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ISOLATION AND CHARACTERIZATION OF *BACILLUS THURINGIENSIS* FROM SOILS IN CONTRASTING AGROECOLOGICAL ZONES OF ETHIOPIA

Zeleke W. Tenssay [1], Mogessie Ashenafi [2], Alexander Eiler [3] and Stefan Bertilson [3]

[1] Institute of Biodiversity Conservation, PO Box 30726
Addis Ababa, Ethiopia. E-mail: otense2002@yahoo.co.uk
[2] Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia
[3] Evolutionary Biology Centre, Department of Limnology/Ecology and Evolution
Uppsala University, Sweden

ABSTRACT: Phenotypic and molecular methods were used to isolate and characterize *B. thuringiensis* from diverse agro-ecological zones of Ethiopia. Bioassays were used to test the insecticidal activity of *B. thuringiensis* strains against the major malaria vector, *Anopheles arabiensis* (Diptera). *B. thuringiensis* were isolated from 32% of the total 503 soil samples collected from the 16 agro-ecological zones. All sequenced isolates were 99%–100% identical to each other and to *B. thuringiensis* entries in Genbank. *B. thuringiensis* with similar 16S rRNA gene sequences from these different zones were characterized with regard to maximum growth rate and temperature optima for growth to test if there was local adaptation in these functional traits. The result showed a narrow temperature range around 30°C for maximal growth rate, and there were no significant differences between agro-ecological zones. Of 110 *Bacillus thuringiensis* isolates analyzed for the presence of crystal protein genes, 7 tested positive for *cry* 4, *cry* 11, and *cyt* toxin genes. Sequencing of these genes in positive strains demonstrated 99–100 % homology to known mosquitocidal *cry* and *cyt* genes in *Bacillus thuringiensis* subsp. *israelensis*. The present study shows that this biotechnologically important species is wide spread in Ethiopian soils and that it does not demonstrate local adaptation to temperature regimes, at least not for basic functions such as growth-temperature response. Our finding also pointed the potential for exploiting this species in vector control programs.

Key words: Agroecological zone, ***Bacillus thuringiensis*,** crystal protein gene, mosquitocidal

INTRODUCTION

B. thuringiensis strains have been isolated worldwide from many habitats, including soil, insects, stored-products, dust and deciduous and coniferous leaves (Carozzi *et al.,* 1991; Smith and Couche, 1991; Chilcott and Wigley, 1993; Yoo, *et al.,* 1996; Hansen *et al.,* 1998; Cavado *et al.*, 2001). A typical method of isolation involves heat treatment to select for spores, sometimes with an acetate enrichment step (Travers *et al.,* 1987), antibiotic selection (Yoo *et al.,* 1996) or non selective agar media (Chilcott and Wigley, 1993).

Ethiopia is considered to be one of the richest genetic resource centers in the world in terms of crop diversity. Although there is a lack of information on microbial diversity, it is believed that Ethiopia's heterogeneous environmental conditions also promote the diversity of microbial communities. Despite the need for information on genetic diversity of indigenous strains of *B. thuringiensis* for potential use in biocontrol programs, studies addressing these issues are scarce in Ethiopia. Some workers tested commercial preparations of *B. thuringiensis* (Dipel) on crop pests against cutworms (*Agrotis spp.*) and Stalkborer (*B. fusca*) in cereals and potato tuber-worm on tomatoes (Alemayehu Refera *et al.*, 1993). A similar study was carried out on *B. thuringiensis var kurstaki* isolates against African bollworm, *Helicoverpa armigera* (Alemayehu Refera *et al.,* 1993). Results from these studies were not encouraging from a biocontrol perspective, but it has been shown that standard *B. thuringiensis* subspecies *israelensis* were active against *Anopheles arabiensis* from Ethiopia (Aklilu Seyoum and Dawit Abate, 1997).

The objective of the present study was to isolate and characterize *B. thuringiensis* from diverse agro-ecological zones of Ethiopia and test the strains against the major malaria vector in Africa.

MATERIALS AND METHODS

Study area

Ethiopia is regionally divided into 18 major agro-ecological zones and 49 sub zones (MOA, 2000). The term ``agro-ecological zone`` is used to describe the broad temperature, moisture, and latitude for different regions. The major zones are sub divided into sub-zones which are more homogenous in terms of climate, physiographic, soils, vegetation, land use, farming system, and animals. Soil samples were collected from 16 accessible major agro-ecological zones (Table 1).

Sampling methods

Surface soil was scraped off to avoid surface contamination and about 200 grams of soil samples were taken from a depth of 4–10 cm with sterile spoons and subsequently transferred into sterile plastic bags. Duplicate soil sample was taken from each selected field in different agro-ecological zones.

Processing of soil samples

Soil samples (10 g) were individually suspended in 100 ml sterile water and homogenized with a magnetic stirrer for 10 minutes. Serial dilutions in tenfold step up to 10^5 were made and the dilutions were heated at 80°C for 10 minutes in a water bath equipped with shaker to destroy non-spore formers and vegetative *Bacillus* cells. Aliquots of 0.1 ml soil slurries were individually plated on Nutrient Agar and incubated aerobically at 28°C for 48–72 hours.

Microscopic examination of culture

After 48 hours of incubation, bacterial colonies on the plates were visually examined. Colonies showing morphological features such as being large, dry, flat, or sticky, characteristics typical for the *B. cereus-B. thuringiensis* group, were picked and transferred to Nutrient broth. Inoculated tubes were incubated aerobically for 4 hours at 28°C and sub-cultured on Nutrient agar to check for purity of the isolate. The Nutrient agar culture was incubated for 48–72 hours aerobically at 28°C. Smears were prepared from the culture, and differentially stained with a method originally reported by Chilcott and Wigley (1988). Phase contrast, and Differential Interference Contrast microscopic observation at 1000x magnification using a Nowarski Optics Microscope fitted with a Hamamatsu Orcca IIIm camera and OPeulab3.0.9 software used to confirm the differential stained observation.

Table 1. Agro-ecological zones where samples were taken, annual average temperature, rainfall and dominant soil type.

Code	Agroecological zone	Sampling Area	Temp	Mean annual Rainfall	Main soil type
A1	Hot to warm arid low land	Afar ,Diredawa	16–21	300–800	Eutric, Regosols,
H1	Hot to humid low land	Derashe	18–24	1200–1500	Dystric Nitosols
H2	Tepid to cool humid high land	Gore, Bedele, Metu, Jimma	11–21	700–2200	Dystric Nitosols
M1	Hot to warm moist low land	AsebeTeferi,Metahara	16–27	600–1600	Orthic Acrisols
M2	Tepid to cool moist mid-high land	Ambo, Dejen, Fiche, *etc*	11–21	1000–1800	Vertisols, Nitosols
M3	Cold to very cold moist sub afroalpine	Quarit, Amhara regions	7.5–11	1000–1800	Phaeozems, Leptisols
PH1	Hot to warm per-humid low land	Bench Maji Zone	24–26	1100–1500	Eutric Fluvisol
PH2	Tepid to cool per-humid mid-high land	Keficho Shekicho Zone	14–25	1100–2200	Dystric Nitosol
SA1	Hot warm sub moist mid-high land	Humera	21–28	300–800	Vertisols
SH1	Hot to warm sub-humid lowland	Bebeka, Arbaminch	16–28	00–1000	Vertisol, Fluvisol
SH2	Tepid to cool sub-humid mid high land	Harari, Alemaya	11–21	700–2200	Dystric Nitosols
SH3	Cold to very cold sub-humid sub afro-alpine	South west Chencha	7.5–15	700–1500	Humic Cambisols
SM1	Hot to warm sub-moist low land	Metema	>21	200–1000	Vertisols ambisols
SM2	Tepid to cool sub-moist mid high land	Nazeret, Mojo, DebreZeit	11–21	700–1200	Vertic andosols
SM3	Cold to very cold sub-miost sub afro-alpine to afro-alpine	Feres Bet, Workamba	ND	ND	Haplic Phaeozems

Note: N.D =Note Determined

16S rRNA gene sequencing

DNA was isolated using the DNeasy DNA extraction kit (Qiagen, Hilden, Germany) following the protocol for gram positive bacteria provided by the manufacturer. 16S rRNA genes were amplified using primer 27f (Vergin *et al*., 1998) and 1492r (Lane, 1991). Between 1ng μl^{-1} and 8 ng μl^{-1} of genomic DNA from each isolate was added to 20 µl reactions containing PCR buffer (buffer specification of DyNAzyme), 100 nM of each primer, 200 mM of each dNTP and 0.25 U DyNAzyme II Polymerase (Finnzymes OY; Espoo, Finnland). PCR conditions were as follows: initial denaturation at 94°C for 3 min followed by 30 cycles of 1 min at 94°C, 1 min annealing at 55°C and 2 min primer extension at 72°C and a final extension at 72°C for 7 min. PCR products were diluted at least 5 times with Q grade water to a final concentration of approximately 6 ng μl^{-1} and sequenced on an ABI 3700 96-capillary sequencer (Applied Biosystems) using primer 27f (Vergin *et al*., 1998) and the BigDye terminator kit vs. 3.1 (Applied Biosystems). This generated high-quality reads of 500–900 bp. The gene sequences were compared to GenBank entries using BLAST (Basic Local Alignment Tool) and imported into ARB and then automatically aligned using the integrated aligner tool and the fast aligner option, followed by manual alignment of the sequences to *B. thuringiensis* entries. A maximum likelihood tree was constructed with ARB (Strunk and Ludwig, 1996). All sequences have been deposited in Genbank under accession numbers: EF 113600-EF 113708.

Temperature dependant growth response

Confirmed *B. thuringiensis* isolates were tested for their growth rate response to temperature. *B. thuringiensis* isolates were cultured on LB medium and incubated at 28°C overnight. Inocula from these cultures were separately transferred to 200 µl LB broth and incubated at 28°C overnight. For each isolate, 2 µl of each isolate was transferred to Bioscreen C multiwell plates for analytical incubations at temperatures ranging from 11°C to 40°C. Incubations and continuous readings of optical density at 600 nm maximal growth recordings were carried out in a Bioscreen C Reader (OY Growth curves AB Ltd, Helsinki, Finland). A flow cytometric determination of bacterial abundance originally devised by delGiorgio *et al.* (1996) was used to estimate abundance of *B. thuringiensis*. Optical density reading versus cell number estimated by flow cytometry was used to plot regression line and from these data maximal growth rate was calculated. Maximal growth rate of isolates from different agro-ecological zones were compared by one way ANOVA using SPSS version 11.0.

Crystal protein gene determination

A loopful of *Bacillus thuringiensis* isolates grown aerobically on LB media overnight at 30° C, were transferred to individual 2ml eppendrof tubes and stored at -80°C for DNA extraction. DNA extraction was carried out using the DNeasy kit from Qiagen (Carlsbad, CA) according to the protocol for isolation of genomic DNA from gram positive bacteria. Reference *Bacillus thuringiensis* strains were obtained from the *Bacillus* Genetic Stock Center (Ohio University, USA). These were *Bacillus thuringiensis* subsp. *kurtstaki* HD 1, *Bacillus thuringiensis* subsp. *kenyae* HD136 (HDB-23), *Bacillus thuringiensis* subsp. *israelensis* ONR60A, and *Bacillus thuringiensis biovar.tenebrionis*.

General primers for crystal protein genes selected from highly conserved regions of the different classes of *cry* genes by multiple alignments of all reported DNA sequences (Ben-Dov *et al*., 1997; Bravo *et al*., 1998) obtained from MWG-Biotech AG. These pairs of primers were gral-*cry*1, gral-*cry* 4, gral-*cry* 8, gral-*cry*11, gral-*cry*-nem (nematocidal *cry* genes), gral-*cyt*, and universal Un-*cry*2, Un-*cry*3 Un-*cry*7 and Un-*cry*8.

Amplification was carried out for 30 cycles in a Stratagene Robocycler, where each 25 µl reaction contained 2µl of DNA extract (1–4 µg template DNA), reaction Buffer (10 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$ 50 mM and KCl 0.1%Triton X100), 150µM of each deoxynucleoside triphosphate, 0.2µM of each primer, and 0.5 U of Dynozyme II DNA polymerase (Finnzymes, Finland). PCR conditions were set according to the original references as given below: for *cry* 1 gene (with general primers, gral-*cry*1) a single initial denaturation at 95°C for 2 minutes, followed by 30 cycles with denaturation at 95°C for 1 minute, annealing at 52°C for 1 minutes, and extension at 72°C for 1 minute, and an additional extension at 72°C for 5 minutes. PCR conditions for the other crystal protein genes were identical except variable annealing temperatures were applied; 49 °C for cry 8, 51°C for *cry* 11 and *cyt*, and 50°C for *cry* nem- primers.

All PCR products obtained were sequenced for confirmation and identification of the specific sub-class of the genes. Briefly, 5 ng of the PCR products were mixed with 1.6 pmol of the respective cry/cyt gene forward primer and sequenced on an ABI 3700 96-capillary sequencer (Applied Biosystems) using the BigDye

terminator kit v.3.1 (Applied Biosystems). Sequences were deposited in GenBank under accession numbers EF649739-EF649757 and compared to Genbank entries using BLAST (Basic Local Alignments Tool; Altschul *et al*., 1997).

Bioassay against An. arabiensis *(Diptera)*

Biological activity of spore-crystal inclusion complexes of *B. thuringiensis* was tested against *An. arabiensis* as described by previous workers (Cavado *et al*., 2001). Briefly, 5 ml of Spore-crystal suspension containing approximately $1.5x10^8$ spores/ml prepared in 0.85 % saline solution was transferred to each duplicate beaker containing 10 larvae in 50ml chlorine free water. The larvae were kept at room temperature for up to 48 hours. *B. thuringiensis* sub-species israelensis (ATCC35646) was used as positive control; and larvae in chlorine free water without spore crystal suspension served as negative control. The dead larvae were recorded at 24 and 48 hours.

RESULTS

Morphological characterization of B. thuringiensis

Mixed bacterial colonies were observed on examination of 48 hour cultures on nutrient agar plates. Gram staining of colonies with big, dry, sticky characteristics, revealed Gram positive rods with refractile spores that do not swell the cells. Differential stain and phase contrast microscopy revealed faint crystal protein inclusion, refractile spores, and vegetative cells (Fig. 1).

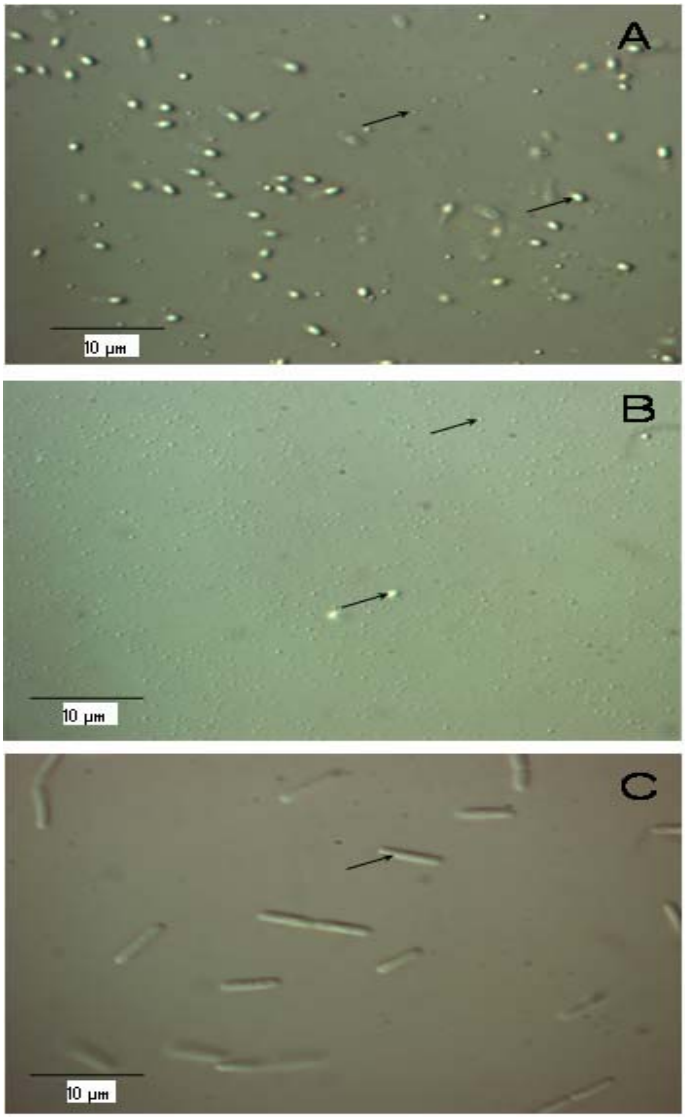

Fig. 1. Differential interference contrast microscopy (1000x) photograph of protein inclusion, vegetative cell and spore of Bt isolates. Arrows A and B indicate spores (bright) and crystal inclusion (faint); C, Vegetative cells (faint).

Molecular characterization of B. thuringiensis by 16S rRNA sequencing

From a total of 161 putative *B. thuringiensis* isolates, 16S rRNA genes sequences were obtained from 110 isolates, with at least one representative from each of the 16 different agro-ecological zones of Ethiopia. For all sequences, BLAST search showed 99–100% similarity to *B. thuringiensis* entries in Genbank. Maximum likelihood analysis revealed that *B. thuringiensis* isolates from agro-ecological zones of Ethiopia were closely related.

Temperature-growth response

B. thuringiensis isolates with similar 16S rRNA gene sequences were tested for growth rate response to temperature. This fundamental physiological trait could be expected to vary between geographical zones as a result of adaptation to prevailing temperature regimes. The average maximal growth rate of isolates from Hot to Warm agro-ecological zones ranged from 2.4 (SM1) to 3.3 generations hour^{-1} (Ph1) and the temperature at which these average maximal growth rates observed was 30˚C (Fig. 2).

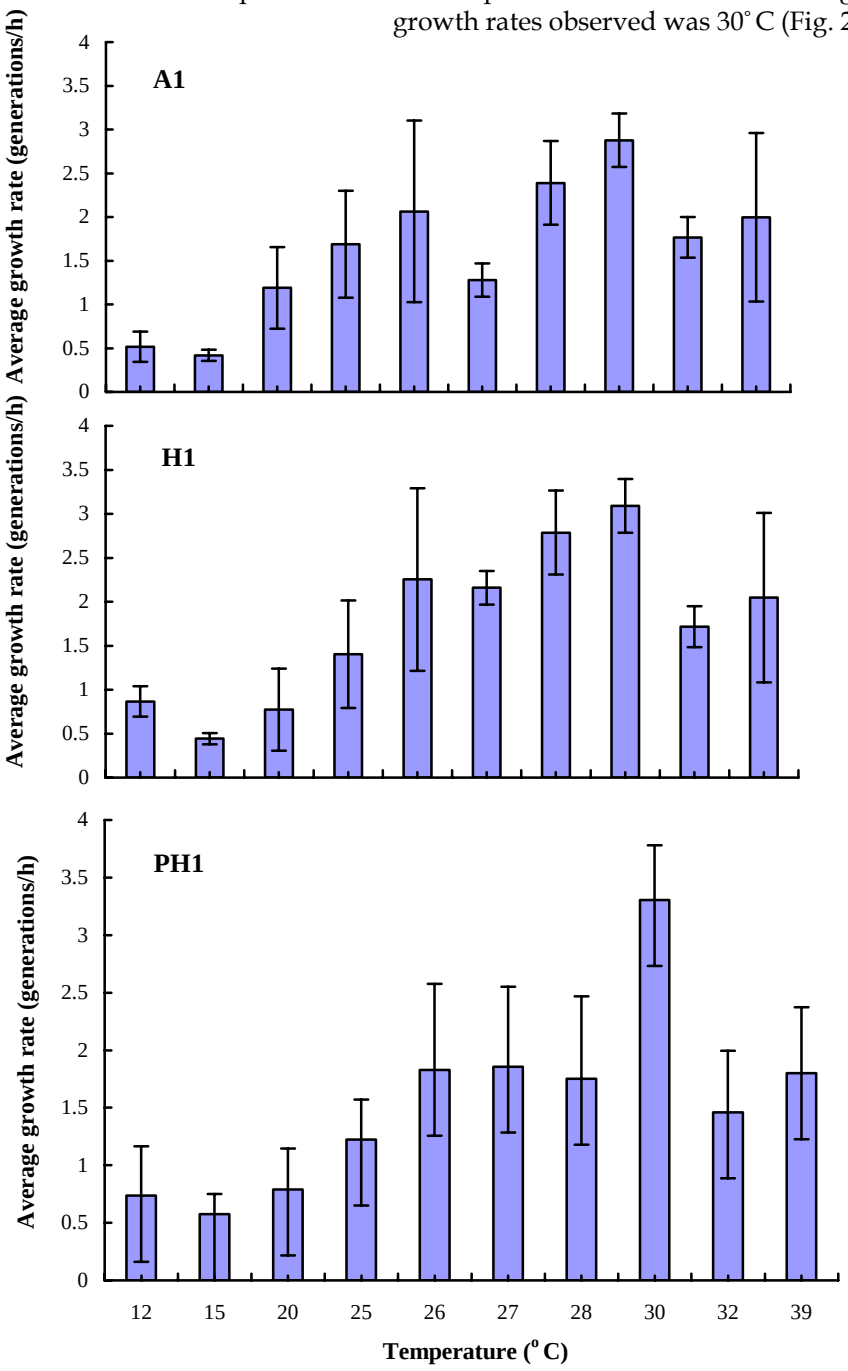

Fig. 2. Growth rate of Bt isolates from hot to warm AEZ of Ethiopia. Growth rate was determined from regression line plotted using data from optical density versus cell number estimated by flow cytometry; AEZ, agro-ecological zone; A1,hot to warm arid lowland; H1,hottowarm humid lowland; Ph1,hot to warm per humid lowland.

The average maximal growth rate for isolates from Tepid to Cool agro-ecological zones ranged from 2.2 (H2) to 2.9 generations hour^{-1} (M2). The temperatures at which these average maximal growth rates were achieved ranged from 26°C to 30°C (Fig. 3). Growth rate of Bt isolates from cold to very cold agroecological zones is shown in Figure 4.

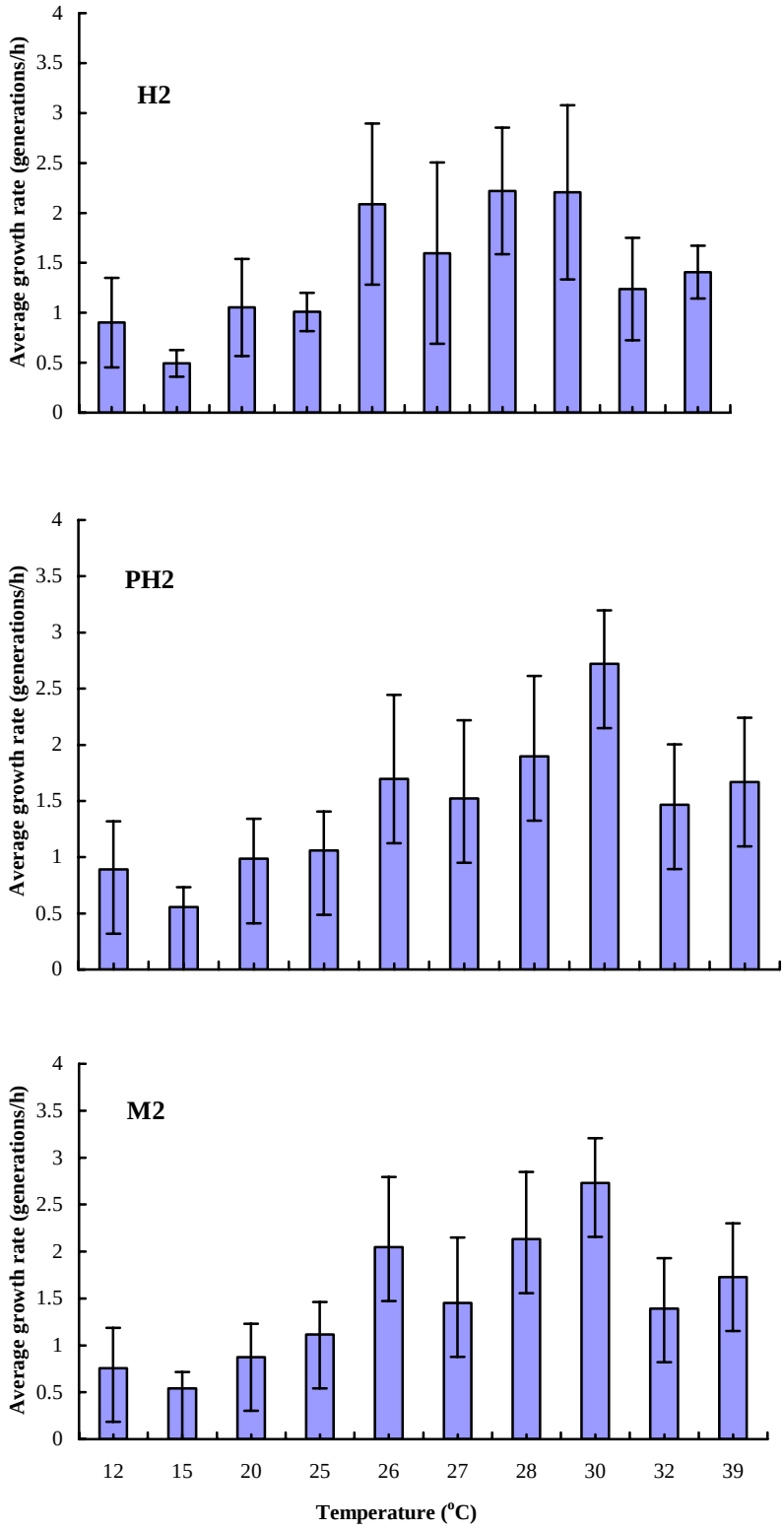

Fig. 3. Growth rate of Bt isolates from tepid to cool AEZ of Ethiopia Growth rate was determined from regression line plotted using data from optical density versus cell number estimated by flow cytometry; H2, tepid to cool humid highland; Ph2, tepid to cool perhumid mid highland; M2, tepid to cool moist mid highland.

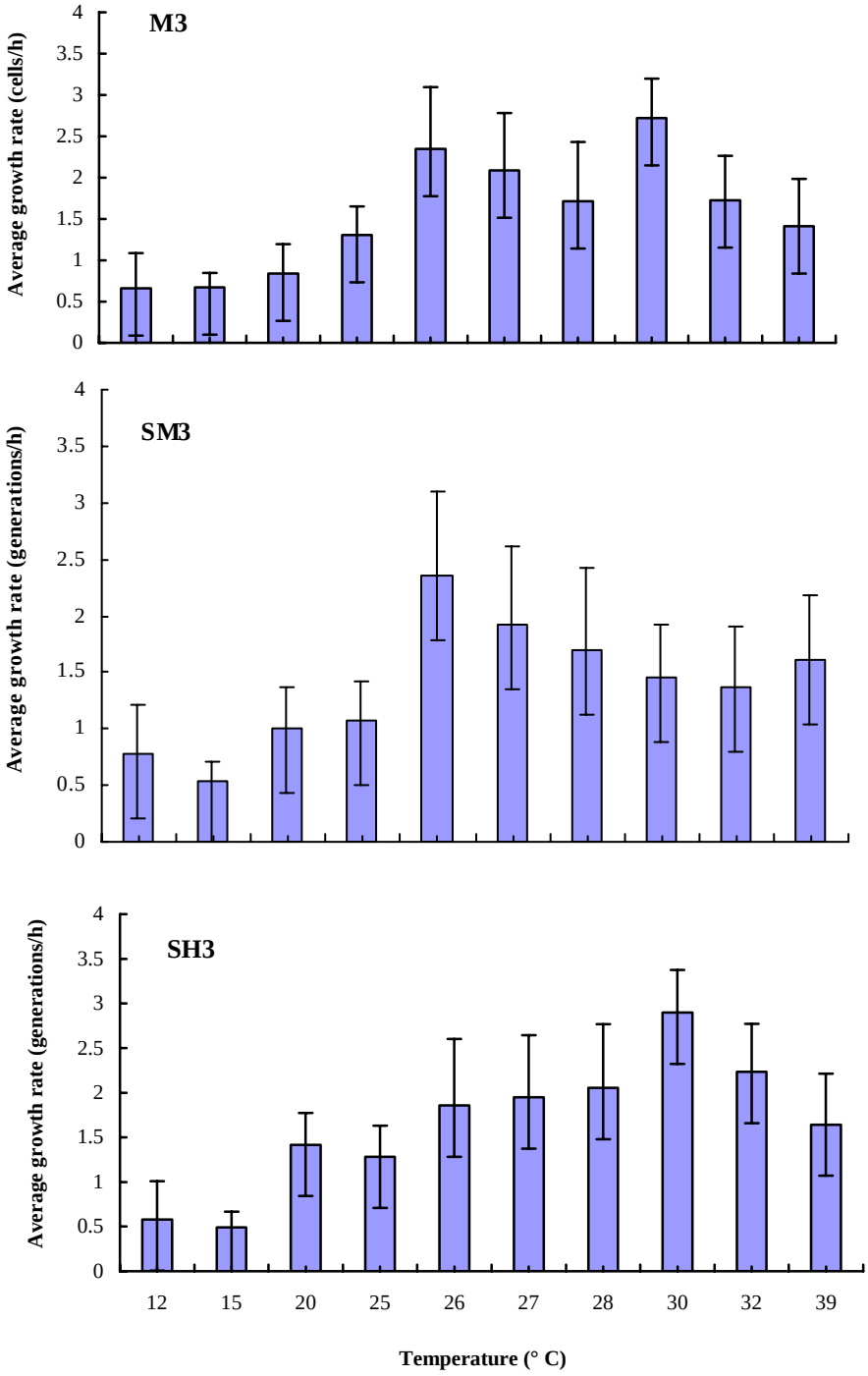

Fig. 4. Growth rate of Bt isolates from cold to very cold AEZ. Growth rate was determined from regression line plotted using data from optical density versus cell number estimated by flow cytometry; M3,cold to very cold moist sub afroalpine; SM3, cold to very cold sub moist sub afroalpine; SH3, cold to very cold sub humid sub afroalpine.

Larvicidal property

A total of 213 *B. thuringiensis-B. cereus* strains were screened for their toxicity against the malaria vector, *A. arabiensis*. Of these, 43 (20.8%) killed 50–100% of the larvae within 48 hours. Out of the 43 strains, 12 killed 100% of the larvae within 24 hours, and 12 killed 100% larvae within 48 hours (Table 2).

Table 2. Activity of Bt against *Anopheles arabiensis* larvae.

AEZ	Bt strain	% larvae killed (24h)	% larvae killed (48 h)
M2	FGW	100	0.0
-	FGB	100	0.0
-	FGJB	40	40.0
-	GDMC2	100	0.0
-	DKF	100	0.0
-	BYW2	100	0.0
-	GMAS	55	65.0
-	FWAW	90	90.0
-	GIAT2	90	90.0
-	GAS	65	80.0
-	GDBm	95	95.0
-	GDFW2	100	0.0
-	GIP	100	0.0
-	ISGP	90	90.0
-	FGJW2	85	85.0
-	KWB2	95	100.0
-	DEP2	100	0.0
-	DDN	100	0.0
-	FGT	100	0.0
-	DGS	100	0.0
-	FWO	100	0.0
SH1	BBGB	45	50.0
-	BBGB2	70	70.0
-	DTAM	75	100.0
-	DTAG	80	100.0
-	AKC	90	100.0
SM3	GDFT	85	95.0
-	GWMN	50	55.0
-	GDFb	70	90.0
-	GWMN2	90	90.0
M3	QDT2	90	95.0
-	QGT2	95	95.0
H2	JGSn	50	95.0
-	IBKT	55	95.0
-	IYM	90	100.0
-	IGDN	65	100.0
-	JDGI2	70	100.0
-	IBKT2	75	95.0
-	JGHS2	95	100.0
-	JGP	55	100.0
-	JGTN	60	100.0
SH2	SDI	90	100.0
-	DMAB2	60	100.0
PH2	SMGB	75	75.0
PH1	BSWC	70	70.0

Ten larvae of *An. arabiensis* were exposed to each Bt strain; and all tests were carried out in duplicate.

Presence of* cry *genes

One hundred and ten *B. thuringiensis* isolates were analyzed for the presence of *cry* genes. Seven isolates were positive for each of *cry* 4, *cry* 11, and *cyt* toxin genes whereas none of the isolates were positive for *cry* 1, *cry*2, *cry*3, *cry*7, *cry*8, and nematocidal *cry* genes. The most frequent *cry* genes sub-class were *cry* 11AA, *cry* 4AA and *cyt* 1AA (Table 3). In most cases, *cry* genes occurred in combination with each other in the present collection of isolates. The sequences of these genes (GenBank Accession numbers EF649739-EF649750) were, with two exceptions, 99–100 % identical to *Bacillus thuringiensis* subsp. *israelensis* mosquitocidal *cry* and *cyt* genes.

Table 3. Crystal protein gene profiles of *Bacillus thuringiensis* isolates from Agro-ecological Zones of Ethiopia.

AEZ	Sample area	Bt Strain	cry11	cry4	cyt	% of larvae killed
M2	Guraketeba, Fiche	FGW2	11 Aa	4AA	1AA	100
M2	Guraketeba, Fiche	FGB	11 AA	4Aa	Ng	100
M2	Enajima, Dejen	DEP2	11 Aa	4AB	1AA	100
M2	Guraketeba, Fiche	FGW	11 AA	4AA	Ng	100
M2	Wuchalejida, Fiche	FWO	11 AA	M39*	1cyt**	100
M2	Dembeza, Dejen	DDN	11 AA	4AA	1AA	100
M2	Yetemen, Bichena	BYW2	11 AA	4AA	1AA	100

cry = crystal protein gene; *cyt* = cytolytic gene; % killed = % *Anopheles arabiensis* killed after 24 h. M39* = 92% identical to mosquitocidal toxin gene subsp. *kurstaki* cry 39, 1cyt** = unclassified cytolytic gene. M2, denotes tepid to cool agro-ecological zone in Ethiopia. Ng = ambiguous sequence; soil samples were collected from crop fields which included wheat, barely, potato, onion except in Dembeza, Dejen which was natural vegetation.

DISCUSSION AND CONCLUSIONS

In the present study, morphological, physiological, and molecular methods coupled with biological activity were used to characterize *B. thuringiensis* isolates from soils collected from contrasting agro-ecological zones of Ethiopia. Based on heat-resistance and colony morphology alone, it was possible to discriminate 213 *B. cereus-B. thuringiensis*-like bacilli. Out of these, 161 isolates contained crystal protein inclusions. Although the use of a single character, such as crystal inclusions, for classification has been criticized (Helgason *et al*., 2000), many studies rely on crystal inclusions as the only characteristic feature that distinguishes *B. thuringiensis* from *B. cereus* (Bernhard *et al*., 1997; Rasko *et al*., 2005). Comparison of the 16S rRNA gene sequences of our isolates with the GenBank BLAST entry showed 99 to 100 % similarity to *B. cereus-B. thuringiensis* group organisms. Blackwood *et al*., (2004) analysed 16S rRNA gene sequences from *Bacillus* species and reported that members of the *B. cereus* group had 100% sequence identity across the 16S rRNA gene. Similarly Goto *et al*. (2000) and Helgason *et al*. (2000) compared 16S rRNA gene sequences of the *B. cereus* group and reported that the sequences were highly conserved, with homologies ranging from 92.2–99.6% identity. These workers indicated that members of *B. cereus* group could not be distinguished from each other. In the present study, we based our identification on a combination of 16S rRNA gene sequencing and microscopic identification of crystal protein inclusions.

When comparing *B. thuringiensis* from different agro-ecological zones of Ethiopia, we expected isolates from colder temperature zones to shift the optimum temperature for growth towards lower temperatures compared to isolates from warmer regions if there was indeed adaptation with regard to this environmental characteristic. Although isolates from different agro-ecological zones grew well across the entire temperature range (12–39°C), almost all isolates showed maximal growth rate around 30°C. The average temperature for reaching maximum growth rates was not significantly different between agro-ecological zones ($P>0.05$). This may be due to either limited physiological potential for adaptation or the result of the typically broad temperature range observed within each agro-ecological zone. The response of our *B. thuringiensis* to a set of temperatures is comparable to observations on enteric bacteria by researchers elsewhere (Bronikowski *et al*., 2001; Shi and Xia, 2003). Bronikowski *et al*.(2001) studied effect of temperature on growth rate in natural isolates of *E. coli* and *Salmonella* from different thermal environments with special interest in questions such as whether optimal temperature and thermal niches of bacterial flora reflected seasonal, geographic or phylogenetic differences in their hosts temperature. Their results suggested that these bacterial species were thermal generalists and did not track fine scale changes in their thermal environments.

In the present study, seven out of 110 isolates tested positive for the presence of crystal protein genes (*cry*4, *cry*11, *cyt*) whereas none tested positive for *cry* 1, *cry* 2, *cry*3, *cry* 7 and 8, and nematocidal *cry* genes. Amplified *cry* genes had 99–100% sequence identity to those of *B. thuringiensis* subsp. *israelensis* except for one strain with *cry* sequence identity of 92 % to *cry* 39 of *B. thuringiensis* subsp. *kurstaki.* The former reference strain carries six genes coding for toxic proteins on a 137 kDa plasmid. The expressed genes include *cry* 4A, *cry* 4B, and *cry* 10A (former cry IVC), *cry* 11A (formerly *cry* IVD), *cyt* 1A, and *cyt* 2A (Rangni *et al*., 1996; Ibarra and Federici., 2003). Each of these *cry*- gene-encoded toxin components in *B. thuringiensis* subsp. *israelensis* is mosquitocidal.

Our result showed that 43(20.8%) of the isolated spore forming bacilli with crystal inclusion morphology killed 50–100% of the tested *An. arabiensis* larvae within 48 hours. Insect species, against which biological activity of *B. thuringiensis* is evaluated, differ from one laboratory to another, so it is difficult to get data of similar insect bioassay to compare mortality level on a large scale. Some workers (Porter *et al*., 1993) compared the susceptibility of mosquito species and indicated that in general species of *Aedes* and *Culex* were more sensitive than species of *Anopheles* to *B. thuringiensis* subsp. *israelensis*. A study from Ethiopia (Aklilu Seyoum, 1995) compared the efficacy of *B. thuringiensis* subsp. *israelensis* against *An. arabiensis* and *Cx. quinquefaciatus* and reported that *An. arabiensis* was more susceptible than *Cx. quinquefaciatus*. Aklilu Seyoum and Dawit Abate (1997) tested the larvicidal activity of a standard strain of *B. thuringiensis* subsp. *israelensis* IPS-82 against *An. arabiensis* larvae in Ethiopia and concluded that the malaria vector was highly susceptible.

The finding of *B. thuringiensis* isolates carrying *cry* protein toxin genes while also expressing insecticidal activity against the malaria vector *An. arabiensis* is promising in the context of indigenous development, production and application of biological control agents against disease vectors in African countries. These isolates may be particularly suited to be applied in these regions as bacteria should be adapted to prevailing environmental conditions as well as the particular insect species targeted. The large number of crystal protein inclusion-containing *B. thuringiensis/cereus* that tested negative for known *cry* and *cyt* genes may indicate the presence of novel toxins or toxin variants. Thus, detailed studies are required to characterize such strains of *Bacillus thuringiensis.*

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