Full Length Research Paper

Isolation and characterization of *Bacillus thuringiensis* (Ernst Berliner) strains indigenous to agricultural soils of Mali

Adounigna Kassogué[1], Kadia Maïga[1], Diakaridia Traoré[1], Amadou Hamadoun Dicko[1], Rokiatou Fané[1], Tiby Guissou[2], Fatouma Alhadji Faradji[1], Fernando Hercos Valicente[3], Abdoulaye Hamadoun[4] and Amadou Hamadoun Babana[1]*

[1]Laboratory of Research in Microbiology and Microbial Biotechnology, Faculty of Science and Technology, University of Sciences, Techniques and Technologies of Bamako, Bamako, BP E320 Mali.
[2]Institut de l'Environnement et de Recherches Agricoles, 03 B.P. 7047 Ouagadougou, Burkina Faso.
[3]Embrapa Milho e Sorgo C.P. 151, CEP: 35701 970 Sete Lagoas, Minas Gerais, Brasil.
[4]Institut d'Economie Rurale (IER), Bamako, Mali.

The objective of this work was to isolate and characterize *Bacillus thuringiensis* from agricultural and other insect breeding sites in Mali. A hundred soil samples were collected from Bamako district, Segou, Sikasso and Timbuktu regions. *B. thuringiensis* (Bt) was isolated from the samples using a heat-acetate method and the isolates were identified and classified using morphological and biochemical tests. The frequency of *B. thuringiensis* in soils was noted. The results showed that, 15 out of the 3111 bacterial isolates were putative Bt. thuringiensis. Most isolates produced parasporal crystals. The average Bt index for all the areas sampled was 5.1%; the highest frequency was recorded for Niono in Segou region (11.7) and the lowest for Bozola in Bamako district (0.5). Contrary the known information on the high content and distribution of B. in soils, the agricultural soils of Mali contain few Bt strains, confirmed by the low Bt index obtained.

Key words: *Bacillus thuringiensis*, isolation, agricultural soils, Mali.

INTRODUCTION

In Sub Saharan Africa and especially for Mali, increased rice and maize production are essential to reduce poverty and food insecurity (Colin et al., 2003; Vitale et al., 2007). They are an important staple food, especially for small-holder families with low incomes (Fofana et al., 2008).

However, the productivity of rice and maize plants is threatened by attack from *Orseolia oryzivora* (Harris and Gagné, 1982) and by *Helicoverpa armigera* (Hamadoun et al., 1998; Hamadoun, 1996; Ratnadass and Ajayi, 1995), which has resulted in a drastic reduction in yield from 20 to 40% of the potential yield (Youdeowei, 1989; Seshu-Reddy and Walker, 1990). It is predicted that this decrease in yield will intensify, if no measures are undertaken (FAO, 2013). Presently, the most effective

method of managing these insect pests by small scale farmers is the use of synthetic pesticides (Carneiro et al., 2014). There is the need to develop alternative management strategies because pesticides and are toxic and expensive, and the major pest on maize in Mali, *H. armigera*, have developed resistance to chemical pesticides (Martin et al., 2002). Also, the growing public concern, stricter environmental regulations, and buildup of resistant biotypes to synthetics pesticides have led to an increased interest in alternative environmentally friendly insect management strategies (Vitale et al., 2007).

Several bacterial species act as biological control agents by enhancing plant growth and suppressing insect pest population (Babana et al., 2011; Bathily et al., 2010; Capalbo et al., 2001; Valicente et al., 2008). Among these bacteria, *Bacillus thuringiensis* (Ernst Berliner). *B. thuringiensis (Bt)* a member of a group of crystalliforeus spore forming aerobic, Gram-positive bacteria (Larison, 2006), is found worldwide in a variety of habitats including soil (Carozzi et al., 1991), insects (Carozzi et al., 1991, stored-product dust (Burges and Hurst, 1977), and some plant leaves (Kaelin et al., 1994). Very little is known about the natural ecology of Bt other than that it occurs naturally in many soils. One study of Entwistle (1993) indicated a little capacity of Bt to move laterally in soil. Other studies of Entwistle (1993) found that Bt was not recovered past a depth of 6 cm after irrigation.

The insecticidal activity of Bt was initially discovered in 1911. Some Bt strains produces parasporal crystals containing one or more Cry proteins that may be toxic for different insect orders including the ones damaging agricultural plants and products (Maruthesh, 2007), but its has only been developed on commercial scales over the last 40 years (Capalbo et al., 2001). Yang and Wang (1998) showed that pest control programs management using Bt pesticides result in a reduction of the use of chemical insecticides. Successful applications have been documented in a variety of agriculturally important crops such as cotton, corn, potato, soybean and many vegetables by Yang and Wang (1998). Bt biopesticides could be an alternative to synthetic insecticides that often have unintended harmful effects on non-target insect species. In spite of the importance of *B. thuringiensis* for the management of major agricultural insect pest, and its high potential for the management of *H. armigera* Hübner in maize and *Orselia orizyvora* in rice, there are little information on the quantity and diversity of *B. thuringiensis* in Malian agricultural soils has not been explored in Mali. That why the authors proposed in collaboration with EMBRAPA a proposal funded by Marketplace Africa-Brazil to develop a biopesticide. There is therefore the need to explore the presence diversity and activity indigenous of Bt and to use the efficient method developed by EMBRAPA (Capalbo et al., 2001; Valicente et al., 2008) to formulate biopesticide based on *B. thuringiensis* (Bt) toxins for the biological control maize and rice pests. Hence, this study aimed at isolating and characterizating *B. thuringiensis* from agricultural and other insect breeding sites in Mali. These could potentially be formulated into biopesticides for the management of insect pest of maize and rice.

MATERIALS AND METHODS

Soil sample collection

The samples were collected by scraping off surface material with a sterile spatula and then obtaining a 10 g sample 2 to 5 cm below the surface. Soil samples were taken from locations as diverse as ditches, wetlands and soils under maize, rice, cotton and bean. All samples were placed in sterile plastic bags aseptically and stored at 4°C until processed.

Isolation and characterization of *B. thuringiensis* strains

Isolation of B. thuringiensis strains

Isolation of *B. thuringiensis* strains was conducted according to the method described by Travers et al. (1987). One gram of each sample was suspended in 10 ml sterile distilled water and pasteurized at 80°C for 30 min. For the selection of *B. thuringiensis,* 1 ml of each suspension was added to 10 ml of Luria-Bertani (Merck, Germany) and broth buffered with 0.25 M sodium acetate at a pH of 6.8. The suspensions were incubated at 30°C for 4 h and then heated at 80°C for 4 min. Suspensions were diluted and plated on T3 medium containing per liter: 3 g tryptone, 2 g tryptose, 1.5 g yeast extract, 0.05 M sodium phosphate of pH 6.8, and 0.005 g of $MnCl_2$. After incubation at 30°C for 24 h, the colonies showing similar morphology were selected and examined under phase-contrast microscope to determine the presence of parasporal inclusions and spores. The reference strains *B. thuringiensis* HD-125 (UNAM, Mexico) and *B. thuringiensis* T09 (Institut Pasteur-France), were supplied by Dr. Fernando Hercos Valicente (Embrapa Milho e sorgho, Brazil).

Characterization of B. thuringiensis strains

Bacterial isolates was characterized according to Laridon (2006).

Colony morphology: The colonies which were found to be white to off-white in colour with smooth edges and flat to slightly raised elevation (Rampersad and Ammons, 2002) were selected and marked on the Petri dish. The selected colonies with those characteristics were categorized as possible Bacillus colonies. Total of 144 colonies were chosen based on colony morphology. These selected colonies were then sub-cultured onto new Nutrient Agar plates and incubated.

The gram stain test: A very small inoculum of bacteria was smeared onto a clean slide using an inoculation loop. The sample was diluted with a drop of sterile distilled water and allowed to air dry. It was then heat-fixed by passing the slide through an open flame. The slide was stained with crystal violet for 1 min and rinsed with sterile water. The slides were then stained with Gram's iodine (1% iodine, 2% potassium iodide in water) for 1 min to fix the dye and then rinsed with sterile water. Excess stain was removed with 95% ethanol and then rinsed with sterile water. Specimens were counterstained with Safranin for 1 min, rinsed with water and then air dried. Slides were viewed using light microscopy under oil

immersion (Provine and Gardner, 1974; Bergey's Manual of Systematic Bacteriology, 1986).

Endospore stain (Schaeffer-Fulton staining method): A small inoculum of bacteria was smeared onto a clean slide using an inoculation loop and diluted with a drop of sterile water. Once it was dry the slides were flooded with Malachite green (made by dissolving 5.0 g in distilled water, made up to 100 ml) and immediately steamed over a water bath for 5 min. After cooling, the slides were rinsed with sterile water. The slides were then counterstained with Safranin O (made by dissolving 0.5 g Safranin O powder in distilled water, made up to 100 ml) for 2 min and then rinsed with sterile water. The specimens were viewed under a compound microscope with oil immersion after the slides had dried (Mormak and Casida, 1985; Bergey's Manual of Systematic Bacteriology, 1986).

Catalase test: The test involved adding hydrogen peroxide to each sample of bacteria. 33 µl of 48 h Bt cultures was smeared onto a clean slide and a drop of 10% hydrogen peroxide solution was alloquoted onto the Bt smear and observed using light microscopy. A slide smeared with inoculum free LB media was used as the negative control, and an inoculum of *B. thuringiensis* HD125 was used as the positive control. The slides were analyzed for the formation of oxygen bubbles and photographed using a digital camera. The presence of bubbles indicated the ability to break down hydrogen peroxide into water and oxygen (Bergey's Manual of Systematic Bacteriology, 1986).

Growth above 45°C: All samples were diluted in order to obtain an optical density (OD) reading of 0.3. Spectrophotometer readings were taken with an absorbance of 600 nm (A600) for each sample prior to incubation, and then once daily for a period of 5 days to determine if growth occurred. Isolated samples were incubated in nutrient broth at a temperature exceeding 45°C for a 5 day period. Cultures that showed signs of growth and thus were capable of reproducing at such high temperatures were assumed not to be *B. thuringiensis* and were eliminated as putative Bt isolates (Laridon, 2006).

Presence of parasporal bodies (δ-endotoxins): The presence of parasporal bodies was confirmed using phase-contrast microscopy. Vegetative cells and parasporal bodies were observed on slides freshly coated with a thin layer of 2% water agar (200 mg biological grade agar diluted in 100 ml sterile water). A drop of culture was placed on the slide and observed using phase-contrast microscopy under a 100X oil immersion objective. Parasporal bodies were characterized as either bipyramidal, spherical, rectangular (cuboid), irregular spherical, or irregularly pointed.

RESULTS AND DISCUSSION

B. thuringiensis isolates

The results of the analysis of the 100 samples are shown in Table 1. Observations under a light microscope showed a total of 3111 Bacillus-like colonies out of which 38 isolates (4.7%) were identified as putative *B. thuringiensis* (Bt). These isolates were observed to show the staining proteinaceous crystals characteristic of Bt species. According to the definition of the Bt index which is considered to be the number of identified Bt colonies divided by the total number of Bacillus like colonies examined, the authors obtained an average Bt index of

1,22 for agricultural soils in Mali (Table 1). The Bt index in Malian agricultural soils is very low as *B. thuringiensis* is qualified as an ubiquitous bacterium, found most abundantly in soil habitats all around the world (Theunis et al., 1998; Martin and Travers, 1989). ICRISAT and Niono sampling sites showed the highest Bt index with 7.4 and 11.76, respectively, followed by Fanidiama, Bougouni, Cinzana, CAA, Samanko et Daoudabougou with 2.9, 2.8, 2.7, 2.5, 2.2, 2.1; respectively. Bozala and Dire sampling sites showed the lowest Bt index with 0.5 and 0.7, respectively (Table 1). These results are not in accordance with that of Bernhard et al. (1997), who found Bt isolates abundantly in plant storage systems, mushrooms, soils, compost as well as deciduous and coniferous leaves

Colony morphology

A total of 3111 Bacillus-like were isolated. Colonies with white to off-white color, smooth edges and flat to slightly raised elevation were isolated and sub cultured in fresh Nutrient Agar plates to obtain single colonies (Figure 1). Thirty-eight colonies with those characteristics was categorized as possible *Bacillus* colonies. In 100 soil samples, we obtained only 38 Bt isolates. Contrary to this result, Laridon (2006), working on 30 soil samples collected in tundra plant comminuties recovered 127 *B. thuringiensis* after the examination of 238 bacterial colonies recovered.

Gram staining

Bacillus species are generally gram positive and rod-shaped (Figure 1). Gram staining was done to differentiate gram positive from the gram negative. Light microscope was used for observations. Isolates which were rod shaped and blue in color indicates a gram positive strain whereas isolates which did not exhibit these characteristics were discarded.

Under the microscope, the vegetative cells of *Bacillus* are thin and long. All samples isolated from morphological characterization and sodium acetate selections tested as Gram positive (Table 2) rod shaped bacteria (Figure 2).

Phase contrast microscopy

Phase contrast is carried out after determining the colonies are gram positive through gram staining. This procedure is important to confirm the isolates are *Bacillus* by viewing the endospore and parasporal bodies. Besides that, phase contrast microscopy was also done for vegetative phase cells to confirm the isolates were rod shape (Figure 2). The authors mounted slides prepared

Table 1. Isolation of *Bacillus thuringiensis* from Malian soil samples.

Sampling site	Bacillus-like isolates	Total Bt isolates	Bt index
Bozola (B)	2181	12	0.5
Daoudabougou (D)	375	8	2.1
Samanko (S)	46	1	2.2
CAA (C)	80	2	2.5
ICRISAT (I)	54	4	7.4
Cinzana (C)	73	2	2.7
Niono (N)	34	4	11.76
Dire (Di)	130	1	0.7
Fanidiama (F)	68	2	2.9
Bougouni (Bo)	70	2	2.8
Total	3111	38	1.22

Table 2. 15 bacteria samples isolated from agricultural were identified as putative *Bacillus thuringiensis* isolates.

Sample name	Source	Mobility	Gram stain	Spore stain	Catalase	Growth over 45°C	Parasporal bodies
D_3G	Soil	+	+	+	+	-	+
B_9G	Soil	+	+	+	+	-	+
CBt_1	Soil	+	+	+	+	-	+
IBt_1	Soil	+	+	+	+	-	+
DBt_2	Soil	+	+	+	+	-	+
SBt_1	Soil	+	+	+	+	-	+
BBt_1	Soil	+	+	+	+	-	+
NBt_1	Soil	+	+	+	+	-	+
NBt_3	Soil	+	+	+	+	-	+
D_1G	Soil	+	+	+	+	-	+
B_1P	Soil	+	+	+	+	-	+
B1G	Soil	+	+	+	+	-	+
B9P	Soil	+	+	+	+	-	+
D4	Soil	+	+	+	+	-	+
B5	Soil	+	+	+	+	-	+

Figure 1. Single creamy and white colonies obtained after sub-culturing of *Bacillus thuringiensis* D3G (right) and B9P (right).

after 4 days of incubation for each isolate. Isolates that showed presence of both endospores and parasporal bodies were selected for further characterization and isolates without those characteristics were discarded (Figure 3). A total of 15 isolates possessed endospores and parasporal bodies.
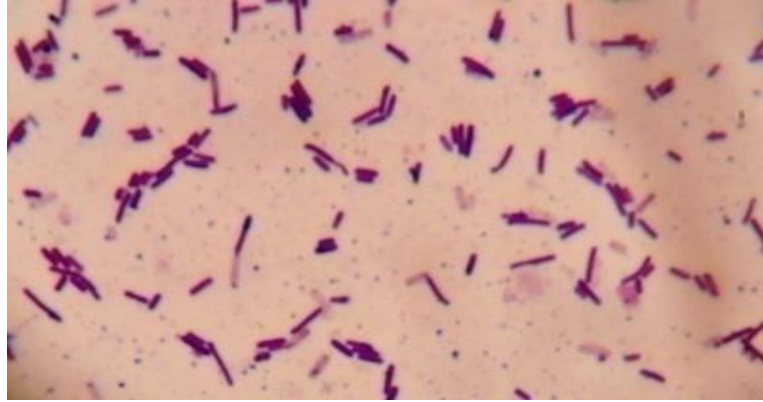
Figure 2. Vegetative form of *Bacillus* (rod shaped and thin) at 1000× magnification. Gram positive (blue).
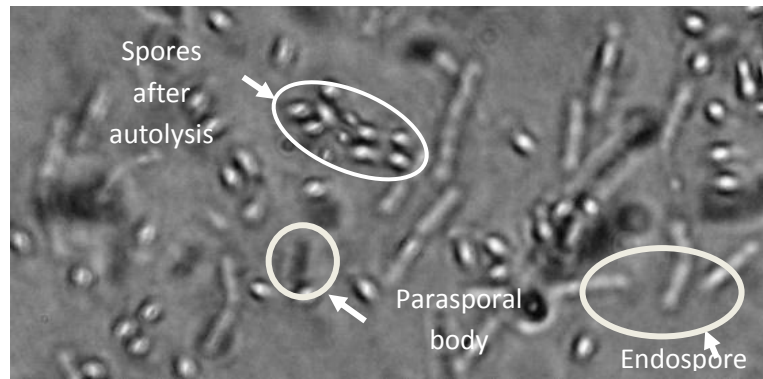

Figure 3. Sporulated form of Bacillus at 1000× magnification. Parasporal body is a dark oval region and endospores are bright rod shape.

Total of 15 isolates were subjected to further screening of parasporal body through coomassie blue staining (Figure 5). This method has a higher resolution compared to phase contrast microscopy. Thus, samples which have parasporal body can be easily identified. All 15 isolates took up the coomassie blue stain (Figure 4) and had parasporal bodies during the sporulated phase and autolysis phase (Table 2).

Growth above 45°C

After 24 h of incubation, 61% of the 39 tested bacteria showed evidence of growth. Growth was not observed in the remaining samples after 24 h; hence, these were excluded from further tests.

Catalase test

The catalase test was performed on all isolates that tested positive for selection tests including the morphological characterization and the sodium acetate selection test (Table 2). All of the samples tested were positive for catalase degradation. After the introduction of hydrogen peroxide to the bacterial smears, samples underwent a violent reaction with bubbles forming at rapid rates (Figure 6). The negative control showed no activity.

In our experiments, we used the sodium acetate test, which proved to be more successful in eliminating most non-wanted bacteria (most sporeforming and nonsporeforming organisms) in the test soil samples. Nevertheless, as observed by Travers et al. (1987), all of the isolates tested positive for Gram stain, endospore stain and catalase test, confirming the necessity to use heat shock to eliminate nosporforming and vegetative cells protected by endospores.

This study used traditional *Bacilli* identification approaches to assign isolates into different groups: aerobic, gram positive, rod shaped bacteria with endospore formation. The different bacteria were further
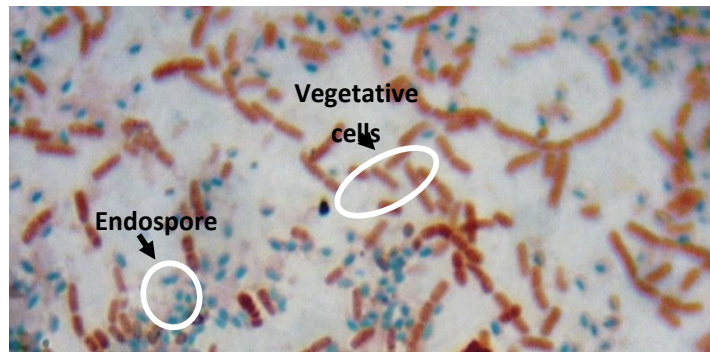
Figure 4. Digital photographs of isolates with green endospores stained with malachite green are distinguishable from the pink safranin O stained vegetative tissue of living bacterial cells (taken at 100x magnification with oil immersion).
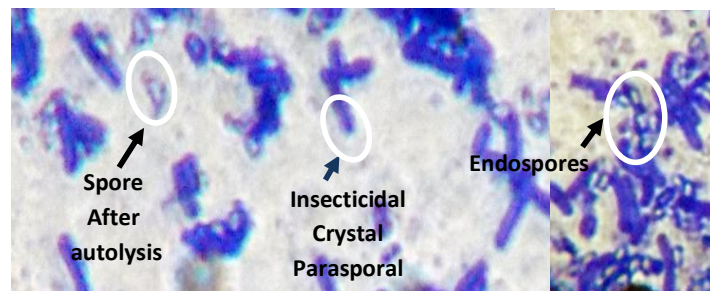

Figure 5. Sporulation phase of Bt. isolates with blue stained parasporal body at 1000X magnification.

Figure 6. Bubble production from bacterial smear in the presence of Hydrogen peroxide.

identified to the species level using physiological and biochemical tests. Also, familiarity with these bacteria was necessary in order to distinguish spore morphologies (Claus and Berkley, 1986). The total bacteria isolated from agricultural soils of Mali were 3111 out of which only 15 were considered as putative *B. thuringiensis*. These results suggest that although many bacteria were isolated from these soils, only few strains could be considered as putative *B. thuringiensis.* Similar results were reported by Deacon (2001) and Zhang et al. (2000) when they isolated many organisms from the natural habitats of insect pests but found only few *B. thuringiensis.*

Also, our result showed Bt indexes were between 0.5 (in Bozola) and 11.7 (in Niono) with an average of 5.1. The higher Bt index in Niono can be explained by the fact that Niono is the highest rice and vegetable production area in Mali. In a *B. thuringiensis* isolation and identification study, Keshavarzi (2008) reported Bt indices between 0 and 5.1 with an average Bt index of 3.2, which was below that reported in the present study.

Conclusion

This work shows that that many bacteria exist in agricultural soils of Mali, but only few strains could be considered as putative *B. thuringiensis*. The Bt index is highly variable in agricultural soils in Mali, but agricultural soils from Niono, the important rice and vegetable production zone in Mali, show the highest Bt index.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

REFERENCES

Babana AH, Bathily H, Samaké F, Maïga K, Traoré D, Dicko A (2011). Microbiological control of bacterial soft rot caused by *Bacillus pumilus* Od23 on potato. Br. Microbiol. Res. J. 1(3):41-48.

Bathily H, Babana AH, Samaké F (2010). *Bacillus pumilus* a new pathogen on potato tubers in Mali. Afr. J. Microbiol. Res. 4(20):2067-2071.

Bergey's Manual of Systematic Bacteriology (1986). Baltimore, London, Los Angeles, Sidney: Williams and Wilkins, 2:965-1599.

Bernhard K, Jarrett P, Meadows M, Butt J, Ellis DJ, Roberts GM Pauli S, Rodgers P, Burges HG (1997). Natural isolates of i: worldwide distribution, and activity against insect pests. J. Invertebr. Pathol. 70:59-68.

Burges HD, Hurst JA (1977). Ecology of *Bacillus thuringiensis* in storage moths. J. Invertebr. Pathol. 30:131-139.

Capalbo DMS, Valicente FH, Moraes IO, Pelizer LH (2001). Solid-state fermentation of *Bacillus thuringiensis tolworthi* to control fall armyworm in maize. EJB Electron. J. Biotechnol. 4(2):112-115.

Carneiro E, Silva L, Maggioni K, dos Santos V, Rodrigues T, Reis S, Pavan B (2014). Evaluation of Insecticides Targeting Control of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). Am. J. Plant Sci. 5:2823-2828.

Carozzi NB, Kramer VC, Warren GW, Evola S, Koziel MG (1991). Prediction of insecticidal activity of *Bacillus thuringiensis* strains by polymerase chain reaction product profiles. Appl. Environ. Microbiol. 57:3057-3061.

Claus D, Berkeley CW (1986). The Genus Bacillus. In: Sneath PHA ed.

Bergey's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore.

Colin T, Lin L, Piesse J (2003). The impact of research-led agricultural productivity growth on poverty reduction in Africa, Asia and Latin America. World Dev. 31(12):1959-1976.

Deacon J (2001). The Microbial World: *Bacillus thuringiensis*. Institute of Cell and Molecular Biology. University of Edinburgh.

Fofana MT, Coulibaly N, Sanogo D, LaRovere R (2009). Characterization of Maize Producing Communities in the Sikasso region of Republic of Mali, DTMA Project Country Report–Community Survey. P. 17.

Harris KM, Gagné RJ (1982). Description of African gall rice midge, Orseolia oryzivora sp. n., with comparative notes on the asian rice gall midge, *Orseolia oryzae* Wood Mason Diptera: Cecidomyiidae). Bull. Entomol. Reserv. 72(3):467-472.

Hamadoun A, Traoré M, Traoré B (1998). Les problèmes phytosanitaires du riz de bas-fond au Mali-sud. In Aménagement et mise en valeur des bas-fonds au Mali. Bilan et perspectives, intérêt pour la zone de savane ouest africaine. Actes de séminaire, Sikasso Mali.

Hamadoun A (1996). Bio écologie des insectes foreurs de tige du riz à l'office du riz. Sahel IPM, n°10. INSA.

Kaelin P, Morel P, Gadani F (1994). Isolation of *Bacillus thuringiensis* from stored tobacco and *Lasioderma serricorne* (F.). Appl. Environ. Microbiol. 60:19-25.

Laridon N (2006). Isolation of a *Bacillus thuringiensis* strain South African soils and the characterization of its cry gene sequence. Dissertation submitted to the Faculty of Science, University of the Witwatersrand, South Africa, P. 135.

Martin T, Chandre F, Ochou OG, Vaissayre W, Fournier D (2002). Pyrethroid resistance mechanisms in the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) from West Africa. Pestic. Biochem. Physiol. 74:17-26.

Martin, PAW, Travers RS (1989). Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. Appl. Environ. Microbiol. 55:2437-2442.

Maruthesh SA (2007). Molecular characterization and efficiency of native isolates of *Bacillus thuringiensis* (Berliner) against cruciferous pests with special reference to diamondback moth. Universi ty of Agr icultural Sciences, Dharwad. Master thesis, P. 81.

Mormak D A, Casida L (1985). Study of Bacillus subtilis endospores in soil by use of a modified endospore stain. App. Environ. Microbiol. 49(6):1356-1360.

Provine H, Gardner P (1974). The Gram's stained smear and its interpretation. Hosp. Pract. 9:85-91.

Rampersad J, Khan A, Ammons D (2002). Usefulness of Staining Parasporal Bodies when Screening for *Bacillus thuringiensis*. J. Invertebr. Pathol. 79:203-204.

Ratnadass A, Ajayi O (1995). Panicle insect pests of sorghum in West Africa. Pages 29-38 *in* Panicle insect pests of sorghum and pearl millet: proceedings of the International Consultative Workshop, ICRISAT Sahelian Center, Niamey, Niger, 4-7 Oct 1993. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Seshu-Reddy KV, Walker PT (1990). A review of the yield losses in graminaceous crops caused by *Chilo* spp. Insect Sci. Applic. 11:563-569.

Theunis W, Aguda RM, Cruz WT, Decock CM, Perferoen M, Lambert B, Bottrel DG, Gould FL, Litsinger JA, Cohen MB (1998). *Bacillus thuringiensis* isolates from the Philippines: Habitat distribution, diversity and toxicity to rice stem borers (LepidopteraL: Pyralidae). Bull. Entimol. Res. 88:335-342.

Travers RS, Martin PA, Reicheferfer CF (1987). Selective process for efficient isolation of soil Bacillus spp. Appl. Environ. Microbiol. 53:1263-126.

Valicente FH, Tuelher ES, Paiva CEC, Gumaraes MRF, Macedo CV, Wolff JLC (2008). A new baculovirus isolate that does not cause the liquefaction of the integument in *Spodoptera frugiperda* dead larvae. Rev. Bras. de Milho e Sorgo 7(1):245-255.

Vitale J, Boyer T, Uaiene R, Sanders JH (2007). The economic impacts of introducing Bt technology in smallholder cotton production systems of West Africa: A case study from Mali. AgBioForum 10(2):71-84.

Yang XM, Wang SS (1998). Development of *Bacillus thuringiensis* fermentation and process control from a practical perspective. Biotechnol. Appl. Biochem. 28:95-98.

Youdeowei A (1989). Major arthropod pests of food and industrial crops of Africa and their economic importance. Biological control: a sustainable solution to crop pest problems in Africa. Yaninek, J.S; Herren, H.R., eds. International Institute of Tropical Agriculture. Ibadan, Nigeria. pp. 31-50.

Zhang H, Yu Z, Deng W (2000). Isolation, distribution and toxicity of *Bacillus thuringiensis* from warehouse in China. Crop Protect. 19:449-454.