### ORIGINAL ARTICLE



# Biodegradation of $\alpha$ -endosulfan via hydrolysis pathway by *Stenotrophomonas maltophilia* OG2

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**Abstract** Stenotrophomonas maltophilia OG2 was isolated from the intestine of cockroaches that was collected from a cow barn contaminated some pesticides belong to pyrethroid and organochlorine groups. OG2 was able to degrade  $\alpha$ -endosulfan in non sulfur medium (NSM) as a sole sulfur source for growth within 10 days of incubation. The effects of some growth parameters on endosulfan biodegradation by OG2 was studied and found that the biodegradation was significantly affected by the endosulfan concentrations, pH and temperature. Experimental results obtained in different conditions show that the optimum concentration of α-endosulfan, pH and temperature were 100 mg/L, 8.0 and 30 °C, respectively. Under these conditions, the bacterium degraded 81.53% of the α-endosulfan after 10 days. The concentration of α-endosulfan and its metabolites was determined by HPLC. Endosulfan ether, endosulfan lactone and endosulfan diol were the main metabolites in culture, but did not produce toxic metabolite, endosulfan sulfate. These results suggested that S. maltophilia OG2 degrades α-endosulfan via a hydrolysis pathway. The present study indicates that strain OG2 may have potential use in the biodegradation of pesticides contaminated environments.

**Keywords** *Stenotrophomonas maltophilia* · Biodegradation · α-Endosulfan · Hydrolysis pathway

#### Introduction

Pesticides are a class of biocide released intentionally into environment to kill living materials such as weeds (herbicides), insects (insecticides), fungus (fungicides), rodents (rodenticides) and others. They are used almost everywhere, not only in agricultural fields, but also in homes, parks, schools, buildings, forests and roads. Recent years use of synthetic pesticides in agriculture is the most widespread method for pest control. It was reported that farmers spend approximately \$40 billion on pesticides annually. The main chemical groups of pesticides are organophosphate pesticides, carbamate pesticides, organochlorinated pesticides and pyretroid pesticides (Popp et al. 2013; Verma et al. 2014). Organochlorinated pesticides (DDT, endosulfan, lindane, aldrin, and dieldrin) are highly toxic and considered a potential risk to both living organisms and the environment. Endosulfan (1,4,5,6,7,7-hexachloro-5-norbornene-2, 3-dimethanol cyclic sulfite) is a chlorinated cyclodiene insecticide which is used extensively throughout the world for the control of numerous pests on a wide range of crops. The intensive use of endosulfan affects public health, beneficial biota and contaminate ecosystem (Arshad et al. 2008; Kataoka and Takagi 2013).

Technical endosulfan is a mixture of two stereoisomers,  $\alpha$  and  $\beta$ -endosulfan in a ratio of 7:3.  $\alpha$ -Endosulfan is more bioaccumulative and about three times more toxic than  $\beta$ -endosulfan (Negro et al. 2012). Previous studies have shown that microbial species prefer  $\beta$ -endosulfan for degradation over  $\alpha$ -endosulfan (Siddique et al. 2003; Goswami and Singh 2009). Biodegradation of endosulfan and its isomers by bacteria and fungi have been previously reported (Singh and Singh 2011; Kong et al. 2013; Silambarasan and Abraham 2013; Kumar et al. 2014; Ozdal



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et al. 2016a). These microorganisms can utilize endosulfan either as carbon or sulfur source or both (Siddique et al. 2003). Although endosulfan is biodegradable in nature, its residues are found in environment (Weber et al. 2010). Biodegradation is affected by a number of physicochemical environmental parameters such as energy source (electron donors), nutrients, pH, temperature, and substrate concentration (Hussain et al. 2009; Singh and Singh 2011; Kong et al. 2013).

There are reports on many microorganisms capable of degrading endosulfan through the action of different of enzymes such as hydrolase, dehydrogenase, lactonase, and monooxygenase (Kataoka and Takagi 2013; Thangadurai and Suresh 2014). Endosulfan is degraded by attacking the sulphite group via either hydrolysis to form the less toxic endosulfan diol or oxidation to toxic endosulfan sulfate, respectively (Kataoka and Takagi 2013). One of the degradation products of endosulfan, endosulfan sulfate, has been found more toxic and more persistent than the endosulfan and its metabolites such as endosulfan diol, endosulfan ether, endosulfan lactone and endosulfan hydroxyether (Weber et al. 2010). Therefore, the isolation of microorganism/s that can degrade the α-endosulfan without the formation this toxic metabolite is very important.

Stenotrophomonas maltophilia is an aerobic, nonfermentative, Gram negative bacterium. S. maltophilia can be found everywhere in nature and is often used for biodegradation studies. Several reports show that S. maltophilia can degrade different compounds, such as the keratin (Bhange et al. 2016), alkane (Hassanshahian et al. 2013), toluene, benzene, ethylbenzene, xylene (Lee et al. 2002), and pesticides (Gur et al. 2014, Ozdal et al. 2016b), and phenol (Basak et al. 2014). S. maltophilia OG2 was isolated from the microflora of cockroaches living in pesticide-contaminated environment. Our previous preliminary study with S. maltophilia OG2 indicated that this strain could utilize  $\alpha$ -cypermethrin (Gur et al. 2014). In this study, biodegradation of α-endosulfan by S. maltophilia and the factors (such as pesticide concentration, pH and temperature) effecting degradation potential were investigated for the first time.

# Materials and methods

#### Microorganism

Stenotrophomonas maltophilia OG2 (GenBank with the accession number KC453991) was isolated during our previous study from the cockroaches (*Blatta orientalis* Linnaeus 1758; Dictyoptera) which live in stables contaminated with pesticides (Gur et al. 2014).



#### Chemicals

 $\alpha\text{-Endosulfan}$   $(C_9H_6Cl_6O_3S),$  endosulfan sulfate  $(C_9H_8-Cl_4S),$  endosulfan diol  $(C_9H_8Cl_6O),$  endosulfan ether  $(C_9-H_6ClO_2),$  endosulfan hydroxyether  $(C_9H_4ClO_2)$  and endosulfan lactone  $(C_9H_4Cl_6O_2)$  were purchased from Sigma-Aldrich (St. Louis, MO). The stock  $\alpha\text{-endosulfan}$  solution was prepared in acetone and used for all the experiments.

#### Biodegradation of α-endosulfan in aqueous medium

Non sulfur medium (NSM) was used for biodegradation studies. The NSM contained (g/L): K<sub>2</sub>HPO<sub>4</sub>, 0.225; KH<sub>2</sub>-PO<sub>4</sub>, 0.225; NH<sub>4</sub>Cl, 0.225; MgCl<sub>2</sub>.6H<sub>2</sub>O, 0.845; CaCO<sub>3</sub>, 0.005; FeCl<sub>2</sub>.4H<sub>2</sub>O, 0.005; glucose, 1 and 1 mL trace element solution. Trace element solution contained (mg/L): MnCl<sub>2</sub>·4H<sub>2</sub>O, 198; ZnCl<sub>2</sub>, 136; CuCl<sub>2</sub>·2H<sub>2</sub>O, 171; CoCl<sub>2</sub>-6H<sub>2</sub>O, 24; and NiCl<sub>2</sub>·6H<sub>2</sub>O, 24 (Siddique et al. 2003). The NSM was autoclaved (121 °C for 20 min), after cooling, NSM was supplemented with α-endosulfan.

#### **Biodegradation assays**

Stenotrophomonas maltophilia OG2 was grown in Nutrient Broth (Merck) aerobically at 30 °C at 150 rpm for 18 h. Cells were obtained by centrifugation (5000 rpm for 6 min at 4 °C), washed two times with sterile saline water (0.9%) and diluted with deionized water to a uniform optical density (O.D<sub>600</sub>) of 0.80. 1 mL of bacterial suspension was aseptically transferred to 50 mL sterile NSM in 250 mL Erlenmeyer flasks containing α-endosulfan and incubated on a rotary shaker. To obtain the optimal condition for  $\alpha$ endosulfan degradation by strain OG2, initial α-endosulfan concentration, the culture pH and temperature of medium were examined. The effect of the different initial substrate concentrations (25, 50, 100 and 200 mg/L) on the α-endosulfan degradation by S. maltophilia OG2 was investigated. The flasks were shaken at 150 rpm at 30 °C for 10 days and a pH of 7.0. To investigate the optimum pH for α- endosulfan biodegradation, the initial pH value of non sulfur medium was adjusted to pH 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 using 0.5 N HCl and 0.5 N NaOH. To investigate the optimum temperature for  $\alpha$ -endosulfan biodegradation, cultures were incubated at 25, 30, 35 and 40 °C.

#### **Analytical methods**

 $\alpha$ -Endosulfan and its degradation products, i.e., endosulfan sulfate, endosulfan diol, endosulfan ether and endosulfan lactone were measured by HPLC.  $\alpha$ -Endosulfan in cultures was extracted using ethyl acetate. The sample was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated with a rotary

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evaporator. Samples were detected by HPLC using ODS  $C_{18}$  Hypersil Column (250 × 4.6 mm, 5 µm) 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. 2009). Retention times for  $\alpha$ -endosulfan, endosulfan sulfate, endosulfan ether, endosulfan lactone and endosulfan diol under analytical conditions were 5.323, 7.873, 8.976, 10.035 and 12.086 min, respectively. Growth was recorded spectrophotometrically at 600 nm. All experiments were conducted in triplicates.

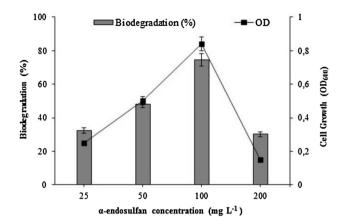
### Results and discussion

## **Organism**

An environment without microorganism is unimaginable and microorganisms are found in almost every habitat in nature. Several interactions (pathogenic, symbiotic and vectoring) can be distinguished between insects and microorganisms (Ozdal et al. 2012; Okay et al. 2013). Many researchers have reported that pesticide degrading microorganisms isolated from pesticide contaminated sites (Singh and Singh 2011; Silambarasan and Abraham 2013; Ito et al. 2016). α-Cypermethrin degrading S. maltophilia OG2 was isolated by Gur et al. (2014) from cockroaches microflora which live in pesticide (cypermethrin and endosulfan) contaminated environment. Ozdal et al. (2016b) reported that  $\alpha$ -endosulfan degrading S. maltophilia strains (FA8, SV3, SV9) were isolated from the body microflora of insects belong to Dermaptera (Forficula auricularia Linnaeus 1758) and Mantodea (Sphodromantis viridis La Greea 1950) orders.

# Effect of $\alpha$ -endosulfan concentrations on the biodegradation and cell growth

The biodegradation of  $\alpha$ -endosulfan and growth of strain OG2 was investigated at concentrations of  $\alpha$ -endosulfan ranging from 25 to 200 mg/L (Fig. 1). After 10 days, 32.5, 48.3, 74.4 and 30.2% biodegradation of  $\alpha$ -endosulfan were achieved at initial concentrations of 25, 50, 100 and 200 mg/L, respectively. Maximum bacterial growth was also obtained at an initial concentration of 100 mg/L. At the highest level of  $\alpha$ -endosulfan (200 mg/L), growth and degradation were inhibited. This is also affirmed by earlier findings that indicated the inhibitory effect of high  $\alpha$ -endosulfan concentrations on growth (Goswami and Singh 2009; Hussain et al. 2009). The presence of  $\alpha$ -endosulfan (>200 mg/L) in the medium had a toxic effect on growth for *Bordetella* sp. B9 (Goswami and Singh 2009) and *Pseudomonas aeruginosa* MN2B14 (Hussain et al. 2009).

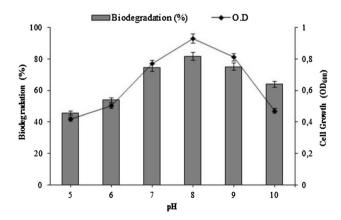


**Fig. 1** Effect of initial  $\alpha$ -endosulfan concentrations on growth profile of *S. maltophilia* OG2 and  $\alpha$ -endosulfan degradation. Fermentation conditions: 30 °C, 150 rpm, 10 days, pH 7. Each value is the mean of three replicates, with *error bars* representing the standard deviation of the mean

Considering the bacterial growth and the degradation rate, 100 mg/L  $\alpha$ -endosulfan concentration was used for further study. *S. maltophilia* OG2 was capable of releasing the sulfite group from  $\alpha$ -endosulfan and utilize it as a source of sulfur for bacterial growth. Previous researchers have reported endosulfan as a sole sulfur source for microbial growth (Kalyani et al. 2009; Yu et al. 2012).

### Effect of pH on the biodegradation and cell growth

Stenotrophomonas maltophilia OG2 was able to growth and degrade  $\alpha$ -endosulfan over relatively wide range of pH (Fig. 2). It was found that change in pH significantly affect the biodegradation rate. Maximum biodegradation of  $\alpha$ -endosulfan by S. maltophila OG2 was observed at an initial pH of 8.0 and minimum at an initial pH of 5.0. The biodegradation  $\alpha$ -endosulfan ranged 45.5–81.53% as the



**Fig. 2** Effect of pH on growth profile of *S. maltophilia* OG2 and  $\alpha$ -endosulfan degradation. Fermentation conditions: 30 °C, 150 rpm, 10 days. Each value is the mean of three replicates, with *error bars* representing the standard deviation of the mean

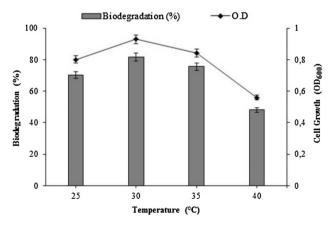


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broth pH increased from 5.0 to 8.0. Biodegradation was inhibited effectively at acidic pH values. At pH higher than 8, growth of S. maltophilia OG2 was slightly inhibited, which resulted to less degradation of α-endosulfan present in the culture. More biodegradation values of endosulfan at higher pH have also been reported by earlier workers (Hussain et al. 2007; Arshad et al. 2008). The slightly alkaline pH range favored the growth of this bacterial strain and the highest O.D<sub>600</sub> (0.93) was observed in liquid culture at an initial pH of 8.0. However, the lowest O.D<sub>600</sub> (0.32) was noted at an initial pH of 5.0 after 10 days of incubation. From the obtained result it was observed that pH of the medium has a significant effect on the growth of the microorganism and ultimately the degradation of the αendosulfan. In general, most bacterial cultures prefer neutral to slightly alkaline conditions rather than acidic conditions for bacterial growth and so maximum degradation was in slightly alkaline pH.

# Effect of temperature on the biodegradation and cell growth

Stenotrophomonas maltophilia OG2 degraded appreciable amounts of  $\alpha$ -endosulfan in the liquid culture at all incubation temperatures (25, 30, 35 and 40 °C) for 10 days of incubation. Optimal temperature used for biodegradation was found to be 30 °C (Fig. 3). The maximum percentage of degradation was found to be 81.53, 75.6 and 70.2 in the temperature range of 30, 35, and 25 °C, respectively. Further increase in incubation temperature to 40 °C decreased the degradation of  $\alpha$ -endosulfan. The highest O.D<sub>600</sub>, 0.93, after 10 days of incubation, was noted in the liquid culture at 30 °C followed by 0.8 and 0.84 at incubation temperatures of 25 and 35 °C, respectively. The results showed that 30 °C was the most favorable



**Fig. 3** Effect of temperature on growth profile of *S. maltophilia* OG2 and  $\alpha$  endosulfan degradation. Fermentation conditions: 30 °C, 150 rpm, 10 days. Each value is the mean of three replicates, with *error bars* representing the standard deviation of the mean



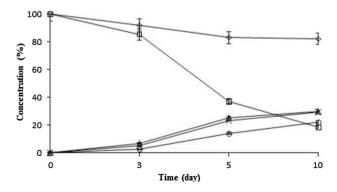
temperature for bacterial growth. Better biodegradation of endosulfan at this temperature have also been described by earlier workers (Hussain et al. 2007; Arshad et al. 2008; Yu et al. 2012). All these reports suggest that 30 °C could be the optimum temperature for the activity of the enzymes that are involved in the  $\alpha$ -endosulfan biodegradation.

# Formation of $\alpha$ -endosulfan metabolites by the cultures

At regular intervals, the degradation of  $\alpha$ -endosulfan and its metabolites (endosulfan diol, hydroxyether and lactone) were analysed using HPLC. As seen in Fig. 4, the rate of biodegradation products increased in NSM with time. *S. maltophilia* OG2 degraded about 14.71, 62.79, 81.53% of  $\alpha$ -endosulfan in 3, 5, 10 days of incubation, respectively.  $\alpha$ -endosulfan degradation was about 17.9% in uninoculated flasks (control) at 10th day of incubation. As seen in Fig. 4, endosulfan diol, endosulfan ether and endosulfan lactone were intermediates in the biodegradation of  $\alpha$ -endosulfan by *S. maltophilia* OG2. Endosulfan ether was the major metabolite in the medium and the endosulfan sulfate was not found at any day.

Microbial degradation of endosulfan often results in the formation of a toxic endosulfan sulfate via oxidation and a less toxic endosulfan diol by hydrolysis. Endosulfan diol can further be transformed by microorganisms to endosulfan ether, endosulfan hydroxyether, endosulfan dialdehyde, and endosulfan lactone. However, these metabolites are less toxic than endosulfan and endosulfan sulfate (Kataoka and Takagi 2013; Thangadurai and Suresh 2014; Kumar et al. 2014). Several soil fungi (*Botryosphaeria laricina* and *Aspergillus tamarii*) have been shown to degrade endosulfan and produce endosulfan sulfate as the metabolic product (Silambarasan and Abraham 2013).

An initial hydrolysis of  $\alpha$ -endosulfan results in the formation of the intermediate metabolite of endosulfan diol, than endosulfan diol was converted to endosulfan ether and



**Fig. 4** Biodegradation of  $\alpha$ -endosulfan and its metabolites in NSM +  $\alpha$ -endosulfan (100 mg L<sup>-1</sup>), control  $(\diamondsuit)$ ,  $\alpha$ -endosulfan  $(\Box)$ , Endosulfan lactone  $(\Delta)$ , Endosulfan ether  $(\times)$ , Endosulfan diol (o)

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**Fig. 5** Proposed pathway for metabolism of  $\alpha$ -endosulfan by *S. maltophila* OG2

endosulfan lactone (Kong et al. 2013). These results implied that OG2 degrades \alpha-endosulfan via hydrolysis (non-oxidative) pathway (Fig. 5). Formation of these metabolites during α-endosulfan degradation has also been reported previously (Weir et al. 2006; Bajaj et al. 2010; Jesitha et al. 2015). Bajaj et al. (2010) reported that after 9 days of incubation; Pseudomonas sp. strain IITR01 was able to degrade 86% of  $\alpha$ -endosulfan. This strain degraded  $\alpha$ -endosulfan to endosulfan sulfate, endosulfan diol, endosulfan ether and endosulfan lactone. Goswami and Singh (2009) reported the isolation of a *Bordetella* sp. B9 that degraded 80% of αendosulfan in 18 days with endosulfan lactone and endosulfan ether as intermediates. Pseudomonas sp. KS-2P (Lee et al. 2006), Achromobacter xylosoxidans C8B (Singh and Singh 2011), S. maltophilia and Rhodococcus erythropolis, and S. maltophilia E4 (Kumar et al. 2007) degraded 49% (7 days), 94.1% (20 days), 73% (14 days) and 46% (14 days) of α-endosulfan, respectively. Recently, endosulfan degrading ability of *Pseudomonas fluorescens* and *P*. aeruginosa immobilised in Ca-alginate beads were investigated by Jesitha et al. (2015) and Pradeep and Subbaiah (2016). In their studies, endosulfan diol, endosulfan ether and endosulfan lactone were detected as hydroxylated products, which indicated that these Pseudomonas species might degrade endosulfan by a non-oxidative pathway.

Different methods such as isolation of pesticide degrading microorganisms, immobilized cells (Jesitha et al. 2015; Pradeep and Subbaiah 2016), determination of

pesticide degradation enzymes (Pradeep and Subbaiah 2016) and genetically engineered microorganisms (Lan et al. 2014) have been developed to reduce the effects of pesticides on the environment and health.

### Conclusion

Stenotrophomonas maltophila OG2 can utilize α-endosulfan as sole sulfur source under sulfur starved condition. Several factors (temperature, pH and endosulfan concentration) can also influence the rate of biodegradation of  $\alpha$ endosulfan by this microorganism. The optimum endosulfan concentration, pH and temperature for maximum biodegradation ability were found to be 100 mg/L, 8 and 30 °C. The S. maltophila OG2 performed well over a fairly wide range of pH and temperature in degrading α-endosulfan in the NSM. Bacterial strain formed less toxic endosulfan diol, endosulfan lactone and endosulfan ether as metabolites during metabolism of  $\alpha$ -endosulfan. The outcome of this research may have practical applications in biodegradation of organochlorine pesticide contaminated soil, waste dump, industrial effluents and water environments. Research should be focused on the pesticide degrading enzyme systems and the location of genes involved in degradation of the pesticide. This isolate should be immobilized using either the isolated enzymes or the whole cells for the detoxification of organochlorine and



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synthetic pyrethroids. Field scale studies should be conducted on the practical use of this bacterium (free or immobilize enzyme/whole cell) in cleaning up pesticides contaminate environments. Moreover, pesticide degrading aerobic and anaerobic microorganisms can be isolated from pesticide resistant insect microflora.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interests regarding the publication of this paper.

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