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Isolation and Characterization of α-Endosulfan Degrading Bacteria from the Microflora of Cockroaches

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Abstract

Extensive applications of organochlorine pesticides like endosulfan have led to the contamination of soil and environments. Five different bacteria were isolated from cockroaches living in pesticide contaminated environments. According to morphological, physiological, biochemical properties, and total cellular fatty acid profile by Fatty Acid Methyl Esters (FAMEs), the isolates were identified as *Pseudomonas aeruginosa* G1, *Stenotrophomonas maltophilia* G2, *Bacillus atrophaeus* G3, *Citrobacter amolonaticus* G4 and *Acinetobacter lwoffii* G5. This is the first study on the bacterial flora of *Blatta orientalis* evaluated for the biodegradation of α-endosulfan. After 10 days of incubation, the biodegradation yields obtained from *P. aeruginosa* G1, *S. maltophilia* G2, *B. atrophaeus* G3, *C. amolonaticus* G4 and *A. lwoffii* G5 were 88.5% , 85.5%, 64.4%, 56.7% and 80.2%, respectively. As a result, these bacterial strains may be utilized for biodegradation of endosulfan polluted soil and environments.

Key words: α-endosulfan, biodegradation, cockroaches, isolation

Introduction

Endosulfan is a chlorinated cyclodiene and broad-spectrum insecticide used in a wide variety of food and non-food crops against many insect pests. Technical grade endosulfan is a mixture of two stereoisomers, *i.e.*, α and β-endosulfan in a ratio of 7:3. Due to its extensive use, endosulfan residues are commonly found in the environment such as atmosphere, soils, sediments, surface and ground waters and foodstuffs (Hussain *et al*., 2007; Kataoka and Takagi, 2013; Kong *et al*., 2014; Kumar *et al*., 2014). Its abundant use poses a threat to environmental quality and public health. Endosulfan affects the central nervous system, kidney, liver, blood chemistry, parathyroid gland and has teratogenic and mutagenic effects (Lu *et al*., 2000).

Several microorganisms, both bacteria and fungi, have been isolated from different sources which have the capability to degrade endosulfan (Goswami and Singh, 2009; Bajaj *et al*., 2010; Bhattacharjee *et al*., 2014). These microorganisms can use endosulfan either as carbon or sulfur source or both (Siddique *et al*., 2003). Such organisms can later be investigated as source of enzymes for further enzymatic reactions in detoxification of endosulfan (Thangadurai and Suresh, 2014). The use of endosulfan as a sulfur source and the subsequent removal of sulfur from the compound considerably reduced its toxicity to mammals (Dorough *et al*., 1978; Goebel *et al*., 1982).

Invertebrates host numerous microorganisms with interactions ranging from symbiosis to pathogenesis, but the microflora of insects is unexplored as yet. The gut microflora plays an important role in pheromone production, pesticide degradation, vitamin synthesis, enzyme synthesis and pathogen prevention (Reeson *et al*., 2003). Cockroaches have been searched as transmitters of pathogenic bacteria and carrries of multiple antibiotic resistance strains (Fotedar *et al*., 1991; Pai *et al*., 2005). No information is available about the intestinal microbes of cockroaches, which live in a pesticide environment. The insect gut provides ideal conditions for gene transfer between bacteria (Dillon and Dillon, 2004). Microorganisms adapt to changing environmental conditions by horizontal gene transfer (De Boever *et al*., 2007; De Gelder *et al*., 2008), conjugative plasmids (De Boever *et al*., 2007) and simple mutations which provides them with new traits (antibiotic-resistance, xenobiotic degradation) so they can survive and colonize their new environment. Considering these points, we suggest that isolated bacterial strains may have

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developed resistance to pesticides. If this is true, we can isolate new and effective α-endosulfan degrading bacteria from cockroaches' microflora and these isolates can be used for the biological treatment of waters and soils polluted with endosulfan and other insecticides.

Experimental

Material and Methods

Chemicals. α-endosulfan and reagents were purchased from Sigma-Aldrich (St Louis, MO, USA). All solvents used were of the highest analytical grade and were employed without further purification. The stock α-endosulfan solution was prepared in acetone and used for all the experiments. The chemical structure and the most important physical parameters of endosulfan are summarized in Fig. 1 and Table I, respectively.

Isolation of endosulfan degrading microorganisms. Cockroaches (*Blatta orientalis* Linnaeus, 1758; Dictyoptera) were collected from stables contaminated with pyrethroid and chlorinated organochlorine pesticide in Samsun (Turkey). Three insects were surface sterilized using 70% ethanol for 1 min and crushed in a sterile mortar containing 10 ml of 0.85% (w/v) saline solution (Okay *et al.*, 2013). Dilutions of 10^{-4} of each sample (1 ml) were suspended in 50 ml liquid non sulfur medium (NSM) containing 100 mg/l α-endosulfan in a 250 ml Erlenmeyer flask, and incubated at 30°C with shaking (150 rpm). The NSM was autoclaved at 121°C for 20 min after which it was aseptically spiked with α-endosulfan dissolved in acetone to yield a final concentration of 100 mg/l. The NSM consisted of (g/l): K_2HPO_4, 0.225; KH_2PO_4, 0.225; NH_4Cl, 0.225; $MgCl_2 \cdot 6H_2O$, 0.845; $CaCO_3$, 0.005; $FeCl_2 \cdot 4H2O$, 0.005; D-glucose, 1.0; and 1 ml of trace element solution per litre. The trace element solution prepared for NSM contained (mg/l): $MnCl_2 \cdot 4H_2O$, 198; $ZnCl_2$, 136; $CuCl_2 \cdot 2H_2O$, 171; $CoCl_2 \cdot 6H_2O$, 24; and $NiCl_2 \cdot 6H_2O$, 24 (Siddique *et al.*, 2003). After 7 days, 5 ml of each culture was re-inoculated into new α-endosulfan-NSM medium and further incubated at 30°C for 7 days. This subculture was repeated under the same culture conditions, and then an aliquot (0.2 ml) from each culture was applied to solid α-endosulfan-NSM for isolation of single colonies.

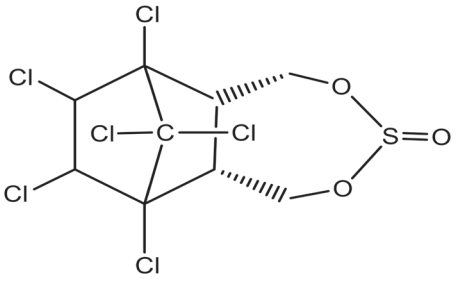

Fig. 1. Molecular structure of α-endosulfan (Fan, 2007).

Table I
Physical parameters of α endosulfan (Fan 2007)

Molecule formula	$C_9H_6Cl_6O_3S$
Molecular weight	406.95 g/mol
Melting point	108–110°C
Water solubility	0.33 µg/L
Density	1.745 g/cm³
Boiling point	<300°C
Color	Yellowish, brown

Identification of endosulfan degrading microorganisms. α-endosulfan degrading bacterial isolates were identified by various tests, such as pigment formation, Gram staining, nitrate reduction, catalase and oxidase tests, and starch hydrolysis. Biochemical reactions were conducted according to Benson (2001). Biochemical activities were determined according to the recommended scheme of Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). Identification was confirmed by the fatty acid analysis for all bacterial isolates. Fatty acid methyl ester (FAME) profiles of each bacterial strain were identified by comparing the commercial databases (Tripticase Soy Broth Agar 40) with the MIS software package. The identity of bacterial strains was revealed by computer comparison of FAME profiles of the unknown test strains with those in the library. FAMEs were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m × 0.2 mm) with cross-linked 5% phenyl methyl silicone.

Preparation of bacterial inoculum for biodegradation studies. Bacterial strains were grown in Nutrient Broth (Merck) to mid log phase of growth. The cells were then centrifuged at 10.000 rpm for 10 min, washed three times with sterile 0.85% saline solution and diluted with sterile water to a uniform optical density (OD_{600}) of 0.75.

Analytical methods. Samples were taken at intervals of 3, 5 and 10 days to determine the levels of bacterial growth and α-endosulfan degradation. The optical densities of the culture media were determined spectrophotometrically by measuring the absorbance at 600 nm. α-endosulfan was measured by HPLC. In the cultures α-endosulfan was extracted using ethyl acetate. The sample was dried with anhydrous Na_2SO_4, and concentrated with a rotary evaporator. Samples were detected by HPLC using ODS Hypersil Column (250 × 4.6 mm) as the stationary phase and acetonitrile: water (70:30, v/v) as the mobile phase. The solutes were determined utilizing UV-VIS detector at 214 nm (Hussain *et al.*, 2007). All experiments were performed in triplicate.

Results

Isolation and identification of α-endosulfan degrading bacteria. Five different bacteria which were able to use α-endosulfan as the sole sulfur source at 100 mg/l concentration were successfully isolated and identified. They were identified based on their morphological, biochemical characteristics (Table II) and the cellular fatty acid compositions (Table III). Different species had various cellular membrane compositions and all strains contained 16:0 fatty acids. Strain *Pseudomonas aeruginosa* G1 was gram negative, catalase and oxidase positive, nitrate reductase negative. Its major fatty acids were 10:0 3OH, 18:1 w7c and 16:0. Strain *Stenotrophomonas maltophilia* G2 was gram negative and catalase negative, oxidase and nitrate reductase positive. The results of fatty acid methyl esters (FAMEs) analysis identified G2 as *S. maltophilia*. As shown Table III, totally 22 different fatty acids were detected in G2 strain. Isopentadecanoic (15:0 iso;13-Methyltetradecanoic) had higher relative mass comparing to remaining FAMEs. Its major fatty acids were 15:0 iso, 16:1 w7c/15:0 iso2OH, 15:0 antesio, 16:0. Strain *Bacillus atrophaeus* G3 was gram positive and catalase, oxidase, nitrate reductase positive. Its major fatty acids were 15:0 antesio, 15:0 iso, 17:0 antesio. Strain *Citrobacter amolonaticus* G4 was gram negative and catalase, oxidase, nitrate reductase positive. Its major fatty acids were 16:0, 18:1 w7c, 16:1 w7c/16:1w6c and 14:0. Strain *Acinetobacter lwoffii* G5 was gram negative and catalase and oxidase negative, nitrate reductase positive. Its major fatty acids were 16:0, 16:1 w7c/15:0 iso2OH, 18:1 w9c and 12:0. *A. lwoffii* possesses fatty acids with almost identical characteristics. The most significant fatty acid was 16:1 w7c/15:0 iso 2OH, with an average proportion of approximately 38.81%. The other three major fatty acids were 12:0 (10.26%), 16:0 (20.58%) and 18:1 w9c (22.85%).

Biodegradation of α-endosulfan. The bacterial growth and α-endosulfan degradation were determined after 3, 5 and 10 days of incubation. The growth performances of test bacteria in liquid NSM+ α-endosulfan were spectrophotometrically monitored by measuring the optical density of the cultures, and the results are shown in Fig. 2. Bacterial strains showed similar growth on the third day of incubation and the cell density increased exponentially with time. At the end of 10th day, the highest optical densities for G1 (1.05), G2 (0.93) and G5 (0.85) were detected in NSM+ α-endosulfan.

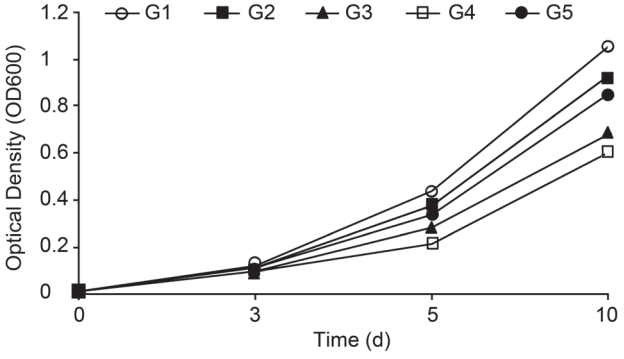

Fig. 2. Bacterial growth monitered by measuring optical densities at 600 nm.

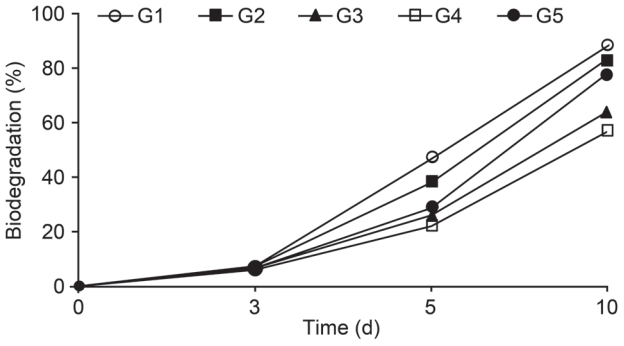

Fig. 3. Biodegradation of α-endosulfan in the broth by isolated bacteria.

As shown in Fig. 3, three strains identified as *P. aeruginosa* G1, *S. maltophilia* G2 and *A. lwoffii* G5 showed the highest potential to degrade α-endosulfan. *P. aeruginosa* G1 exhibited the highest degradation rate of 88.5%. The lowest percentage of degradation (56.7%) was observed with *C. analyticus* G4 strain. The result also demonstrated thatwhen growth of the bacterial cell increased, α-endosulfan degradation also

Table II
The morphological and biochemical characteristics of bacterial isolates of *Blatta orientalis*

Tests	*P. aeruginosa* G1	*S. maltophilia* G2	*B. atrophaeus* G3	*C. amolonaticus* G4	*A. lwoffii* G5
Pigment	Blue-green	Bright yellow	–	Yellow	–
Shape	Bacilli	Bacilli	Bacilli	Bacilli	Coccobacilli
Gram stain	–	–	+	–	–
Catalase	+	–	+	+	–
Oxidase	+	+	–	–	–
Nitrate reduction	–	+	+	–	+

Table III
The fatty acid profiles of bacterial isolates using Microbial Identification System

Fatty acids	*P. aeruginosa* G1	*S. maltophilia* G2	*B. atrophaeus* G3	*C. amalonaticus* G4	*A. lwoffii* G5
10:0		0.59			
11:0 iso		2.87			
11:0 iso 3OH		1.51			
10:0 3OH	3.75				
12:0	4.7			3.79	10.26
12:0 2OH	3.70				
12:0 3OH	3.64	2.93			3.55
13:0 iso		0.39			
14:0 iso		0.67			
14:0		4.23		5.46	
13:0 iso 3OH		2.52			
13:0 2OH		0.36			
15:1 iso F		1.22			
15:0 iso		32.27	39.21		
15:0 Antesio		12.33	46.70		
15:0		0.47			
16:0 iso		0.88			
16:1 w7c			0.96		
16:1 w11c			0.79		
16:0	22.84	7.58	0.72	25.84	20.58
16:1 w9c		3.87			
17:1 iso w9c		4.35			
17:0 cyclo				1.88	
17:1 iso w10c			2.00		
17:0 iso		2.63	1.09		
17:0 antesio		0.31	4.77		
18:1 w7c	44.46	0.99		26.18	3.94
18:1 w9c		1.89			22.85
18:0	0.87				
17:1 iso/antesio B			3.76		
16:1 w7c/15:0 iso2OH		14.06			38.81
16:1w7c/16:1w6c				29.51	
14:0 3OH/16:1 iso 1				6.42	

relatively increased. All isolates were capable of utilizing α-endosulfan as a sulfur source and degraded 56.7–88.5% of α-endosulfan after 10 days.

Discussion

Invertebrates are known to harbour a rich and complex community of microorganisms in their guts and other body regions (Dillon and Dillon, 2004; Ozdal *et al*., 2012). Insect gut symbiotic microbiota allow us to find novel biocatalysts. Many researchers have reported pesticide degrading bacteria isolated from pesticide contaminated soil (Goswami and Singh, 2009; Thangadurai and Suresh, 2014). The present study describes the isolation and identification of bacterial strains capable of degrading α-endosulfan from cockroaches body microflora. Kikuchi *et al*. (2012) and Werren (2012) showed that infection with an insecticide degrading bacterial symbiont immediately establishes insecticide resistance in pest insects as chemical detoxifying agents. Also, although our livers get much of the credit as a toxin degrading organ, our gut microbiome is likely a major player as well (Gill *et al*., 2006). Therefore, the chance of isolating novel microorganisms capable of degrading α-endosulfan from the insect microflora is very high.

A total of five bacterial isolates were isolated and screened for their ability to utilize α-endosulfan as

a sole source of sulfur. These isolates were identified as *P. aeruginosa* G1, *S. maltophilia* G2, *B. atrophaeus* G3, *C. amolonaticus* G4 and *A. lwoffii* G5. Mainly, different applied methods are used for the identification of unknown microorganisms. The first method is based on biochemical, physiological and morphological criteria. The second method is based on chemotaxonomy. The chemotaxonomic approach for obtaining bacterial fatty acid profiles is based on analysis of the methyl esters of their fatty acids by gas chromatography (Basile *et al*., 1998; Fang *et al*., 2000). Whole cellular fatty acid methyl esters content is a stable bacterial profile, the analysis method is rapid, cheap, simple to perform and highly automated, and has long been used for microbial diagnostics and taxonomy (Giacomini *et al*., 2000). *Pseudomonas* species have 3-OH 10:0, 3-OH 12:0 and 12:0, in addition to 16:1 w9c, 16:0 and 18:1 w7c, as their cellular fatty acids, as well as the Q-9 ubiquinone (Ikemoto *et al*., 1978; Vancanneyt *et al*., 1996). *S. maltophilia* has a complex fatty acid profile and different OH-acids detected in the sample. Similiar results were reported by David *et al*. (2008). The predominance of terminally methylbranched iso and anteiso fatty acids having 12 to 17 carbons is a characteristic observed in all *Bacillus* species. The normal fatty acids such as 14:0 and 16:0, the most common fatty acids in the majority of organisms, are generally minor constituents in the genus *Bacillus* (Kaneda, 1977). The major cellular fatty acids of *Citrobacter* are C16:0, C14:0, C18:1 w7c, C16:1 w7c/16:1 w6c and 14:0 3OH/16:1 iso (Whittaker *et al*., 2007). The major fatty acids of *Acinetobacter* strains were 18:1 w9c, 16:1 w7c, 16:0. Each strain of *Acinetobacter*, except *A. lwoffii*, also contained a small amount of 12:0 2-OH (Moss *et al*., 1988).

Previous researchers have reported that endosulfan can be used as the sole sulfur source (Hussain *et al*., 2009; Kataoka and Takagi, 2013; Kumar *et al*., 2014) or the sole carbon source for microbial growth (Sutherland *et al*., 2000; Kumar *et al*., 2007; Castillo *et al*., 2011). In this study, the isolates were screened for their ability to utilize α-endosulfan as the sole sulfur source. Endosulfan is a poor biological energy source, as it contains only six potential reducing electrons. However, it has a relatively reactive cyclic sulfite diester group and can serve as a good sulfur source (Sutherland *et al*., 2000). The removal of sulfur moiety from the endosulfan substantially reduced its vertabrate toxicity and therefore helped in detoxification of the compound (Goebel *et al*., 1982; Singh and Singh, 2011).

The isolated bacterial strains could degrade about 56.7–88.5% of 100 mg/l of α-endosulfan in 10 days of incubation. The difference in degradation capability of various strains may be due to difference in enzyme system and/or difference in their growth rate (Bhattacharjee *et al*., 2014).

There was a very little α-endosulfan biodegradation during the first 3 days, because there was possibly a lag phase. As the incubation continued, the biodegradation rates of α-endosulfan then accelerated. This positive effect may be due to the induction/activation of the enzymes required for degradation (Verma *et al*., 2011). Optical density of the isolates increased with time. This demonstrated that α-endosulfan was degraded by the isolates for their growth. This effect is due to a greater number of organisms that can more easily tolerate the toxic compounds (Hussain *et al*., 2007; Gur *et al*., 2014).

To the best of our knowledge, G3 and G4 have not yet been reported as α-endosulfan degraders. However, in this study, we demonstrated that our strains could degrade α-endosulfan much higher or similiar than of previously reported bacterial strains. *S. maltophilia* E4, *Rhodococcus erythropolis* E5 (Kumar *et al*., 2007), *P. aeruginosa* MN2B14 (Hussain *et al*., 2009) degraded 46% (15 days), 24% (15 days) and 95% (14 days) of α-endosulfan, respectively. In the case of the experiment done by Yu *et al*. (2012), *Stenotrophomonas*. sp. LD-6 completely degraded of α-endosulfan and β-endosulfan (100 mg/l) after 10 days.

Conclusions. Strains of *P. aeruginosa* G1, *S. maltophilia* G2, *B. atrophaeus* G3, *C. analyticus* G4 and *A. lwoffii* G5, able to use α-endosulfan as the only sulfur source, were isolated from cockroaches. Pesticide tolerance in these bacteria may be important in the bioremediation of polluted environments by the pesticides. In this study, we showed that pesticide degrading bacteria can be isolated from insects. Other insects (Colorado potato beetle, fleas and termites) which lives in pesticide contaminated environments should be researched to isolate different pesticide degrading bacteria as well. We demonstrated that the α-endosulfan degrading bacterial strains establish a particular and beneficial symbiosis with the cockroaches.

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Literature

Bajaj A., A. Pathak, M.R. Mudiam, S. Mayilraj and N. Manickam. 2010. Isolation and characterization of a *Pseudomonas* sp. strain IITR01 capable of degrading α-endosulfan and endosulfan sulfate. *J. Appl. Microbiol.* 109: 2135–2143.

Basile F., M.B. Beverly and K.J. Voorhees. 1998. Pathogenic bacteria: their detection and differentiation by rapid lipid profiling with pyrolysis mass spectrometry. *Trends Analyt. Chem.* 17: 95–109.

Benson H.J. 2001. Microbiological Applications Laboratory Manual. Laboratory Manual for General Microbiology. Eighth Edition. The McGraw-Hill Companies, New York.

Bhattacharjee K., S. Banerjee, L. Bawitlung, D. Krishnappa and S.R. Joshi. 2014. A study on parameters optimization for degradation of endosulfan by bacterial consortia isolated from contaminated soil. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci*. 84: 657–6676.

Castillo J.M., J. Casas and E. Romero. 2011. Isolation of an endosulfan-degrading bacterium from a coffee farm soil: Persistence and inhibitory effect on its biological functions. *Sci. Total Environ*. 412: 20–27.

David F., B. Tienpont and P. Sandra. 2008. Chemotaxonomy of bacteria by comprehensive GC and GC-MS in electron impact and chemical ionisation mode. *J. Sep. Sci.* 31: 3395–3403.

De Boever P., P. Ilyin, V. Forget-Hanus, G. Van der Auwera, J. Mahillon and M. Mergeay. 2007. Conjugation-mediated plasmid exchange between bacteria grown under space flight conditions. *Microgravity Sci. Technol*. 19: 5–6.

De Gelder L., J.J. Williams, J.M. Ponciano, M. Sota and E.M. Top. 2008. Adaptive plasmid evolution results in host-range expansion of a broad-host-range plasmid. *Genetics* 178: 2179–2190.

Dillon R.J. and V.M. Dillon. 2004. The gut bacteria of insects: nonpathogenic interactions. *Annu. Rev. Entomol.* 49: 71–92.

Dorough, H.W., K. Huhtanen, T.C. Marshall and H.E. Bryant. 1978. Fate of endosulfan in rats and toxicological considerations of apolar metabolites. *Pestic Biochem. Physiol.* 8: 241–252.

Fan S. 2007. Draft Endosulfan Risk Characterization Document: Volume III – Environmental Fate. Department of Pesticide Regulation, Environmental Monitoring Branch, California Environmental Protection Agency: Sacramento, CA, USA.

Fang J., M.J. Barcelona and P.J.J. Alvarez. 2000. A direct comparison between fatty acid analysis and intact phospholipid profiling for microbial identification. *Org. Geochem.* 31: 881–887.

Fotedar R., U.B. Shriniwas and A. Verma. 1991. Cockroaches (*Blattella germanica*) as carriers of microorganisms of medical importance in hospitals. *Epidemiol. Infect.* 107: 181–187.

Giacomini M., C. Ruggiero and L. Calegari. 2000. Artificial neural network based identification of environmental bacteria by gas-chromatographic and electrophoretic data. *J. Microbiol. Methods* 43: 45–54.

Gill S.R., M. Pop, R.T. DeBoy, P.B. Eckburg, P.J. Turnbaugh, B.S. Samuel, J.I. Gordon, D.A. Relman, C.M. Fraser-Liggett and K.E. Nelson. 2006. Metagenomic analysis of the human distal gut microbiome. *Science* 312: 1355–1359.

Goebel, H., Gorbach, S., Knauf, W., Rimpau, R.H. and H. Huttenbach. 1982. Properties, effects, residues and analytics of the insecticide endosulfan. *Residue Rev.* 83: 40–41.

Goswami S. and D.K. Singh. 2009. Biodegradation of a and b endosulfan in broth medium and soil microcosm by bacterial strain *Bordetella* sp. B9. *Biodegradation* 20: 199–207.

Gur O., M. Ozdal and O.F. Algur. 2014. Biodegradation of the synthetic pyrethroid insecticide α-cypermethrin by *Stenotrophomonas maltophilia* OG2. *Turk. J. Biol.* 38: 684–689.

Holt J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams. 1994. Bergey's Manual of Determinative Bacteriology, 9th ed. Lippincott Williams and Wilkins, Baltimore.

Hussain S., M. Arshad, M. Saleem and A. Khalid. 2007. Biodegradation of α- and β-endosulfan by soil bacteria. *Biodegradation* 18: 731–740.

Hussain S., M. Arshad, B. Shaharoona, M. Saleem and A. Khalid. 2009. Concentration dependent growth/non-growth linked kinetics of endosulfan biodegradation by *Pseudomonas aeruginosa. World J. Microbiol. Biotechnol.* 25: 853–858.

Ikemoto S., H. Kuraishi, K. Komagata, R. Azuma, T. Suto and H. Muroka. 1978. Cellular fatty acid composition in *Pseudomonas* species. *J. Gen. Appl. Microbiol.* 24: 199–213.

Kaneda T. 1977. Fatty Acids of the Genus *Bacillus*: an Example of Branched-Chain Preference. *Bacteriol Rev.* 41: 391–418.

Kataoka R and K. Takagi. 2013. Biodegradability and biodegradation pathways of endosulfan and endosulfan sulphate. *Appl. Microbiol. Biotechnol.* 97: 3285–3292.

Kikuchi Y., M. Hayatsu, T. Hosokawa, A. Nagayama, K. Tago and T. Fukatsu. 2012. Symbiont-mediated insecticide resistance. *Proc. Natl. Acad. Sci.* 109: 8618–8622.

Kong L., S. Zhu, L. Zhu, H. Xie, K. Su, T. Yan, J. Wang, J. Wang, F. Wang and F. Sun. 2013. Biodegradation of organochlorine pesticide endosulfan by bacterial strain *Alcaligenes faecalis* JBW4. *J. Environ. Sci.* 25: 2257–2264.

Kumar K., S.S. Devi, K. Krishnamurthi, G.S. Kanade and T. Chakrabarti. 2007. Enrichment and isolation of endosulfan degrading and detoxifying bacteria. *Chemosphere* 68: 317–322.

Kumar A., N. Bhoot, I. Soni and P.J. John. 2014. Isolation and characterization of a *Bacillus subtilis* strain that degrades endosulfan and endosulfan sulfate. *3 Biotech.* 4: 467–475.

Lu Y., K. Morimoto, T. Takeshita, T. Takeuchi and T. Saito. 2000. Genotoxic effects of α-endosulfan and β-endosulfan on human HepG2 cells. *Environ. Health Perspect.* 108: 559–561.

Moss C.W., P.L. Wallace, D.G. Hollis and R.E. Weaver. 1988. Cultural and chemical characterization of CDC groups EO-2, M-5, and M-6, *Moraxella* (*Moraxella*) species, *Oligella urethralis*, *Acinetobacter* species, and *Psychrobacter immobilis*. *J. Clin. Microbiol*. 26: 484–492.

Okay S., M. Ozdal and E.B. Kurbanoğlu. 2013. Characterization, antifungal activity and cell immobilization of a chitinase from *Serratia marcescens* MO-1. *Turk. J. Biol.* 37: 639–644.

Ozdal M., U. Incekara, A. Polat, O. Gur, E.B. Kurbanoğlu and G.E. Tasar. 2012. Isolation of filamentous fungi associated with two common edible aquatic insects, *Hydrophilus piceus* and *Dytiscus marginalis*. *J. Microbiol. Biotechnol. Food Sci.* 2: 95–105.

Pai H., W.C. Chen and C.F. Peng. 2005. Isolation of bacteria with antibiotic resistance from household cockroaches (*Periplaneta americana* and *Blattella germanica*). *Acta Trop*. 93: 259–265.

Reeson A.F., T. Jankovic, M.L. Kasper, S. Rogers and A.D. Austin. 2003. Application of 16S rDNA-DGGE to examine the microbial ecology associated with a social wasp *Vespula germanica. Insect Mol. Biol.* 12: 85–91.

Siddique T., B.C. Okeke, M. Arshad and W.T.J. Frankenberger. 2003. Enrichment and isolation of endosulfan degrading microorganisms. *J. Environ. Qual.* 32: 47–54.

Singh N.S. and D.K. Singh. 2011. Biodegradation of endosulfan and endosulfan sulfate by *Achromobacter xylosoxidans* strain C8B in broth medium. *Biodegradation* 22: 845–857.

Sutherland T.D., I. Horne, M.J. Lacey, R.L. Harcourt, R.J. Russell and J.G. Oakeshott. 2000. Enrichment of an endosulfan-degrading mixed bacterial culture. *Appl. Environ. Microbiol*. 66: 2822–2828.

Thangadurai P. and S. Suresh. 2014. Biodegradation of endosulfan by soil bacterial cultures. *Int. Biodeterior. Biodegradation* 94: 38–47.

Vancanneyt M., S. Witt, W.R. Abraham, K. Kersters and H.L. Fredrickson. 1996. Fatty acid content in wholecell hycrolysates and phospholipid fractions of pseudomonads: A taxonomic evaluation. *Syst. Appl. Microbiol.* 19: 528–540.

Verma A., D. Ali, M. Farooq, A.B. Pant, R.S. Ray and R.K. Hans. 2011. Expression and inducibility of endosulfan metabolizing gene in *Rhodococcus* strain isolated from earthworm gut microflora for its application in bioremediation. *Bioresour. Technol.* 102: 2979–2984.

Werren J.H. 2012. Symbionts provide pesticide detoxification. *Proc. Natl. Acad. Sci.* 109: 8364–8365.

Whittaker P., C.E. Keys, E.W. Brown and F.S. Fry. 2007. Differentiation of *Enterobacter sakazakii* from closely related *Enterobacter* and *Citrobacter* species using fatty acid profiles. *J. Agric. Food Chem.* 55: 4617–4623.

Yu F.B., W.A. Shinawar, J.Y. Sun and L.P. Luo. 2012. Isolation and characterization of an endosulfan degrading strain, *Stenotrophomonas* sp. LD-6, and its potential in soil bioremediation. *Pol. J. Microbiol.* 61: 257–262.