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REVIEW ARTICLE

Streptomyces sp. is a powerful biotechnological tool for the biodegradation of HCH isomers: biochemical and molecular basis

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ABSTRACT

Actinobacteria are well-known degraders of toxic materials that have the ability to tolerate and remove organochloride pesticides; thus, they are used for bioremediation. The biodegradation of organochlorines by actinobacteria has been demonstrated in pure and mixed cultures with the concomitant production of metabolic intermediates including γ-pentachlorocyclohexene (γ-PCCH); 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN); 1,2-dichlorobenzene (1,2-DCB), 1,3-dichlorobenzene (1,3-DCB), or 1,4-dichlorobenzene (1,4-DCB); 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,4-trichlorobenzene (1,2,4-TCB), or 1,3,5-trichlorobenzene (1,3,5-TCB); 1,3-DCB; and 1,2-DCB. Chromatography coupled to mass spectrometric detection, especially GC–MS, is typically used to determine HCH-isomer metabolites. The important enzymes involved in HCH isomer degradation metabolic pathways include hexachlorocyclohexane dehydrochlorinase (LinA), haloalkane dehalogenase (LinB), and alcohol dehydrogenase (LinC). The metabolic versatility of these enzymes is known. Advances have been made in the identification of actinobacterial haloalkane dehydrogenase, which is encoded by *linB*. This knowledge will permit future improvements in biodegradation processes using Actinobacteria. The enzymatic and genetic characterizations of the molecular mechanisms involved in these processes have not been fully elucidated, necessitating further studies. New advances in this area suggest promising results. The scope of this paper encompasses the following: (i) the aerobic degradation pathways of hexachlorocyclohexane (HCH) isomers; (ii) the important genes and enzymes involved in the metabolic pathways of HCH isomer degradation; and (iii) the identification and quantification of intermediate metabolites through gas chromatography coupled to mass spectrometry (GC–MS).

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Introduction

During the past few centuries, specific organochlorine pesticides (OPs) were widely have been used for agricultural pest control as well as for medical purposes. However, the use of these compounds is now prohibited due to their toxicity, environmental persistence, and bioaccumulation in the food chain, as reported by Barber et al. [1] and Bempah and Donkor [2]. Aerial dissemination is another concern as it increases the potential human risks associated with OPs, according to Jia et al. [3]. Thus, in 2009, OPs were added to the UNEP Stockholm Convention list [4] as persistent organic pollutants [5]. Within this group, γ-hexachlorocyclohexane (γ-HCH; also called lindane) is a highly halogenated organic insecticide that has been used worldwide. For many decades due to its low cost and high effectiveness, lindane has been used for crop protection as well as the prevention of vector-borne diseases. However, several years after its initial use, studies determined the negative impact of lindane on the environment and humans. Hermanowicz et al. [6] reported that workers directly exposed to lindane for 12–30 years had a higher presence of infectious diseases, particularly upper respiratory tract infections such as tonsillitis, bronchitis, or pharyngitis, compared to a control population.

Based on toxicological studies, HCH isomers were determined to cause damage to the central nervous system, liver, kidneys, and reproductive system [7–12]. Moreover, the HCH synthesis mixture contains eight possible stereoisomers of which four (α-, β-, γ-, and δ-HCH) predominate in the technical product; γ-HCH is the most well-known and effective insecticidal component of HCH, although it only represents 9–18% of the total synthesis product. These isomers are highly hydrophobic, persistent, and widespread in the environment.

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They accumulate in the food chain [13] due to their lipophilic properties, which leads to toxicity [14], as determined by Liu et al. [15]. Additionally, Manickam et al. [13] and Quintero et al. [16] detected the presence of HCH isomers in the environment that are transported by the atmosphere, thereby producing greater pollution.

Preliminary reports on the aerobic degradation of γ-HCH have included studies in Gram-negative bacteria such as *Sphingomonas* [17] and in the white-rot fungi *Trametes hirsutus*, *Phanerochaete chrysosporium*, *Cyathus bulleri*, and *Phanerochaete sordida* [18,19]. There is little information available regarding the OP biotransformation abilities of Gram-positive microorganisms, particularly those of Actinobacterial species, which are the main group of bacteria present in soils and sediments [20,21]. However, some studies demonstrated that these interesting microorganisms have a great capacity to degrade HCHs, as reported by Alvarez et al. [22].

Studies carried out by Manna et al. [12] reported that the enzymes responsible for the first step in HCH degradation were dehalogenases from the Lin family of enzymes, which have been shown to metabolize HCHs to less toxic compounds via two distinctive mechanisms. LinA dehydrochlorinates HCH substrates via an elimination mechanism, whereas LinB catalyzes hydrolytic dehalogenation via the S_N2 pathway.

In this review, we summarize current knowledge related to possible routes of HCH isomer degradation and the metabolic intermediates produced by *Streptomyces*. Additionally, future perspectives for genetic improvements that have the potential to make these microorganisms more efficient and reliable strains for decontamination processes are considered.

HCH-degrading Actinobacteria

In general, environmental pollution can be remediated with microorganisms. Both bacteria and fungi have coexisted for millions of years with a great variety of pollutants, and they continue to adapt their genetic machinery to degrade organic and inorganic compounds, thereby producing different intermediates and perpetuating themselves over time [23]. For example, in the case of heavy metals, the main mechanisms of microbial remediation include active transport mediated by efflux pumps, intra- and extracellular sequestration, enzymatic transformation into other chemical species through redox reactions, methylation or alkylation/dealkylation, and/or reduction [24,25]. As noted above, Actinobacteria can biodegrade several compounds, including pesticides and heavy metals. However, as this is not the main objective of the present review, more information regarding the biodegradation of heavy metals and other pesticides is available in an excellent review by Alvarez et al. [26].

Different groups of microorganisms have been found to be able to degrade pesticides with different chemical structures including organochlorides, *s*-triazines, triazinones, carbamates, organophosphates, organophosphonates, acetanilides, and sulfonylureas. However, a limited number of these xenobiotics can be mineralized to carbon dioxide and water using these microorganisms. Among the organic pollutants, studies have indicated that halogenated compounds (aliphatic or aromatic), specifically organochlorines, such as lindane (γ-HCH), or hexachlorocyclohexane isomers (α-, β-HCH), such as metoxichlor and chlordane [27,28], can be fully degraded. In this context, Actinobacteria are an interesting group of microorganisms that are important members of microbial communities in soils. These microorganisms have great metabolic diversity and specific growth characteristics; therefore, they are well suited to biodegrade environmental pollutants [29]. Given their capacity to produce extracellular enzymes applicable for the degradation of a wide range of chemical compounds, as shown by Polti et al. [29], Actinobacteria have been well characterized. Moreover, their filamentous growth favors soil colonization [30]. In this context, Actinobacteria have been evaluated in mixed cultures with *Pseudomonas* for the degradation of polycyclic aromatic hydrocarbons [31]. The mixed cultures were reported to be able to extensively degrade naphthalene, phenanthrene, and pyrene. Moreover, Briceño et al. [32] demonstrated that *Streptomyces* sp. strains AC7 and AC5 were capable of degrading approximately 90% of chlorpyrifos at 25 mg kg^{-1}, and their main metabolite was 3,5,6-trichloro-2-pyrinidol (TCP). The biodegradation of polychlorinated-biphenyl by Actinobacteria has also been reported. Papale et al. [33] reported that Actinobacteria from the genera *Salinibacterium*, *Microbacterium*, and *Nacardioides* isolated from water and sediments in high Arctic Norway removed more than 90% of Aroclor 1242 and PCB congeners. Specifically, HCH isomer degradation by Actinobacteria has also been reported. De Paolis et al. [34] demonstrated that *A. giacomelloi* is the most effective species, after 72 h incubation it degraded 88% of α-HCH, 60% of β-HCH, and 56% of γ-HCH, more than *A. fluorescens* (Table 1). Manickam et al. [13] reported other microorganisms within the Actinobacteria with degradation capacity, including the *Microbacterium* sp. ITRC1 strain, which utilizes HCH (α, β, γ, and δ) isomers as its sole carbon and energy source under aerobic conditions in liquid media. In other studies, Sineli et al. [41] showed that the γ-, α-, and β-HCH isomers were

Table 1. Main genera of HCH isomers-degrading Actinobacteria.

Studied microorganism	γ-HCH	α-HCH	β-HCH	Reference
Arthrobacter fluorescens	+ Pentachlorocyclohexenes, tetrachlorocyclohexenes	+ Pentachlorocyclohexenes, tetrachlorocyclohexenes	+ Pentachlorocyclohexenes, tetrachlorocyclohexenes	[34]
Arthrobacter giacomelloi	+ Pentachlorocyclohexenes, tetrachlorocyclohexenes	+ Not reported	+ Not reported	[34]
Arthrobacter sp.	+ Not reported	+ Not reported	+ Not reported	[35]
Rhodococcus erythropolis S-7	+	+	+	[36]
Rhodococcus sp. m15-3	Not reported	Not reported	Not reported	
Streptomyces sp. M7	+ γ-PCCH 1,4-TCDN	+ 1,2-Dichlorobenzene 1,3 or 1,4-Dichlorobenzene Trichlorobenzene isomers Pentachlorocyclohexene	+ Dichlorobenzene isomers Tetrachlorocyclohexene Dichlorobenzene isomers	[27,37]
Microbacterium sp. ITRC1	+ γ-Pentachlorocyclohexene, 2,5-dichlorophenol (2,5-DCP)	+ Not reported	+ Not reported	[38]
Mycobacterium tuberculosis	+ Not reported	+ Not reported	+ Not reported	[39]
Achrobacter citreus B1-100	γ-1,3,4,5,6-Pentachlorocyclohexene, tetrachlorocyclohexene, trichlorocyclohexa-diene, 2-chlorophenol, phenol, and catechol	+ Not reported	+ Not reported	[40]

(+) Means degradability of HCH isomers.

effectively metabolized by *Streptomyces* sp. M7, with 95, 80, and 78% degradation, respectively, after 7 d of incubation individually as well as in combination. These results demonstrate the high metabolic capacity of *Streptomyces* sp. M7. Additionally, the simultaneous biodegradation of Cr(IV) and lindane has been reported. Polti et al. [29] reported that *Streptomyces* spp. A5, A11, M7 and MC1 and *Amycolatopsis tucumanensis* DSM 45259 were capable of removing both compounds in sterile and non-sterile soils. In the sterile soil microcosms, the consortium was able to grow similarly in the different textured soils (clay silty loam, sandy, and loam), both contaminated or uncontaminated with the OP mixture. The *Streptomyces* consortium was able to remove all of the OPs in the sterile soil microcosms (removal order: clay silty loam > loam > sandy). In the non-sterile clay silty loam soil (CSLS) microcosms, higher rates were observed for lindane (11%). In the CSLS slurries, the consortium exhibited similar growth levels in the presence or absence of the OPs [42].

Metabolism of HCH in Actinobacteria

Microbial degradation of HCH isomers involves the removal of chlorine atoms from these molecules through the actions of dehalogenase enzymes. Ravel et al. [43] indicated that these enzymes play a central role in the biodegradation process of this compound. There are several reports on dehalogenase activities in different species of Gram-positive and Gram-negative microorganisms, such as *Pseudomonas* sp., *Xanthobacter* sp. and *Moraxella* sp. [18], *Mycobacterium tuberculosis* [39], *Bacillus* and *Micrococcus* [44], *Rhodococcus* sp. [45], *Microbacterium* sp. ITRC1 [16], *Streptomyces* sp. M7 [27,28], *Microbacterium lindanitolerans* sp. nov. [46], and *Corynebacterium* sp. [47]. In *Corynebacterium*, Yokota et al. [47] determined that the specific substrate of these dehalogenase enzymes was 1-chlorobutane, though it is important to highlight that these enzymes are able to act on a wide range of halogenated aliphatic compounds. Therefore, Nagata et al. [17] demonstrated the importance of identifying the metabolite intermediates that result from organochlorine compound degradation and to compare the GC–MS spectra obtained with electron-impact fragmentation spectra previously reported to infer the degradation ability of OPs by these microorganisms.

Several lindane-degrading microorganisms and their metabolic intermediates have been described by Camacho-Pérez et al. [48]. Nagata et al. [17] pioneered the first studies of the HCH degradation pathway in Gram-negative microorganisms, and γ-PCCH (γ-pentachlorocyclohexene) was the first reported compound in the lindane degradation pathway of *Sphingobium japonicum* UT26. This intermediate is one of the metabolites most commonly found in the aerobic degradation pathway of HCH, in addition to 2,5-DCHQ (2,5-dichlorohydroquinone), CHQ (chlorohydroquinone), chlorophenol, and phenol [48]. Geueke et al. [49] detected the presence of γ-PCCH and 1,2,4-TCB during the aerobic

degradation of different HCH isomers by *Sphingobium* strains isolated from contaminated sites. Other authors also reported 1,4-DCB (1,4-dichlorobenzene) or 1,2-DCB (1,2-dichlorobenzene) as products of lindane degradation [48]. Manickam et al. [13] reported that *Microbacterium* sp. ITRC1 strain utilizes HCH (α, β, γ, and δ) isomers as its sole carbon and energy source under aerobic conditions in liquid medium during γ-HCH degradation, and the product formed was identified as 2,5-dichlorophenol (2,5-DCP). This knowledge of the degradation route has led to its consideration as a model for the study of the degradation pathway in Gram-positive bacteria. Another unusual feature of the *lin* system (constituted by the *lin* genes) is that it has not been found predominantly in a single genus but is distributed across several genera. Horizontal transfer of the *lin* genes among HCH degraders has been reported and is mainly associated with plasmids and IS6100 elements. In addition, Actinobacteria are known for their metabolic versatility and have received substantial interest globally for several biotechnological applications [50].

Therefore, many Actinobacteria are well-known degraders of toxic materials that are used for bioremediation, and *Streptomyces* is the most abundant genus within this group. *Streptomyces* sp. M7, an Actinobacteria with the ability to tolerate and remove organochloride pesticides [51–53], was able to degrade lindane when it was present in the culture medium as the sole carbon source. Cuozzo et al. [27] determined the metabolic intermediates produced by *Streptomyces* sp. M7, including: γ-PCCH and 1,4-TCDN (1,3,4,6-tetrachloro-1,4-cyclohexadiene). The metabolite γ-PCCH has also been detected during HCH degradation by *Sphingomonas* sp. [13] and other microorganisms [54], and dehalogenase enzymes were found to participate in the degradation pathway. These intermediary metabolites are products of the γ-HCH catabolism pathway and are obtained by the action of dechlorinase enzymes. The relative abundances of γ-PCCH and 1,4-TCDN were increased 1.5-fold at 96 h compared with 48 h of growth. Highlighting the importance of not only detecting metabolic intermediaries but also investigating the toxic effects of these metabolites that are produced during the remediation process is necessary. For instance, the intermediate 1,2-DCB is known to cause acute and chronic toxicity in fish, amphibians, bacteria, algae, and aquatic invertebrates, even though its half-life is approximately 3 weeks, which is much shorter than its parental compound lindane ($t_{1/2} = 2.6$ years) [55,56]. However, to corroborate the toxicity level of the products obtained from the biodegradation of this toxic pesticide by *Streptomyces* sp. M7, several toxicity studies have been carried out, including phytotoxicity tests. For example, Calvelo et al. [57] used *Lactuca sativa* for this type of test because numerous physiological processes are known to occur during its first few days of seedling development. In this context, the presence of a toxic compound may disturb the survival and normal development of a plant, indicating that it is a time of great sensitivity to adverse external factors. Therefore, the evaluation of radicle and hypocotyl development is a representative indicator of the ability of the plant to establish and develop [58]. Similarly, Saez et al. [59] demonstrated that systems contaminated with lindane that were bioremediated by *Streptomyces* sp. M7 were less toxic than systems not remediated by this microorganism via phytotoxicity tests. The results confirmed that metabolites more toxic than lindane were not released into the medium during the bioremediation process (Figure 1).

Conversely, the identification of metabolites during the degradation of other HCH isomers was determined by Sineli et al. [37,41], who studied the metabolism of the α-HCH isomer by *Streptomyces* sp. M7. Three peaks were detected in the GC–MS chromatogram, the first of which was identified as 1,2-DCB, an intermediate was also detected by Quintero et al. [9] in a study of the anaerobic degradation of HCH isomers in liquid and soil slurry systems. The second peak was either 1,3-dichlorobenzene (1,3-DCB) or 1,4-dichlorobenzene. The third peak was 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,4-trichlorobenzene (1,2,4-TCB), or 1,3,5-trichlorobenzene (1,3,5-TCB), which was also detected by Manickam et al. [13] and Geueke et al. [49] in studies with *Sphingobium*. The other intermediate products found in cell-free extracts of *Streptomyces* sp. M7 were PCCH and dichlorobenzene isomers from α-HCH degradation and tetrachlorocyclohexene (TCCH) and dichlorobenzene from β-HCH degradation. The PCCH and TCCH metabolites were detected from α- and β-HCH degradation by *Arthrobacter fluorescens* and *Arthrobacter giacomelloi*, respectively [41], and they were also found to be metabolic intermediates of the degradation pathways of *Sphingobium japonicum* UT26 [17]. Moreover, other intermediate products identified from the degradation of α-HCH by *Streptomyces* sp. M7 included PCCH and dichlorobenzene isomers (1,3-DCB and 1,2-DCB), which are produced by action of dehydrochlorinase, according to the anaerobic mechanism proposed by Quintero et al. [9]. All of the intermediates produced by *Streptomyces* sp. M7, which are produced through the lower pathway of the lindane degradation process described by Nagata et al. [17], have lower toxicity or no toxicity compared to the original substrate, as phenolic compounds were not detected in any studies after 7 d of incubation (Table 1).

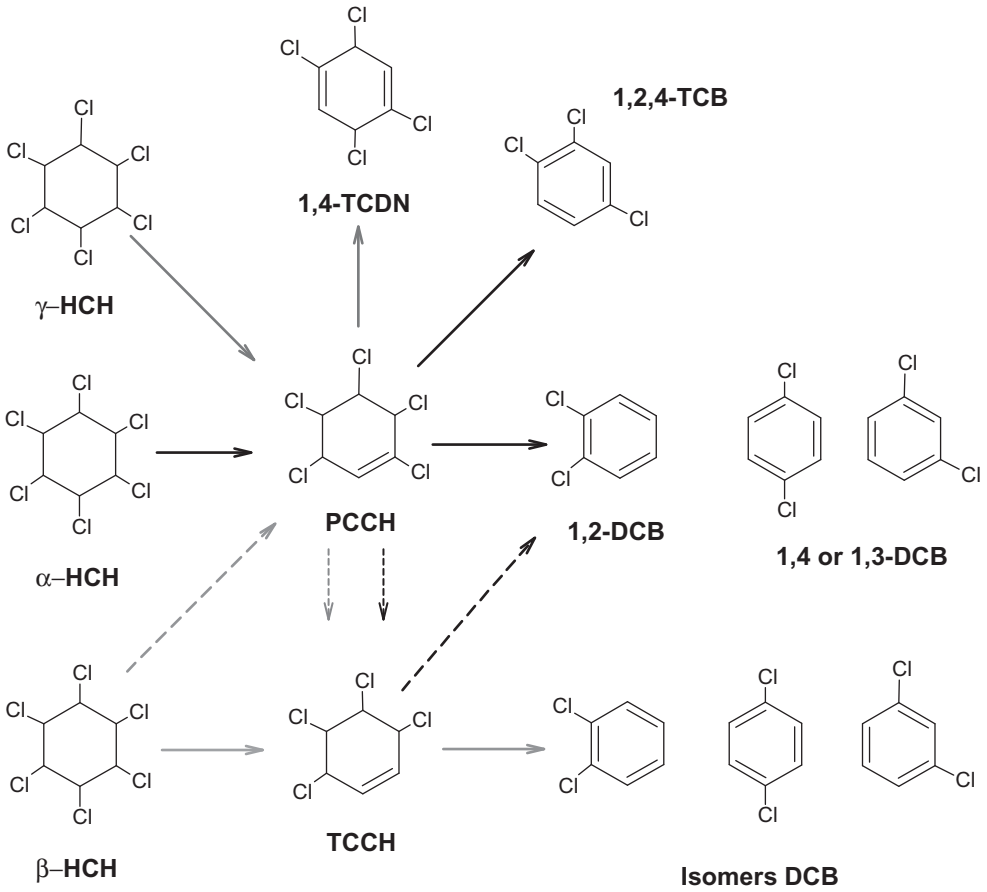

Figure 1. Possible partial degradation pathways of α-HCH (black lines), β-HCH (gray lines) and γ-HCH (dark gray lines) by *Streptomyces* sp. M7, according to the identification of metabolic intermediates by GC/MS [37,27]. Dashed lines refer to steps not established.

The β-HCH isomer is known to be the most recalcitrant of all the HCH isomers. However, Sineli et al. [37] identified intermediates of its metabolism in culture supernatants of *Streptomyces* sp. M7 grown in the presence of the β-HCH isomer as a carbon source. Dichlorobenzene isomers were detected via GC–MS. *Streptomyces* sp. M7 showed the same metabolite profile as *Sphingobium japonicum* UT26 in its degradation of β-HCH, producing PCCH and TCB according to the mechanism proposed by Pearce et al. [60]. The degradation process of β-HCH isomers by *Streptomyces* sp. M7 follows the low degradation pathway proposed by Nagata et al. [17]. Sineli et al. [37] did not detect phenolic compounds in any of the conditions studied, indicating that the degradation process continues, and the benzene-ring containing compounds are not the final degradation products (Table 1). Studies by Manickam et al. [13] also demonstrated that *Microbacterium* sp. ITRC1 strain can grow on a broad spectrum of chlorinated compounds. In these studies, they did not observe any growth on 2,5-DCP. It is possible that this strain has a degradation pathway similar to that of *Sphingobium japonicum* UT26 in which 2,5-DCP is a dead-end product [61].

Important enzymes involved in the metabolic pathways of HCH degradation

Dehalogenases catalyze the cleavage of the carbon–halogen bond in organohalogen compounds. Recently, they have attracted a great deal of attention due to their potential applications in the chemical industry and in bioremediation. Kurihara and Esaki [62] described the occurrence, reaction mechanisms, and applications of bacterial hydrolytic dehalogenases and related enzymes, particularly L-2-haloacid dehalogenase, DL-2-haloacid dehalogenase, fluoroacetate dehalogenase, and 2-haloacrylate reductase. L-2-Haloacid dehalogenase is a representative enzyme of the haloacid dehalogenase (HAD) superfamily, which includes the P-type ATPases and other hydrolases.

Dehalogenases enzymes are key in the degradation of various halogenated pesticides through their cleavage of the carbon chlorine stable bond. Furthermore, as HCH isomers have six chlorine atoms per molecule, dechlorination is a significant step in their degradation process [34,41]. Similarly, in *Sphingobium japonicum* UT26, three different dehalogenases enzymes, LinA, LinB, and LinC, are involved in the degradation of γ-HCH, as established by Nagata et al. [17] and Cuozzo et al. [27].

We next discuss special types of dehalogenases involved in aerobic degradation pathways of chlorinated organic compounds that are relevant to bioaugmentation with specialized bacteria such as Actinobacteria.

Hexachlorocyclohexane dehydrochlorinase (LinA)

LinA belongs to a class of enzymes known as the dehydrohalogenases. It readily degrades α-HCH, γ-HCH, and δ-HCH isomers and has been shown to have very low but detectable activity towards β-HCH. Dehydrochlorinases are able to eliminate HCl from a substrate molecule, leading to the formation of a double bond.

The work of Manickam et al. [38] established that LinA catalyzes two steps of dehydrochlorination from γ-HCH to 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) via γ-PCCH. 1,4-TCDN is proposed to convert 1,2,4-TCB non-enzymatically because 1,4-TCDN has an unstable diene-type structure, and 1,2,4-TCB has a stable aromatic ring.

The overall three-dimensional structure of LinA predicted by computer modeling was verified by site-directed mutagenesis. Additionally, D25, H73, and R129 were shown to be catalytically important residues of LinA that are essential for its activity, as determined by Nagata et al. [63].

By comparative analysis with the protein from *Rhodococcus equi*, that is responsible for the cleavage of the Cl–H, the active site residues of LinA were determined to be conserved, including D-aspartic acid at residues 24 and 25 and Histidine at residue 72. As in *Streptomyces*, only the amino acids in the active site are stable.

Haloalkane dehalogenase (LinB)

Haloalkane dehalogenase is a key enzyme in the degradation of synthetic haloalkanes that are soil pollutants. A member of the α/β-hydrolase family, haloalkane dehalogenase, catalyzes dehalogenation through a hydrolytic mechanism. The 1,4-TCDN halidohydrolase (LinB), which is involved in the biochemical pathway responsible for utilization of the halogenated organic insecticide γ-HCH (also called γ-benzenehexachloride) in *Sphingobium japonicum* UT26 [64], also belongs to the haloalkane dehalogenase family [32,33]. LinB not only converts 1,4-TCDN to 2,5-dichloro-2,5-cyclohexadiene-1-ol but also converts various kinds of haloalkanes to their corresponding alcohols. A catalytic triad (i.e. nucleophile-histidine-acid) is essential for the reactions catalyzed by members of the γ/β-hydrolase family. Based on a site-directed mutagenesis analysis, the catalytic site amino acid residues of LinB were proposed to be D108, H272, and E132 [64].

The putative LinB protein sequence of *Streptomyces* was compared with that of *Mycobacterium tuberculosis* H37Rv [64], and through this analysis, the active site was determined to be E (glutamic acid) 129, D (aspartic acid) 100 and H (Histidine) 290, which indicates with high probability that *Streptomyces* LinB possesses this active site. However, the function and features of these enzymes are still under investigation. Moreover, the studies of Jesenska et al. [65] aimed to find haloalkane dehalogenase genes in *M. tuberculosis* H37Rv and dehalogenating activity in 13 different *Mycobacterium* species. For safety reasons, *M. avium* was used for cloning and overexpression of an identified mycobacterial haloalkane dehalogenase gene to enable its characterization. The genome of *M. avium* 104 has been partially sequenced, and in it, sequences of genes encoding known and putative haloalkane dehalogenases has been identified. The translation of one of these gene fragments revealed a 106 amino acid protein (later designated DhmA) with 36.7% sequence identity to the haloalkane dehalogenase DhlA of *Xanthobacter autotrophicus* GJ10, 45.5% sequence identity to the putative haloalkane dehalogenase Cc1175 of *Caulobacter crescentus* CB15, and 82.4% sequence identity to the putative haloalkane dehalogenase Rv2296 of *M. tuberculosis* H37Rv. Nagata et al. [64] proposed that the putative catalytic triad of DhmA consists of Asp123, His279, and Asp250 and that the putative oxyanion hole consists of Glu55 and Trp124, the latter of which is likely involved in substrate binding and product (halide) stabilization. The substrate specificity of DhmA is unlike that of the LinB, DhaA, and DhlA dehalogenases.

Dehydrogenase (LinC)

The third enzyme in the HCH high degradation pathway is LinC, which is considered to be a 2,5-DDOL dehydrogenase in the short-chain alcohol dehydrogenase family (Oxidoreductase superfamily). Lal et al. [66] proposed a general catalytic mechanism for these enzymes, which

involves the participation of a conserved Ser-Tyr-Lys catalytic site plus one NAD cofactor that is necessary for the reaction. Specifically, the tyrosine hydroxyl is stabilized in a deprotonated state by the amino group of the lysine. Finally, hydride transfer from the substrate to NAD forms NADPH and the reduced product, i.e. the conversion of 2,5-DDOL to 2,5-DCHQ.

As the transformation of lindane can lead to many different intermediate compounds, we must also consider the key enzymes involved in further transformations/degradation of the most commonly reported metabolites of lindane. Datta et al. [40] reported that the aerobic lindane degradation intermediates of *Achrobacter citreus* included phenol, catechol, and a few chlorinated phenols.

The first step in the aerobic metabolism of phenol is hydroxylation to catechol by NADPH-dependent phenol hydroxylase. Catechol (ortho-dihydroxybenzene) is a central intermediate in the degradation pathways of various aromatic and non-aromatic parent compounds, and it enables the continuation of the degradation pathway. Recently, we identified a putative *linC* gene sequence in the *Streptomyces* genome, which clearly indicates that this essential step is carried out in the mineralization process to obtain less toxic products. The sequence in *Streptomyces* shows a similarity of 56% to that of the *linC* gene present in *Sphingobium*. Furthermore, *linC* genes have been identified in the chromosomes of several HCH-degrading sphingomonads in a single copy. Variants of *linC* have also been amplified directly from HCH contaminated soils [46].

A putative ABC transporter essential for the utilization of γ-HCH

Recently, Sallis et al. [45] identified genes encoding a putative ABC-type transporter essential for γ-HCH utilization in *Rhodococcus* and *Streptomyces*; several ABC systems were identified, but it is still unknown whether any of these transporters are specific for the transportation HCH. From the literature, it is known that the *linK*, *linL*, *linM*, and *linN* genes encode a permease, ATPase, periplasmic protein, and lipoprotein, respectively, which together form a putative ABC-type transporter system, as demonstrated by Endo et al. [67]. This type of transporter system is required for the utilization of γ-HCH, probably because it confers tolerance to toxic dead-end metabolites such as 2,5-DCP. Endo et al. [67] also detected a homologous ABC-type transporter system with high levels of similarity to the *linKLMN* genes; this new transporter has only been found in *Sphingobium*, suggesting that such systems may be important for its high metabolic activity of a range of xenobiotic compounds. As halos form around *Streptomyces* colonies grown on an agar medium with HCH isomers, degradation occurs inside the cell, indicating the presence of an ABC transporter system [27].

Conclusions and future perspectives

Typical anaerobic pathways of HCH isomer degradation are well understood for a few selected microbial strains. However, the particular routes of degradation have not been fully elucidated for *Streptomyces*, which has demonstrated effective degradation of these pollutants.

In an analysis of the *Streptomyces* genome, IS6100 insertion sequences were not detected, which indicates that genes were not transferred horizontally in this genus as they have been in *Sphingobium*. We cannot be certain that these strains did not acquire some *lin* genes, however, because they are known to be highly mobile due to IS6100-mediated genome rearrangements [68].

Nutrient recycling in the terrestrial environment requires the concerted action of a community of microorganisms in which Actinobacteria are important primary degraders of chemical synthesis compounds. For this reason, enzymatic and genetic characterizations of the molecular mechanisms involved in the early stages of degradation are important, especially the rupture of the C–Cl bonds, which are the most stable bonds in these compounds. Therefore, future work is needed to elucidate the relevant catalytic mechanisms.

More information is also needed to understand the HCH degradation and metabolic pathways in soil under field conditions, where several factors may influence biodegradation (soil type, environmental factors, and the presence of other microorganisms, among others). The general biodegradation of HCH isomers has been evaluated for individual compounds as well as for combinations of HCH isomers, but evaluating the biodegradation capacity of Actinobacteria in the presence of other pollutants is necessary, because environmental pollution often occurs as a mix of pollutants in actuality. Moreover, the use of metal nanoparticles has recently been proposed for the degradation of HCH isomers [69–71], but little information exists regarding whether the interactions of Actinobacteria and metal nanoparticles during HCH degradation would be negative or positive or if they would affect degradative metabolic pathways.

The findings discussed in this review strongly imply that *Streptomyces* spp. are HCH-degrading "specialists" that potentially have the physiological background to support the degradation of various recalcitrant compounds.

Disclosure statement

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