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ORIGINAL ARTICLE

Evaluation of endosulfan degradation capacity by six pure strains isolated from a horticulture soil

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Abstract

Endosulfan is an organochlorine pesticide included in the Stockholm Convention for Persistent Organic Compounds. The utilization of endosulfan as the sole source of carbon and its mineralization was evaluated using pure strains of *Bacillus subtilis*, *Bacillus pseudomycoides*, *Peribacillus simplex*, *Enterobacter cloacae*, *Achromobacter spanius*, and *Pseudomonas putida*, isolated from soil with historical pesticide use. The consumption of the α isomer of endosulfan by five of the six strains studied was higher than 95%, while *B. subtilis* degraded only 76% of the initial concentration (14 mg/L). On the other hand, the degradation of the β isomer was approximately 86% of the initial concentration (6 mg/L) by *B. subtilis*, *P. simplex*, and *B. pseudomycoides* and 95% by *P. putida*, *E. cloacae*, and *A. spanius*. The ability of *A. spanius*, *P. simplex*, and *B. pseudomycoides* to degrade endosulfan has not been previously reported. The production of endosulfan lactone by the *Bacillus* strains, as well as *A. spanius* and *P. putida*, indicated that endosulfan was degraded by the hydrolytic pathway.

Introduction

In 2018, the Food Agriculture Organization (FAO) registered the use of 4.1 million tons of pesticides worldwide (FAO 2020). In Latin America, Argentine and Mexico are the second- and third-largest pesticide consumers, with 172.9 and 53.1 thousand tons, respectively (FAO 2020). The indiscriminate use of pesticides, mainly organophosphates and organochlorine, has surpassed its benefits, invoking health and environmental problems (Mahmood et al. 2016). Organochlorine pesticides are classified as persistent organic pollutants (POPs) that bioaccumulate in aquatic and terrestrial organisms (Jayaraj et al. 2016).

Endosulfan is the common name for pesticide 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide. The commercial preparation of endosulfan (a mixture of the α and β stereoisomers in a ratio of 7:3) was used worldwide until its inclusion in the Stockholm Convention in 2011. Endosulfan is commonly used in coffee-producing countries for the control of coffee borers (*Hypothenemus hampei*) and insects, mainly, *Nezara viridula* and caterpillars, which affect soybean crops. It is worth noticing that soybean production is one of the most profitable activities in the Argentine economy, as coffee production is in Mexico (González et al. 2009). Endosulfan is ubiquitous, it has a half-life in soil of 60 and 800 days for *α* and β isomers; therefore, it can persist in the environment for several months (Kataoka and Takagi 2013). The presence of endosulfan in the environment affects the biodiversity and fertility of soils (Khan 2012). The α isomer is asymmetric and thermodynamically stable, while the β isomer is symmetric and easily transforms into the α isomer. β-Endosulfan has been reported to be more toxic than its α counterpart, indicating enzymatic specificity (Kwon et al. 2005). The degradations rates of the two isomers in the environment can vary from hours to months depending

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on the soil type, pH, temperature, and microbial activity (Schmidt et al. 1997; Singh et al. 2014).

In the environment, endosulfan isomers can be transformed abiotically by the attack of the sulfite group or biotically by the action of microorganisms (Kataoka et al. 2011). Biological degradation can occur via hydrolytic and oxidative pathways either through consecutive oxidation and hydrolysis reactions or hydrolysis (Kamei et al. 2011; Kataoka et al. 2010; Katayama and Matsumura 1993; Kullman and Matsumura 1996; Shetty et al. 2000). The primary metabolites produced by oxidation and hydrolysis are endosulfan sulfate and endosulfan diol, respectively (Sutherland et al. 2004). Endosulfan monoalcohol is produced by further oxidation of endosulfan sulfate by the enzymatic action of monooxygenases. In contrast, the hydrolysis of endosulfan by some microorganisms produces endosulfan diol (Kwon et al. 2005). Endosulfan sulfate has been reported to be even more toxic than endosulfan, whereas endosulfan diol can be further transformed into less toxic metabolites such as endosulfan ether, endosulfan hydroxyether, endosulfan lactone, and hydroycarbolate of endosulfan (Verma et al. 2011).

Some microorganisms have been reported to use endosulfan as a carbon or sulfur source or both (Siddique et al. 2003). *Pseudomonas putida* is commonly used in bioremediation processes because of its ability to degrade a wide range of compounds, including endosulfan (Loh and Cao 2008). A *P. putida* strain isolated from contaminated soil from a coffee-cultivated area has been reported to degrade 11.66 mg/L per day of endosulfan with the production of endosulfan sulfate, endosulfan diol, and endosulfan lactone (Sunitha et al. 2012). *Pseudomonas* sp. KS-2P has been reported to degrade 2.5 mg/L per day of endosulfan and 2.9 mg/L per day of endosulfan sulfate (Lee et al. 2003). The degradation and mineralization of endosulfan by the *Enterobacter* genus have also been reported (Abraham and Silambarasan 2014). In particular, *Enterobacter cloacae* has been reported to tolerate up to 1300 mg/L and degrade 100 mg/kg per day of endosulfan and 111.11 mg/kg per day of endosulfan sulfate (Abraham and Silambarasan 2015). Furthermore, a strain of *E. cloacae* isolated from agricultural soil degraded both isomers with a degradation rate of 0.143 mg/L per day and 0.085 mg /L per day for α and β-endosulfan, respectively (Jimenez-Torres et al. 2016). On the other hand, a study of utilization of endosulfan as the energy source by *Bacillus subtilis* reported a degradation rate of 6.72 mg/L per day with the production of endosulfan diol, endosulfan lactone, and endosulfan sulfate, as well as the consumption of 8.3 mg/L per day of α-endosulfan and 9.7 mg/L per day of β-endosulfan with the production of 1,2,3,4,7,7-hexachloro-5,6-dihydroxybicyclo[2.2.1]-2-heptene and 1,2,3,4,7,7-hexachloro-formaldehyde-6-methylbi-cyclo[2.2.1]-2-heptene (Ishag et al. 2017; Kumar et al. 2014).

Peribacillus simplex, *Bacillus pseudomycoides*, and *Achromobacter spanius* were also studied in this work, and to the best of our knowledge, these microorganisms have not been previously reported as degraders of endosulfan. However, *Achromobacter* sp. degraded endosulfan and *Peribacillus simplex* (formerly *Bacillus simplex*) has been studied for metal absorption and degradation of metolachlor and trifluralin (Erguven et al. 2016; Munoz et al. 2011; Patel and Gupta 2020; Sunitha et al. 2012; Valentine et al. 1996). *Bacillus pseudomycoides* has been reported as a malathion and azo dye acid black 24 degrader (Li et al. 2016; Tamer and Medhat 2013). *Achromobacter spanius* has been known to degrade kerosene and TNT (Gumuscu et al. 2015; Stancu 2020).

Therefore, the objective of this study was to evaluate the ability of *P. putida*, *E. cloacae*, *A. spanius*, *B. subtilis*, *P. simplex*, and *B. pseudomycoides* to degrade and mineralize endosulfan.

Table 1 Physicochemical characteristics of the soil

Parameter	Value
CEC (meq/100 g)	16.8
EC (meq/100 g)	11.89
Mg	3.1
K	2.67
Na	0.79
ES (%)	4.7
pH	6.8
TOC	2.04
N total (%)	0.23
FC (%)	59.85

CEC cation exchange capacity, *EC* exchangeable cations, *ES* exchangeable sodium, *TOC* total organic carbon, *FC* field capacity

Materials and methods

Soil sample collection and characterization

Soil samples were collected from the horticulture region known as "Cinturón hortícola Platense" in Buenos Aires, Argentine, where tomato, pepper, aubergine, parsley, broccoli, cabbage, lettuce, and artichoke, among others, have been cultivated traditionally for more than 20 years with intensive use of organic and inorganic fertilizers and pesticides, including endosulfan. Five soil samples were collected randomly from a depth of up to 15 cm and preserved at 5 °C until analysis.

The soil was classified as loamy with a composition of 33% sand, 46.64% silt, and 20.36% clay; their physicochemical characteristics are shown in Table 1 (Cabrera et al. 2018). Endosulfan sulfate was detected in the soil indicating the previous presence of endosulfan (data not shown).

Isolation, purification, selection, and acclimation of strains

Strain isolation was performed using 25 g of soil and 250 mL of mineral medium (in g/L) 5.97 Na_2HPO_4, 0.01 $CaCl_2{\cdot}H_2O$, 2.27 KH_2PO_4, 0.99 $(NH4)_2SO_4$, 0.025 $FeSO_4{\cdot}7H_2O$, and 0.5 $MgSO_4{\cdot}7H_2O$ (Jimenez-Torres et al. 2016), and 22 mg/L or 2000 mg/L of commercial endosulfan (Tridane 350) was added. The culture was incubated at room temperature (24–28 °C) and 150 rpm. After 7 days, 50 mL of the cultures were mixed with 200 mL of fresh mineral medium and the corresponding amount of endosulfan to restore the concentrations. After another 7 days, an aliquot of each culture was streaked in plates with Luria–Bertani agar (LB) or potato dextrose agar (PDA) and incubated at 30 °C for 48 h. The cultivated colonies were observed under a microscope and classified according to their morphology, color, edge, and surface. Different colonies were successively cultured for strain purification under the same conditions.

Inhibition tests in the presence of endosulfan were performed on the seven pure strains obtained following a previously reported protocol (Hernández-Ramos et al. 2019). The strains that did not exhibit inhibition were selected for acclimation, identification, and degradation tests, as described in the following paragraph.

In the acclimation process, the selected strains were cultured in LB broth and 2.5 mg/L of endosulfan at 30 °C and 150 rpm for 72 h and reseeded three times using 10% (v/v) of inoculum each time. The final culture cells were centrifuged three times. The pellet was washed with sterile water each time. The final pellet was resuspended in a known volume of sterile water and then cryo-preserved at −4 °C in a 20% (v/v) glycerol solution. The purified strains are deposited and available in the internal UAM collection of microorganisms.

The inoculum for biodegradation tests was prepared in 250 mL of LB broth using 1.5 mL of the preserved strains.

DNA extraction, amplification of 16S rRNA gene, and sequence analysis

Total DNA was extracted from the pure strains following the method described by (Lawson et al. 2001). The quality of the extracts was verified on 1% (wt/vol) agarose-TBE 1X gels. The gels were stained with ethidium bromide (10 ng/mL) and documented using the Chemidoc system (Bio-Rad, Richmond, CA). DNA was quantified using a spectrophotometer (Epoch, BioTek, USA). The 16S rRNA gene sequence was amplified with pA 5-AGA GTT TGA TCC TGG CTC AG 3′ and pH 3-′AAG GAG GTG ATC CAG CCG CA 5′ primers. The PCR was performed in a Thermocycler T-Personal Combi (Biometra, Germany) using the following conditions: preheating at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 1 min; and a final extension step at 72 °C for 5 min. The amplified products were examined by electrophoresis on a 1% agarose gel, as previously described.

The amplicons were purified using the MONTAGE GENOMICS Kit, according to the manufacturer's instructions. Sequencing was performed at the "Laboratorio de Secuenciación Genómica de la Biodiversidad y de la Salud" at the Universidad Nacional Autónoma de México. The quality of the sequences was verified using the Chromas software (version 2.6.6) and then analyzed for bacterial identification in the National Center for Biotechnology Information (NCBI) database using the online BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei 1987). The bootstrap consensus tree constructed from 1000 replicates was used to represent the analyzed taxa's evolutionary history (Felsenstein 1985). Evolutionary analyses were conducted using MEGA X (Kumar et al. 2018). The analysis involved seven nucleotide sequences. The included codon positions were the 1st + 2nd + 3rd + noncoding positions. All ambiguous positions were removed for each sequence pair (pairwise deletion option). For the root identification, *Sulfolobus acidocaldarius*, an Archaea, was included as a phylogenetically distant microorganism (outgroup) as the phyla of the studied microorganisms were Proteobacteria and Firmicutes.

Degradation tests

Degradation tests were performed in triplicate for each strain in hermetic amber flasks (125 mL) using 27 mL of mineral media (previously described), 3 mL of inoculum, and 20 mg/L of endosulfan (Sigma-Aldrich, isomers α and β, ratio 7:3, 99.9% purity). They were incubated at 150 rpm and 30 °C for approximately 800 h. The flasks were provided with a precision sampling valve (Mininert®) that allowed periodical headspace sampling. The final content of each flask was analyzed for residual endosulfan, metabolites, and biomass, as described in the next section.

Analytical methods

Carbon dioxide quantification

Headspace gas samples (100 µL) were analyzed in a gas chromatograph (550 Gow-Mac series, USA) with a thermal conductivity detector (GC-TCD) using a CTR1 column (Alltech) and helium as a carrier gas, as described previously (Hernández-Ramos et al. 2019).

Biomass quantification

After the test period, the complete content of each flask was membrane-filtered (0.45 µm) to recover the final biomass. Solvent washing was performed for endosulfan recovery. The membranes were dried at 50 °C until a constant weight was achieved. The mass was quantified by the difference between the wet and dry weights. The recovered liquid phase was analyzed for residual endosulfan and metabolites as follows.

Liquid–liquid extraction

The liquid phase recovered after filtration was subjected to a liquid–liquid extraction procedure (US EPA 1996; 3510C). Briefly, 33 mL of dichloromethane was added, followed by 10 min of magnetic agitation and phase decantation. The final volume of the organic phase, collected after repeating this procedure three times, was concentrated. The solvent was changed to hexane by rotoevaporation (Hernández-Ramos et al. 2019).

Quantification of residual endosulfan

The concentrated extracts were analyzed by US EPA Method 8270D using gas chromatography with a mass spectrum detector (Agilent 6890 N, MSD 5975B, USA) with a 5MS SGE capillary column. The initial and final oven temperatures were 90 °C and 250 °C; the temperature was increased at a rate of 5 °C min^{-1}, and helium was used as the carrier gas. The detector and injector temperatures were 250 °C and 220 °C, respectively. For metabolite analysis, a scan method in the range of 50–450 z/m at 70 eV was performed, while the identification was performed using the NIST05 Mass Spectral Library.

Data analysis

The CO_2 experimental data were fitted by the Gompertz model using OriginPro 8 software to obtain kinetic parameters, such as the maximum production rate (Vmax) and production rate (k), which are related to the degradation of endosulfan. One-way analysis of variance (ANOVA) with a 95% confidence level and post hoc (Tukey) tests were performed to establish the differences between tests and controls and between strains in the IBM SPSS 22 software.

Results and discussion

Isolation and identification of strains

Seven strains were isolated on media containing endosulfan. The inhibition tests indicated that one gram-positive strain exhibited moderate growth inhibition in the presence of endosulfan. Therefore, no further experiments were performed using this strain. Three strains were isolated from media containing 2000 mg endosulfan per L of culture (B1, B2, and C2 in Table 2), and three from the culture with endosulfan concentration 22 mg/L (A1, A2, and C1 in Table 2). There are few reports where endosulfan concentrations of 500, 1000, and 2100 mg/L were used for isolation of fungal and bacterial strains or even in degradation tests (Bhalerao and Puranik 2007; Kumar and Philip 2006; Silambarasan and Abraham 2014). The identification of the six isolated strains, using 16S rRNA gene sequencing, is presented in Table 2.

Degradation of endosulfan isomers and specific rates

The α-endosulfan consumption was 76% for *B. subtilis* and above 95% for the other strains (initial concentration 14 m/L). On the other hand, the β isomer (initial concentration 6 mg/L) was approximately 95% for *P. putida*, *E. cloacae*, and *A. spanius*, and 86% for *B. subtilis*, *P. simplex*, and *B. pseudomycoides*. These results are consistent with those reported in the literature, where the β isomer has been reported as being more toxic due to the specificity of the enzymes required for its degradation (Kwon et al. 2002).

The specific degradation rates (i.e., the mass of the isomer eliminated per volume of culture per biomass) for

Table 2 Identification of bacterial isolates from soil using 16S rRNA gene sequencing

Isolate label	Organism	Identity (%)	Sequence length (bp)	Accession number
A1	*Pseudomonas putida*	99.00	1378	MZ147607
A2	*Enterobacter cloacae*	98.00	1483	MZ147605
B1	*Achromobacter spanius*	98.00	1451	MZ147606
B2	*Bacillus subtilis*	98.00	1449	MZ147602
C1	*Peribacillus simplex*	99.65	1419	MZ147604
C2	*Bacillus pseudomycoides*	99.70	1437	MZ147603

α-and β-endosulfan are presented in Fig. 1a, b, respectively. The biomass of the tests was corrected using the biomass quantified from the endogenous controls of each strain. The specific degradation rate for both isomers by *P. putida* was significantly ($p > 0.05$) higher, four times higher than those of *E. cloacae* and *B. subtilis*, three times higher than that of *P. simplex*, and two times higher than those of *A. spanius* and *B. pseudomycoides.*

Global degradation

The volumetric rates (mg/L per day) of endosulfan degradation and the degradation of its isomers are presented in Table 3. The highest volumetric degradation rate was observed for *E. cloacae*, which was approximately twice those of *P. putida* and *B. pseudomycoides*. However, no significant differences were observed for *E. cloacae*, *A. spanius*, *P. simplex*, and *B. subtilis*. The rate of total endosulfan obtained for *P. putida* was four times lower than that reported by (Sunitha et al. 2012). The total endosulfan rate for *B. subtilis* observed in this study was similar to the values of 6.72 mg/L per day and 2.5 times lower than those previously reported (Ishag et al. 2017; Kumar et al. 2014). On the other hand, the *E. cloacae* values for both isomers were one order of magnitude higher than those reported by (Jimenez-Torres et al. 2016).

As previously mentioned, endosulfan degradation *by A. spanius*, *P. simplex*, and *B. pseudomycoides* has not been previously reported. However, the observed rates are comparable to those reported for *P. aeruginosa*, *B. megaterium*, and *Klebsiella pneumoniae* (Kumar 2011; Kwon et al. 2002; Ozdal et al. 2016; Seralathan et al. 2014).

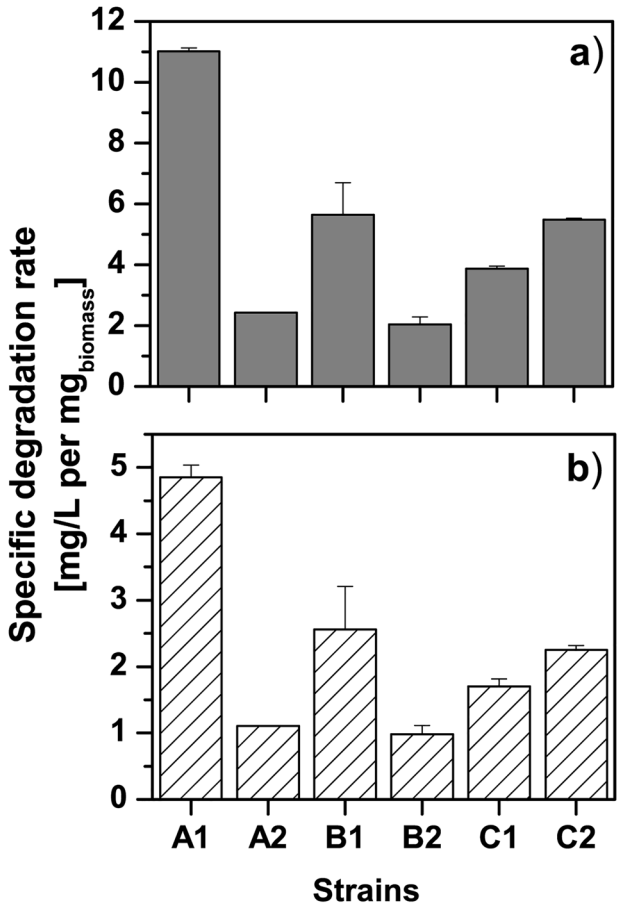

Fig. 1 Specific degradation rate for **a** α—endosulfan and **b** β—endosulfan for the isolated strains. A1 *Pseudomonas putida*, A2 *Enterobacter cloacae*, B1 *Achromobacter spanius*, B2 *Bacillus subtilis*, C1 *Peribacillus simplex*, and C2 *Bacillus pseudomycoides*. Bars represent the standard deviation of $n = 6$

CO_2 production as an indirect measure of microbial activity

The microbial activities related to CO_2 production for each strain are shown in Fig. 2a–f. Except for *B. pseudomycoides*, in all cases, the CO_2 produced was significantly higher ($P = 0.05$) in the tests than in the endogenous controls, indicating the utilization of endosulfan isomers as the sole carbon source and its mineralization. The CO_2 production by *B. pseudomycoides* indicated that the microorganism utilized endosulfan for growth or metabolite production rather than CO_2 production, which was consistent with its highest biomass production among all the studied strains.

The rate values (k) obtained by fitting the CO_2 production by the Gompertz model for *E. cloacae*, *A. spanius*, and *B. subtilis* were approximately 0.012 h^{-1}. While, the rates for *P. putida*, *P. simplex*, and *B. pseudomycoides* were approximately half, suggesting that the first mineralize the endosulfan twice as fast despite the specific rates of *P. putida* being the highest. Furthermore, the values of V_{max} (~0.1 mg/h) obtained for *A. spanius* and *B. subtilis* were significantly higher ($P = 0.05$) than those obtained for the other strains (Table 4).

Table 3 Volumetric degradation rate of total endosulfan and its α- and β-isomers

Strain	Degradation rate (mg/L per day)		
	Total	α-Isomer	β-Isomer
Pseudomonas putida	2.80 ± 0.06	1.99 ± 0.04	1.06 ± 0.15
Enterobacter cloacae	5.58 ± 0.08	4.61 ± 0.04	1.97 ± 0.03
Achromobacter spanius	5.43 ± 0.21	3.80 ± 0.16	1.62 ± 0.05
Bacillus subtilis	4.79 ± 0.88	3.99 ± 0.36	1.55 ± 0.36
Peribacillus simplex	5.19 ± 0.34	2.07 ± 0.08	0.81 ± 0.11
Bacillus pseudomycoides	2.66 ± 0.11	1.99 ± 0.04	0.68 ± 0.07

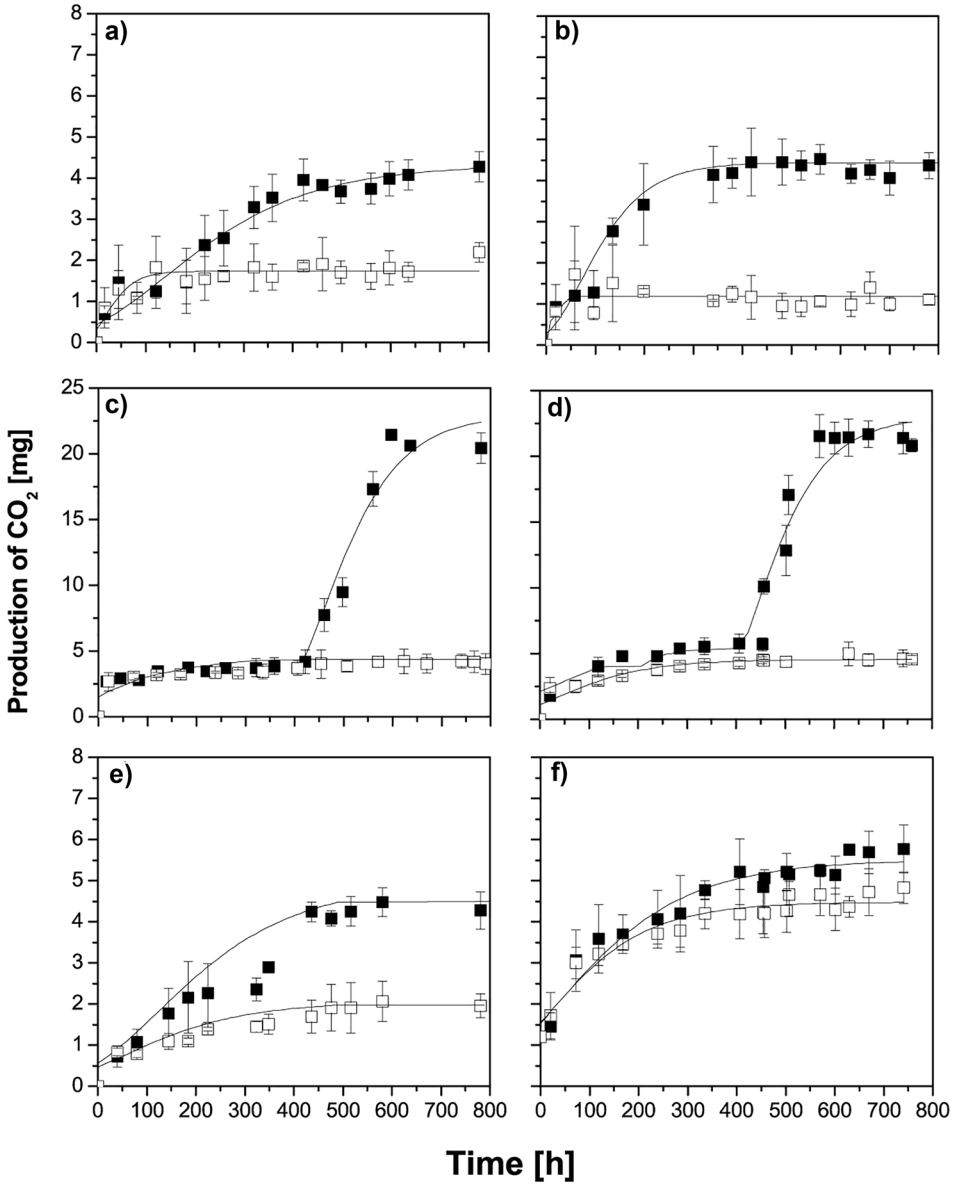

Fig. 2 CO_2 production for **a** *Pseudomonas putida*, **b** *Enterobacter cloacae*, **c** *Achromobacter spanius*, **d** *Bacillus subtilis*, **e** *Peribacillus simplex*, and **f** *Bacillus pseudomycoides*. Black square indicates the addition of endosulfan, white square endogenous control (without endosulfan), and (—) the Gompertz model fit. Bars represent the standard deviation of $n = 6$

Evolutionary relationships of taxa

The evolutionary taxa relationship analysis had a total of 1557 positions in the final dataset. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Fig. 3). These data indicate that at least 63% of the replications for *B. subtilis* and *B. pseudomycoides* belonged to the same common ancestor and a common ancestor of *B. pseudomycoides*, *B. subtilis*, and *P. simplex* existed in 100% of the replications. The branches corresponding to the partitions, reproduced in less than 50% of the bootstrap replicas, collapsed (*A. spanius* and *E. cloacae*). As expected, the *Bacillus* spp. genus strains were more closely related. The *A. spanius* strain was not evolutionarily related to any of the other strains.

Finally, the evolutionary distances of the strains did not indicate any relationship with the parameters studied

Table 4 Kinetics parameters for CO_2 obtained by Gompertz fitting

Organism	k (h^{-1})	V_{max} (mg/h)
Pseudomonas putida	0.0059 ± 0.0009	0.0095 ± 0.0002
Enterobacter cloacae	0.0138 ± 0.0012	0.0225 ± 0.0002
Achromobacter spanius	0.0116 ± 0.0028	0.0978 ± 0.0003
Bacillus subtilis	0.0127 ± 0.0033	0.1077 ± 0.0053
Peribacillus simplex	0.0063 ± 0.0016	0.0115 ± 0.0008
Bacillus pseudomycoides	0.0067 ± 0.0011	0.0135 ± 0.0017

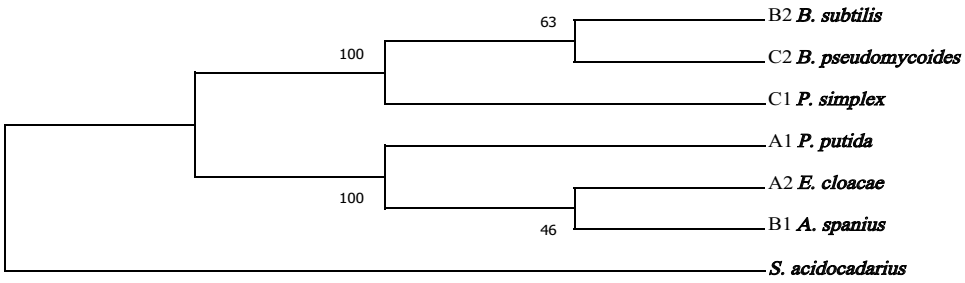

Fig. 3 Identification of strains isolated from soil. Neighbor-joining dendrogram constructed by analysis and comparison of partial sequences of 16S rRNA gene from soil strains. *Sulfolobus acidocaldarius*: outgroup microorganism. Bootstrap = 1000

(volumetric or specific rates and production of CO_2). For *E. cloacae* and *A. spanius*, the bootstrap percentages lower than 50% had the highest endosulfan degradation rates. However, due to their lower replication percentage, the relationship between their evolutionary line and degrading capabilities could not be established. Therefore, the ability to degrade endosulfan is related to processes of the envelope of the organisms through natural selection, which allows them to adapt to the conditions imposed by the environment, including the presence of pollutants; in this case, endosulfan.

Metabolite identification

The metabolites identified for each strain are presented in Table 5. Although endosulfan sulfate is one of the most commonly reported metabolites, it was not found in any of the tested strains (Kataoka and Takagi 2013; Sutherland et al. 2004; Thangadurai and Suresh 2014). For *E. cloacae*, the identified compounds (not shown) in the final samples were also present in the abiotic control samples. Therefore, they were not related to biological degradation. On the other hand, endosulfan diol (retention time (RT) = 20.124) and endosulfan ether (RT = 20.143 min) were detected in the *P. putida* and *B. pseudomycoides* degradation tests, indicating a hydrolytic pathway consistent with previous reports of pure and mixed *P. putida* cultures (Sunitha et al. 2012).

Endosulfan lactone (RT = 20.474 min) was detected in the final *B. subtilis* samples of the degradation tests, in agreement with previous studies (Kumar et al. 2014). However, this metabolite was also found in *P. simplex* and *B. pseudomycoides* strains that have not been reported for endosulfan degradation (Ahmad 2020; Ishag et al. 2017). This compound is produced from endosulfan hydroxyether by endosulfan hydroxyether dehydrogenase, suggesting degradation by the hydrolytic pathway (Lee et al. 2003).

Table 5 Identified metabolites on final samples of the degradation experiments

RT (min)	Compound	Formula	#CAS	Quality (%)
Pseudomonas putida				
20.124	Bicyclo (2.2.1) hept-5-ene-2,3-dimethanol, 1,4,5,6,7,7-hexachloro-(endosulfan diol)	$C_9H_8Cl_6O_2$	002,157–19-9	90
20.143	4,7-methanoisobenzofuran, 4,5,6,7,8,8-hexachloro-1,3,3a,4,7,7a-hexahydro-(endosulfan ether)	$C_9H_6Cl_6O$	003,369–52-6	84
Achromobacter spanius				
22.210	1,2-benzenedicarbocylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	017,851–53-5	93
Bacillus subtilis				
22.210	1,2-benzenedicarbocylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	017,851–53-5	93
25.474	4,7-methanoisobenzofuran-1[3H]-one, 4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-(endosulfan lactone)	$C_9H_4Cl_6O_2$	003,868–61-9	90
Peribacillus simplex				
25.458	4,7-methanoisobenzofuran-1[3H]-one, 4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-(endosulfan lactone)	$C_9H_4Cl_6O_2$	003,868–61-9	86
Bacillus pseudomycoides				
20.124	Bicyclo (2.2.1) hept-5-ene-2,3-dimethanol, 1,4,5,6,7,7-hexachloro-(endosulfan diol)	$C_9H_8Cl_6O_2$	002,157–19-9	89
20.143	4,7-methanoisobenzofuran, 4,5,6,7,8,8-hexachloro-1,3,3a,4,7,7a-hexahydro-(endosulfan ether)	$C_9H_6Cl_6O$	003,369–52-6	92
25.458	4,7-methanoisobenzofuran-1[3H]-one, 4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-(endosulfan lactone)	$C_9H_4Cl_6O_2$	003,868–61-9	90

RT residence time

Conclusions

Five of the six isolated strains showed the capacity to degrade both isomers of endosulfan and mineralize them (i.e., conversion to CO_2). Furthermore, *A. spanius*, *P. simplex*, and *B. pseudomycoides* have not been previously reported for endosulfan degradation. However, *B. pseudomycoides* utilizes endosulfan for growth or metabolite production rather than CO_2 production. The degradation rates observed for the α isomer were higher than those for the β isomer, concurring with its higher toxicity. The presence of endosulfan lactone indicated that the *Bacillus* strains (*B. subtilis*, *P. simplex*, and *B. pseudomycoides*), as well as *A. spanius* and *P. putida*, degraded endosulfan by the hydrolytic pathway.

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Author contribution A. Casanova carried out the research, analyzed the data, prepared the visuals, contributed to the first draft, and read and approved the final manuscript. S. Cabrera carried out the sampling and characterization of the soil, isolation and purification of the strains, preliminary degradation tests, and read and approved the final manuscript. G. Díaz-Ruiz carried out the identification of strains, contributed to the first draft, and read and approved the final manuscript. S. Hernández supervised the experimentation, analyzed the data, contributed to the final draft, and read and approved the final manuscript. C. Wacher analyzed the data and read and approved the final manuscript. M. Zubillaga analyzed the data and read and approved the final manuscript. I. Ortíz performed the conceptualization, supervised the research, analyzed the data, and wrote and approved the final draft.

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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