A Malian rice seed endophyte bacteria control efficiently the growth of *Xanthomonas oryzae* pv *oryzae* and *Xanthomonas oryzae* pv *oryzicola*

ABSTRACT

Aims:
In Mali, rice (*Oryza sativa* L.) is a very important cereal in the food system and important losses in rice production are caused by bacterial diseases. Despite the important rice losses in production and the proven ability of some bacteria to control rice pathogens, in Mali very few studies have been carried out in biocontrol. With the aim of improving food security, the objective of this work is to select, at least one endophyte bacteria with great capacity to inhibit the growth *Xanthomonas oryzae* pv *oryzae* and *Xanthomonas oryzae* pv *oryzicola*.
Study design: this experimental research involving a Malian local rice cultivar named Kogoni and biocontrol endophyte bacteria isolated from rice in Mali
Pace and duration of study: This research was carried out in greenhouse at LaboREM-Biotech and in the field at the Office of irrigated perimeters of Baguineda (OPIB) for 3 years
Study design
To achieve this objective, pathogenic bacteria (*Xoo* and *Xoc*) circulating in rice fields of Mali were identified, endophyte bacteria from the bacterial collection of the LaboREM with high antagonistic activities against the identified pathogens were selected, and the efficacy of the selected endophytes to inhibit the growth of these pathogens in vitro were tested.
Results: A total of five isolates, including 3 from *Xoo* and 2 from *Xoc*, were isolated in Niono from two varieties of rice: Kogoni91-1 and Adny11. No isolate characteristic of *Xanthomonas oryzae* was observed on samples from Baguineda. The three selected rice endophytes were tested in vitro to assess their effectiveness in controlling *Xoo* and *Xoc*. Following this test, 100% of the endophytes showed significant antimicrobial activity against *Xoo* and *Xoc* with an inhibition diameter varying between 6 and 28.5mm. endo Ad9 was selected for its strong ability to inhibit the growth of both pathogens at the same time.

Keywords: Rice, *Xanthomonas oryzae* pv *oryzae, Xanthomonas oryzae* pv *orizycola*, endophytes, growth inhibition

1. INTRODUCTION

Rice (*Oryza sativa* L.), is the main cereal crop in the world [1]. It is the only staple cereal that is almost exclusively reserved for human food [2]. and essential for poverty and reduction of food insecurity in sub-Saharan Africa in general, and in Mali in particular. Rice production, which was 2,268,054 tons in 2010 [3], is

expected to increase due to the growing demand for food in Mali and the sub-Saharan region. In Mali, although the country has significant rice potential and a rapidly growing production, demand for rice still exceeds national supply forcing the country to import from Asia about a quarter of the rice consumed in the country (Diarra et al. 2014).

Taking into account the different biotic and abiotic constraints negatively impacting production, to meet national demand 430,000 tons of additional rice would be needed in 2020 and 640,000 tons in 2025 [4]. Unfortunately, the bacterial wilt or "Bacterial Leaf Blight (BLB)" and the bacterial leaf streak or "Bacterial Leaf Streak (BLS)" respectively caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) [5] constitute one of the main constraints limiting the intensification of Malian rice cultivation [6], [7].

Despite the important rice losses caused by these bacteria and the proven ability of certain rhizospheric microorganisms to control these bacteria, in Mali very few studies have been carried out in this direction. With the aim of improving food security, the objective of this work is to formulate, at least one effective and easy to use biopesticide against bacterial leaf blight (or bacterial wilt) and/or bacterial leaf streak of rice in Mali.

2. MATERIAL AND METHODS

2.1 Material:

The materials used to conduct this study are:
A reference strain of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) which was selected from the collection of the Laboratory of Microbiology and Microbial Biotechnology (LaboREM). Three (3) rice endophyte isolates (REM7, REM8 and Adny11) were selected from the bacterial strain collection of the LaboREM. Four (4) pairs of primers (back and forth): Xoo80; Xoc3866 and Xoc3864; Xo3756 were used for molecular identification of the pathogens. Seeds of two (2) rice variety (Adny11 and Kogoni 91-1) were kindly provided to us by the Institute of Rural Economy.

2.2 Methods:

2.2.1 Sampling

For pathogen isolation, randomly selected samples of thirty rice leaves exhibiting typical symptoms of bacterial blight were collected from infested rice fields of the ″Office du Niger (ON) ″ and the ″Office des Périmètres Irrigués de Baguineda (OPIB)″. These samples were placed in plastic bags appropriately labeled and stored at 4ºC for further analysis.

2.2.2 Isolation of *Xanthomonas oryzae:*

- Isolation of *Xanthomonas oryzae* from collected 30 samples collected from the ″Office du Niger″ and OPIB was carried out at the LaboREM-Biotech according to the method described by [8]. Leaf pieces measuring 3x3mm in size were excised from the

second and 1% bleach for one minute, under aseptic conditions. Following a quick rinse in sterile distilled water (SDW), seven leaf pieces were blotted dry on sterile blotting papers and transferred to Petri plates containing peptone-sucrose glutamic acid agar medium; containing 10g Peptone, 10g Sucrose, 1g glutamic acid and 16g agar; using a laminar flow unit. Plates were incubated at 27^{o}C for 3 days. Mucoid, doomed, yellow colonies on YDC plates were considered as Xanthomonads. These bacterial isolates were sub-cultured to obtain pure culture which was stored for further use. Selected isolates were named as follows*: Xoo* (*Xanthomonas oryzae oryzae*) or *Xoc* (*Xanthomonas oryzae oryzicola*), followed by the first two letters of the rice variety used to isolate the bacteria and the number of the sample. Cultures were suspended in SDW or grown on PSG slants, and stored at 4^{o}C for short-term preservation. For long-term use, cultures were preserved as stated in soil by [9], by pipetting approximately 0.5 ml of inoculated, heavy nutrient broth suspension onto approximately 3 g of sterile loamy garden soil in small, test tubes (75x10 mm) or bijou bottles. The soil was air-dried, passed through a 2mm mesh sieve to obtain finer particles, autoclaved thrice at 121^{o}C for 1 h and stored at 4oC. Bacteria were recovered by plating the infested soil onto PSG agar plates whenever needed.

2.2.3 Common characters

2.2.3.1 Mobility:

In order to verify the mobility of the isolates, each isolate was examined under a microscope in the fresh state. A small mass of bacteria from 48 hours incubation of each isolate was suspended in two (2) drops of sterile distilled water placed on a sterile slide. The suspension thus obtained was covered with a coverslip and observed under a phase contrast microscope.

2.2.3.2 Gram staining

The Gram staining was done according to [10]. It consisted of subjecting a thin bacterial film on a glass slide to aqueous Crystal Violet, Iodine, Ethanol and Safranin solutions for various periods of time and washing with tap water. Gram negative stains give red whereas Gram positive bacteria give blue-blue black color. Gram staining results were confirmed with reaction to Potassium hydroxide (3% KOH) test [11]. During this lest, a loopful of bacteria was stirred in 3% KOI 1 and any change in the viscosity was recorded. Gram negative bacteria forms thread like slime when picked with a tooth pick while gram positive bacteria disperse and forms no slime.

2.2.3.3 Catalase and oxidase tests

Catalase and oxidase enzymes were determined according to the technique described by [12]. The oxidase test was carried out as described in [9]. Briefly, one day old bacterial colony, grown on nutrient agar supplemented with 1% glucose was used in this assay. A loopful of the inoculum was rubbed onto a filter paper impregnated with 1% (w/v) freshly prepared aqueous solution of tetramethyl-p-phenylene diamine dihydrochloride. The isolate was rated oxidase-positive if a purple color developed within 10 seconds, delayed positive if coloration developed within 10-60 seconds; and negative if no color developed after 60 seconds [13].

Likewise, the fluorescence test on King B solid medium to differentiate *Xanthomonas* genus from *Pseudomonas* genus was carried out according to the method described by [14].

The oxidation / fermentation test, meanwhile, aims to determine by which metabolic pathway (oxidative or fermentative) according to the protocol of New and Misra (1994).

The ability of the isolates to hydrolyze starch was determined according to the method described by [12], while the ability of isolates to hydrolyze gelatin was determined by the method of [15].

2.2.4 Differential characters:

2.2.4.1 Growth on 0.001% copper nitrate solid media

Strains of *Xanthomonas oryzae oryzae* (*Xoo*) and *Xanthomonas oryzae oryzicola* (*Xoc*) were identified and differentiated on PSG medium containing 0.001% of copper nitrate. For that, the copper nitrate was diluted in 1.5 ml of 70% ethanol and then filtered. The obtained solution was mixed with a cooled sterile PSG medium. The solidified medium was inoculated with the different isolates. After ten (10) days of incubation at 28°C, the presence of colonies indicates the presence of *Xoo* and the absence indicates the presence of *Xoc* [10].

2.2.4.2 Growth on 0.2% casamino acid solid media

To test the capacity of the isolates to growth on casamino-acid medium without vitamins, each isolate was inoculated on PSG minimum medium with 0.2% casamino acid and then incubated at 28°C for ten (10) days. The presence of colonies indicates the presence of Xoc and the absence indicates the presence of Xoo [10].

2.2.4.3 Identification of Xanthomonas oryzae pathovars by multiplex PCR

The DNA amplification of the isolates was carried out according to the method described by [16] with the use of four (4) pairs of primers specific to *Xanthomonas oryzae* (*Xo*) and its pathovars *Xoo* and *Xoc* [17]. Amplification of bacterial DNA was performed in an Applied Biosystems 2720 Thermal Cycler according to the thermal program described by [17]. The amplification cycle (denaturation, hybridization, and elongation) was repeated thirty-five (35) times during PCR and the resulting product was stored at 4°C. The electrophoresis of the amplification products was performed on a 1.5% agarose gel prepared from 0.5X TBE and ethidium bromide (0.3 µg / ml). Ten (10) microliters of each product were housed and run over the gel for 1 hour 30 minutes at 80 volts. The gel was then photographed using Gel Doc E-BoX, to visualize the DNA fragments as bands under Ultraviolet (UV) light.

2.2.4.3 Selection of endophytes with high antimicrobial activity against Xanthomonas oryzae pv. oryzae and Xanthomonas oryzae pv. Oryzicola

The antimicrobial activity of endophytes against *Xoo* and *Xoc* was determined using the modified [18] protocol described as follows: Three rice endophytic bacteria, preselected for their antibacterial activities, were drawn at random from the LaboREM-Biotech bacterial collection, and tested against confirmed isolates such as *Xoo* and *Xoc*. Pathogens (*Xoo* and *Xoc*) and endophytes; were reactivated on nutrient agar with 1% glucose, and incubated at 28°C for 48 hours. A bacterial suspension of each isolate was prepared in sterile physiological water at a concentration of 10^8 CFU/mL. One milliliter of the bacterial inoculum from each isolate was seeded evenly with a swab on 25 mL of solidified PSA medium. A four (4 mm) diameter portion of each endophyte was cut using a cookie cutter and gently placed on the PSA medium already inoculated with the pathogen. Three replicates and one control were done for each isolate. The inoculated dishes were then incubated at 28 ° C for 72 hours. After incubation, the zones of inhibitions were measured in mm and the antimicrobial

activity (D) was determined by making the difference between the diameter of inhibition (d1) and the diameter endophytes (diameter of the punch) (d2): D = d1- d2 [18]. Thus, the antimicrobial activity D of endophytes has been classified into four (4) categories: If D ≥ 20 mm, it is marked +++; D ≥ 10-19 mm, ++; D ≥ 5 -9 mm, +; and D <5 mm, no antimicrobial activity [18].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Bacterial isolates

Out of thirty (30) samples treated, five (5) from the ″Office du Niger (ON) ″ showed colonies with characteristic appearances of *Xanthomonas oryzae*: pale yellow, shiny, of a circular shape with regular outline, convex, of viscous and mucous consistency, with a diameter of 1 mm (Figures 1A and 1B).

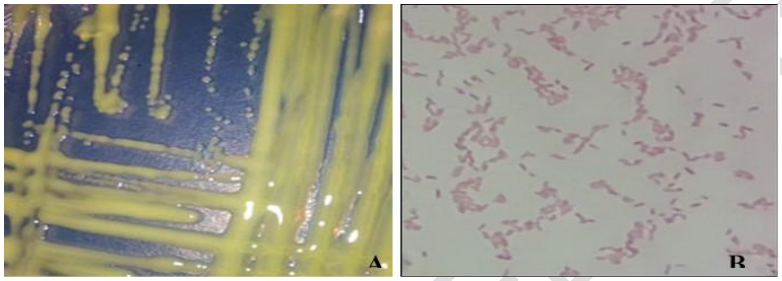

Figure 1. Macroscopic (A) and microscopic (B) aspects of bacterial cells.

No isolates with the macroscopic characteristics of *Xanthomonas oryzae* was observed in OPIB samples. Of the 5 isolates, we had three (3) isolates from the rice variety Adny11, one isolate from the variety Kogoni 91.1 and another from wild rice, *Oryza longistaminata* (Table 1).

Table 1: Sample number, sampling sites, isolate code, rice varieties and symptoms observed on samples from which the corresponding bacteria was isolated.

Sample numbers	Sampling sites	Isolates code	Rice varieties	Observed symptoms
9	Niono	Xoo Ad9	Adny11	Rice bacterial blight
10	Niono	Xoo Ad10	Adny11	Rice bacterial blight
20	Niono	Xoo Ad20	Adny11	Rice bacterial blight
12	Niono	Xoc Ko12	Kogoni 91.1	Bacterial leaf strike
13	Niono	Xoc Lo13	*Oryza Longistaminata*	Bacterial leaf strike

3.1.2. Identification of the isolates

3.1.2.1 Common biochemical characteristics

Common biochemical characteristics of the isolates are presented in Table 2. Analysis of the table 2 showed that all isolates are catalase, amylase, gelatinase and oxidase positive. On

the contrary, they are all non-fluorescent and oxidase negative with the exception of *Xoo*-Ad20 which is more or less oxidase positive

Table 2: Results of biochemical characteristic tests of the isolates

Isolates	Common biochemical characteristics						
	*KOH	[1]King B	Oxydase	Catalase	Amylase	Gelatinase	O/F
***Xoo* Ad9**	+	-	-	+	+	+	O
***Xoo* Ad10**	+	-	-	+	+	+	O
***Xoo*-Ad20**	+	-	±	+	+	+	O
***Xoc* Ko12**	+	-	-	+	+	+	O
***Xoc* Lo13**	+	-	-	+	+	+	O

*= solubilisation dans KOH, [1]= fluorescéine, + = positive ; - = negative; ± = Slightly oxidase positive, O/F=Oxydative/fermentative.

3.1.2.2 Differential biochemical characteristics:

The results of the isolate susceptibility test on 0.001% copper nitrate medium and growth test on 0.2% casamino acid medium without vitamin summarized in Table 3. The results of the isolate susceptibility tests on the 0.001% copper nitrate media and growth tests on the 0.2% casamino acid medium without vitamin made it possible to differentiate the isolates as *Xoo* and *Xoc*. These tests revealed that the isolates Xoo Ad9, Xoo Ad10, Xoo Ad20 are *Xanthomonas oryzae pv oryzae* and the isolates Xoc Ko12, Xoc Lo13 are *Xanthomonas oryzae pv oryzicola*.

Table 3 : Résults of the sensibility test of the isolates to nitrate with copper at 0,001% et au test de croissance à 0.2% d'acide casaminique en l'absence de vitamine.

Isolats	Sensitivity on 0,001% copper nitrate	Growth on à 0.2% casamino acid without vitamins	Interpretation
Xoo Ad9	+	-	*Xoo*
Xoo Ad10	+	-	*Xoo*
Xoo Ad20	+	-	*Xoo*
Xoc Ko12	-	+	*Xoc*
Xoc Lo13	-	+	*Xoc*

3.1.2.3 Identification des isolats par la PCR

After amplification and migration of the DNA of the isolates, two (2) migration patterns were observed: profile 1 showed three bands of respective sizes 331, 691 and 945 bp for the strains Xoc Ko12, Xoc Lo13; while profile 2 showed two bands of sizes 331 and 162 bp for the strains Xoo-Ad9, Xoo-Ad10 and Xoo-Ad20 (figure 2).

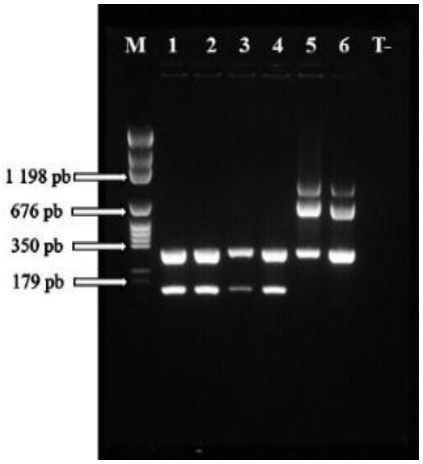

Figure 2. Photo of electrophoresis gel. **M**: Marker, **1**: Control *Xoo*, **2**: Ad9, **3**: Ad10, **4:** Ad20, **5**: Ko12; **6**: Lo13; **T-**: Negative control.

3.1.2.4 Endophytes with high antimicrobial activity against Xanthomonas oryzae pv. Oryzae and Xanthomonas oryzae pv. Oryzicola

After confirming the identities of the strains by PCR, the antimicrobial activity of the endophytes ADNY9, REM8, NIE6 against all strains Xoo and Xoc was shown to be effective, in vitro (Table 4).

Table 4. Effects of the endophyte bacteria tested against *Xanthomonas oryzae* pv. *Oryzae* et *Xanthomonas oryzae* pv. *oryzicola*

Isolates	**Tested endophytes**		
	ENDO-ADNY9	**ENDO-REM8**	**ENDO-NIE6**
Xoo-Adny9	+++	++	+
Xoo-Adny10	+++	++	+
Xoo-Adny20	+++	++	+
Xoc-Kogoni12	++	++	+
Xoc-Longis13	++	++	+

+ = 5mm≤D≤9 mm ; ++ = 10mm≤ D≤19mm ; +++ = D > 20 mm

The 3 strains of endophytes tested, showed significant antibacterial activity against Xoo and/or Xoc with at least an inhibition diameter of 6 mm. Compared to other endophytes, the

ADNY9 endophyte was shown to be more effective against all Xoo and Xoc strains with a maximum zone of inhibition of 28.5 mm (Figure 3). On the other hand, the endophyte ENDO-NIE6 has been shown to be less effective with a maximum inhibition zone of 11mm and a minimum of 6mm.

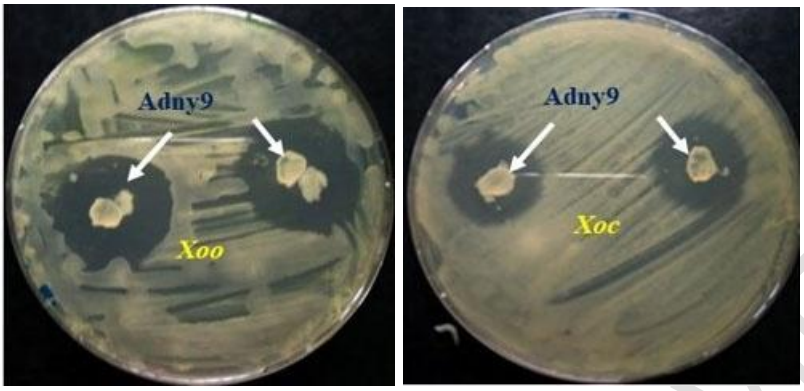

Figure 3. Antimicrobial activities of endophyte Adny9 against *Xanthomonas oryzae* pv. *oryzae and Xanthomonas oryzae* pv. *Oryzicola*.

3.2 Discussions:

Several techniques and isolation media have been developed to isolate *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola*. These techniques are essentially based on (i) the addition to the isolation media of antibiotics and antifungals (which can inhibit the growth of other saprophytic microorganisms close to *Xanthomonas* genus and (ii) isolation from a solution consisting of a portion of infected leaves macerated in sterile distilled water [19], [6]. Xoo and Xoc strains.

This isolation technique showed that out of a total of thirty (30) samples collected with symptoms of BLB or BLS, 12.66% showed colonies that looked like *X. oryzae*. These isolates were obtained from 5 different samples, all taken from the fields of the Office du Niger. On the other hand, no isolates with the macroscopic characteristics of *X. oryzae* were observed from the samples collected in Baguineda. According to [19], when Xoo or Xoc could not be isolated from symptomatic leaves, isolation should be resumed. As a result, isolation of the remains of twenty-five (25) samples that tested negative was resumed. The results of these re-isolations were consistent with those previously observed. [16] reported similar results in Mali, out of 90 samples collected with symptoms of BLB and / or BLS, approximately 37% gave X. oryzae-appearing colonies on the PSA culture medium. This result can be explained by the following various reasons: (i) use of phytosanitary products which can accumulate in the rice fields and affect the beneficial and pathogenic microorganisms they harbor and (ii) taking symptomatic samples at stages of advanced bacterial infection. [19] reported that at advanced stages of infection, isolation of bacteria may fail. Likewise, [20], also reported that in the pale-yellow stage of the leaves, bacteria are not present on the leaves but rather on the stem, which may explain the failure of isolation on the leaves at these stages.

The isolates obtained were first characterized and identified which revealed that all the isolates are of *X. oryzae* identified three (3) strains of Xoo and two (2) strains of Xoc. The microbiological and biochemical characteristics obtained from our isolates are similar to those described by [10]. These results are not consistent with those of [20], who in a study conducted in Iran obtained opposite results with regard to the production of catalase and amylase. These authors have isolated Xoo strains which do not produce catalase or amylase. Such contradictory results may explain the diversity of African strains compared to those of Asia. The identification of the isolates from their microbiological and biochemical characteristics as Xoc and Xoo, was confirmed by PCR technique. In our study, the two X oryzae pathovars were distinguished by multiplex PCR with four specific primer pairs first used by [17]. An expected profile composed of three (3) bands was obtained from the strains Xoc Ko12, Xoc Lo13 and another expected profile and composed of two (2) bands was obtained from the strains Xoo-Ad9, Xoo Ad10 and Xoo Ad20. These results obtained correlate with those of [17] and [6] who obtained the same strains. Despite the contradictory results of the amylase, catalase tests compared to those of [21], the PCR confirmed that Xoo-Ad9, Xoo Ad10 and Xoo Ad20 are Xoo strains. Indeed, the use of multiplex PCR remains the safest and fastest way to identify the two pathovars. As for the pathogenesis test, it also differentiated the pathovars of X. oryzae by observing the symptoms. Indeed, Xoo-Ad9, Xoo Ad10 and Xoo Ad20 induced symptoms of bacterial wilt and Xoc Ko12, Xoc Lo13 induced symptoms of leaf streak. These results prove that all the strains isolated are pathogenic and confirm the results obtained by microbiological and biochemical identification and by PCR. In this study, we note that all the Xoo strains were isolated from the variety Adny11 and those of Xoc from the variety Kogoni 91-1 and *Longistaminata*. [16] reported similar ones, but he also isolated strains of Xoo and Xoc on the same varieties in the Niger office which was not the case in our study. In Mali, Adny 11 and Kogoni 91-1 are among the most cultivated rice cultivars.

4. CONCLUSION

Two rice bacterial pathogens, *Xanthomonas oryzae* pv. *Oryzae (Xoo) and Xanthomonas oryzae* pv. *Oryzicola (Xoc)*, were isolated and identified in rice fields in Mali. An endophyte bacterium Adny9 isolated from Adny seeds showed high capacity to control the growth of the two isolated rice pathogens. This endophyte can be used to formulate an efficient and low-cost biopesticide to improve rice production by minimizing the impact of *Xoo* and *Xoc.*

REFERENCES

[1] Khan JA, Siddiq R, Arshad HMI, Anwar, HS, Saleem, Jamil FF. Chemical control of bacterial leaf blight of rice caused by Xanthomonas oryzae pv. oryzae. Pakistan Journal of Phytopathology. 2012; 24(2): 97-100.

[2] Nagendran K, Karthikeyan G, Peeran MF, Raveedran M, Prabakar K. Management of Bacterial Leaf Blight disease in rice with endophytic bacteria. World Applied Sciences Journal. 2013 ; 28 (12) : 2229-2241.

[3] PNIP – SA. Plan National d'Investissement Prioritaire dans le Secteur Agricole au Mali. Rapport d'Aout 2010..

[4] Baris P, Zaslavsky J, Perin S. La filière riz au Mali : compétitivité et Perspectives de marché, 2005, 63p.

[5] Nino-liu DO, Ronald PC, Bogdanove AJ. Xanthomonas oryzae pathovars: model pathogens of a model crop. Molecular plant pathology. 2006; 7(5): 303-324

[6] Wonni I, Cottyn B, Detemmerman L, Dao S, Ouedraogo L, Sarra S, Tekete C, Poussier S, Corral R, Triplett L, Koita O, Koebnik R, Leach J, Szurek B, Maes M, Verdier V. Analysis of Xanthomonas oryzae pv. oryzicola population in Mali and Burkina Faso reveals a high level of genetic and Pathogenic Diversity. Phytopathology 2014; 104(5): 520-531.
[7] Sarra S, Diarra, L, Dembele, M., Coulibaly, M.M, Séré, Y. 2010. Characterization of bacterial leaf blight epidemic in the Office du Niger (Mali) and search for a sustainable resistance against the pathogen.
[8] Mallé I, Kassogué A, Babana A H, Abreu C, Ivodio I. A Malian native Azospirillum sp, Az6-based biofertilizer improves growth and yield of both rice (Oryza sativa L,) and maize (Zea mays L,) AJMR. 2020; 14(7): 286-293.
[9] Rafi A, Hameed A, Akhtar MA, Tullah F, Sohail K, Shahid M, Fahim M. Identification and characterization of Xanthomonas oryzae pv. Oryzae in North-West of Pakistan, Sarhad J. Agric. 2013; 29(3): 423-431
[10] Mew TW, Misra JK. A manual of rice seed health testing. Int. Rice Res. Inst., 1994.
[11] Jabeen R, Ifthikar E, Batool H. Characterization, preservation and pathogenicity test of Xanthomonas oryzae pv. oryzae causing BLB disease in rice. Pak. J. Bot. 2012; 44(1): 261-265.
[12] Ouedraogo SL, Somda I, Wonni I, Seré Y ,2007. Etude de la Résistance au flétrissement bactérien de lignés inter- et intra spécifiques de riz de bas-fonds en conditions d'infestation artificielles. African Crop Science Journal. 2007; 15: 191-199.
[13] Kovacs N. Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature, Lond. 1956 178 : 703.
[14] Ouedraogo SL, Mortensen CN. Quelques méthodes de détection de Xanthomonas campestris pv vesicatoria dans les semences de la tomate et de l'aubergine, 2003, 12p.
[15] Gerhardt P. (Ed.). Manual of Methods of General Bacteriology. Amer. Soc. Of Microbiol Washington, D.C., 1981.
[16] Dao S. Contribution à l'amélioration de la résistance du riz au Xanthomonas oryzae au Mali. Mémoire du diplôme d'étude approfondie, Université des Sciences, des Techniques et des Technologies de Bamako, Faculté des Sciences et des Techniques. 2013; 57 p.
[17] Lang J, M Hamilton JP, Diaz MGQ, Van SMA, Burgos, M. R. G., Vera-Cruz, C.M., Buell C. R., Tisserat, N, A., Leach, J. E. Genomics-based diagnostic marker development for Xanthomonas oryzae pv. oryzae and X. oryzae pv. oryzicola. Plant disease. 2010; 94(3): 311-319.
[18] Hastuti RD, Lestarri Y, Suwanto A, Saraswati R. Endophytic Streptomyces spp. as biocontrol agents of rice Bacterial Leaf Blight pathogen (Xanthomonas oryzae pv. oryzae). HAYATI Journal of Biosciences. 2012; 19 (4): 155-162.
[19] OEPP/EPPO. Xanthomas oryzae: Diagnostics, 11p Bulletin OEPP/EPPO Bulletin 37, 2007, 543–553 p
[20] Sawadogo WM. Criblage de variétés et lignées isogoniques pour la résistance à Xanthomonas oryzae pv oryzae et étude du développement de l'épidémie flétrissement bactérien du riz sur le périmètre irrigue de barge (burkina faso), 2008, 191p.
[21] Ghasemie E, Kazempour MN, Padasht F. Isolation and identification of Xathomonas oryzae pv. oryzae the causal agent of bacterial blight of rice in Iran. Journal of Plant Protection Research 48(1), 2008.