Localization of HCH catabolic genes (*lin* genes) in *Sphingobium indicum* B90A

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Abstract The locations of hexachlorocyclohexane (HCH) catabolic (*lin*) genes were investigated in HCH degrading sphingomonad, *Sphingobium indicum* B90A (that was isolated from India). Southern blot analysis revealed the presence of *linA1*, *linC*, *linDER* and *linX* (*linX1* and *linX2*) on the plasmid DNA in *Sphingobium indicum* B90A.

Keywords Plasmid · *lin* gene · IS6100 · sphingomonads

Introduction

Sphingobium indicum (formerly *Sphingomonas paucimobilis*) B90A was isolated from the rhizosphere soil of hexachlorocyclohexane (HCH)-treated sugarcane in India and was reported to metabolize γ -HCH under aerobic conditions¹. Although several HCH degrading bacteria are known to date², the pathway for γ -HCH degradation has been elucidated in detail only in *S. japonicum* UT26^{3,4}. All the HCH catabolic genes (called *lin* genes) involved in the degradation of γ -HCH isomer have been identified in *S. japonicum* UT26^{3,4}. The primary enzyme in γ -HCH degradation is HCH dehydrochlorinase encoded by *linA*⁵. The remaining genes of the γ -HCH degradative pathway are *linB*⁶, *linC*⁷, *linD*⁸, *linE/linEb*^{9,10}, *linF*¹⁰, *linGH*⁴ and *linJ*⁴ encoding haloalkane dehalogenase, dehydrogenase, reductive dechlorinase, ring-cleavage oxygenase, maleylacetate reductase, acyl-CoA transferase and thiolase, respectively. Two regulatory genes, *linR*¹¹ and *linI*⁴ are reported to be necessary for the degradation of γ -HCH in *S. japonicum* UT26. Additionally, a *linX* gene, encoding a protein that has activity similar to that of *linC* (but probably is not involved in the degradation pathway of γ -HCH), was also characterized⁷. Recent studies have shown that both LinA and LinB are also involved in the degradation of the recalcitrant β - and δ -HCH isomers^{12–14}.

Nearly identical *lin* genes were identified in *S. indicum* B90A¹⁵. However, *S. indicum* B90A was found to contain two copies of *linA* (*linA1* and *linA2*) and three copies of *linX* (*linX1*, *linX2* and *linX3*) compared to only one copy of these genes in *S. japonicum* UT26¹⁵. Furthermore, *S. indicum* B90A was reported to contain several copies of an insertion sequence, IS6100 (originally isolated from a Gram-positive bacterium, *Mycobacterium fortuitum* FCI)¹⁶, which were

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found to be associated with most of the *lin* genes in this strain¹⁵. Recently, several HCH-degrading sphingomonads have been isolated from the HCH contaminated soils from different geographical locations^{17–22}. These strains were found to contain *lin* genes highly similar to UT26 and B90A^{14, 17–19}. Most of the *lin* genes in these organisms were also found to be associated with IS6100 element^{17–19,22}. The finding of virtually identical *lin* genes and IS6100 in sphingomonads, isolated from different parts of the globe, is suggestive of recent horizontal gene transfer (HGT). The spread of *lin* genes and IS6100 among sphingomonads can further be facilitated by their association with plasmids, but the presence of *lin* genes and IS6100 on plasmids in *S. indicum* B90A has not been explored as yet. Recent studies have, however, revealed the presence of *lin* genes on plasmids in *S. japonicum* UT26²³ and in the HCH degrading isolate, *Sphingobium francense* Sp⁺¹⁸.

The present study was undertaken to explore the locations of *lin* genes in *S. indicum* B90A genome.

Materials and Methods

S. indicum B90A was grown at 28°C in Luria-Bertani (LB) medium containing 1% tryptone, 0.5% yeast extract, and 0.5% sodium chloride. Solid media was prepared by addition of 1.5% agar to LB broth.

Plasmid DNA from *S. indicum* B90A was detected from crude lysate using a method described by Ramos-González *et al.*²⁴. To isolate pure plasmid DNA from B90A, a modified method as described by Kieser²⁵ was used. For this purpose, a 50 ml culture in LB medium was grown to stationary phase and cells were harvested by centrifugation (8,000 r.p.m. for 10 min at 4°C). The cells were suspended in 27 ml of freshly prepared lysozyme buffer (25 mM Tris hydrochloride, pH 8.0; 25 mM EDTA, pH 8.0; 300 mM sucrose) and 3 ml of freshly prepared lysozyme mix (lysozyme, 10 mg/ml, in lysozyme buffer) followed by thorough mixing of the cell suspension. The cell suspension was incubated at 37°C for 1 h. The cells were lysed by adding 20 ml of freshly prepared alkaline solution containing 0.2 N NaOH and 4% (w/v) sodium dodecyl sulphate (SDS). The contents were mixed by inverting the tube gently and the resulting clear viscous suspension was kept at 55°C for 30 min. Chromosomal DNA was removed by adding 50 ml of 3 M sodium acetate (pH 4.8) and incubation on ice for 20 min. After centrifugation (10,000 r.p.m. for 20 min at 4°C), the filtered supernatant containing the plasmid DNA was precipitated by adding equal volume of isopropanol. The precipitated plasmid DNA was collected by centrifugation (10,000 r.p.m. for 20 min at 4°C) and the dried DNA pellet was suspended in 500 µl of TE buffer (10 mM Tris hydro-

chloride, pH 8.0; 1 mM EDTA, pH 8.0) followed by further purification by phenolization²⁶. The DNA sample was analyzed by 0.8% (w/v) agarose gel electrophoresis.

Genomic DNA from *S. indicum* B90A was isolated by the procedure described in SuperCos 1 Cosmid Vector Kit, Instruction manual, Stratagene (www.stratagene.com).

Results

In order to determine the distribution of *lin* genes, the plasmid as well as the genomic DNA of *S. indicum* B90A was digested with *Bcl*I and *Hind*III (Fig. 1A) (enzymes which do not cut within *lin* genes) and hybridized separately with [α -³²P]dATP (BRIT, Hyderabad, India)-labeled *lin* gene probes under high-stringency conditions as described previously¹⁵. DNA-DNA hybridization data revealed the presence of *linA1* (corresponding to the ~1.4 kb *Bcl*I and ~3.5 kb *Hind*III fragment) (Fig. 1B), *linC* (Fig. 1C), and two copies of *linX* gene (*linX1* and *linX2* represented by a ~3.5-kb *Hind*III fragment) (Fig. 1D) on the plasmid DNA in B90A. In our previous study, we had demonstrated the presence of two copies of *linX* (*linX1* and *linX2*), *linA1* and *linC* genes on a 41-kb contiguous fragment of a cosmid clone, pLINA57 indicating that these findings are in agreement with the organization of these genes¹⁵. This further suggests that the insert in cosmid, pLINA57, is a part of plasmid DNA. Both *linA2* and *linX3* genes were localized on the chromosome (corresponding to the ~5.7-kb *Hind*III fragment) (Figs. 1B and D). In our previous study, three cosmid clones (cosmids 39, 43 and 53), which had both the *linA2* and *linX3* genes, were isolated¹⁵, indicating that these findings are also consistent with the organization of these genes. Further, Southern blot analysis revealed that the *linD* gene is located on the plasmid DNA (Fig. 1E). As expected (since *linDER* genes are located near each other in B90A)¹⁵, *linE* (Fig. 1F) and *linR* (Fig. 1G) genes were also localized on the plasmid. No hybridization signal was detected on the plasmid DNA with *linB* gene (data not shown).

DNA-DNA hybridization data of *Bam*HI (enzyme which does not cut within IS6100)-digested plasmid and genomic DNA revealed the presence of IS6100 element on the plasmid in B90A (data not shown). Our earlier studies had revealed the presence of, at least, 11 copies of IS6100 on the genome of B90A¹⁵ out of which, at least, 8 copies were now found to be present on the plasmid DNA.

Conclusion

In the present study, the locations of the *lin* genes were investigated in the HCH degrading isolate *S. indicum* B90A.

Fig. 1 (A) Plasmid and genomic DNA of *S. indicum* B90A was digested with *Bcl*I (lanes 1 and 2) and *Hind*III (lanes 3 and 4), prepared for Southern hybridization, and hybridized separately against the *linA* (B), *linC* (C), *linX* (D), *linD* (E), *linE* (F) and *linR* (G) probes. M, molecular mass marker. Note that in A-G, lanes 1 and 3 represent digested plasmid DNA, and lanes 2 and 4 represent digested genomic DNA

Southern hybridization studies revealed that *lin* genes are present on the plasmid (*linA1*, *linC*, *linDER* and *linX* [*linX1* and *linX2*]) and genomic DNA (*linA2*, *linB* and *linX3*) in *S. indicum* B90A. However, the possibility of locating *lin* genes (*linA2*, *linB* and *linX3*) on an unrecoverable plasmid, such as megaplasmid cannot be excluded.

It is evident from the present study that *lin* genes are scattered within the *S. indicum* B90A genome, a situation similar for a number of catabolic genes found in sphingomonads^{27, 28}. In *S. japonicum* UT26, *lin* genes were found to be dispersed on three circular replicons (*linA-linC* on chromosome I, *linF* on chromosome II, and *linDER* on the

conjugative plasmid pCHQ1)²³. In *S. francense* Sp⁺, *lin* genes (*linA*, *linB*, *linE* and *linX*) were shown to be located on three plasmids¹⁸, showing dispersal within the genome. The presence of *lin* genes on plasmids suggests the possible role of plasmids in the spread of *lin* genes among HCH-degrading sphingomonads, thus representing a remarkable example of horizontal transfer of catabolic genes.

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