Structure and conservation of a polyethylene glycol-degradative operon in sphingomonads

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INTRODUCTION

Polyethylene glycols (PEGs) and their derivatives are industrially mass-produced xenobiotics used as surfactants, dispersants, cosmetics and lubricants in many fields. Since PEGs and their derivatives are water-soluble and used in dilute solution, it is difficult to recover them from waste and from watercourses. Hence, microbiological degradation appears to be the only means to decompose this group of polymers.

We have been studying the mechanism of PEG degradation in sphingomonads, which have the ability to degrade PEG up to a molecular mass of 20,000 Da (Kawai et al., 1978).

PEG molecules are degraded by successive oxidation of terminal hydroxyl groups. A hydroxyl group is oxidized to an aldehyde and further to a carboxylic acid, and then one glycol unit is split by PEG-carboxylic acid dehydrogenase (Enokibara & Kawai, 1997). The first step of degradation is catalysed by PEG dehydrogenase (PEG-DH). The gene (pegA) encoding PEG-DH has been cloned and sequenced from Sphingomonas terrae (Sugimoto et al., 2001) [reidentified as Sphingopyxis terrae (Takeuchi et al., 2001)]. Recombinant PEG-DH has been purified and characterized as a novel flavoprotein alcohol dehydrogenase.

Sphingomonads are known to degrade a wide range of environmental pollutants (Kilbane et al., 2002; Tiirila et al., 2002; Wattiau et al., 2001). Intensive studies of the genetic basis of this ability have been made, from which it is clear that members of this genus have the ability to adapt to and efficiently degrade new compounds in the environment, and this trait makes the study of sphingomonads attractive and interesting. As a molecular basis for this ability, it should be noted that many xenobiotic-degrading sphingomonads contain large degradative plasmids (Basta et al., 2004). A large plasmid, pNL1, derived from a degrader of various aromatic compounds, Sphingomonas aromaticivorans F199, has been sequenced and its conjugative transfer into other Sphingomonas species demonstrated (Romine et al., 1999).

Sphingopyxis terrae, and Sphingopyxis macrogoltabida strains 103 and 203, can degrade polyethylene glycols (PEGs). They differ in the following respects: (i) different substrate specificities (chain length) of assimilable PEG, (ii) PEG-inducible or constitutive PEG-degradative proteins, and (iii) symbiotic or axenic degradation of PEG. S. terrae was able to incorporate PEG 6000, but strain 103 could not incorporate more than PEG 4000, suggesting that the difference in assimilable PEG chain length depends on the ability to take up substrate. PEG-degradative genes (pegB, C, D, A, E and R) from these strains were cloned. Their primary structures shared a high homology of more than 99%. The peg genes encode a TonB-dependent receptor (pegB), a PEG-aldehyde dehydrogenase (pegC), a permease (pegD), a PEG dehydrogenase (pegA) and an acyl-CoA ligase (pegE), and in the opposite orientation, an AraC-type transcription regulator (pegR). The peg operon was flanked by two different sets of transposases. These three strains contained large plasmids and the operon was located in one of the large plasmids in S. terrae. The peg genes could be detected in other PEG-degrading sphingomonads. These results suggest that the peg genes have evolved in a plasmid-mediated manner. An insertion of a transposon gene (pegF) between pegD and pegA in strain 203 was found, which caused the constitutive expression of pegA in this strain.

Abbreviation: PEG-DH, PEG dehydrogenase.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are AB196775 (S. macrogoltabida strain 103), AB239080 (S. macrogoltabida strain 203) and AB239603 (S. terrae).

Details of the primers used in this study, the annotation of ORFs located within the peg gene cluster and its flanking region in S. macrogoltabida strain 103, the inducibility of PEG-DH in S. macrogoltabida strains 103 and 203, and Western blots using anti-PEG-DH antibody are available as supplementary data with the online version of this paper.

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Therefore, it is suggested that the dissemination of degradation ability is mediated by such large plasmids and by conjugation between different sphingomonads. Basta et al. (2004) have revealed the existence of two plasmids (150 and 450 kb) in a PEG degrader, Sphingomonas macrogoltabida strain 203 (DMS 8826, IFO 15033) and that the 450 kb plasmid contains genes encoding replication initiation proteins (Basta et al., 2005). However, it is not known whether the genes for PEG degradation are encoded on such plasmids and whether the other PEG-degrading sphingomonads contain plasmids.

From our collection of PEG-degrading sphingomonads, we selected three strains, namely S. terrae, and S. macrogoltabida strains 103 and 203 (Takeuchi et al., 1993), for our studies. Both species have been reidentified as Sphingopyxis terrae and Sphingopyxis macrogoltabida, respectively (Takeuchi et al., 2001). They differ from one other in the following respects: (i) strains 103 and 203 can assimilate PEG 4000, and S. terrae can assimilate PEG up to 20000 (Kawai et al., 1978); (ii) PEG-DH is constitutively formed in strain 203, whereas it is induced by PEG in strain 103 and S. terrae (Yamanaka & Kawai, 1989); and (iii) strains 103 and 203 can grow in axenic cultures, but S. terrae can grow on PEG only in a symbiotic culture with Rhizobium sp., and metabolism of glyoxylate has been suggested to be a key to this symbiosis (Kawai & Yamanaka, 1986). At present, the molecular basis for these differences is unclear. In this paper, we show that the inability of strain 103 to grow on PEG 20000 is due to a deficiency of the uptake of longer substrates. We also describe the cloning of the peg operon, a comparison of the operon structures among five different PEG-degrading sphingomonads, and the localization of the operon on a large plasmid. Furthermore, we show that the different inducibility of PEG-DH among the three strains is due to the transposon insertion in strain 203 alone in the peg operon.

METHODS

Microbial strains and culture conditions. S. terrae, and S. macrogoltabida strains 103 and 203 (Takeuchi et al., 2001), were grown at 28°C in glucose medium or PEG 4000 medium (Kawai et al., 1985). For routine cloning purposes, Escherichia coli DH5a was grown in Luria–Bertani (LB) medium (Sambrook et al., 1989) at 37°C for 12–15 h for use as a host. Ampicillin was used at a final concentration of 50 μg ml⁻¹, when necessary.

Substrate uptake analysis. S. macrogoltabida strain 103 and a symbiotic culture of S. terrae and Rhizobium sp. were cultivated in 100 ml PEG 4000 and 6000 medium, respectively. The cells of late-exponential to early stationary phase were collected by centrifugation at 8000 g for 10 min, washed twice with cold 50 mM phosphate buffer (pH 7.0), and suspended in 10 ml 50 mM phosphate buffer (pH 7.0) containing 0.5% (v/v) PEG 4000 or PEG 6000. The samples were taken at 2-day intervals and centrifuged at 8000 g for 10 min to remove cells. The supernatant was analysed by HPLC (Tosoh CCPM-II liquid chromatograph). The experiment was done in triplicate. The analytical conditions were as follows: detection, Tosoh RI-6020; columns, Tosoh TSK-GEL2500PW (7.5 × 300 mm) connected with TSK-GEL3000PW (7.5 × 300 mm); eluent, 0.3 M NaNO₃; flow rate, 1 ml min⁻¹; and column temperature, 40°C. Molecular masses were measured using ethylene glycol, its oligomers, PEGs and TSK standard polyethylene oxides (Tosoh).

DNA manipulations, PCR, and nucleotide sequence analysis. Standard protocols were used for DNA cloning and transformation (Sambrook et al., 1989). Total DNAs of S. terrae, and of S. macrogoltabida strains 103 and 203, were extracted by the method of Marmur (1961). Restriction enzymes and other DNA-modifying enzymes were purchased from TOYOBO or Takara Bio and used as specified by the manufacturers. A MagExtractor DNA purification kit (TOYOBO) was used to purify DNA fragments from agarose gels. Oligonucleotides for PCRs were obtained from Qiagen. PCR mixtures (25 μl) contained 0.4 pmol μl⁻¹ each primer, 2 mM MgCl₂, 0.25 mM each deoxynucleotide, 1 × TAq DNA polymerase buffer, and 0.5 U LA Taq DNA polymerase (Takara Bio). All PCR products were cloned into the pCR-2.1 TOPO vector (Invitrogen). DNA sequencing was done on double-stranded DNA, using an ABI3100 Genetic Analyser and a BigDye Cycle Sequencing kit version 1.1 (Applied Biosystems). The gaps between contigs were sequenced using a primer-walking method with appropriately synthesized primers (Supplementary Table S1). Sequence assembly and computer analysis of the DNA sequences were done with the GENETYX software.

Cloning of pegA-flanking regions from S. terrae, and from S. macrogoltabida strains 103 and 203. In our previous paper (Sugimoto et al., 2001), we reported a 3.3 kb DNA sequence (accession no. AB050784) containing pegA that encodes S. terrae PEG-DH (Fig. 1). The sequence also contains the partial 3′ region of a putative permease-encoding gene and the 5′ region of the acyl-CoA ligase gene. Using primers designed on the basis of the 3.3 kb DNA sequence of S. terrae, we were able to amplify a DNA fragment from strain 103 of the same size as that from S. terrae, and sequence analysis revealed that the fragment had the same gene cluster. Then, we selected strain 103 for further sequencing. Using the upstream region of the obtained fragment as a probe, a 2.1 kb SalI fragment, and subsequently a 5.4 kb PstI fragment, were cloned by the colony hybridization method (Sambrook et al., 1989) using pBluescript II SK+ (Stratagene) as a cloning vector. For the downstream region, the 1.1 kb SalI fragment and a further 2.9 kb SacI fragment were cloned using the same method. The obtained plasmids were appropriately subcloned and sequenced. Next, based on the sequence obtained from S. macrogoltabida strain 103, we amplified the corresponding regions from S. terrae and S. macrogoltabida strain 203 by PCR. Using primers TonB-N and P3/PEGDH (Fig. 1, Supplementary Table S1), we amplified 6.5 and 8.0 kb fragments as pegA upstream regions from S. terrae and strain 203, respectively. For downstream regions, primers 4-F and Sc1 (Fig. 1, Supplementary Table S1) were used, and 6.0 kb fragments were obtained from the two strains. The PCR products were cloned into the pCR-2.1 TOPO vector and sequenced with the appropriate subcloning technique.

Detection of large plasmids. Preparation and detection of large plasmid DNA from PEG-degrading sphingomonads were done following the method reported by Ka & Tiedje (1994). After electrophoresis, the gel was subjected to Southern blot hybridization (Southern, 1975), using the AlkPhos Direct kit (Amersham) for probe synthesis and detection. The pegB fragment (2.2 kb) that was generated by PCR using primers 1-F and 1-R (Fig. 1, Supplementary Table S1) was used as a probe and a positive control.

PCR experiments to detect peg genes in other sphingomonads. To detect the peg genes from other sphingomonads, we used the pairs of primers listed in Supplementary Table S1 for PCR amplification. The total genomic DNAs from PEG-degrading...
sphingomonads, namely PEG 20 000 degrader *Sphingomonas* sp. N6 (IFO15866) and PEG 4000 degrader *Sphingomonas* sp. K1 (IFO15867) (Kawai & Takeuchi, 1996), were used as templates. The following PCR programme was used: an initial denaturation (3 min, 94°C) was followed by 25 cycles consisting of annealing at 53°C (30 s), polymerization at 72°C (2 min), and denaturation at 94°C (30 s). The PCR products were electrophoresed in 0.7 % (w/v) agarose gels and the band patterns were compared to those from *S.* macrogoltabida strain 103.

**Preparation of total RNA and RT-PCR.** *S. macrogoltabida* strains 103 and 203 were grown at 28°C to OD₆₀₀ ~ 0.3. Total RNA was isolated using ISOGEN (Nippon Gene). The RNA samples were treated with DNase I (Invitrogen). RT-PCR was performed with a One-Step RT-PCR kit (Qiagen) according to the supplier’s instructions. Positive-control experiments were done with genomic DNA as a template. Negative-control experiments were prepared by omitting the reverse-transcription step. The positions and sequences of primers used for each PCR amplification are indicated in Fig. 1 and Supplementary Table S1, respectively.

**RNA dot-blot hybridization.** Three micrograms of total RNA from each sample was spotted and heat-fixed on nylon membranes (Hybond-N+; Amersham Biosciences) at 80°C for 120 min. Probes were generated by amplifying the DNA fragments of the ORFs of each gene with appropriate primers, listed in Supplementary Table S1. The probes were labelled as described in the manual for the DIG system (Roche). The conditions used for pre-hybridization, hybridization, washing and detection followed the instructions of the manufacturer.

**Purification of an anti-PEG-DH antibody.** The recombinant *S. terrae* PEG-DH protein that was expressed as a histidine-tagged protein in *E. coli* (Sugimoto *et al.*, 2001) was used as an antigen. We used two 2-month-old female Japan White rabbits for immunization. One millilitre of the antigen solution (1.5 mg recombinant PEG-DH ml⁻¹ in PBS: 137 mM NaCl, 8.1 mM Na₂HPO₄, 2.68 mM KCl, 1.47 mM KH₂PO₄, pH 8.0) was mixed with 1 ml Complete Freund’s Adjuvant (Difco). A total of four intradermal injections of 200 µl each were administered at 14-day intervals. Two weeks after the last injection, blood was collected, from which antibody was purified. The protein concentration of the blood was adjusted to 5 mg ml⁻¹ with PBS, and ammonium sulfate was added to 25 % saturation. The sample was centrifuged to remove precipitate (8000 g for 15 min), and ammonium sulfate was added up to 45 % saturation. The precipitated protein was collected by centrifugation (8000 g for 15 min) and dialysed against PBS overnight at 4°C. The antibody was further purified using a HiTrap rProteinA column (Amersham) under the conditions recommended by the manufacturer.

**Inducibility of PEG-DH protein.** Strains 103 and 203 were cultivated in PEG 4000 medium and glucose medium until the OD₆₀₀ reached 1.0. Cells were harvested by centrifugation (8000 g for 20 min at 4°C), suspended in lysis buffer (50 mM Tris/HCl, pH 8.8, 500 mM NaCl, 10 %, v/v, glycerol) and disrupted by sonication (ultrasonic disruptor UD-200, Tomy Seiko) with cooling intervals. Cell debris was removed by centrifugation (8000 g for 20 min at 4°C). The supernatants designated cell-free extracts were used immediately for measuring PEG-DH activity, as described previously (Kawai *et al.*, 1985). Protein concentration was measured with the Bio-Rad protein assay kit, using BSA as a standard. The cell-free extracts were run on SDS-PAGE gels (Laemmli, 1970), and further subjected to Western blotting analysis using the anti-PEG-DH antibody and ECL Advance Western Blotting Detection kit (Amersham), employing manufacturer’s protocols.
RESULTS AND DISCUSSION

S. terrae could incorporate PEG 6000, but S. macrogoltabida strain 103 could not incorporate greater than PEG 4000

S. terrae can grow on PEG 20 000, but S. macrogoltabida strains 103 and 203 cannot grow on greater than PEG 4000 (Kawai et al., 1978). This fact led us to study their substrate-uptake ability. As shown in Fig. 2, washed cells of S. terrae could incorporate PEG 4000 and PEG 6000 efficiently, but those of strain 103 could not incorporate PEG 6000. HPLC peaks of substrates did not show any distinct change in their molecular sizes (data not shown), suggesting that metabolites do not accumulate in the supernatant. Thus, the difference in chain length of assimilable PEG between the strains is suggested to depend on a difference of substrate-uptake machinery, probably in the outer membrane. In addition, efficient uptake of PEG by washed cells suggested that the uptake is independent of energy-producing systems such as ATPases.

Operon structures in three sphingomonads

We cloned four DNA fragments flanking pegA from the total DNA of S. macrogoltabida strain 103 (Fig. 1, Supplementary Table S2). The overall sequence covered 13.9 kb. The DNA sequence contained nine genes that encoded transposase B (orf1), transposase A (orf2), TonB-dependent receptor (pegB), PEG-aldehyde dehydrogenase (pegC), permease (pegD), PEG-DH (pegA), acyl-CoA ligase (pegE), AraC-type regulator (pegR), another transposon A (orf3) and another partial transposon B (orf4). pegBCDAE was revealed to form an operon (peg operon) by RT-PCR analysis, as reported previously (Charoenpanich et al., 2006). The GC content of these genes (pegB, C, D, A, E and R) is far lower (~46–54 %) than the mean genomic GC contents of S. macrogoltabida (~63–65 %), S. terrae (~62–68 %) (Takeuchi et al., 1993). This suggests that the peg operon originated in a different genus. The same gene structure was conserved in S. terrae and S. macrogoltabida strain 203. Another transposon gene (pegF) was found in the intergenic region between pegD and pegA in strain 203, but not in other strains. The gene structure was conserved among the three strains (except pegF in strain 203), and the genes shared 99.1–99.8 % homology with one other. As a minor difference, a total of 33 nucleotide differences were found in the cloned regions among the three strains. These mismatches were neither gene specific nor strain specific.

Since PEG is not a natural compound but a synthetic polymer, the genes responsible for its metabolism should be a mosaic of genes evolved from genes with other cellular metabolic functions. PEG-DH (pegA) has already been characterized as a novel type of FAD-containing alcohol dehydrogenase that belongs to the GMC oxidoreductase group (Sugimoto et al., 2001). This protein is interesting in that it seems to be a hybrid of alcohol dehydrogenases and oxidases. The aldehyde dehydrogenase encoded by pegC is a novel NADP-containing nicotinoprotein aldehyde dehydrogenase active toward PEG-aldehyde (Ohta et al., 2005).

A general role of acyl-CoA ligase (pegE) is to activate carboxylic acids by ligating carboxylic acids and coenzyme A. The fact that the gene was PEG inducible suggested its involvement in PEG degradation (Charoenpanich et al., 2006). We have already confirmed that PegE catalyses the ligation of PEG-carboxylate and CoA (A. Tani and others, unpublished results). These results showed the involvement of an acyl-CoA ligase (PEG-carboxylate-CoA ligase) in PEG degradation. The localization analysis of the protein (Supplementary Table S2) and the necessity of ATP for the reaction suggested that the protein is a cytoplasmic membrane protein that faces the cytoplasm. The PEG-carboxylate dehydrogenase has been purified from S. terrae (although its gene has not yet been cloned) and suggested to be the third enzyme involved in the cycle of PEG metabolism (Enokibara & Kawai, 1997). The role of PEG-carboxylate-CoA formation in PEG metabolism is unclear, because such compounds cannot be metabolized via β-oxidation due to the ether bond. The protein may play an important role in decreasing the concentration of carboxylates, and also in enhancing the dehydrogenation of reactive and toxic aldehyde compounds.
The pegB-encoded TonB-dependent receptor is known to be involved in siderophore and vitamin B₁₂ uptake into the periplasmic space (Ferguson & Deisenhofer, 2002). The siderophore containing ferric ion is further transported through the inner membrane by an ATP-binding cassette (ABC) transporter (Ferguson & Deisenhofer, 2002). Some receptors are known to act as signal transducers, and the signal finally activates iron-transport genes (Schalk et al., 2004). A gene for the TonB-dependent receptor stfP encoded in the sts–sft gene cluster for sulfate ester metabolism in Pseudomonas putida has been implied to be a possible substrate translocator (Kahnert et al., 2002). Related proteins have also been identified as a possible alginate transporter in Sphingomonas sp. Al (Hashimoto et al., 2005) and as a maltodextrin transporter in Caulobacter crescentus (Neugebauer et al., 2005). Thus, the TonB-dependent receptor encoded by pegB might be a PEG translocator and/or a signal transducer. In general, the molecular limit for incorporation into the cytoplasm is considered to be about 1000 Da, while the peg operon is induced by PEG 4000–20 000. Thus, there should be a specific PEG-sensing or -translocating system in the cells, and this receptor protein is a possible candidate for the system. Otherwise, the receptor is in fact a siderophore transporter to supply ferric ions to the PEG metabolic pathway or to a related pathway that requires ferric ions.

The permease (encoded by pegD) was homologous to glycoside/pentoside/hexuronide cation symporters from various sources. Since glycoside compounds contain ether bonds in their structures, pegD might be recruited for transport of PEG (or shortened PEG compounds) from its original function as a glycoside transporter.

An AraC-type transcription regulator (pegR) was revealed to be a positive regulator of the peg operon. Expression of this gene was repressed by a GalR-type regulator with the help of the histone-like protein HU, and de-repression occurred in the presence of PEG 4000 as an inducer. PegR regulates the expression of the peg operon positively through its binding to the pegB promoter within the region that contains the activator-binding motif aral (Charoenpanich et al., 2006).

The transposon gene (pegF) inserted between pegE and pegA in strain 203 was homologous to IS256 family transposons (Kozitskaya et al., 2004). IS256 is known as a mutator transposon. The relatively high frequency of transposition of IS256 and the conservation of peg genes among three strains suggested that pegF was inserted into the peg operon of strain 203 by chance. This insertion caused constitutive expression of pegA only in strain 203, as described below.

Two sets of transposase A and B genes found up- and downstream of the peg operon in strain 103 shared 38.3 and 34.2 % homology, respectively, between the A genes and the B genes. Gene transfer by transposons is based on a set of homologous transposases (Nojiri et al., 2004; Tan, 1999). Whether or not the low homology suggests the existence of another transposon counterpart for each transposon should be further elucidated.

Detection of large plasmids and localization of the peg operon

As shown in Fig. 3, we detected one large and one small plasmid in S. macrogoltabida strain 103, and two large plasmids in S. macrogoltabida strain 203 and S. terrae. Judging from their mobility, the two plasmids in S. macrogoltabida strain 203 correspond to the 150 and 450 kb plasmids already detected by Basta et al. (2004). By hybridization, the peg operon was found to be located in the smaller plasmid in S. terrae. Thus, most probably, the peg operon has been distributed to PEG-utilizing sphingomonads via conjugative transfer of plasmids, as seen in pNL1 from Sphingomonas aromativorans F199 to other sphingomonads (Romine et al., 1999). The peg operon did not seem to contain all of the genes that confer complete degradation of PEG. Therefore, the regions further up- and
downstream of the operon or the whole plasmid, which has yet to be sequenced, may be required.

**Possible existence of the peg operon in other bacteria**

PCR with genomic DNA from other PEG degraders, *Sphingomonas* sp. strains K1 and N6 (Kawai & Takeuchi, 1996), gave specifically amplified bands of peg genes (data not shown, and pegF not tested). The 16S rDNA sequences of these two strains were most homologous to that of *S. macrogoltabida*. Subsequently, in databases, we found PEG-DH homologues, which included a (PEG-DH-like) putative alcohol dehydrogenase from *Rhodopseudomonas palustris* strain CGA009 (accession no. NC_005296), an oxidoreductase from *Bradyrhizobium japonicum* (BA000040), a dehydrogenase from *Mesorhizobium loti* (BA000012), an alcohol dehydrogenase from *P. putida* (AB100375) and a GMC family oxidoreductase from *Sericibacter pomeroyi* (NC_003911). Comparison of the surrounding regions of the above homologues with those of the peg operon suggested that there was no structure in the reported sequences that was similar to those of the five PEG-utilizing sphingomonads. These results suggested that, at present, the peg operon may only be found in strains of *S. terrae*, *S. macrogoltabida* and their close relatives. These species are taxonomically close, based on their 16S rDNA sequences, and form a cluster with *Sphingopyxis alaskensis* and *Sphingopyxis taejonensis* (Yabuuchi et al., 2002). A survey of the distribution of the peg operon among sphingomonads will provide a key to understand the evolutionary organization of the operon and the host range of the large plasmids that contain the peg operon.

**pegA was induced only by PEG in strain 103**

To investigate whether short-chain glycols acted as inducers, di-, tri- and tetraethylene glycols were used as carbon sources, and induction of pegA was checked by RT-PCR and dot-blot hybridization. These carbon sources failed to induce pegA (Fig. 4). This result suggested that the GalR-type regulator does not respond to such short ethylene glycols to de-repress the pegA promoter, although the pegB promoter was induced by these compounds (Charoenpanich et al., 2006).

**The activity of PEG-DH in strain 203 is constitutive, due to insertion of a transposon**

Since pegF was found to be inserted in between pegD and pegA in strain 203, we investigated its effect on the activity, protein expression and mRNA expression levels of pegA. As shown in Supplementary Table S3, PEG-DH activity was clearly inducible in strain 103, but constitutive in strain 203. This result was confirmed using the anti-PEG-DH antibody (Supplementary Fig. S1). Furthermore, we analysed expression of pegA and pegD by dot-blot hybridization and RT-PCR using RNA samples from strain 203 grown on glucose and PEG 4000. The result showed that transcription of pegD was induced by PEG but that transcription of pegA was constitutive (Fig. 5). These results suggested that the transposon insertion disrupted the promoter region, resulting in the constitutive transcription of pegA in strain 203. GalR is thought to be a negative regulator for the pegA promoter in the presence of the histone-like protein HU, and the regulation can be de-repressed in the presence of PEG as an inducer, as reported previously (Charoenpanich et al., 2006). Therefore, it is possible that the insertion of a transposon in the pegA promoter of strain 203 interferes with the binding of GalR to its operator or with the binding of the HU protein that is necessary for stabilizing the repressosome structure (Semsey et al., 2006).
Many insertion elements have been shown to activate the expression of neighbouring genes, and in such cases they possess outwardly directed −35 promoter hexamers located in the terminal inverted repeats (Mahillon & Chandler, 1998). pegF may have targeted the 8 bp sequence (CCTGCGAC) between the putative −35 and −10 regions of pegA. Furthermore, a putative outwardly directed −35 region was found in the transposon sequence, which seemed to serve as a −35 region for pegA transcription (Fig. 6). Thus, the constitutive expression of pegA in strain 203 is considered to be due to a lack of GalR binding and the occurrence of constitutive transcription from the promoter present in the transposon.

In this study we have revealed that PEG-degrading sphingomonads possess an operon responsible for degradation of this high-molecular-mass synthetic polymer. This is believed to be the first report on the structure of the peg operon and its distribution among sphingomonads. The operon consists of PEG-degradative genes and a putative PEG-translocating system, as well as its regulatory machinery. Since industrial PEG utilization started about 30 years ago, investigation of the structure and function of the peg operon is significant for the development of PEG-degrading sphingomonads as organisms for industrial use.
before the isolates were found, these sphingomonads have been able to evolve this xenobiotic-degradative operon. Very possibly, transposases and conjugative plasmids have permitted the organization of the operon from existing sources. The constitutive expression of PEG-DH in strain 203 alone is explained by the genetic structure of the operon in this strain. The complete sequencing of the large plasmids and comparison of their structure with that of related plasmids will reveal the evolution and origin of the operon, as well as the reasons for the differences of substrate-uptake specificity and symbiosis among PEG-degrading sphingomonads.

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