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Screening of rice endophytic natives biofertilizers with plant growth-promoting characteristics

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In Mali, rice cultivation is faced with declining yields due to insufficient soil nutrients and diseases with yield losses of up to 80%. The use of endophytic bacteria and fungi is a more ecological and effective alternative compared to chemicals. This study aims to select at least one endophytic rice bacterium in combination with mycorrhizal fungi to improve the rice production in Mali and effectively control the growth of *Xanthamos oryzae.* This selection focused on: (i) the isolation of endophytes and (ii) the identification of PGP traits and their enzymatic productions. Thirty seven (37) endophytic bacteria were successfully isolated from rice roots (10 isolates) and seeds (27 isolates) (Table 1). Among the strains isolated, 24 and 29 isolates showed antibiosis activities, respectively against XOO and XOOC, 24 and 12 isolates showed, respectively chitin and melanin activities, and 20 isolates produced siderophore. All inoculated rice roots with the endophytic bacteria showed a rice growth-promoting ability. *In vitro* selection made it possible to retain 6 isolates (DK1, DK2, DK3, DK4, REM2 and REM9). An 18.4% increase in grain weight per plant of rice and 17.14% in yield per hectare for the treatments inoculated with DK4. The size of the plants, the number of fertile thalli, the biomass (fresh and dry) were respectively improved by 8.48, 10.1, 11.82 and 18.43% for the treatments inoculated with DK4 followed by REM9. All agronomy parameters measured were significantly increased by bacterial endophytes compared to the controls. These results showed that bacterial endophytes with high PGP activities improve host-plant growth traits and can be used as biofertilizers.

Key words: Bacterial endophytes, rice, biofertilizer, plant growth, Mali.

INTRODUCTION

Rice is a crucial and strategic cereal crop for more than half of the population in sub-Saharan Africa, particularly in Mali. Its per capita consumption is estimated at 81.61 kg/person/year and it represents around 12% of the agricultural value (Diallo et al., 2014). The common problems encountered by rice farmers in West Africa are high cost of inputs, lack of postharvest facilities, pest and diseases (Tekete, 2019). Several studies also showed

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that pests and diseases significantly affect rice production. Among the cereal which contributes significantly to food security, rice cultivation is faced with a drop-in yield due to diseases such as bacterial blight, rice yellow mottle virus and insect diseases. Some of these diseases such as rice yellow mottle virus (RYMV) can cause an incidence of 80 to 100% to rice grain yield (Diallo et al., 2014).

The measures of control are generally cultural practices, new varieties, chemical and biological control (Niño-Liu et al., 2006). Despite some of these control methods have proven dangerous or/and effective, the use of resistant varieties may not be effective due to the great diversity of diseases; the chemical used are dangerous for the environment and can cause bacterial resistance (Azman et al., 2017; Hastuti et al., 2012).

Due to these cited constraints, it has been reported by several-research studies that biological control constitutes a safer, more promising, more ecological and more profitable alternative than chemicals (Velusamy et al., 2006; Ni et al., 2015; Azman et al., 2017; Dicko et al., 2018; Hastuti et al., 2012; Bertani et al., 2016; Borah et al., 2018).

Bacteria that reside in the tissue of living plants and do not visibly harm the plants are known as endophytic bacteria. This relationship appears to provide a protective environment that helps a potential endophyte by limiting exposure to competition from the indigenous microbial population. Endophytic bacteria, which have been shown to be plant growth-promoting or pathogen-suppressing or to activate plant defense systems can benefit plants through enhanced resistance to biotic and abiotic stresses and plant growth promotion. The use of these bacteria for disease management has great potential in the agricultural systems of West Africa. However, to date, production and use of endophytic bacteria biopesticides has not been scaled-up for use by farmers in West Africa. Endophytes are endosymbiotic microorganisms that colonize plants tissues (Ravelomanantsoa, 2014). In Mali, Dicko et al. (2013) conducted a biological control study against Xoo using streptomyces *in vitro*. The main interaction between rhizosphere microbe's and plants mainly bacteria, fungus and arbuscular mycorrhiza fungi plays an important role for water and nutrients update from the rhizospheric zone and soil (Seneviratne et al., 2010).

The objective of this study is to select Malian native endophyte bacteria that can improve growth and control rice diseases.

MATERIALS AND METHODS

Isolation of endophyte bacteria and spores of mycorrhizal fungi

Isolation of endophyte bacteria

Endophyte bacteria have been isolated from roots, seeds and leaves of rice. For root and leaves, a section of 2 to 3 cm was cut with a sterile scalpel. The stem samples were first weighed and surface sterilized with hydrogen peroxide (20%) for 10 min, rinsed four times with 0.02 M potassium phosphate buffer (pH 7.0). The root and leaf samples were surface disinfected with sodium hypochlorite (1.05%) and washed four times with 0.02 M phosphate buffer solution. 0.1 ml aliquot from the final wash buffer was removed and transferred to 9.9 ml of trypticase soy broth to serve as a sterile control. Each sample was diluted in 9.9 ml of sterile physiologic water and spread on Tryptic Soya Agar (TSA Sigma-Aldrich) then incubated at 28°C for 72 h. The different colonies were transferred to fresh TSA to obtain pure cultures.

Extraction and production of mycorrhizal fungi by the on-farm technique

The extraction of spores from the soil coming from the rice perimeters was carried out using the sieving and decantation wet technique of Gerdermann and Nicolson (1963) described in the work of Amutha and Shamini (2014). The spores were identified in accordance with Schenck and Perez's (1990) ACV fungi identification manual. The INVAM worksheet was used to diagnose spores.

Mycorrhizal inoculum were produced by the AMF on-farm technique described by Doud et al. (2015) using two plants (Brachiaria and Maize) in conical plastic pots filled with a 1: 3 mixture of soil: sand (ratio). In order to avoid the introduction of pathogens, the soils were sterilized.

Selection of endophytic bacteria with PGP activity

Antibacterial activity

The antibacterial activity of the different isolated endophytes was evaluated on two rice pathogens *Xanthomonas oryzae pv. oryzae* (Xoo) and *X. oryzae pv. oryzicola* (Xoc) according to the method described in Dicko et al. (2018). This method consists of pouring 25 ml of Bennett agar medium (HiMedia laboratories) onto 200 µl of a suspension of the pathogenic bacteria from sterile physiological water. After solidification, 10 µl of each isolate, adjusted to 10^8 ufc/ml of bacteria, were spot-inoculated then incubated at 28°C for 48 h. The inhibition halo was measured in millimeters and the averages were calculated.

Enzymatic activity of isolates

To test the chitinolytic of each isolate, it was spot-inoculated by loop at the center of the basal salt with the following composition (in g/L of distilled water): K_2HPO_4 0.7, KH_2PO_4 0.3, $MgSO_4$ 0.5, $FeSO_4$ 0.01, $ZnSO_4$ 0.001, $MnSO_4$ 0.001, $(NH_4)_2SO_4$ 0.25, and yeast extract 1.0 agar plates containing 1.0% colloidal chitin. Plates were incubated at 30°C for 4 days and then the agar plate was flooded with Congo red solution (0.1% (w/v)) for 40 min. The Congo red solution was washed with 1 M NaCl for 20 min. The clear halo around each isolate was measured (Nagpure et al., 2014).

The production of the cellulase was carried out by the method described in Dicko et al. (2018). After 4 days of incubation, the dishes were flooded with a 0.1% Congo red solution for 20 min. The dishes were then washed with 1 ml of NaCl (1M). The appearance of a clear halo around the bacterial colonies shows that the bacteria produce cellulase, so hard if the area around the colonies turns red, shows that the bacteria does not produce cellulase.

Test of siderophore production

For each isolate, siderophores production was detected

Table 1. Distribution of isolated bacterial endophytes.

Origin	Designations	Number of endophytes isolated	Percentage
Root	REM	10	29
Seed	ADNY, BRIE, DJA, DK, NERI	27	71
Leaf	-	0	0

qualitatively by using the universal Chrome Azurol Sulphonate assay (CAS) (Schwyn and Neilands, 1987) as described in Nagpur et al. (2014) modified. This medium was poured onto sterile Petri dishes, then each isolate (10 ul) was spot-inoculated onto the blue agar and incubated at 30°C for 4 days. The results were interpreted based on the colour change.

Rice seeds germination test (Vigor index) in vitro

The germination of rice seeds inoculated with endophytic bacteria *in vitro* was carried out using the blotting paper method described by Noumavo et al. (2013). After 7 days of cultivation, the height of plant and roots were measured and the vigor index determined using the following formula:

Vigor index = (average height of roots + average height of shoots) × percentage of germination rate.

Determination of the effect of endophytic rhizobacteria and mycorrhizal fungi in station

To determine the effect of endophytic bacteria inoculation on rice growth and yield, a split plot design with main factor treatments (DK1: T1; DK2: T2; DK4: T3; REM2: T4; ENDO9: T5; MYCO: T6 and CONTROL: T0) were established with three (3) repetitions. Each treatment was combined with MYCO (*Glomus* species) treatments and distributed at random. Each treatment was installed on a 2 m^2 wooden frame board. The variety used is rain-fed (Nerica L1) and has a life cycle of around 90 days. The following agronomic parameters were evaluated: the number of thallus at flowering, the height of each plant at maturity, the number of fertile thallus, the weight of 1000 seeds, the yield per plant, the yield per hectare and the weight of fresh and dry biomass.

Data analysis

Bartlett's test was used to verify the consistency of the variance of the means for the different parameters measured. The treatments with non-homogeneous means underwent a transformation before analysis.

Analysis (ANOVA) for the various measured agronomic parameters was performed for all data using the procedures of the General Linear Model (GLM) of the Statistical Analysis System (SAS). For all parameters where the F was found to be significant, we compared the data using the Fischer protected Smallest Difference (LSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Isolation and selection of endophytic bacteria from rice

Thirty-seven (37) endophytic bacteria were isolated; 10 isolates from rice root and 27 isolates from seed (Table 1). Among the strains isolated, 24 and 29 isolates showed antibiosis activities, respectively against *XOO* and *XOOC*, 24 isolates showed chitin activities, 12 isolates produce melanin and 20 isolates showed siderophore (Table 2).

Finally, 6 endophytic bacterial were selected for their performance by improving significantly the growth of rice (Table 2).

A total of 37 endophytes were isolated from seed (27 isolates) and root (10 isolates) samples. No bacterial endophytes have been isolated from rice leaves.

Isolates DK1, DK2, DK3, DK4, REM2 and REM9 showed a marked improvement in vigor index compared to uninoculated controls. The most efficient strain is DK1 with a vigor index of 1929 compared to the uninoculated control with an index of 286. These selected isolates were used for further studies.

Determination of the effect of endophytic rhizobacteria in station

The inoculation of rice seeds with bacterial endophytes combined with mycorrhizal fungi and/or alone showed a significant effect on growth and as well as on grain production (yield) (Table 3).

Table 3 shows that at the level of treatments, the difference is very significant for all the parameters evaluated. The replicates are also significant for all the parameters evaluated except the fresh and dry biomass as well as the weight of 1000 seeds.

Table 4 shows that there is a significant difference between the treatments in terms compared to the non-inoculated controls. The best results of the inoculation effect were observed with isolate DK4 and REM9, respectively. An increase of 18.4% in grain weight per plant of rice and 17.14% in yield per hectare for the treatments inoculated with DK4. The plant height, the number of fertile thalli, the fresh biomass and the dry biomass were, respectively improved by 8.48, 10.1, 11.82 and 18.43% for the treatments inoculated with DK4 compared to the non-inoculated controls. The treatment inoculated with MYCO only have significantly improved the number of fertile thalli, but the fresh and dry biomass values obtained are less compared to the other treatments with PGPR.

The correlations between grain weights per hectare and the weight of 1000 seeds are not uniform for all

Table 2. Identification of antibacterial activities and Plant Growth Promoting (PGP) traits of isolated endophytes.

Isolate	Antimicrobial and PGP activities					
	XOO (mm)	XOC (mm)	Chitin (mm)	Melanin (mm)	Siderophores*	Vigor index
REM1	4.85	6.26	0.00	0.00	2.00	1636
REM2	23.95	16.28	27.00	18.70	2.00	1760
REM3	8.27	10.24	21.40	22.20	0.00	1538
REM4	10.7	9.12	24.20	22.20	2.00	1300.81
REM5	0	3.91	0.00	0.00	1.00	1219.2
REM6	0.8	6.28	20.70	19.70	1.00	1316
REM7	4.76	3.97	20.00	0.00	1.00	1304
REM8	0	4.23	23.10	0.00	2.00	1013
REM9	29.34	26.89	37.00	17.80	3.00	1480
REM10	10.34	5.83	30.10	0.00	0.00	286
BRIE2	3.325	8.44	24.60	0.00	0.00	1548
BRIE4	9.26	6.13	13.00	0.00	0.00	1356.8
BRIE7	3.81	9.56	0.00	0.00	0.00	915
ADNY4	3.36	8.67	18.90	0.00	0.00	593.6
ADNY8	13.35	4.96	9.00	0.00	2.00	1697
ADNY9	12.44	10.08	16.20	17.80	1.00	915
ADNY10	12.09	9.38	27.10	0.00	0.00	1389
DK1	24.89	16.27	25.70	18.10	2.00	1929
DK2	26.34	15.18	30.70	19.70	2.00	1885
DK3	27.12	16.88	28.40	0.00	3.00	1770
DK4	29.04	24.81	41.80	19.89	3.00	1830
DJA1	8.7	10.78	21.80	0.00	3.00	915
DJA3	3.48	4.90	5.70	8.10	1.00	915
DJA7	0	12.91	10.30	29.20	1.00	1242
DJA8	13.27	10.45	15.60	0.00	0.00	1689
NERI2	12.32	4.81	18.10	25.00	2.00	1114
NERI3	0	5.63	18.60	0.00	0.00	1450
NERI6	4.89	10.33	0.00	0.00	2.00	1338
NERI7	4.89	10.33	0.00	0.00	2.00	1090
CONTROL	0	0	0	0	0	286

*((0): No production, (1): poor production, (2): production medium et (3): production intense). DK1, DK2, DK3, Dk4, REM2, REM4 and REM9 isolates showed strong abilities to control the growth of XOO and XOC and significant efficient PGP activities.

Table 3. Result of ANOVA test for all parameter analysed.

Source of variance	F-value								
	DF	N Thallus	Plant heigh	Fertile Thalius	Fresh biomass	Dry biomass	W1000	Yield/plant	Yield/ha
Treatment	7	4.29***	24.97***	6.95***	46.14***	34.95***	76.6***	21.4***	21.4***
Repetition	2	6.06**	2.53*	7.44**	1.07 NS	0.58 NS	0.57NS	6.64**	6.64**
Coef. Var.		31.5308	11.80048	31.63252	13.31496	11.47062	17.40099	37.56421	37.56421

DF: Degre of free, N: number, Coef. Var.: coefficient of variance.

treatments. The correlation is only relevant at the treatment level (Figure 1B). Figure 1A shows a correlation between the number of thalli and the size of the rice plants.

DISCUSSION

From a total of 27 endophytic isolates, 6 were isolated endophytic bacterial isolates selected for their strong

Table 4. Result of the effect of inoculation of different bacterial and mycorrhizal endophytes on rice production in station.

Treatment	N Thallus	Plant height	Fertile Thali	Fresh biomass	Dry biomass	Yield/plant	Yield/ha
DK1	3.4343[ba]	4.57466[bc]	2.9929[a]	4.9997[c]	3.16079[c]	3.9322[c]	5.5416[c]
DK2	3.3124[ba]	4.50755[c]	3.0304[a]	4.50176[c]	3.32361[b]	4.0741[bc]	5.6835[bc]
DK3	3.4840[a]	4.60409[bc]	3.1608[a]	4.47727[c]	3.34771[b]	4.0972[bc]	5.7067[bc]
DK4	3.4486[ba]	4.77208[a]	3.1775[a]	5.00063[a]	3.50620[a]	4.8010[a]	6.4105[a]
REM2	2.8420[c]	4.49639[c]	2.4784[b]	4.47939[c]	3.35111[b]	3.6794[c]	5.2888[c]
REM9	3.0785[bc]	4.69569[ba]	2.9073[a]	4.55867[c]	3.42293[ba]	4.802[ba]	6.4105[a]
MYCO	3.2543[ba]	4.52163[c]	3.1923[a]	4.71074[b]	3.49324[a]	3.0870[d]	6.0987[ba]
CONTROL	2,7140[c]	3.92325[d]	2.0778[c]	3.81775[d]	2.63792[d]	2.9572e	4.6964[d]
LSD	0.3883	0.1240	0.3757	0.1268	0.1157	0.5251	0.5251

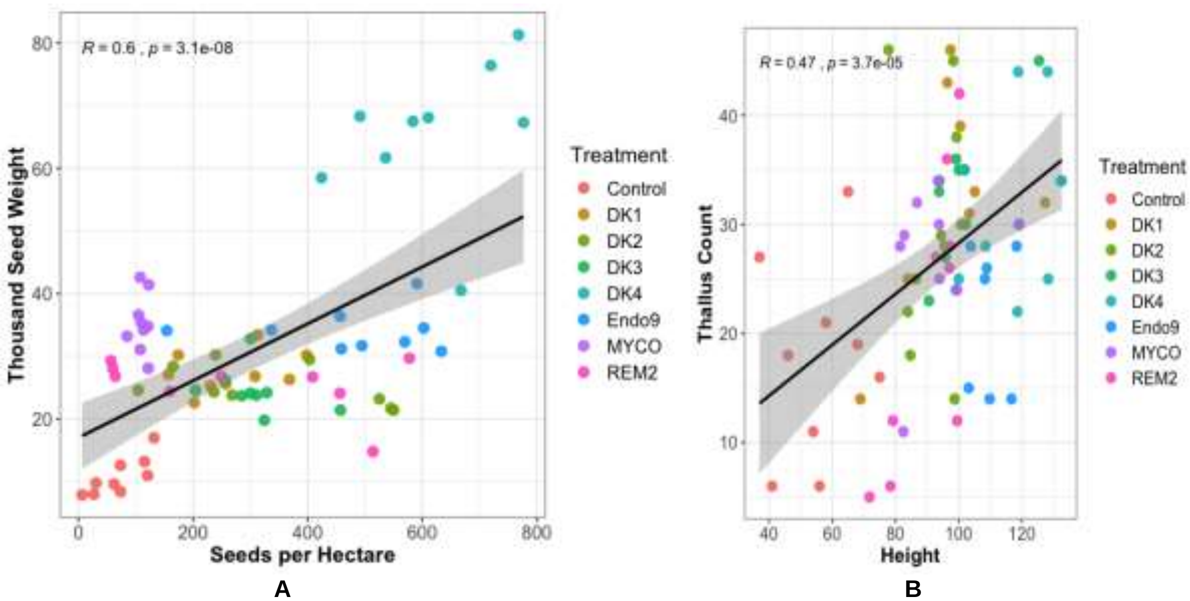

Figure 1. Correlation between seed per hectare and 1000 seed weight (A), and correlation between number of thalli and size (B).

power PGP. These results correlated with those obtained by Bertani et al. (2016) who isolated 21 endophytes among them, 7 isolates showed PGP activities. Similar results have also been found in the work of Borah et al. (2018). The bacterial rings formed by the root exudates of B510-inoculated plants were slightly larger than those of the non-inoculated control, suggesting that the B510 inoculation stimulated chemoattractant production by roots (Naher et al., 2018). Previous studies have found endophytic bacteria from root, leaves and stem of plant with a potentiality to control disease and enhance growth (Adhikari et al., 2001; Gyaneshwar et al., 2001; Hironobu and Hisao, 2008; Malle et al., 2020).

A significant improvement on growth and yield of the rice was observed for each treatments compared to the controls. The results obtained in this study may be explained by the ability of each endophyte to control the growth of pathogens, especially the strong PGP activities which mediate growth and increase yield. These results confirm those of some researchers (Malle et al., 2020; Lyu et al., 2019; Zhang et al., 2019a, b; Dicko et al., 2018; Verma et al., 2018; Babana et al., 2016; Santoyo et al., 2016; Dicko and Verma, 2014) who indicated that the production of enzymes (chitinase, amylase, antimicrobial compounds and siderophores) by microorganisms improve plant growth by promoting root initiation and modification of specific gene expression under stressful conditions thus impacting crop yields.

Significant increases in the plant height, the weight of 1000 seeds, the yield per hectare and, the fresh and dry biomass obtained in this experiment were observed in DK4 and REM9 compared to the uninoculated control. Similar results were obtained by certain researchers (Noumavo et al., 2013; Dicko et al., 2018; Kassogué et al., 2016) who respectively inoculated maize with *Azospirillum lipoferum* DSM 1691 and *Pseudomonas fluoresens* DSM 50090; *P. fluoresens* species, *Actinomycetes* species H7 and *Bacillus* species BI4' and BI4. These results confirm those of many authors who have shown that inoculation of rice with PGP bacteria could lead to a significant increase in plant height, root and aerial biomass (Subba Rao et al., 1979; Balasubramania and Kumar, 1987; Babana et al., 2016; Sev et al., 2016; Majeed et al., 2018; Lyu et al., 2019; Malle et al., 2020). Improvements are similar to those obtained by some researchers on other crops like wheat (Babana et al., 2016; Gholami et al., 2009; Babana and Antoun, 2006), with corn (Dicko et al., 2018; Kassogué et al., 2016), tomato (Khan et al., 2015), and cannabis (Lyu et al., 2019).

Among beneficial microbes, plant growth promoting bacteria offers excellent opportunities for their wide utilization in agriculture to manage soil quality and other factors which correspond to limited growth and yield output of major field crops.

Conclusion

This study confirmed the promoting effect of the different endophytic isolates on the germination, growth and yield of rice. The results showed clearly that Growth Promoting Endophytic Bacterial selected in combination with mycorrhizae fungi had a better effect on rice growth and yield production than control non-inoculated with an increase in rice yield between 10 and 18%. As perspective, such bacterial endophytes will be tested at the field level to assess their potential as a bio-fertilizer, in synergism with plant growth promoting rhizobacteria or in combination with other biofertilizers. Considering the beneficial effect of PGPR in terms of biofertilization and biocontrol activities, all treatment showed a positive influence on crop productivity and ecosystem functioning, encouragement should be given to its implementation in Malian agriculture.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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