Journal of Environmental Sciences Vol. 17, No. 1, pp. 123—125, 2005

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2005)01-0123-03 **CLC number**: X17 **Document code**: A

Streptomyces avermitilis from marine

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Abstract: The insecticidal strain 173 was isolated from marine source and its activity was explored by the bioassay of brine shrimp and *Helicoverpa armigera*. Based on morphological, physiological and molecular properties, the insecticidal strain 173 was identified as *Streptomycetes avermitilis*, which is the best insecticidal microorganism found in the terrestrial environment. The taxonomy of the strain 173, insecticidal spectrum and properties of the corresponding insecticidal antibiotics are reported.

Keywords: insecticidal microorganism; marine; taxonomy; *Streptomyces avermitilis*

Introduction

The intensive use of chemical pesticides is one of the factors contributing to high crop yields and lower commodity prices. It is estimated that 2.5 million tons of pesticides are applied to agricultural crops worldwide annually. However, in most cases, the amount of pesticide coming in direct contact with target pests is an extremely small percentage of the amount applied, usually less than 0.3%, and thus 99.7% pesticides went "somewhere else" in the environment (Pimentel, 1995). Contamination by chemical pesticides has affected the quality of soil, water and air and, consequently, may pose a risk to human, flora and fauna (Laura, 2004). Fortunately, biological pesticides are distinguished from conventional chemical pesticides by their natural occurrence, non-toxic mode of action to the target pests, safety for human and other non-target organisms, reduction of pesticide residues in food and environment, preservation of other natural enemies, and increased biodiversity in managed ecosystems (Mike, 2000; Lacey, 2001).

The past two decades have seen significant research interest in attempts to discover and develop environmental friendly pesticides from nature origin (Prem, 2000). So far, most of the insecticidal microorganisms have been isolated from entomopathogens and terrestrial environment (Leonard, 2000). Ocean is a huge drug resource, nearly 6000 kinds of novel chemical compounds have been identified from marine living organisms, among which about 10000 compounds exert biologically activities, such as anti-tumor, anti-microbe and anti-virus, etc. (Fenical, 1993; Davidson, 1995). However, the marine environment is still rarely explored for the development of biological pesticides and little information about marine microorganisms possessing insecticidal activities has been reported (Takahashi, 1989; Hu, 2000).

In the course of screening for new insecticidal metabolites from marine microorganisms, the strain 173 was found to have strong insecticidal activity against both brine shrimp and *Helicoverpa armigra*, as we previously reported (Xiong, 2004). In this paper, the taxonomy of the strain 173, insecticidal spectrum and properties of the corresponding insecticidal antibiotics are reported in this paper.

1 Materials and methods

1.1 Microorganism

The strain 173 was isolated and maintained as previously described (Xiong, 2004).

1.2 Taxonomy

The insecticidal strain 173 was characterized by its morphological and physiological properties (Yan, 1975), as well as 16S rDNA sequencing. Extraction of genomic DNA, PCR amplification of 16S rDNA and purification of the PCR products was performed following the procedure of Chun & Goodfellow (Chun, 1995), Kim (Kim, 1996) and Lu (Lu, 2001) respectively. The 16S rDNA of the strain was aligned manually with the available Streptomyces nucleotide sequence that retrieved from EMBL/Genebank and RDP (Ribosomal Database Project; Maidak, 1997) using Clustal *1.8 program (Thomposon, 1997).

1.3 Laboratory bioassay of some important insect pests

Helicoverpa armigera, *Plutella xylostlla* L., aphids and *Spodoptera exigua*, which are serious pests of many important crops and claim major share in crop losses every year, are used as the test pests.

Test pests: 3-instar larvae of *Helicoverpa armigera*, *Plutella xylostlla* L., aphids and *Spodoptera exigua*; Positive control: 1 ppm and 10 ppm avermectin B_{1a}; Test solution: the supernatant of fermentation broth of the strain 173; The bioassay method was the same as previously described (Xiong, 2004).

1.4 Properties of the insecticidal substances of the strain

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The pH of eleven portions of the fermentation broth were adjusted to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 respectively for the test of pH sensitivity. The fermentation broth were heated at 40℃, 60℃, 80℃, 100℃, 121℃ for 30 min respectively for the test of thermo-stability. The insecticidal activities for the samples obtained from the above treatments were examined by brine shrimp bioassay (Xiong, 2004).

1.5 HPLC analysis

The supernatant and mycelia of fermentation broth were extracted respectively with ethyl acetate for three times. The ethyl acetate extracts of the supernatant and the mycelia were concentrated under diminished pressure to dryness. The two residues were dissolved in methanol. The avermectin B_{1a} (92%) was used as authentic sample. HPLC analysis was carried out on Shimadzu LC-3A according to the method of Thomas (Thomas, 1979).

2 Results

2.1 Taxonomic studies of the strain 173

The cultural and physiological characteristics of strain 173 grown on various media at 2℃ for 14 d are shown in Table 1 and Table 2 respectively. The organism is aerobic, mesophilic and gram-positive. The spore chains were rectiflexibiles and spores were subsperical to oval in shape and smooth.

Table 1 Cultural characteristics of strain 173

Medium	Growth	Aerial mycelium	Reverse side color
Glycerol-asparagine agar	Poor	Light yellow	White to yellow
Yeast extract-malt extract agar	Good	Light brown and grey	Yellow to brown
Inorganic salts-starch agar	Poor	Very little	Light yellow
Gauses 1	Moderate	Light yellow	Light yellow
Starch hydrolysis	Poor	White to grey	Yellow
PDA agar	Moderate	Yellow to grey	Cinnamon
Yeast-starch agar	Moderate	White to grey	Light cinnamon

Table 2 Physiological properties of strain 173

Property	Result	Property	Result
Xylose	+	Glucose	+
Mannitol	+	Esculin	−
Mannose	+	Salicin	−
Arabinose	+	Surcose	−
Raffinose	−	Melanoid pigment	−
Inositol	+	Peptonization of milk	+
Galactose	+	Cellulose	−
Inulin	−	Starch hydrolysis	+
Fruitcose	+	NO_3^- reduce	+
Sorbit	−	Gelation liquefaction	+
Rhamnose	+	H_2S production	−

The 16S rDNA sequence of the strain 173 showed 100% similarity to that of the type strain *Streptomyces avermitilis* and the strain 173 was identified as *Streptomyces avermitilis*.

2.2 Bioassay of some important insect pests

The bioassay results are shown in Table 3. The strain 173 showed strong activity against *Heliothis armigera* larvae, *Plutella xylostlla* L., *Spodoptera exigua* and aphids. The insecticidal activity of fermentation broth of the strain 173 was similar to that of 10 ppm avermectin B_1 in view of the death time and mortality, indicating that the strain 173 is a very promising marine insecticidal *Streptomyces avermitilis*.

Table 3 Lab bioassay of 3-instar larvae

Treatments		*t*, 24 h		*t*, 48 h		*t*, 72 h	
		Adjustment mortality, %	Mortality, %	Adjustment mortality, %	Mortality, %	Adjustmen mortality, %	Mortality, %
Ck		0	2.78	0	5.56	0	8.33
Helicoverpa	1 ppm avermectin	18.05	20.83	40.27	45.83	79.17	87.50
armigera	10 ppm avermectin	76.39	79.17	81.94	87.50	91.67	100
	Strain 173	84.72	87.50	90.27	95.83	91.67	100
Plutella	1 ppm avermectin	15.27	18.05	44.44	50.00	81.95	90.28
xylostlla L.	10 ppm avermectin	76.38	79.16	91.66	97.22	91.67	100
	Strain 173	79.16	81.94	84.71	90.27	90.28	98.61
Spodoptera	1 ppm avermectin	9.73	12.51	22.22	27.78	37.5	45.83
exigua	10 ppm avermectin	40.27	43.05	51.39	56.95	69.48	77.81
	Strain 173	66.66	69.44	79.16	84.72	83.34	91.67
Aphids	1 ppm avermectin	44.44	47.22	90.27	95.83	91.67	100
	10 ppm avermectin	93.05	95.83	94.44	100	91.67	100
	Strain 173	70.82	73.60	90.27	95.83	91.67	100

2.3 Properties of the insecticidal antibiotics of the strain

The insecticidal substances showed a good stability in a broad pH range from 3 to 12, and good thermo-stability from 40℃ to 80℃, so they are good candidates for industrial applications.

2.4 HPLC results

HPLC analysis indicated that both the supernatant and mycelia of fermentation broth contain avermectin B_{1a}. This is different from the related report (Richard, 1979) that claimed avermectin B_1 mainly exists in mycelia. It is well known that avermectin B_1, the most powerful avermectin, is broadly effective against most important agricultural pests (Mrozik, 1989).

3 Discussion

By comparison with the taxonomic results of *Streptomyces avermitilis* (Richard, 1979), it was found that there were many obvious differences between the marine insecticidal strain 173 and *Streptomyces avermitilis* from terrestrial environment, such as morphology of sporophores, soluble pigment, utilization of raffinose and sucrose, and H_2S production etc. It can be deduced that many dissimilarities can be made by the *Streptomyces avermitilis* from marine and the *Streptomyces avermitilis* from terrestrial environment.

Avermectins, isolated originally from the culture broth of *Streptomyces avermitilis* from soil, are a series of unique 16-membered macrolides (Egfrton, 1979). Owing to high efficiency, broad spectrum (Mrozik, 1989), low residue (Forbes, 1993), rapid degradation in the environment (Bruce, 1993) and safe for non-target organisms (Payne, 1993), the avermectin natural products are the best biological pesticide ever found and they can replace the highly toxic chemicals. Thus, the producing strain 173 from marine source is a very promising and less polluted bio-pesticide with significant importance for environmental protection.

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(Received for review March 31, 2004. Accepted May 14, 2004)