Biological activity of entomopathogenic actinomycetes against lepidopteran insects (Noctuidae: Lepidoptera)

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Vijayabharathi, R., Kumari, B. R., Sathya, A., Srinivas, V., Abhishek, R., Sharma, H. C. and Gopalakrishnan, S. 2014. **Biological activity of entomopathogenic actinomycetes against lepidopteran insects (Noctuidae: Lepidoptera)**. Can. J. Plant Sci. **94**: 759–769. The aim of the present study was to identify an efficient broad-spectrum bio-pesticide for the control of lepidopteran insects from microbes in various ecological niches. A total of 111 microbes isolated from various herbal vermi-composts and organically cultivated fields were evaluated for their intracellular metabolites (ICM), extracellular metabolites (ECM) and whole culture (WC) against early instars of lepidopteran insects. Fifteen actinomycete isolates which showed insecticidal activity against 2nd instar *Helicoverpa armigera* were selected and further screened against *Spodoptera litura* and *Chilo partellus*. A significant broad spectrum insecticidal activity was found in the order ECM > ICM > WC against all the insects under laboratory conditions. All these actinomycete isolates also registered significant activity under greenhouse conditions on 2nd instar *H. armigera*. The actinomycete isolates were identified by 16S rDNA sequencing and matched with *Streptomyces* species using BLAST search. Among all the 15 isolates, SAI-25 (*S. griseoplanus*), CAI-155 (*S. bacillaris*) and BCA-698 (*S. albolongus*) showed consistent entomopathogenic activity against all the three insects suggesting their potential as broad-spectrum biocontrol agents against other lepidopterans.

Key words: Entomopathogenic microbes, actinomycetes, bioassays, Lepidoptera, pest control

Vijayabharathi, R., Kumari, B. R., Sathya, A., Srinivas, V., Abhishek, R., Sharma, H. C. et Gopalakrishnan, S. 2014. **Activité biologique des actinomycètes entomopathogènes contre les lépidoptères (Noctuidae : Lepidoptera)**. Can. J. Plant Sci. **94**: 759–769. Cette étude devait permettre l'identification d'un pesticide biologique à spectre large efficace contre les lépidoptères à partir des microorganismes occupant diverses niches écologiques. Cent onze microorganismes ont été isolés de plusieurs lombricomposts d'herbacées et cultures biologiques, puis on a évalué l'efficacité de leurs métabolites intracellulaires (MIC), de leurs métabolites extracellulaires (MEC) et de la culture complète (CC) contre les premiers instars de lépidoptères. Quinze isolats d'actinomycètes présentant une activité insecticide contre le deuxième instar de *Helicoverpa armigera* ont été retenus et ont fait l'objet de tests plus poussés avec *Spodoptera litura* et *Chilo partellus*. Un effet insecticide à spectre large appréciable a été observé dans l'ordre MEC > MIC > CC contre tous les insectes, dans des conditions de laboratoire. Les isolats d'actinomycètes se caractérisent aussi par une importante activité contre le deuxième instar de *H. armigera* en serre. Les isolats ont été identifiés par séquençage de l'ADNr 16S, puis associés à l'espèce de *Streptomyces* par une recherche BLAST. Parmi les 15 isolats, SAI-25 (*S. griseoplanus*), CAI-155 (*S. bacillaris*) et BCA-698 (*S. albolongus*) illustrent une activité entomopathogène soutenue contre les trois insectes étudiés, ce qui laisse supposer qu'ils pourraient servir d'agent de lutte biologique à spectre large contre d'autres lépidoptères.

Mots clés: Microorganismes entomopathogènes, actinomycètes, analyse biologique, lépidoptères, lutte antiparasitaire

Food security has become a global issue due to a convergence of factors such as increasing population and food prices and reduced agricultural practices (Food and Agriculture Organization 2010). However, crop losses of 20 to 40% (valued at US$120 billion) occur annually due to insect pests (Zhou 2001). Approximately 70 000 different insect species damage food crops across the world. Among them, the species belonging to Lepidoptera are the major cause of crop losses (Pimental 2009). Specifically, the cotton bollworm/legume pod borer, *Helicoverpa armigera* (Hubner), is the most damaging pest, due to its characteristics, such as polyphagy, high mobility, high fecundity, and facultative diapause. It feeds on over 181 plant species of economically important crops (Ali et al. 2009). The second most important polyphagus lepidopteran pest, *Spodoptera litura*, causes 25–100% yield loss on economically important crops such as cotton, groundnut, chilli, tobacco, caster, okra and

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Abbreviations: **AC**, acetonitrile control; **DAT**, days after treatment; **ECM**, extracellular metabolite; **ICM**, intracellular metabolite; **MC**, media control; **NC**, normal control; **WC**, whole cultures

various pulse crops (Armes et al. 1997). The sorghum/maize stem borer, *Chilo partellus* (Swinhoe), is another polyphagus pest and cause significant damage to crops such as maize, sorghum, rice, wheat, sugarcane etc. (Ben-Yakir et al. 2013).

Control of these pests becomes a challenging task in Southeast Asian countries such as Japan, China, and India, and in many other parts of the world, where they are designated as economically important insect pests. Chemical pesticides belonging to the class of organophosphates, carbamates and pyrethroids are quite effective in controlling these lepidopteran pests. The incessant use of these pesticides has led to subsequent problems of insect resistance and reduction in natural enemies followed by sporadic out breaks and crop failures. In addition, their slow degradability pollutes the ecosystem and affects humans through the food chain leading to various health issues. So the need to develop new pesticides with safe, sustainable and economic control measures mainly relies on natural products such as plant/microbial compounds (Copping and Menn 2000; Rimando and Duke 2006; Bale et al. 2008).

Microorganisms are ubiquitous in nature and more diverse than higher-order of organisms. Hence, they constitute an infinite pool for novel metabolites/compounds with medicinal, agricultural and industrial importance (Omura 2011; Subramani and Aalbersberg 2012). Among the agriculturally favourable traits, production of fungicidal/bactericidal/insecticidal compounds such as blasticidin-S, kasugamycin, natamycin, mildiomycin, oxytetracycline, polyoxins, streptomycin, validamycin, avermectins, milbemycin, spinosad and harpin protein (Copping and Duke 2007) fills the void for the need of biological control agents. The insecticidal activities of numerous microbes on lepidopteran and dipteran insect pests have been studied extensively by various researchers (Bream et al. 2001; Hussain et al. 2002; Sundarapandian et al. 2002; Gadelhak et al. 2005; Huamei et al. 2008; Dhanasekaran et al. 2010). Not all microorganisms possess the capability of producing useful secondary metabolites. Specifically, filamentous actinomycetes produce a wide-range of active metabolites, but they have not been explored thoroughly (Donadio et al. 2002), so exploration of the microbial diversity can provide an overwhelming reservoir of potentially active compounds.

The current study was based on our previous laboratory studies on the biowash (washings of herbal vermi-composts) of various botanicals and plant growth-promoting bacteria, which revealed their insecticidal activity against *H. armigera* and *S. litura* (Gopalakrishnan et al. 2011). In view of the aforementioned facts, the present study was aimed to screen microorganisms isolated from herbal vermi-composts and organically cultivated fields at the ICRISAT farm for insecticidal activity against three lepidopteran insects, *H. armigera*, *S. litura* and *C. partellus*.

MATERIALS AND METHODS

Insect Rearing and Maintenance

Helicoverpa armigera and *S. litura* larvae were reared using a chickpea flour based semi-synthetic diet [Fraction A: chickpea flour, 300 g; ascorbic acid, 4.7 g; methyl *p*-hydroxybenzoate, 5 g; sorbic acid, 3 g; auromycin powder, 11.5 g; vitamin solution (nicotinic acid, 1.528 g; calcium pantothenate, 1.528 g; riboflavin, 0.764 g; aneurine hydrochloride, 0.382 g; pyridoxine hydrochloride, 0.382 g; folic acid, 0.382 g; D-biotin, 0.305 g; cyanocobalamin, 0.003 g; water, 500 mL), 10 mL; water, 450 mL; Fraction B: yeast, 48 g; agar-agar, 17.3 g; water, 800 mL] (Armes et al. 1992). The insects were reared using the standard protocols of Narayanamma et al. (2007) at laboratory temperature ($27 \pm 3^{\circ}$C) with a relative humidity of 65–70%. *Chilo partellus* larvae were reared at $28 \pm 1^{\circ}$C with a relative humidity of 60–70% as per the protocols of Sharma et al. (1992) using sorghum leaf powder based diet (Fraction A: water, 2000 mL, kabuli chickpea flour, 438.4 g, yeast, 32 g, sorbic acid, 4 g, vitamin E, 4.6 g, methyl *p*-hydroxy benzoate, 6.4 g, ascorbic acid, 10.4 g, sorghum leaf powder, 160 g. Fraction B: agar-agar, 40.8 g, water, 1600 mL, formaldehyde (40%), 3.2 mL). In both diet preparations, ingredients for fraction A were mixed and blended separately, while fraction B ingredients were boiled in the respective volumes of water, except yeast/formaldehyde, which is added at 40°C. Finally, both fractions were blended thoroughly, poured in to jars and allowed to cool.

Screening of Microorganisms for Insecticidal Activity

A total of 15 bacteria and 96 actinomycetes, previously isolated from various herbal vermi-composts and organically cultivated fields at ICRISAT, were used in this study. The bacterial and actinomycete isolates were cultured on Luria Bertani agar and starch casein agar plates for 3 and 8 d, respectively, under aerobic conditions. All the 111 isolates were screened for their intracellular and extracellular metabolites for insecticidal activity against 2nd instar *H. armigera* larvae using the diet impregnation assay (Narayanamma et al. 2007). Promising isolates were further tested for their efficacy against 3rd instar *H. armigera* and *S. litura* larvae and 7-d-old *C. partellus* larvae as detailed below.

Sample Preparation

The bacterial and actinomycete isolates were cultured on Luria Bertani broth (1 L) for 3 d and starch casein broth (1 L) for 8 d, respectively, at 28°C. At the end of the incubation period, the cultures were centrifuged at $10\,000 \times g$ for 10 min, and the supernatant containing the extracellular metabolites was concentrated on a rotary evaporator at 35°C. The obtained extract was reconstituted in MilliQ water (10 mL; 0.6–0.7 g), labelled as extracellular metabolites (ECM) and stored

for further studies at −20°C. Intracellular metabolites were obtained by extracting the pellet (containing biomass) with acetonitrile (100%) and concentrated at 35°C on a rotary evaporator. The obtained extract was reconstituted in acetonitrile (10 mL; 0.35–0.5 g), labelled as intracellular metabolites (ICM), and stored at −20°C for further studies. ICM samples were diluted to 30% with distilled water before used in the assay. Whole cultures (WC) containing actively growing cells (OD_{600} = 0.6) were also analysed for their efficacy. Un-inoculated medium [denoted media control (MC)] for ECM and WC, and acetonitrile [denoted acetonitrile control (AC)] for ICM were used as controls. Insects treated with none of the above (samples, media or acetonitrile) were considered as normal control (NC) and used for comparative analyses.

Biocontrol Studies

Diet Impregnation Assay

In brief, 2 mL of the artificial diet was poured into a 24-well plate and allowed to dry. At the end of drying, 300 μL of the sample (ECM/ICM/WC/MC/AC) was added to the diet in each well and air dried (3 h). The larvae (*H. armigera, S. litura* and *C. partellus*) were pre-starved for 6 h before being released (one each) into the treated 24-well plates. There were four replications for each treatment (sample), and each replication consisted of 24 larvae. The experiment was conducted three times, in three different batches. The insect mortality was recorded 3 and 6 d after treatment (DAT) for all the three insects.

Detached Leaf Bioassay

The ECM and ICM of the selected isolates were further tested against 2nd instar *H. armigera* larvae by detached leaf bioassay (Sharma et al. 2005). Ten milliliters of 3% agar was poured into plastic cups maintained at an angle of 45°. Chickpea terminal branches with four leaflets along with the terminal bud were washed thoroughly in normal water, followed by distilled water to avoid the interference of the exudates released by the plant. These branches were dipped in 5 mL of the ECM and ICM extracts for 5 min, allowed to dry and inserted into agar. Healthy larvae (pre-starved for 6 h) of similar weight were taken for the experiment. Three replications (10 larvae replication^{-1}) were maintained for each treatment. The experiment was conducted three times, in three different batches. Observations were recorded 3 and 6 DAT.

Greenhouse Experiments

The efficacy of the ECM and ICM of all the selected isolates were further analysed under greenhouse conditions on cotton plants against 2nd instar *H. armigera* larvae. Cotton plants (21 d old) were sprayed with the ECM/ICM extracts (5 mL plant^{-1}). Ten healthy 2nd instar *H. armigera* larvae (pre-starved for 6 h) were released onto each treated plant in a pot and covered with meshed plastic containers to restrict the movement of the larvae. The greenhouse was maintained at 25 ± 2°C under natural lighting conditions. The experiment was conducted three times, in three different batches. Observations were taken 3 and 6 DAT. For each treatment, three replications were maintained in a completely randomised design.

Molecular Identification of the Promising Isolates

Pure cultures of the potential actinomycetes were grown in starch casein broth for 5 d. Genomic DNA was isolated as per the protocols of Bazzicalupo and Fani (1995). The amplification of 16S rDNA gene was performed with the universal primers 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) and 27F (5′-AGA GTT TGA TCMTGG CTC AG-3′) according to the conditions described by Pandey et al. (2005). The polymerase chain reaction product was sequenced at Macrogen Inc. (Seoul, Korea). The obtained nucleic acid sequences were compared with those from GenBank using the BLAST program (Altschul et al. 1990) and aligned with the help of Clustal W software (Thompson et al. 1994). The phylogenetic trees were interpreted using the Neighbor-Joining method (Saitou and Nei 1987). Bootstrap analyses were performed to estimate the statistical stability of the branches in the cluster (1000 replicates) using MEGA version 5.0 programme (Tamura et al. 2011). The sequences were submitted to the GenBank database and accession numbers were obtained.

Statistical Analyses

Experiments conducted with ECM, ICM and WC for the 15 actinomycete isolates were laid out in a completely randomised design. The data had multiple measurements of the response variable on the same experimental unit. Data were recorded 3 and 6 DAT. For each response variable, data were analysed by repeated measures of analysis of variance using the SAS Mixed Procedure (SAS Institute, Inc. 2013, SAS V 9.3). Here, genotypes were between-subjects factor, the time within-subjects factor, and both of the factors considered as fixed effects. A first-order auto regressive [AR(1)] covariance structure was used for these data, which were selected on the basis of the Akaike Information Criteria and Bayesian Information Criteria. For each response, variable LSMeans and Pdiff were also calculated.

RESULTS

Biocontrol Studies

Diet Impregnation Assay

Preliminary studies with 15 bacterial and 96 actinomycete isolates tested for their intra- and extracellular extracts against the 2nd instar *H. armigera* larvae revealed that 15 actinomycetes were insecticidal, whereas none of the bacteria showed the activity. ECM, ICM and WC of these 15 isolates were further evaluated for

insecticidal activity against 3rd instar *H. armigera,* 3rd instar *S. litura* and 7-d-old *C. partellus*.

Among the 15 actinomycete isolates, ECM extract of seven isolates (BCA-508, BCA-546, BCA-659, BCA-667, BCA-698, CAI-85 and SAI-25) were found to have 100% mortality on 2nd instar *H. armigera* on 6 DAT, whereas the remaining isolates showed 55–99% ($P < 0.001$) mortality (Table 1). In the case of ICM, four isolates (BCA-546, BCA-667, CAI-155 and SAI-25) showed 100% mortality, while the remaining isolates showed 55–92% mortality significantly different than the control ($P < 0.001$). Insignificant mortality was also registered for BCA-659 and BCA-689. With WC, five isolates (BCA-546, BCA-659, CAI-87, CAI-155 and SAI-25) registered 100% larval mortality. Isolates BCA-667, BCA-689, BCA-698 and CAI-132 registered no activity in WC, though they showed significant mortality by their ECM/ICM or both (Table 1). As a whole, the pooled mean value of insecticidal activity was found in the order of ECM (92%) > ICM (77%) > WC (60%).

Similar results were observed for 3rd instar *H. armigera*. ECM extract of BCA-659, BCA-689, ICM extract of BCA-546, BCA-659, BCA-698, CAI-133 and WC of BCA-508, BCA-546, CAI-8 showed insignificant insecticidal activity, though the same isolates registered significant mortality rates on 2nd instar *H. armigera*. The isolates SAI-25 and CAI-155 have greater mortality rates by all of their samples (ECM/ICM/WC) analysed (Table 1). From the pooled mean value, insecticidal activity was observed in the order of ECM (84%) > ICM (67%) > WC (45%).

For *S. litura*, four isolates (BCA-546, BCA-667, BCA-698 and CAI-155) documented 100% mortality by ECM on 6 DAT, whereas other isolates registered 38–91% mortality ($P < 0.001$). For ICM, only BCA-698 showed 100% mortality, while nine isolates showed 30–93% significant mortality ($P < 0.01/0.001$). The remaining five isolates were not effective (Table 2). From the pooled mean values, insecticidal activity was found in the order of ECM (70%) > ICM (45%) > WC (27%).

On *C. partellus* larvae, ECM of three isolates (BCA-546, BCA-667 and BCA-689) showed significant ($P < 0.001$) mortality of 56–98%, while the remaining isolates registered 100% mortality at 6 DAT. ICM of eight isolates (BCA-508, BCA-659, CAI-8, CAI-70, CAI-85, CAI-87, CAI-155, SAI-25) and WC of seven isolates (CAI-13, CAI-70, CAI-85, CAI-87, CAI-133, CAI-155, SAI-25) were registered with 100% mortality. The remaining isolates documented lower, but significant insecticidal activity ($P < 0.05$, 0.01, 0.001). All the three samples i.e., ECM, ICM and WC of the isolates CAI-70, CAI-85, CAI-87, CAI-155 and SAI-25 showed 100% mortality (Table 2). As a whole, insecticidal activity was found in the order of ECM (94%) > ICM (74%) > WC (61%). The results of two-way variance analysis between the isolates, time and their interaction on insecticidal activity clearly demonstrated that all three lepidopteran insects were severely affected by ECM, ICM and WC of selected actinomycete isolates analysed by diet impregnation assay (Tables 1 and 2).

Detached Leaf Bioassay

When the 15 isolates were evaluated for insecticidal activity via detached leaf bioassay, 100% mortality was observed in both ECM (BCA-546, BCA-659, CAI-13, CAI-87, CAI-132, CAI-133, CAI-155 and SAI-25) and ICM (BCA-689, BCA-698, CAI-8, CAI-13, CAI-70, CAI-85, CAI-132 and SAI-25) of eight isolates. Other than the isolate BCA-508, the remaining 14 isolates registered significant ($P < 0.001$) activity at 6 DAT against 2nd instar *H. armigera* (Table 3). The pooled mean value of insecticidal activity was in the trend of ECM (99%) > ICM (95%). Two-way variance analysis performed for the data obtained documented that *H. armigera* was severely affected by ECM and ICM of selected actinomycete isolates, which was shown by the *P* values on isolates, time and their interaction (Table 3).

Greenhouse Experiments

Under greenhouse conditions, the experiments on the bioactivity of 15 isolates showed 68–89% mortality by ECM and 59–71% mortality by ICM. It is interesting to note that all the isolates showed statistically significant ($P < 0.001$) insecticidal activity (Table 4). From the pooled mean value, the overall insecticidal activity was observed in the order of ECM (80%) > ICM (66%). Two-way variance analysis on insecticidal activity under greenhouse conditions found higher significance ($P < 0.001$) for isolates and time. Interaction analysis of isolates and time showed higher significance ($P < 0.001$) for ICM and borderline significance ($P < 0.06$) for ECM.

Molecular Identification of the Promising Isolates

The phylogenetic relationship of the 15 promising actinomycete isolates (approximately 1500 bp of sequence data) is depicted in Fig. 1. They were identified as BCA-508: *S. cavourensis* subsp. *cavourensis* (GenBank acc. no: KF770887), BCA-546; *S. cyaneofuscatus* (GenBank acc. no: KF770898), BCA-659; *S. cavourensis* subsp. *cavourensis* (GenBank acc. no: KF770889), BCA-667; *S. albolongus* (GenBank acc. no: KF770888), BCA-689; *S. hydrogenans* (GenBank acc. no: KF770899), BCA-698; *S. albolongus* (GenBank acc. no: KF770900), CAI-8: *Streptomyces* sp. (GenBank acc. no: KF770890), CAI-13; *S. cavourensis* subsp. *cavourensis* (GenBank acc. no: KF770891), CAI-70; *S. antibioticus* (GenBank acc. no: KF770892), CAI-85; *S. antibioticus* (GenBank acc. no: KF770897), CAI-87; *S. cyaneofuscatus* (GenBank acc. no: KF770893), CAI-132; *S. antibioticus* (GenBank acc. no: KF770894), CAI-133: *S. carpaticus* (GenBank acc. no: KF770895), CAI-155; *S. bacillaris* (GenBank acc. no: KF770896) and SAI-25; *S. griseoplanus* (GenBank acc. no: KF770901).

Table 1. Insecticidal activity of selected actinomycetes on 2nd and 3rd instar *H. armigera* larvae by diet impregnation assay

	% Mortality of *H. armigera* (2nd Instar)[z]						% Mortality of *H. armigera* (3rd Instar)[z]					
	ECM		ICM		WC		ECM		ICM		WC	
Isolates	3 DAT	6 DAT	3 DAT	6 DAT	3 DAT	6 DAT	3 DAT	6 DAT	3 DAT	6 DAT	3 DAT	6 DAT
BCA-508	80***	100***	70***	85***	24*	25*	65***	89***	74***	100***	3NS	5NS
BCA-546	100***	100***	55***	100***	100***	100***	100***	100***	0NS	13NS	0NS	4NS
BCA-659	100***	100***	22NS	31NS	100***	100***	0NS	30NS	0NS	21NS	58***	100***
BCA-667	100***	100***	71***	100***	0NS	0NS	95***	100***	78***	78***	0NS	0NS
BCA-689	87***	87***	0NS	9NS	0NS	0NS	13NS	13NS	0NS	21NS	0NS	0NS
BCA-698	100***	100***	91***	91***	0NS	0NS	100***	100***	4NS	17NS	0NS	4NS
CAI-8	77***	95***	83***	85***	71***	87***	57***	100***	83***	100***	0NS	4NS
CAI-13	42***	68***	88***	92***	79***	92***	32*	100***	74***	74***	38*	100***
CAI-70	97***	99***	62***	66***	33*	71***	92***	100***	94***	100***	22NS	71***
CAI-85	90***	100***	91***	91***	0NS	34*	99***	100***	95***	100***	0NS	25*
CAI-87	27NS	55***	57***	61***	92***	100***	97***	100***	81***	96***	37*	83***
CAI-132	85***	94***	53***	55***	0NS	0NS	49***	74***	51***	57***	0NS	0NS
CAI-133	45***	87***	78***	91***	83***	92***	40***	61***	15NS	28NS	84***	84***
CAI-155	72***	91***	100***	100***	100***	100***	94***	99***	100***	100***	100***	100***
SAI-25	100***	100***	100***	100***	100***	100***	100***	100***	100***	100***	100***	100***
MC for ECM, WC & AC for ICM	4NS	15NS	0NS	9NS	0NS	0NS	0NS	18NS	0NS	9NS	0NS	0NS
NC	0	5	0	5	0	4	0	9	0	9	0	9
SEM	0.106	0.106	0.104	0.104	0.11	0.11	0.09	0.09	0.08	0.08	0.09	0.09

Effect of isolates and time on insecticidal activity

Treatment	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value
Isolates	16	40.87***	16	21.48***	16	58.32***	16	76.55***	16	60.38***	16	64.35***
Time	1	96.51***	1	54.71***	1	12.28**	1	119.66***	1	89.82***	1	110.62***
Isolates × Time	16	6.23***	16	9.51***	16	1.66NS	16	8.15***	16	5.94***	16	17.97***

[z]Values are LSMeans. ECM, extracellular metabolites; ICM, intracellular metabolites; WC, whole cultures; DAT, days after treatment; MC, media control; AC, acetonitrile control; NC, normal control; SEM, standard error mean.

*, **, *** Values are statistically significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, compared with the control group (NC); NS, nonsignificant.

Table 2. Insecticidal activity of selected actinomycetes on *S. litura* and *C. partellus* by diet impregnation assay

	% Mortality of *S. litura* (3rd Instar)[z]						% Mortality of *C. partellus* (7-d-old larvae)[z]					
	ECM		ICM		WC		ECM		ICM		WC	
Isolates	3 DAT[z]	6 DAT	3 DAT	6 DAT	3 DAT	6 DAT	3 DAT	6 DAT	3 DAT	6 DAT	3 DAT	6 DAT
BCA-508	21NS	59***	2NS	13NS	13NS	21NS	100***	100***	100***	100***	37**	79***
BCA-546	92***	100***	36**	78***	8NS	38**	0NS	56***	22NS	68***	0NS	0NS
BCA-659	50***	63***	6NS	13NS	0NS	17NS	98***	100***	67***	100***	0NS	0NS
BCA-667	44***	100***	50***	61***	0NS	17NS	73***	98***	39NS	51*	0NS	0NS
BCA-689	67***	91***	23*	44***	9NS	29NS	17NS	58***	0NS	28NS	0NS	0NS
BCA-698	96***	100***	68***	100***	25*	46***	96***	100***	0NS	10NS	0NS	0NS
CAI-8	46***	74***	7NS	30**	4NS	29*	100***	100***	100***	100***	25*	75***
CAI-13	34***	83***	30**	52***	0NS	25NS	100***	100***	64**	70***	41**	100***
CAI-70	37**	55***	0NS	11NS	9NS	17NS	100***	100***	100***	100***	54***	100***
CAI-85	19NS	47***	38***	56***	0NS	14NS	100***	100***	100***	100***	79***	100***
CAI-87	19NS	38**	11NS	33**	4NS	8NS	100***	100***	100***	100***	100***	100***
CAI-132	0NS	21NS	0NS	17NS	0NS	29*	100***	100***	0NS	25NS	10NS	67***
CAI-133	25NS	55***	0NS	11NS	21*	59***	97***	100***	44*	64**	25*	100***
CAI-155	50***	100***	85***	93***	29*	34**	100***	100***	100***	100***	100***	100***
SAI-25	19NS	57***	22NS	62***	8NS	21NS	100***	100***	100***	100***	100***	100***
MC for ECM, WC & AC for ICM	0NS	5NS	0NS	0NS	0NS	0NS	0NS	8NS	0NS	0NS	0NS	13NS
NC	0	0	0	0	0	0	0	0	0	0	0	0
SEM	0.09	0.09	0.08	0.08	0.07	0.07	0.11	0.11	0.14	0.14	0.082	0.082
Effect of isolates and time on insecticidal activity												
Treatment	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value
Isolates	16	32.81***	16	30.5***	16	3.76***	16	48.41***	16	23.56***	16	116.17***
Time	1	220.5***	1	206.36***	1	149.89***	1	32.25***	1	54.06***	1	184.55***
Isolates × Time	16	7.53***	16	10.3***	16	4.27***	16	4.94***	16	6.82***	16	19.73***

[z]Values are LSMeans; ECM, extracellular metabolites; ICM, intracellular metabolites; WC, whole cultures; DAT, days after treatment; MC, media control; AC, acetonitrile control; NC, normal control; SEM, standard error mean.

*, **, *** Values are statistically significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, compared with the control group (NC); NS, nonsignificant.

Table 3. Insecticidal activity of selected actinomycetes on 2nd instar *H. armigera* larvae through the detached leaf bioassay

	Mortality (%)[z]			
	ECM		ICM	
Isolates	3 DAT	6 DAT	3 DAT	6 DAT
BCA-508	30NS	99***	24NS	37[NS]
BCA-546	63***	100***	87***	99***
BCA-659	54***	100***	43*	99***
BCA-667	40*	99***	34NS	99***
BCA-689	24NS	99***	41NS	100***
BCA-698	47**	99***	48*	100***
CAI-8	54***	95***	54**	100***
CAI-13	24NS	100***	54**	100***
CAI-70	40*	99***	74***	100***
CAI-85	27NS	90***	61**	100***
CAI-87	53**	100***	20NS	99***
CAI-132	20NS	100***	64***	100***
CAI-133	34NS	100***	60**	95***
CAI-155	47**	100***	40NS	99***
SAI-25	78***	100***	72***	100***
MC for ECM & AC for ICM	17NS	30NS	20NS	33NS
NC	10	27	10	27
SEM	0.10	0.10	0.13	0.13
Effect of isolates and time on insecticidal activity				
Treatment	df	F value	df	F value
Isolates	16	2.93**	16	4.54***
Time	1	1072.27***	1	527.96***
Isolates × Time	16	2.3*	16	3.18**

[z]Values are LSMeans; ECM, extracellular metabolites; ICM, intracellular metabolites; MC, media control; DAT, days after treatment; AC, acetonitrile control; NC, normal control; SEM, standard error mean. *, **, *** Values are statistically significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively, compared with the control group (NC); NS, nonsignificant.

Table 4. Insecticidal activity of selected actinomycetes against 2nd instar *H. armigera* larvae under greenhouse conditions

	Mortality (%)[z]			
	ECM		ICM	
Isolates	3 DAT	6 DAT	3 DAT	6 DAT
BCA-508	66***	79***	53***	70***
BCA-546	59***	75***	51***	66***
BCA-659	72***	80***	45***	59***
BCA-667	57***	68***	47***	62***
BCA-689	59***	72***	55***	67***
BCA-698	66***	81***	48***	65***
CAI-8	61***	85***	53***	69***
CAI-13	60***	77***	49***	62***
CAI-70	66***	89***	52***	68***
CAI-85	66***	85***	54***	69***
CAI-87	61***	76***	55***	70***
CAI-132	63***	82***	56***	70***
CAI-133	55***	73***	50***	71***
CAI-155	74***	86***	49***	61***
SAI-25	69***	87***	54***	68***
MC for ECM & AC for ICM	12NS	14NS	11NS	15NS
NC	11	11	12	12
SEM	0.09	0.09	0.093	0.093
Effect of isolates and time on insecticidal activity				
Treatment	df	F value	df	F value
Isolates	16	8.17***	16	7.48***
Time	1	132.59***	1	389.06***
Isolates × Time	16	1.86NS	16	3.37**

[z]Values are LSMeans; ECM, extracellular metabolites; ICM, intracellular metabolites; DAT, days after treatment; MC, media control; AC, acetonitrile control; NC, normal control; SEM, standard error mean. *** Values are statistically significant at $P<0.001$ compared with the control group (NC); NS nonsignificant.

DISCUSSION

Biopesticides have gained considerable importance among researchers and the farming community because of their safety towards the environment, non-target organisms, including mammals, and reduced effect on emergence of natural and cross resistance. Hence, the search for novel biopesticides is gaining importance for pest management in agriculture. In the present study, we made an attempt to screen for microorganisms from various herbal vermi-composts and organically cultivated fields for insecticidal activity against lepidopteran insect pests, i.e., *H. armigera*, *S. litura* and *C. partellus*.

Among the 111 microbes isolated from herbal vermicomposts (CAI isolates) (with earthworm *Eisenia foetida*) and organically cultivated fields (SAI and BCA isolates), 15 isolates were initially selected with insecticidal activity against 2nd instar *H. armigera*. Similarly, many reports are available for insecticidal activity of microbes and/or their products (Bream et al. 2001; Liao et al. 2002; Xiong et al. 2004; Yousefnezhad-Irani and Asghar 2007; Deng et al. 2008; El-khawaga and Megahed 2012). It is interesting to note that all the selected isolates in the present study were actinomycetes, and none of the bacterial isolates displayed significant insecticidal activity. Our results are corroborated with those of Mishra et al. (1987) who found 832 actinomycetes, 70 fungus and 40 bacterial isolates from various soil types and reported the predominantly active 27 strains with insecticidal and nematicidal traits as actinomycetes. Our results are further supported by Polyanskaya et al. (1996), who isolated antibiotic heliomycin producing actinomycete *S. olivocinereus* from earthworm *E. foetida* and Reilly et al. (2013), who observed higher microbial communities and activities in organic fields compared with farming systems utilising conventional agriculture.

As a whole, ECM has exhibited higher insecticidal activity followed by ICM and WC in all of the in vitro and greenhouse studies. This indicates that though intracellular metabolites and whole cells exhibit the insecticidal activity, the extracellular metabolites have significant

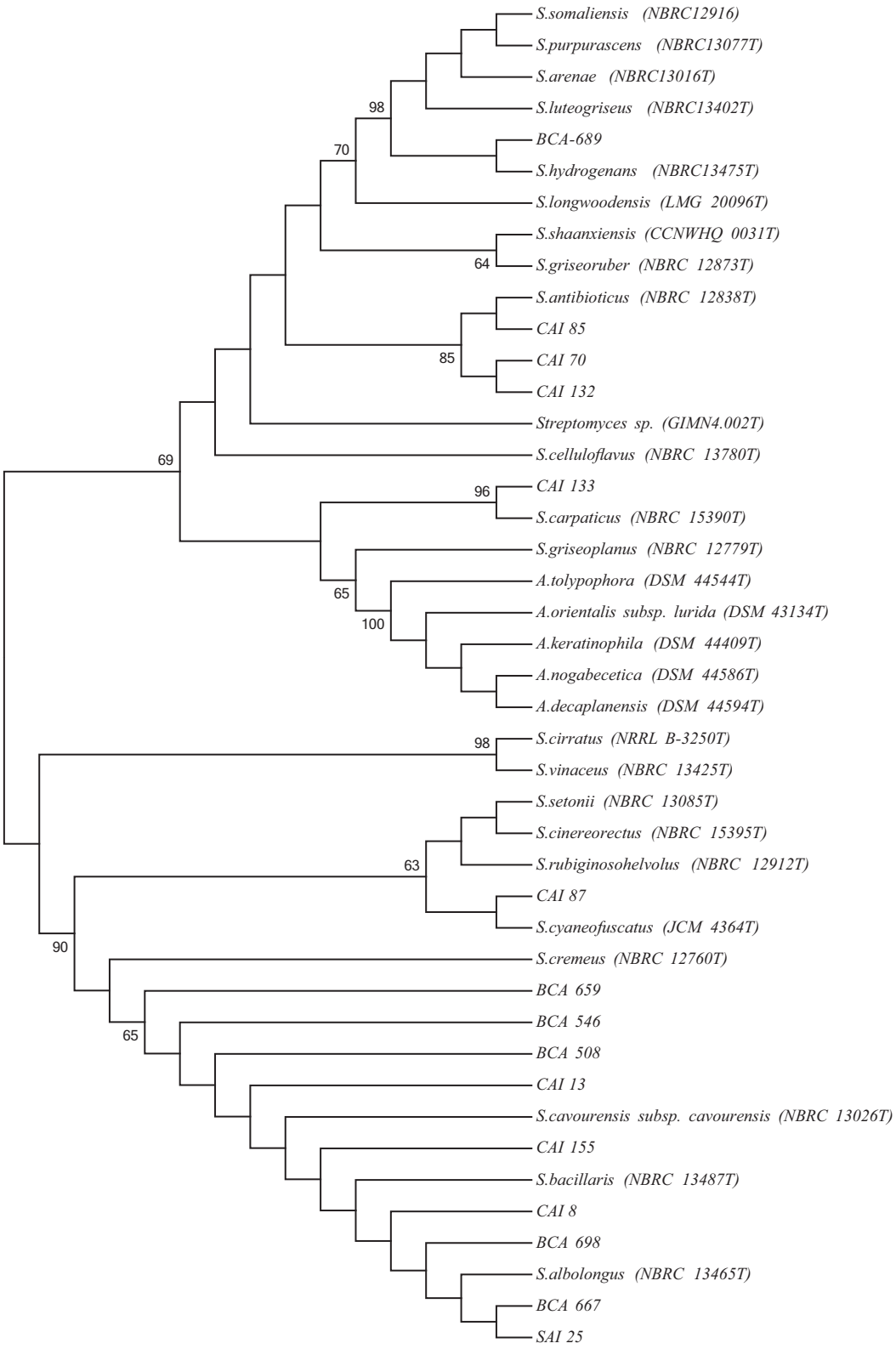

Fig. 1. Phylogenetic relationships of the 15 selected isolates based on 16S rDNA gene sequences. The numbers are the estimated confidence levels, expressed as percentages, for the positions of the branches, determined by bootstrap analysis. The scale bar indicates the evolutionary distance value between the sequences.

functionality. Similarly, Frisvad (1989), Arasu et al. (2008) and Jaiswal et al. (2011) demonstrated the efficiency of extracellular secondary metabolites from bacteria, fungi and actinomycetes as biocidal compounds. Recently, Omura (2011) has reviewed the wide range of microbial metabolites isolated and identified by his research group, most of which have been obtained as extracellular metabolites from bacterial, fungus and actinomycete isolates.

In the present investigation, it was observed that the 2nd instar larvae of *H. armigera* was more susceptible than the 3rd instar larvae. Similarly, Aggarwal et al. (2006) found higher insecticidal activity on 2nd instar *H. armigera* and *S. exigua* than their 4th instar larvae by botanical pesticides (Neem Azal-T/S: NA, and *Quassia amara*: QA), biopesticides (*Bacillus thuringiensis* subsp. *Aizawai*: Bta) and their combination (Bta + NA). Another lepidopteran larvae, *Ocinara varians*, also exhibited higher sensitivity on 3rd and 4th instar larvae than their 5th instar larvae against the entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea*) (Hussain et al. 2009). In the present study the reverse phenomenon was also observed, i.e., the 3rd instar *H. armigera* larva was more susceptible to some of the CAI isolates (CAI-8, CAI-13, CAI-70 and CAI-87), than the 2nd instar larvae. Similar observations were reported by Vandenberg et al. (1998), in which increased susceptibility of 3rd and 4th instar larvae of the diamondback moth *Plutella xylostella* compared with their 2nd instar larvae against the entomopathogenic fungi *Beauveria* spp. In the present study, 100% mortality exhibited by SAI-25 irrespective of the larval age of *H. armigera* was similar to the previous findings of Cooper (1984) on *H. punctigera* by live cells of *Bacillus thuringiensis* Berliner (Thuricide® HPSC WP). Hence, it can be concluded that susceptibility of the insects varies with their developmental stages.

Based on molecular characterization, all the selected isolates belonged to the genus *Streptomyces,* an economically important group of actinomycetes, and a pivotal source of copious amounts of biologically active compounds. Approximately three-quarters of all known commercially and medicinally useful antibiotics and several agriculturally important compounds, e.g., antibacterial (Ramesh and Mathivanan 2009), antifungal (Berdy 2005), insecticidal, anti-parasitic (Pimentel-Elardo et al. 2010), anti-inflammatory (Renner et al. 1999), antiviral (Sacramento et al. 2004), antitumour and enzyme inhibitors (Hong et al. 2009), have been obtained from *Streptomyces* spp. (Subramani and Aalbersberg 2012). Moreover, approximately 60% of the antibiotics discovered in the year 1990 and most of the antibiotics used in agriculture are from the genus *Streptomyces* (Tanaka and Omura 1993).

The results obtained in the present study on the insecticidal activity of the selected isolates in vitro and under greenhouse conditions indicate that SAI-25 (*S. griseoplanus*) is a promising candidate among the 15 isolates, since it has reliable activity against 2nd and 3rd instar *H. armigera* larvae, 3rd instar *S. litura* larvae and 7-d-old *C. partellus* larvae. Results of the present study and our concurrent research activity on the identification of the active compounds is fairly supported a recent study of Arasu et al. (2013) who identified a novel polyketide metabolite with antifeedant, larvicidal and growth inhibitory properties on *H. armigera* and *S. litura* from a marine actinomycete *Streptomyces* sp. AP-123. However, further studies on efficacy testing of identified active compound under glasshouse and field conditions are required.

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