Isolation and Characterization of Seed-Borne Pathogenic Bacteria from Rice (*Oryza sativa* L.) in Tanzania

[1]Ashura* L.K., R.B. Mabagala[1] and C. N. Mortensen[2]

[1]Department of Crop Science and Production, Sokoine University of Agriculture, P.O. Box 3005, Morogoro, Tanzania

[2]Danish Government Institute of Seed Pathology for Developing Countries, Ryvangs Alle 78, DK-2900 Hellerup, Copenhagen, Denmark

Abstract

*A study was conducted to isolate and characterize seed-borne bacterial pathogens from sixty rice (**Oryza sativa** L.) varieties in Tanzania. Various methods, including the slide cassette holder test, liquid assay and direct plating, were used to isolate bacterial pathogens. The isolated bacterial strains were identified based on colony morphology, biochemical, serological and pathogenicity tests. **Acidovorax avenae** subsp. **avenae**, the causal agent of brown stripe was detected in 63% of the seed samples tested indicating that this pathogen is widely distributed in Tanzania. Other pathogens identified were **Pantoea agglomerans** causing palea browning, **Xanthomonas oryzae** pv. **oryzae** causing bacterial blight and **Burkholderia glumae** which causes grain rot of rice. The presence of these seed-borne bacterial pathogens in rice indicates the importance and the need for designing effective control measures of such diseases in order to limit their spread to various rice growing regions in Tanzania where they may not be present.*

Key words:Bacteria, Disease, *Oryza sativa*, Seed-borne, *Acidovorax avenae* subs. *avenae*, *Pantoea agglomerans*

Introduction

There have been records of occurance of various bacterial diseases of rice (*Oryza sativa* L.) that affect the grain, seedling and flag leaf sheath (Azegami *et al.*, 1987, Zeigler *et al.*, 1987; Zeigler and Alvarez 1990; Miyajima *et al.*, 1983; Shakya *et al.*, 1985; Shakya and Chung, 1985; Rott *et al.*, 1989; and Jaunet *et al.*, 1995). These diseases seem to be prevalent in tropical countries of Asia and Africa and cause discoloration of the flag leaf sheath and discoloration of the grain (Cottyn *et al.*, 1996).

Discoloured and poorly filled grains cause economic losses to rice farmers. The use of certified rice seed in Tanzania is very limited. Farmers still retain their seed from the previous season for use in the next season. This means that the pathogens which are seed-borne are multiplied with the seed. Several bacterial pathogens are known that affect the sheath, leaves, panicles and grain of the rice plant (Cottyn *et al.*, 1996; Rott *et al.*, 1989, Zeigler *et al.*, 1987). These include *Xanthomonas oryzae* pv *oryzae* causing bacterial leaf blight, *Acidovorax avenae* subsp *avenae* causing brown stripe *Pseudomonas fuscovagnae* sheath brown rot, *Pseudomonas syringae* causing sheath brown rot, *Pseudomonas plantarii* causing seedling blight, *Pantoea agglomerans* causing palea browning and *Burkholderia glumae* causing grain rot.

Although the above pathogens have been reported from many tropical countries, this information is lacking in Tanzania. This study was therefore conducted to investigate the presence

*Corresponding author

of the pathogenic bacteria in rice seed varieties grown in Tanzania.

Materials and Methods

Materials

The rice seed samples which were used in this study were from 60 cultivars and breeding lines obtained from rice research programmes at Sokoine University of Agriculture and Agricultural Research Institute (ARI), KATRIN, Ifakara. Samples from ARI-KATRIN which is the coordinating centre for rice research in Tanzania included some cultivars collected from other regions in the country (Tables 1 and 2). The seed samples were harvested during 1995 and 1996 growing seasons.

Isolation of bacteria

The isolation of plant pathogenic bacteria was done by using the slide cassette holder method (Shakya and Chung, 1983) direct plating of seeds (Zeigle, *et al.*, 1987) and liquid assay (Tsushima *et al.*, 1986; Di *et al.*, 1991).

For the slide cassette holder method, 200 rice seeds were placed between pairs of filter papers inserted in each slide holder of the cassettes. The slide cassettes holding the

Table 1: Percentage of seedlings showing symptoms of various bacterial pathogens and pathogen isolated using the seedling symptom Slide Cassette-Holder Method (rice seeds from SUA, Morogoro)

Acc. No.	Variety/Line	Source	Percentage of Germinated seed	Percentage of Seedlings w/Symptoms	Pathogen(s) Isolated (Biolog similarity)
40551	Themanini	Ifakara	83.0	54.2	*A. avenae* subsp. *avenae* (0.907)
40552	Limota	Ifakara	95.5	15.7	*P. agglomerans* (0.277)
40554	Shingo ya mwali	Ifakara	62.0	28.0	*P. agglomerans* (0.47)
40555	Mbawa mbili	Ifakara	93.0	43.0	*A. avenae* subsp. *avenae*, *Xanthomonas* sp.
40556	Faya mafuta	Ifakara	94.5	28.5	*A. avenae* subsp. *avenae* (0.869)
40557	Chakula na Bwana	Ifakara	86.5	13.0	No pathogen identified
40559	Afaa Kikangaga	Ifakara	88.0	7.5	*A. avenae* subsp. *avenae* (0.759)
40560	Rangi mbili	Ifakara	92.5	67.0	*A. a.* subsp. *avenae* (0.869), *P. agglomerans* (0.182)
40561	Kaling'anaula	Ifakara	95.0	35.0	No pathogen identified
40562	Faya-Mzinga	Ifakara	84.0	43.5	*A. a.* subsp. *avenae* (0.875), *Xanthomonas maltophilia* (0.769)
40564	Mwarabu	Ifakara	95.5	3.0	No pathogen identified
40565	Loya	Ifakara	84.0	14.5	*A. a.* subsp. *avenae* (0.864), *X. maltiphilia* (0.849)
40566	Supa India	Ifakara	83.0	27.5	*S. plymuthica* (0.712), *P. agglomerans* (0.698)
40568	Pishori	Mwanza	97.5	26.0	*P. agglomerans* (0.423)
40569	Kula na Bwana	Ifakara	82.0	36.0	*A. avenae* subsp. *avenae* (0.908)
40570	Dakawa 83	Ifakara	78.0	38.5	*A. avenae* subsp. *avenae* (0.555)
40572	Afaa Mwanza	Ifakara	77.5	20.5	No pathogen Identified
40573	Usiniguse	Ifakara	66.0	28.0	*P. agglomerans*, *Serratia plymuthica* (0.619)
40574	Supa	Mwanza	84.5	38.5	*A. avenae* subsp. *avenae* (0.658)
40575	Supa India	Dakawa	94.5	27.0	*A. avenae* subsp. *avenae* (0.719)
40576	Honda	Ifakara	61.0	50.0	*A. avenae* subsp. *avenae*
40589	Lac 23	Ifakara	88.0	28.5	No pathogenic bacteria detected
40590	Yogoyamuchi	Bagamoyo	79.5	15.0	No pathogen bacteria detected
40591	ML 215	Zanzibar	53.0	26.5	*A. avenae* subsp. *avenae* (0.693)
40592	Wabis 18	Ifakara	88.5	2.0	*A. avenae* subsp. *avenae* (0.792)
40593	CT 6447-7-1-1-7	Ifakara	83.5	29.0	*A. avenae* subsp. *avenae* (0.693)
40594	BKN 3036B	Zanzibar	96.0	60.0	*A. avenae* subsp. *avenae* (0.824)
Table 2.	continued.	Bagamoyo	73.0	35.0	*X. streltizia* (0.585)
40595	Koshinikari				
40597	Jaribu 220	Ifakara	67.5	16.5	No pathogenic bacteria detected
40598	BKN 3036A	Zanzibar	89.5	56.0	*A.a.* subsp. *avenae* (0.840), *X. maltophilia* (0.920)
40599	Chianung Sen Yu	Zanzibar	90.0	44.5	*A.a.* subsp. *avenae* (0.847), *P. putida* (0.706)
40600	Colombia 25592	Zanzibar	0.01	0	No pathogenic bacteria detected
40601	IR 47686-15-1-1	Ifakara	90.5	33.0	*X. oryzae* pv. *oryzae* (0.895)
40602	Subarmati	Zanzibar	85.0	48.5	*A. avenae* subsp. *avenae* (0.566)
40603	Wabis 844	Ifakara	90.5	45.0	*P. agglomerans* (0.516)
40604	TXD 276-4-5-11	Ifakara	92.5	41.0	*A. avenae* subsp. *avenae* (0.561)
40605	Mangetsumochi	Bagamoyo	87.0	0	No pathogenic bacteria detected
40609	IR 13429-2-1-3	Ifakara	72.5	8.0	No pathogenic bacteria detected
40610	BU 380-2	Ifakara	71.5	5.5	No pathogenic bacteria detected

Table 2: Percentage of seedlings showing symptoms of various bacterial pathogens and pathogens isolated using the seedling symptom Slide-Cassette Holder Method (rice from ARI, KATRIN, Ifakara)

Acc. No.	Variety/Line	Source	Percentage of Germinated seed	Percentage of Seedlings w/Symptoms	Pathogen(s) Isolated(Biolog similarity)
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40609	IR 13429-2-1-3	Ifakara	72.5	8.0	No pathogenic bacteria detected
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seeds were then placed in plastic trays containing 230 ppm nitrogen until about 1cm of the filter paper was covered by the solution. The nitrogen solution was made by dissolving 230 mg of urea (H_2NCONH_2) in 1000 ml of distilled water. Four rice seeds were placed between each pair of moistured filter papers. The trays with cassettes were incubated in a humid chamber and kept in growth rooms at 95-98% relative humidity, 25^0-30^{0}C and 12 daylight for 10 to 14 days.

Germinating seedlings were observed for the presence of typical bacterial stripe symptoms and other symptoms. The seedlings with symptoms were counted and recorded.

Four to five seedlings showing typical bacterial brown stripe symptoms on the coleoptile, leaf blade and leaf sheaths were taken for isolation. Small areas of the diseased tissue including health portions of the neighbouring tissue were placed on a flame sterilized glass slide in a sterile drop of water. A cover slip was put on a drop and the sample was examined for bacterial streaming under a compound microscope at 40x to 100x magnification. Isolations were made from leaf tissues which were positive for bacterial streaming. Cover slips were removed, and the leaf tissues were teased apart in a few drops of sterile distilled water using a glass rod and left to stand for 10-15 minutes to allow the bac-

terial cells to move out of the macerated tissues. Using a bacterial drop, bacterial suspensions were streaked a number of times onto medium B (KB) of King *et al.*, (1954) in triplicate. Inoculated plates were incubated at 25°C to 30°C for 2 to 3 days. The bacterial colonies were purified by a series of single colonies transfer on KB and nutrient agar (NA) for further characterization (Agarwal *et al.*, 1989).

For the liquid assay method, three media were used for the detection of *Xanthomonas oryzae* pv *oryzae*, *Burkholderia glumae* and *Pseudomonas fuscovaginae*. In this method 400 seeds were ground into course flour using a blender and suspended in 200 ml of sterile saline solution for 2 hours. Four ten-fold serial dilutions were made in nutrient broth test tubes. Aliquots of 0.01 ml of the resulting suspension were plated onto mXOS selective medium for *X.o.* pv. *oryzae*, (Di *et al.*, 1991) S-PG semi selective medium for *B. glumae* (Tsushima *et al.*, 1986) and Miyajima's semi-selective medium for *P. fuscovaginae* (Miyajima, 1983). The inoculated plates were incubated for 3-5 days at 28°C.

Direct plating onto King's medium B and Miyajima's selective medium (Miyajima, 1983) was done for detection of *Pseudomonas fuscovaginae* as described by Zeigler *et al.*, 1987. In this assay 200 surface sterilized seeds were collected in a cheese cloth and washed in running tap water for 4 hours and then dried on filter papers. The washed seeds were plated by partially embedding them in the King's medium B and Miyajima's medium. Twenty five seeds were plated in each petri dish and incubated at 27°C for 24-48 hours. The plates were observed under Near Ultra Violet (NUV) light (under UV lamp) and loopfuls of fluorescing bacterial colonies were transferred to Miyajima's medium or nutrient agar and incubated for 2 days at 27°C.

Identification of bacterial isolates

Pure bacterial isolates from the seedlings were identified based on colony morphology on NA, pigment production on KB, Gram reaction based on Potassium hydroxide solubility tests (Lelliot and Stead, 1987), oxidase reaction (Kovac, 1959, Schaad, 1988, Hildebrand and Schroth, 1968), arginine dihydrolase, levan production, gelatin hydrolysis, nitrate reduction (Fahy and Persley, 1983; Schaad, 1988), tobacco hypersensitivity reaction (Klement, 1983) pathogenicity tests (Shakya and Chung, 1983, Kauffman *et al.*, 1973), and Biolog identification system (Biolog, Inc, 1993, Bouchner, 1989).

Colonies suspected to be *X. oryzae* pv *oryzae* were also tested serologically by ELISA (Agden Diagnostic system), PCR and pathogenicity on rice seedlings of IR 64 variety.

Results

Symptoms of seedlings germinated under the slide cassette method

Results of the percentage of germinated rice seed and seedlings with bacterial disease symptoms are shown in tables 1 and 2. The germination percentage of all rice varieties were above 50% except accession no. 40600 where only two seedlings germinated out of 200 seeds which were plated. The percentage of seeds with bacterial disease symptoms varied from 0 for accessions 40600 and 40606 to 67% for accession no. 40560.

The symptoms which were observed on the seedlings included brown lesions on the coleoptile, leaf sheaths and sometimes the stripes extended to the leaf blades. Yellowing of the leaves, stunted seedlings and brown lesions on the leaf tips were also observed.

Bacteria isolated from seedlings of the slide cassette holder methods

Out of 4-5 individual seedlings used for the isolation of bacteria, 5-8 isolates were obtained depending on the type of symptoms observed. The bacterial pathogens isolated were mostly *Acidovorax avenae* subsp. *avenae* (38 samples); *Pantoea agglomerans* (10 samples) and *Xanthomonas spp.* (5 samples) [Tables 1 and 2]. No pathogenic bacteria was isolated from accession nos. 40553, 40557, 40561, 40572, 40589, 40590, 40597, 40600, 40605, 40609 and 40610.

In some instances, *Acidovorax avenae* subsp. *avenae* was detected in association with

other pathogens such as *P. agglomerans* and *Xanthomonas spp.* The colonies of *A. a.* subsp. *avenae* were cream to white, raised, entire, circular and non-fluorescent on KB. The isolates gave a strong positive reaction to tobacco and pepper plants, 24 hours after inoculation. The isolates were also highly pathogenic to rice plants giving brown stripe on leaf sheath extending to the midrib of the leaf.

Some yellow bacterial isolates which were identified by as *X. maltophilia* were neither fermentative nor oxidative positive. They produced a brown diffusible pigment on KB medium and had a variable hypersensitive reaction on tobacco and pepper. However these were not pathogenic on rice seedlings using the stem inoculation methods.

Isolates identified as *P. agglomerans* had colonies that were yellow, circular, entire and fast growing. They were gram-negative and facultative anaerobes. The suspected colonies gave negative results for nitrate reduction, gelatin liquefaction and starch hydrolysis.

Fluorescent isolates isolated by the seedling symptom-slide cassette holder method induced a hypersensitive reaction in pepper only and not in tobacco. The Biolog identified them as *P. putida* type A1. Some of the fluorescent isolates did not give a hypersensitive reaction to tobacco and pepper and were thus discarded. The characteristics of bacteria isolated from rice seed by slide cassette holder method are summarised in Table 3.

Bacteria isolated using the liquid assay method

No bacterial colonies were recovered on Miyajima's semi-selective medium. Phyto-pathogenic bacteria isolated on mXOS medium are shown in Table 4. Colonies suspected to be *X.o. oryzae* were identified based on the comparison with typical morphology of the reference *X.o. oryzae*. The isolated colonies were mucoid and yellow on NBY medium. All the tested isolates were oxidase negative for oxidative metabolism of glucose (Table 4). Twelve isolates were identified as *X.o.* pv. *oryzae* F by Biolog and three were identified as *X. campestris* pv. *dieffenbachiae*. In addition, these bacterial isolates induced a hypersensitive reaction in pepper and a few in tobacco as well. Pathogenicity test by `clip' inoculation of IR 64 seedlings produced water-soaked lesions on the clipped end and yellow discoloration which extended downwards.

On S-PG medium bacterial isolates were recovere from accession no. 40555 (Variety Mbawa Mbili). These had a biochemical reaction similar to *B. glumae* reference isolate. Colonies of *B. glumae* isolates on S-PG medium closely resembled type B colonies. They produced a non-fluorescent yellowish-green

Table 3: Selected biochemical characteristics of the bacterial strains identified using seedling symptoms slide cassette-holder method

		Biochemical tests								
No. of samples	Biolog identity	Fluore/ colour pigment	Kovac's oxidase	O/F	NR	GL	SH	LS	HR T/P	Pathog on rice
38	*A. avenae* subs *avenae*	-/white	+	+/-	+	-	-	-	+/+	+
10	*P. agglomerans*	-/yellow	-	+/+	+	-	-	-	+/+	+
2	*P. dispersa*	-/yellow	-	+/+	+	+	-	-	+	-
1	*C. acidovorans*	-/white	+	+/-	-	-	-	-	+/+	-
3	*X. maltophilia*	-/yellow	-	-/-	+	-	-	-	+/+	-
1	*P. corrugata*	-/white	+	-/-	+	+	-	-	+/-	-
3	*S. plymuthica*	-/white	+	+/-	+	-	-	-	+/-	-
1	*X. campestris* pv *strelitizia*	-/yellow	-	+/-	+	+	-	-	-/+	-
1	*P. putida* type A1	+/white	+	+/-	+	+	-	-	-/+	-
1	*X. o.* pv *oryzae*	-/yellow	-	+/-	+	+	+	-	+/+	+

O/F = Oxidase/Fermentative, NR- Nitrate reduction, GL = Gelatine liquefaction, SH-starch hydrolysis, LS = Levan production from sucrose, HR = Hypersensitivity reaction, T/P = Tobacco/Pepper, Pathog = Pathogenesis

Table 4: Selected morphological and biochemical characteristics of bacteria isolates from rice seed as detected by the Liquid Assay on mXOS.

Acc. No./ Isolate(s)	Fluorescent/ Colour of Pigment	Kovac's Oxidase	O/F	NR	GL	SH	LS	H R T/P	Pathog on Rice	Biolog Identification (Similarity)
40555/a	-/yellow	-	+/-	-	+	+	-	-/+	+	*X.o.* pv. *oryzae* F(0.790)
40555/b	-/yellow	-	+/-	-	+	+	-	+/+	+	*X.o.* pv. *oryzae* F(0.847)
40555/c	-/yellow	-	+/-	-	+	+	-	+/+	+	*X.o.* pv. *oryzae* F(0.887)
40555/g	-/yellow	-	+/-	-	+	+	-	+/+	-	*X.c.* pv. *dieffenbachiae* (0.882)
40562/b	-/yellow	-	+/-	-	+	+	-	-/+	+	*X.o.* pv. *oryzae* (0.784)
40562/c	-/yellow	-	+/-	-	+	+	-	-/+	+	*X.c.* pv. *dieffenbachiae* (0.867)
40562/e	-/yellow	-	+/-	-	+	+	-	-/+	+	*X.o.* pv. *oryzae* (0.367)
40562/j	-/yellow	-	+/-	-	+	+	-	-/+	+	*X.o.* pv. *oryzae* (0.533)
40566/c	-/yellow	-	+/-	-	+	+	-	-/+	+	*X.o.* pv. *oryzae* (0.886)
40567/b	-/yellow	-	+/-	-	-	+	-	-/+	-	No identification
40567/d	-/yellow	-	+/-	-	-	+	-	-/+	-	*X.o.* pv. *oryzae* F(0.442)
40567/h	-/yellow	-	+/-	-	+	+	-	-/+	-	No identification
40567/I	-/yellow	-	+/-	-	+	+	-	-/+	-	No identification
40587/2	-/yellow	-	+/-	-	+	+	-	-/+	+	*X.o.* pv. *oryzae* F(0.327)
40587/3	-/yellow	-	+/-	-	+	+	-	-/+	+	*X.o.* pv. *oryzae* F(0.648)
40587/5	-/yellow	-	+/-	-	+	+	-	-/+	+	*X.o.* pv. *oryzae* F(0.687)
40587/6	-/yellow	-	+/-	-	+	+	-	-/+	-	*X.o.* pv. *oryzae* (0.511)
40587/8	-/yellow	-	+/-	-	+	+	-	-/+	-	*X.c.* pv. *dieffenbachiae* (0.839)
Ref. *X.o.o.*	-/yellow	-	+/-	-	+	+	-	+/+	+	*X.o.* pv. *oryzae* (0.763)

O/F = Oxidase/Fermentative; NR = Nitrate Reduction: GL = Gelatine Liquefaction; SH = Starch Hydrolase; LS = Levan from Sucrose; HR = Hypersensitive Reaction; T/P = Tobacco/Pepper; Paths. = Pathogenicity

Table 5: Selected morphological and biological characteristics of bacteria isolated from rice seed as by the Liquid Assay on S-PG Selective Medium

Acc. No./ Isolate(s)	Fluorescent/ Colour of Pigment	Kovac's Oxidase	O/F	NR	GL	SH	LS	H R T/P	Pathog on Rice	Biolog Identification (Similarity)
40555/a$_1$	-/white	-	+/-	+	+	nd	nd	+/+	+	No identification
40555/b$_1$	-/white	-	+/-	+	+	nd	nd	+/+	+	No identification
40555/c$_1$	-/white	-	+/-	+	+	nd	nd	+/+	+	No identification
40555/d$_1$	-/white	-	+/-	+	+	nd	nd	+/+	+	*Burkholderia gladioli* (0.685)
40555/f$_1$	-/white	-	+/-	+	-	-	-	+/+	+	*A. avenae* subsp. *avenae* (0.658)
40562/4	-/white	+	+/-	+	-	-	-	+/+	+	*A. avenae* subsp. *avenae* (0.733)
40562/8	-/white	+	+/-	+	-	-	-	+/+	+	*A. avenae* subsp. *avenae* (0.864)
40566/C	-/white	+	+/-	+	-	-	-	+/+	+	*A. avenae* subsp. *avenae* (0.759)
40587/d	-/white	+	+/-	+	-	-	-	+/+	+	*A. avenae* subsp. *avenae* (0.564)

O/F=Oxide/Fermentive; NR = Nitrate Reaction; GL = Gelatine Liquefaction; SH = Starch Hydrolysis; HR = Hypersensitive Reaction; T/P = Tobacco/Papper; Pathog = Pathogenicity; ND = Not done.

Table 6: Selected morphological and biochemical characteristics of bacteria isolates from rice seed as detected by Direct Planting Technique on Mayajima's semi selective medium

Acc. No./ Isolate(s)	Fluorescent/ Colour of Pigment	Kovac's Oxidase	O/F	NR	GL	SH	LS	AD	2-Keto Gluc.	HR T/P	P/R	Biolog Identification (Similarity)
40590/a	+/white	+	+/-	-	-	-	-	+	+	-/+	-	No identification
40590/d	-/white	+	+/-	-	-	-	-	-	-	-/+	-	No identification
40593/a	-/yellow	+	+/-	-	+	-	-	+	-	+/+	+	No identification
40593/b	+/white	+	+/-	-	-	-	-	+	-	-/+	-	P. putida type A1 (0.577)
40593/c	+/white	+	+/-	-	+	-	-	+	-	-/+	-	P. putida type A1 (0.517)
Ref P. fuscovaginae	+/white	+	+/-	+	-	-	-	+	+	+/+	+	P. fuscovaginae (0.79)

O/F = Odidase/Fermentative; NR = Nitrate Reduction; GL = Gelatine Liquefaction; SH = Starch Hydrolysis; LS = Levan from Sucrose; AD = Arginine Dihydrolase; 2-Keto Gluc. = 2-Keto Gluconate; HR = Hypersensitive Reaction; T/P = Tobacco/Pepper; P/R = Pathogenicity on Rice

were also isolated from S-PG medium and were clearly identified by Biolog with high similarity values. Their colonies were cream white, circular, entire, smooth and raised with biochemical profile typical of the species (Table 5).

Bacteria isolated using the direct plating method

No pathogenic bacteria was isolated from seeds directly plated on KB medium. Instead, a lot of fungi and non-pathogenic bacteria (fluorescent and non-fluorescent) grew on the medium. Out of 13 samples tested, bacteria isolates which induced Hyper sensitive reaction (HR) were isolated from accessions nos. 40590 and 40593 plated onto Miyajima's agar medium. Three isolates were fluorescing and were identified as *P. putida* type A1. One whitish isolate was identified as *A.a. avenae* while a yellow non-fermentative isolate was not identified.

Pseudomonas reference isolate was positive for 2-ketogluconate production. The positive reaction was also given by one isolate from accession no. 40590. The other fluorescent isolates gave negative results (Table 6).

Discussion

Several bacterial pathogens were isolated and identified by using morphological characteristics, biochemical and serological tests as well as by Biolog identification system.

Seeds of ten varieties viz. Kihogo red, Afaa Mwanza, Chakula na Bwana, Kaling'aula, Lac 23, Yogoyamochi, Jaribu 220, Mangetsumochi, Mutant No. 8, IR 13429-2-1-3 were found to be free of pathogenic bacteria.

Acidovorax avenae subsp. *avenae* was detected in 63% of the seed samples. The *A.a.* subsp. *avenae* isolates had biochemical profile typical of the type species. The colony morphology of all the isolates were similar to that of the reference isolate and Biolog identification gave high similarity indices. Similar results have been reported by Cottyn *et al.*, (1996).

Detection and identification *A.a.* subsp. *Avenae* in most of the seed samples, indicated that this pathogen may be widely distributed in Tanzania. The pathogen has been shown to have a wide distribution in other countries (Shakya *et al.*, 1985; Shakya & Chung, 1985). Presence of *A.a.* subsp. *avenae* in the tested samples indicates the importance of designing measures to control this pathogen which affect seedling vigour. Most of the farmers in Tanzania retain seed from the previous for planting the next season. In addition, the bacterium can survive in rice seed up to 8 years at 5^{0}C (Shakya *et al.*, 1985). This means that the disease can spread very fast if control measures are not taken.

Effective control of brown stripe can be achieved by the use of pathogen-free rice seeds, hot-air treatment (Zeigler *et al.*, 1987) and the use of resistant varieties. Although some bactericides such as Kasugamycin have been used to control brown stripe in rice (Kadota &

Ohuchi, 1990), such an approach may prove to be very expensive for the resource poor farmers in Tanzania. The use of chemicals is also limited by the risk of environmental pollution and an increase in production cost especially for small-holder farmers.

Pantoea agglomerans was detected in 10 out of sixty rice samples, especially those infected with *A.a* subsp. *avenae*. This pathogen is known to affect grain quality by causing grain (palea) discolouration and reducing 1000-seed weight (Azegami *et al.*, 1983). Isolates suspected to be *P. agglomerans* induced a strong hypersensitive reaction to tobacco and pepper seedlings. These facultative anaerobes were oxidase negative, did not reduce nitrate and did not hydrolyse starch. However, the pathogenicity test on rice by stem inoculation produced a weak reaction. This is the first report of *P. agglomerans* in rice seeds in Tanzania. The pathogen was first reported in Japan by Azegami *et al.*, (1983).

About 30 isolates from 5 samples isolated by liquid assay were classified in the genus *Xanthomonas*. Besides, 3 isolates detected by slide cassette-holder method also belonged to this genus. Based on ELISA results, morphological characters, biochemical profile and Biolog identification, the 30 isolates were all *X.o.* pv. *Oryzae*. Colonies of isolates identified as *X.o.* pv. *oryzae* and of the reference bacterium on NBY, were yellow and mucoid as expected. They were, however, positive for starch hydrolysis, which contradicts results reported by Bradbury, 1986. On the other hand, Vera Cruz *et al.*, (1984) reported positive starch hydrolysis from *X.o.* pv. *oryzae* isolates. Further studies are needed to confirm the presence of *X.o.* pv. *oryzae* in rice seed samples from Tanzania.

Burkholderia glumae was isolated from var. Mbawa Mbili. The morphological and biochemical characteristics of these isolates conformed to the features of the species based on the reference bacterium. The detection of *B. glumae* in Tanzania rice samples is a threat to rice production in the country. The pathogen causes both seedling and grain rot and its presence in the seeds should be a major concern, especially in areas where direct seeding is practised.

The isolates obtained from accessions 40590 and 40593, were identified as *Pseudomonas putida* Type A1 by Biolog, which is a fluorescent pseudomonad known to multiply in the rhizosphere of paddy rice plants and has been implicated in `suffocation disease'. The disease arises under conditions of poor drainage (Bradbury, 1986).

The variety Mbawa mbili (acc. 40555), which was obtained from a farmer's store at Ifakara was infected by most of the detected seed-borne pathogens. The pathogens isolated from this variety were *A.a.* subsp. *avenae, B. glumae* and *X.o.* pv. *oryzae*. The farmer is likely to plant the same seeds the following year, thus further multiplying the pathogens. Efforts should be made to give simple recommendations to the farmers on the importance of using healthy seeds when establishing a new crop.

Results from this study indicate that there is a strong need to establish seed certification programmes in the country to ensure that certified rice seeds are available for farmers.

Indeed in Tanzania, there is a need to take precautionary control measures, including the use of resistant varieties and cultural practices, such as elimination of volunteer plants and weed hosts between crops. Apart from that, selection of disease-free seeds and restriction of movement of seeds from one area to another to limit the spread of the disease should be encouraged.

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