Phylogenetic Investigation Of Lin Genes Involved In Degradation Of Hexachlorocyclohexane (Hch)

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Citation

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Abstract

Hexachlorocyclohexane (HCH) is a non-degradable compound extensively used against agricultural pests and various public health programs for controlling mosquitoes. It imbibes into the food chain from crop to animals; biodegradable enzymes present in the genes of some bacteria degrade HCH in soil. Mainly, two genes linA and linB encode dehydrochlorinase and dehalogenase enzymes involved in degradation of HCH. Bioinformatics strategies were used to find other homologous genes and proteins, which can also degrade HCH present in various bacteria for controlling of pollution and offer clue to prevent its entry in the food chain. Total 38 nucleotides and 36 proteins sequences of linA and linB were used for constructing the phylogeny. In addition, analysis on culture dependent and culture independent bacteria were done, which might play a vital role in the degradation of the non-degradable compounds. These bacteria possibly will facilitate in the safety of soil pollution, improving fertility of the soil and prevent entry of HCH in the food chain.

INTRODUCTION

Xenobiotics are chemicals, which did not exist in nature. These are synthetic compounds viz. pesticides, fungicides, herbicides, insecticides, disinfectant and toxic present in the nature. They take action by transferring with microbial reactions in the target organisms. A wide range of halogenated organic compounds were used as solvents, agricultural chemicals, and insulating liquids. Most of them are toxic and recalcitrant in nature, cause serious contamination problems. Generally, these insert in soil and can affect the microorganisms that were important in maintaining fertility of the soil. Some organisms detoxify pesticides in soil gradually. Environmental factors also influence to degrade the compound which includes pH, temperature, bioactivity, nutrient supply and oxygen availability.

In India Hexchlorocyclohexane (HCH) is also known as benzene hexachloride (BHC). It is more toxic than DDT and was used mainly in public health program. HCH was introduced for controlling agricultural pests and vector-borne diseases. It was used extensively around the world and their toxic effect on target and nontarget organisms appeared in the 1980s ($_{1}$). These reports finally resulted in prohibition or restrict the use of HCH in various countries. The problem arises in the uptake of HCH residues from soil by crops, from where it enters into the food products through food chain ($_{2}$). The decontamination program for HCH polluted soils would diminish the risk posed by HCH residues to human, plant and animal health. The prospect for decontamination is spontaneous or induced microbial degradation of HCH proceeds gradually by number of bacteria ($_{3}$). Degradation of HCH was carried out commonly by strains of Sphingomonas paucimobilis and Rhodanobacter lindaniclasticus. HCH-degrading strains were isolated from different parts of the world; and S. paucimobilis SS86 has been isolated from Japan ($_{4}$). On the basis of 16S rDNA phylogenetic analysis of bacteria was done. The ribosomal operons mainly 16S rDNA has proven to be a stable and specific molecular marker for bacterial identification. The copy number of 16S rDNA genes might fluctuate from 1 to 15 among bacterial genomes; it is generally believed that all the copies in an organism are identical or nearly identical in nucleotide sequence. The 16S rDNA is present in scattered form in the entire genome of bacteria. These ribosomal sequences was functional for the phylogenetic analysis and molecular taxonomic of any bacteria. Recently, metagenomics a new approach to identify culturable and non culturable bacteria with the help of 16S rDNA sequences isolate total DNA from HCH contaminated soil and amplified 16S rDNA through universal primers and confirmed its total population with denaturing gradient gel electrophoresis and sequencing. A culture-independent PCR-

based detection method for specific primers targeting the Sphingomonas 16S rRNA gene combined with DGGE was developed to assess Sphingomonas diversity in polycyclic aromatic hydrocarbons (PAH) contaminated soils; PCR using the new primer pair on a set of template DNAs of different bacterial genera showed that the method was selective for bacteria belonging to the family Sphingomonadaceae. Single-band DGGE profiles were obtained in most of the Sphingomonas strains tested. Strains belonging to the same species had identical DGGE fingerprints, and in most cases, these fingerprints were typical for one species. Sequence analysis of cloned PCR products amplified from soil DNA revealed new 16S rRNA gene Sphingomonas sequences significantly different from sequences from known cultivated isolates ($_5$).

Therefore, bacteria which encode linA ($_6$) and linB ($_7$) involve in the degradation of HCH also encode halidohydrolase, a dehydrogenase, respectively. Several bioinformatics tactics were used to find the open reading frame and evolution of linA and linB genes in the bacterial diversity of culturable and non culturable bacteria.

The pesticide used to control carriers of vector borne disease viz. malaria, sleeping sickness, Dengue fever, yellow fever etc. It has majority of organochlorides as non degradable and accumulates in the environment thus, causes soil pollution, from where it enter the food chain; their concentration heed as they move in the food chain.

This approach is useful in distinguishing other bacteria which comprises the same homologous gene which could biodegrade HCH. Here, emphasis on identification of culture dependent and culture independent bacteria which involved in degrading the non-degradable compounds was done.

MATERIAL AND METHODS

COLLECTION OF SEQUENCES

The complete nucleotide and protein sequences of linA and LinB from different source of bacteria extracted from biological database, viz. National Centre for Biotechnology Information (NCBI) cited at http://www.ncbi.nlm.nih.gov.The open reading frame of linA and LinB encoding proteins was also analyzed by NCBI.

BLAST

The relatedness of sequences deposited in databases was evaluated by BLAST (Basic Local Alignment Search Tool) implemented via the NCBI website [www.ncbi.nlm.nih.gov/blast/] against the complete training dataset which is extracted from Genbank database. The BlastP (protein query – protein database comparison) in which conditional composition score adjustment having no filters of BLOSUM 62 matrix with threshold expect value 10 were used.

CONSTRUCTION OF PHYLOGENETIC TREE

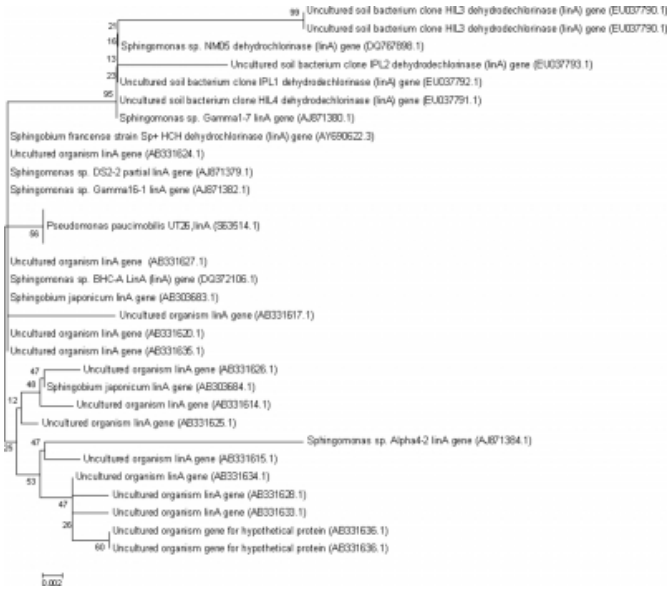
The nucleotide sequences of linA and linB were compared with sequences deposited in the Genbank database using the BLAST programme. The sequences were aligned in CLUSTALX 1.83. The computed alignment was then manually checked and corrected. Pairwise evolutionary distances were computed using the Jukes and Cantor equation implemented in the MEGA 3.1 program and a phylogenetic tree was constructed by the neighbour-joining method programs package available online. Protein sequences were also used for construction of phylogenetic tree.

RESULTS AND DISCUSSION

In the present study, total twenty nine linA genes having complete open reading frame (ORF) taken from different bacterial source were used. The phylogenetic relationships as demonstrated on the basis of all these structure nucleotide sequences. The homology of these sequences was mainly from three types of bacterial diversity viz Sphingomonas spp, Pseudomonas and uncultuarable bacteria comprise genes for linA, which involve in the degradation of HCH. In the phylogenetic tree (Fig 1) total 100 bootstrapped values were sampled to determine a measure for support of each node on consensus tree. Jukes and Contor algorithm was used. Recently, the nomenclature of genus Sphingomonas spp has been changed and its current genus is Sphingobium spp.

Figure 1

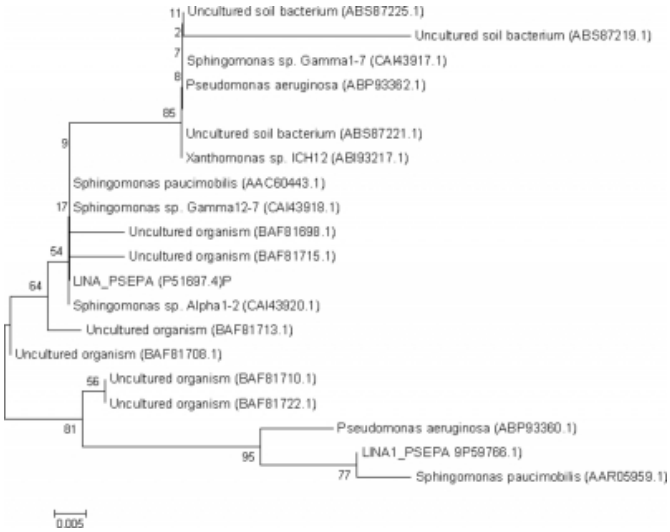
Figure1: Phylogenetic tree based on the Lin A gene sequences with NCBI accession number using neighbor-joining method and Jukes & Cantor algorithm.



The evolutionary distribution of Lin A gene was present in the whole diversity of bacteria. In spite of nucleotide, we also used Lin A protein sequences for constructing phylogeny. Total nineteen protein sequences of either HCH degrading or almost similar homologous with this protein were used. We observed homology of these sequences mainly from four types belonging to bacterial diversity like Sphingomonas spp, Pseudomonas, Xanthomonas and uncultuarable bacteria, which may involve in the degradation of HCH. The phylogenetic tree was given in Fig: 2. A total 100 bootstrapped values were sampled to determine a measure of the support for each node on the consensus tree. Passion correction algorithm was used.

Figure 2

Figure 2: Phylogenetic tree based on the Lin A protein sequences with NCBI accession number using neighbor-joining method and Passion correction algorithm.



In this study, identification of HCH degrading bacteria on the basis of 16S rDNA gene in the genome was used. Based on 16S rDNA analysis, strain DS3-1 was closely related to Sphingomonas taejonensis, while strains DS2 and DS2-2 were closely related to Sphingomonas flava and seven HCH-degrading strains recently isolated from HCH-contaminated Spanish soil, geographic origin of strains was not reflected in their phylogenetic affiliation. Subsequently, Lin genes involved in HCH degradation, virtually identical to those from Sphingomonas paucimobilis strains UT26 from Japan and B90A of India, strains from Spain DS3-1, DS2 and DS2-2 were identified. The conserved lin gene sequence and structural organization, in close association with IS6100, suggest a shared lin gene origin and recent horizontal gene transfer among phylogenetically diverged Sphingomonas strains in remote geographic locations ($_{8}$).

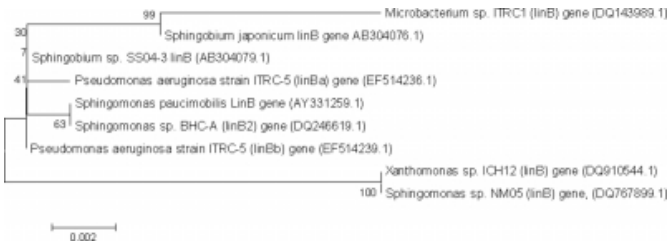
It was reported that Pseudomonas paucimobilis UT26 is capable of growing on hexachlorocyclohexane. A genomic library of P. paucimobilis UT26 was constructed in Pseudomonas putida using broad-host-range cosmid vector pKS13. Nucleotide sequence analysis revealed an open reading frame (linA) of 465 bp within the fragment ($_{6}$). The nucleotide sequence of linA gene and amino acid sequence showed no similarity to any known sequences. The product of the linA gene was 16.5 kDa according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Recently, hexachlorocyclohexane a halogenated organic insecticide cause serious environmental problems was identified. The aerobic degradation pathway of HCH was extensively reveal

in bacterial strain Sphingobium japonicum UT26 gamma-HCH is transformed to 2,5-dichlorohydroquinone through sequential reactions catalyzed by LinA, LinB, and LinC, and then 2,5-dichlorohydroquinone is further metabolized by LinD, LinE, LinF, LinGH, and LinJ to succinyl-CoA and acetyl-CoA, which metabolized in the citrate/tricarboxylic acid cycle. In addition to these catalytic enzymes, a putative ABC-type transporter system encoded by Lin is essential for gamma-HCH utilization in UT26. It reveals that two dehalogenases, LinA and LinB, had variants with small number of amino acid differences, and they showed dramatic functional differences for the degradation of HCH isomers, indicating these enzymes still evolve at high speed ($_{9}$). Variability in the degradation rate of the phenyl-urea herbicide isoproturon (IPU) [3-(4-isopropylphenyl)-1, 1-dimethylurea] has been shown to occur within agricultural fields, implications for longevity of the compound in the soil, and its movement to ground- and surface water. Using enrichment techniques, an IPU-degrading bacterial culture (designated strain F35) was isolated from fast-degrading soil, and partial 16S rRNA sequencing placed it within the Sphingomonas group. Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified bacterial community 16S rRNA revealed two bands that increased in intensity in soil during growth-linked metabolism of IPU, and sequencing of the excised bands showed high sequence homology to the Sphingomonas group while F35 was not closely related to either DGGE band, one of the DGGE bands showed 100% partial 16S rRNA sequence homology to an IPU-degrading Sphingomonas sp. (strain SRS2) isolated ($_{10}$). The culturable bacteria can be identified easily, carry gene for linA. However, there was not any standardized culture media presently available to culture the unculturable bacteria. Therefore, the coding gene for linA and linA similar protein may contribute in the degradation of HCH.

In this study, another gene linB, also involved in the degradation of HCH was used to construct the phylogenetic tree. Total four types of bacteria viz Sphingomonas, Pseudomonas, Xanthomanas and Microbacterium having the linB gene were analyzed. The homology of these sequences, which might contributes in the biodegradation of HCH given in Fig: 3.

Figure 3

Figure3: Phylogenetic tree based on the linB gene sequences.

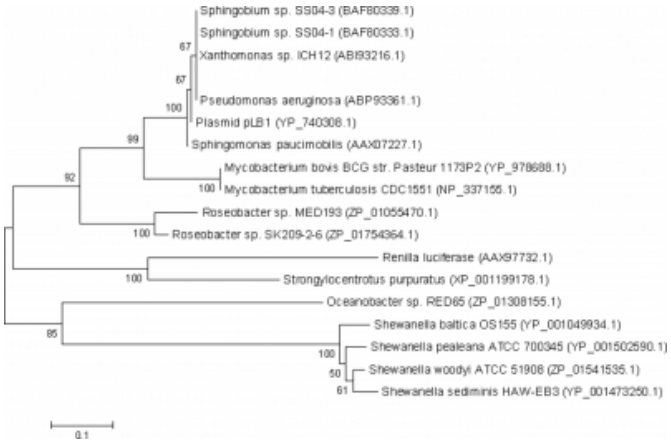


A gram-positive Microbacterium sp. strain, ITRC1, able to degrade the persistent and toxic hexachlorocyclohexane (HCH) isomers was isolated and characterized. This strain has the capacity to degrade all four major isomers of HCH present in both liquid cultures and aged contaminated soil. DNA fragments corresponding to two initial genes involved in gamma-HCH degradative pathway, encoding enzymes for gamma-pentachlorocyclohexene hydrolytic dehalogenase (linB) and a 2, 5-dichloro-2, 5-cyclohexadiene-1, 4-diol dehydrogenase (linC), were amplified by PCR and sequenced. Both 16S rRNA sequencing and phylogenetic analysis resulted in the identification of the bacteria as a Microbacterium sp. We assume that these HCH-degrading bacteria evolved independently but possessed genes similar to S. paucimobilis UT26 ($_{11}$). Total seventeen protein sequences of linB or homologous protein from diverse bacteria, also involved in the HCH degradation, have got the homology of these sequences from variety of bacterial diversity like Sphingomonas spp, Pseudomonas, Xanthomonas, Mycobacterium, Roseobactor, Renilla,Stronglycentrotus, Oceanobactor, Shewanella and plasmid.

Fig: 4. A total of 100 bootstrapped values were sampled to determine a measure of support for each node on the consensus tree. Passion correction algorithm was used. In this Dendogram, we also got other bacteria having HCH degrading encoding gene.

Figure 4

Figure 4: Phylogenetic tree based on the Lin B protein sequences.



Bacteria degrade HCH isomers also been isolated from HCH contaminated soils from different geographical locations of the family Sphingomonadaceae. All these bacteria encompass nearly identical Lin genes to degrade HCH, which diverge to perform several catabolic functions. The organization and diversity of Lin genes were studied among several sphingomonads, and have been found associated with plasmids and IS6100, both of which appear to have significant role in their horizontal transfer. The organization of Lin genes and IS6100 was studied in three strains of Sphingomonas paucimobilis, degrade HCH isomers but isolated at different geographical locations. The copy number and sequence of genes of the HCH degradative pathway (linB, linC, linD, and linE) were nearly the same in all strains. The evidence gathered in this study coupled with the observation that GC contents of linA genes was lower than of remaining DNA sequence of S. paucimobilis, it strongly suggests that these strains acquired the linA gene through horizontal gene transfer mediated by IS6100. The association of IS6100 with the rest of the Lin genes further suggests that it played a major role in shaping the current Lin gene organization ($_{12}$). HCH consist of assortment of isomers, alpha, beta, gamma and delta. All these isomers were toxic and recalcitrant pollutants. Sphingobium sp. strain BHC-A is able to degrade all four HCH isomers. Eight lin genes responsible for the degradation of gamma-HCH in BHC-A were cloned and analyzed for their role in the degradation of delta-HCH, and the initial conversion steps in delta-HCH catabolism by LinA and LinB in BHC-A were found ($_{13}$).

The two linA- and linB-like genes coding, respectively, for a gamma-HCH dehydrochlorinase and a dehalogenase were characterized using a PCR strategy based on sequence homologies with previously published sequences from Sphingomonas paucimobilis UT26. Nucleotide sequence analysis of the linA-like region revealed the presence of 472 bp open reading frame exhibiting high homology with the linA gene from S. paucimobilis, while preliminary study indicates strong homology among two linB genes. All enzymes involved in the gamma-HCH degradative pathway appear to be extracellular and encoded by genes located on the chromosome ($_{14}$). One more study was done on use of HCH. It has been extensively used against agricultural pests and public health programs for mosquito control. It was investigated that degradation of HCH isomers by Sphingomonas paucimobilis strain B90 and characterized the lin genes encoding enzymes from strain B90 responsible for the degradation of HCH isomers ($_{15}$). Two bacterial isolates (LIN-1 and LIN-3) grow on gamma-HCH as a sole source of carbon and energy isolated from enrichment culture. In liquid cultures of LIN-1 and LIN-3, 25.0 and 45.5% removal of gamma-HCH, respectively, were achieved in 2 weeks. LIN-3 was identified as Pandoraea sp. by 16S rRNA gene sequence analysis ($_{16}$).

In conclusion, the investigations of phylogenetic relationship approach on the basis of Lin A and Lin B genes and proteins were useful for other bacteria having the same homologous gene and furthermore used to degrade HCH. We also identified culture dependent and culture independent bacteria that involved in degradation of the non-degradable compounds. In future these bacteria may play a vital role for the safety of soil pollution and thus, improving soil fertility by preventing entry of HCH in food chain for the safety of animal health.

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