocols, applications and overviews. 1997; 151-171.

[10]   Schwab, Stefan, Leonardo Araujo Terra, and José Ivo Baldani.Genomic   characterization   of   Nitrospirillum amazonense strain CBAmC, a nitrogen-fixing bacterium isolated from surface-sterilized sugarcane stems. Molecular Genetics and Genomics. 2018;1-20.

[11] Shlipak MG, Matsushita K, Ärnlöv J, Inker LA, Katz R, Polkinghorne KR, Rothenbacher D, Sarnak MJ, Astor BC, Coresh J, Levey AS. Cystatin C versus creatinine in determining risk based on kidney function. N. Engl. J. Med. 2013; 369(10): 932-943.

[12] Shokrani, Masih, Jamie Phan, Judith Lukaszuk. Assessment of Relationship between Hemoglobin A1c and Highly Sensitive C-Reactive Protein Levels in Pre-Diabetic Individuals. Clinical Laboratory Science. 2016; 29(3).

[13] Sirisathaworn, Thanyaluk, et al. Evaluation of mating type distribution and genetic diversity of three Magnaporthe oryzae avirulence genes, PWL-2, AVR-Pii and Avr-Piz-t, in Thailand rice blast isolates. Agriculture and Natural Resources. 2017; 51(1): 7-14.

[14] Tabañag, Ian Dominic Flormata, et al. The role of yeast-surface-display techniques in creating biocatalysts for consolidated bioprocessing. Catalysts. 8(3): 94.

[15] Theo, Wai Lip, et al. ''Review of pre-combustion capture and ionic liquid in carbon capture and store.'' Applied energy. 2016; 183: 1633-1663.

[16] Schwab, Stefan, Leonardo Araujo Terra, and José Ivo Baldani.Genomic characterization of Nitrospirillum amazonense strain CBAmC, a nitrogen-fixing bacterium isolated from surface-sterilized sugarcane stems. Molecular Genetics and Genomics. 2018; 1-20.

[17] Shlipak MG, Matsushita K, Ärnlöv J, Inker LA, Katz R, Polkinghorne KR, Rothenbacher D, Sarnak MJ, Astor BC, Coresh J, Levey AS. Cystatin C versus creatinine in determining risk based on kidney function. N. Engl. J. Med. 2013; 369(10): 932-943.

[18] Shokrani, Masih, Jamie Phan, Judith Lukaszuk. Assessment of Relationship between Hemoglobin A1c and Highly Sensitive C-Reactive Protein Levels in Pre-Diabetic Individuals. Clinical Laboratory Science. 2016; 29(3).

[19] Sirisathaworn, Thanyaluk, et al. Evaluation of mating type distribution and genetic diversity of three Magnaporthe oryzae avirulence genes, PWL-2, AVR-Pii and Avr-Piz-t, in Thailand rice blast isolates. Agriculture and Natural Resources. 2017; 51(1): 7-14.

[20] Tabañag, Ian Dominic Flormata, et al. The role of yeast-surface-display techniques in creating biocatalysts for consolidated bioprocessing. Catalysts. 8(3): 94.

[21] Theo, Wai Lip, et al. Review of pre-combustion capture and ionic liquid in carbon capture and store. Applied energy. 2016; 183: 1633-1663.

[22] Tokpah DP, Li H, Wang L, Liu X, Mulbah QS, Liu H. An assessment system for screening effective bacteria as biological control agents against Magnaporthe grisea on rice. Biol. Control 103:21-29.2016 Review of pre-combustion capture and ionic liquid in carbon capture and storage. Applied energy. 2016; 183: 1633-1663.

[23] Tokpah, David P., et al. 'Biological control of potential antagonistic bacteria isolates to restrict Magnaporthe grisea infection on rice. African Journal of Microbiology Research. 2017; 11(27): 1108-1119.

[24] Tomkins J. Alleged Human Chromosome 2 "Fusion Site" Encodes an Active DNA Binding Domain Inside a Complex and Highly Expressed Gene-Negating Fusion. Answers Res. J. 2013; 6: 367-375.

[25] Truyens, Sascha, et al. Changes in the population of seed bacteria of transgenerationally Cd-exposed A rabidopsis thaliana. Plant Biology. 2013; 15(6): 971-981.

[26] Wang JC, JC Correll, Yulin Jia. Characterization of rice blast resistance genes in rice germplasm with monogenic lines and pathogenicity assays. Crop protection. 2015; 72: 132-138.

[27] Yang JH, Liu HX, Zhu GM, Pan YL, Xu LP, Guo JH. Diversity analysis of antagonists from rice-associated bacteria and their application in biocontrol of rice diseases. Journal of Applied Microbiology. 2008; 104: 91-104.

[28] Zaidi A, Ahmad E, Khan MS. Role of Phosphate-Solubilizing Microbes in the Management of Plant Diseases. In. Phosphate Solubilizing Microorganisms. Springer International Publishing. 2014; 225-256.