Effective Changes in Microbial Diversity of Activated Sludge Consortium in Presence of Benzene, Toluene & Xylene

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Activated sludge was adapted sequentially to benzene, toluene and o-xylene to study the effects on the change in the microbial community. Sludge suitable for benzene, toluene and o-xylene each degraded separately by different levels in a following order; toluene > o-xylene > benzene. Degradation rates were increased after exposure to points or repeated substrates. Eleven types of sludge were prepared by the benzene, toluene and o-xylene combination or sequential adaptations. Analysis of the concentration revealed that the acclimated sludge had different characteristics from non acclimated sludge and could be grouped according to their prior treatment. The sludge profile was affected by the final point of the substrate adaptation irrespective of the sequence adaptive tracking. In the sludge, adapted to 50 ppm toluene, *Nitrosomonas* sp. and bacteria were dominated, but these bands were not dominant in benzene and benzene and after toluene adaptations. Instead, *Flexibacter* sp. was dominant in these cultures. *Dechloromonas* ssp was dominant in culture adapted to 50 ppm benzene. *Thauera* sp. was the main band in the sludge adapted to 50 ppm xylene, but became vaguer as the concentration of xylene was increased. Instead, *Flexibacter* sp. dominated in the sludge adapted to 100 ppm xylene, but not in the culture adapted to 250 ppm xylene. Two bacterial species dominated in sludge suitable for 250 ppm xylene, and they also existed in the slurry suitable to achieve 250 ppm xylene, toluene and benzene.

**Keywords:** Activated Sludge, Benzene, Microbial Diversity, Toluene, Xylene

INTRODUCTION

Anthropogenic activities aimed at industrial and agricultural advancement have resulted in the nonjudicious production and usage of chemical compounds. Consequently, the environment has become heavily contaminated with chemical pollutants that are toxic both to the environment and to human health (1). However, with increasing awareness about the hazardous effects of these chemical pollutants, a polyphasic approach has been proposed to overcome this situation. This approach includes (1) stringent regulations for the production and usage of complex chemicals; (2) pre-treatment and safer disposal of toxic chemical wastes; and (3) restoration of contaminated sites and environments (4). The first two approaches are of a preventive nature and concentrate on minimizing further damage, while the latter offers a curative mechanism. Several recent research activities have focused on the use of different physico-chemical and/or biological means for the decontamination of polluted environments (5). These studies have led to a general acceptance of bioremediation as being an environmentally benign, efficient and economic measure.
for pollutant removal and restoration of contaminated sites (6). Bioremediation methods are based on the exploitation of metabolic potential for attenuation of the toxic effects of the pollutant(s) by (1) transformation to lesser toxic products; (2) complete mineralization of pollutants; and (3) immobilization of the pollutant (7). Most of the living beings including plants and higher animals exhibit a minimal basal level of detoxification ability that is expressed via the above mechanisms; however, microorganisms have been studied in greater detail for carrying out the detoxification activities (8). Microorganisms in general and bacteria in particular, harbor enormous metabolic diversity, allowing them to utilize the complex chemicals as energy sources (9). Further, their ability to undergo rapid genetic evolution also enhances their chance to acquire new metabolic potential for degradation of the recently introduced xenobiotic chemicals (10). Conventionally, studies on microbial degradation of chemical pollutants have followed a reductive approach based on the isolation and characterization of a single bacterial strain or a syntrophic bacterial consortium (which could bring together different degradative potentials) for carrying out the desired degradation under controlled laboratory conditions (11). The other major thrust area of bioremediation studies has been the characterization of metabolic pathways and their respective molecular regulations (12). Some of the relatively recent studies have also attempted to address questions related to the finer details of the biodegradation process for example transcriptional regulation, kinetic behavior and the structure–function relation of the enzyme involved in the processes, etc. (13). The advent of whole-genome sequencing and related genomics methods has also given rise to new avenues for genome-wide screening of degradative genetic elements and regulatory sequences among the pollutant-degrading strains (14). In activated sludge reactors and bioremediation of contaminated sites where microbial communities are degrading and treating wastewaters and toxic chemicals, only a subset of the microorganisms are important for the transformation of hazardous materials (15). Since pollutant fate is largely controlled by the native microbiota, a more complete understanding of community structure and activity should provide for better prediction and process control (16). Since active microorganisms in the systems are in a dynamic state and most microbes cannot be cultured by conventional culture methods, the molecular biology techniques have been useful to monitor microbial community dynamics and to investigate their activities (17). Many fingerprint methods, such as denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism, or clone libraries have gained much recent attention as useful molecular techniques for the analysis of microbial communities (18). Denaturing Gradient Gel Electrophoresis is a quick and easy method that provides a useful tool for monitoring change in microbial communities, and it can be used to evaluate the generic diversity and dynamics of the community in question (19). The change in structure of a microbial sludge community associated with benzene, toluene & xylene degradation has been investigated using denaturing gradient gel electrophoresis (19). The object of this study was to better understand the dynamics of a sludge consortium when benzene, toluene & xylene substrates were applied in different sequences. After acclimating activated sludge to each benzene, toluene & xylene compound, it was then adapted to different substrates sequentially, and the community changes were analyzed by denaturing gradient gel electrophoresis. Cluster analysis and dimensioning techniques were used to explain the denaturing gradient gel electrophoresis profile. To our knowledge, this is the first time that the effect of subsequent feeding of benzene, toluene & xylene on a microbial community has been studied.

**MATERIALS AND METHODS**

**Degradation of Benzene, Toluene & Xylene**

Activated sludge was operated in aerobic condition and washed twice with distilled water. Five ml of activated sludge was washed and inoculated to 250 ml flask with 100 ml of saline. Composition of saline was K₂HPO₄ 1.55 g / L, KH₂PO₄ · H₂O 0.83 g / l, (NH₄)₂SO₄ 2 g / l MgCl₂ · 6H₂O 100 mg / l, yeast extract 0.2 g / l, and 0.2% (v / v) of a mineral salt solution. The mineral EDTA salt solution was 5 g / l; ZnSO₄ · 7H₂O was 1 g / l; CaCl₂ · 2H₂O was 0.5 g / l; FeSO₄ · 7H₂O was 1.25 g / l; Na₂MoO₄ · 2H₂O was 0.1 g / l; CuSO₄ · 5H₂O was 0.1 g / l; CoCl₂ · 6H₂O 0.2 g / l, and MnCl₂ · 4H₂O was 0.5 g / l (19). The FeSO₄ · 7H₂O solution added separately. Benzene, toluene and o-xylene were added to cultures. The concentration of each substrate was 50 ppm (w / v) and all the cultures were incubated at 25 °C at 150 rpm. Abiotic controls were appropriate following the same procedure of corresponding cultures except inoculating activated sludge. Incubation bottles were conical flasks with gas permeable silicone caps for culturing in an open system making the condition during aerobic degradation, and cylindrical chlorobutyl bottles with rubber stoppers and aluminum serum caps for cultures in a closed system to prevent volatile benzene, toluene and xylene to evaporate out and drain into the air. In the closed system, the caps were opened and the cultures were stirred twice per day in order to maintain it in an aerobic condition. The analysis of benzene, toluene and xylene concentration was measured by gas chromatography equipped with a 60 m x 0.53 mm capillary column of MXT-VOL, a “purge and trap” blank trap, and a Tenax-GR trap installed. Carrier gas was helium at a flow rate of 10 ml/min, and detected by FID detector. The sample volume was 1 ml,
and a linear temperature program from 40οC to 180οC at the rate of 10οC/min with a temperature program for purge and trap was applied.

**Sludge Acclimatization**

Cultures were inoculated with activated sludge which was prepared, and BTX at concentrations 50, 100, and 250 ppm were fed daily to each culture separately for two weeks. Activated sludge samples were taken from each culture and analysis of microbial community structure was done. Three sludge cultures treated with toluene at different concentrations were transferred into benzene, containing media and adapted for two weeks. After sampling, these were adapted to xylene for another two weeks.

**Nucleic Acid Extraction**

After harvesting cells by centrifugation from the consortium, genomic DNAs of microorganisms were extracted by the following method as described by Muyzer et al. (17). The bacterial cells were suspended in 567 ml of TE buffer (10mM Tris–HCl, 1mM EDTA, pH 8.0), and 30 ml of 10% sodium dodecyl sulfate and 3 ml of 20 mg/ml proteinase K was added and incubated at 37 °C for 1 h. Subsequently, 100ml of 5M NaCl and 80 ml of 10% hexadecyltrimethyl ammonium bromide in 0.7M NaCl solution was added and incubated at 65°C for 10 min. An equal volume of chloroform–isoamyl alcohol (24:1 vol/vol) was added and mixed. After centrifugation, the aqueous phase was transferred to a new tube and extracted with an equal volume of phenol–chloroform–isoamyl alcohol (25:24:1 vol/vol). A 0.6 volume of iso-propanol was added to the aqueous phase transferred to a new tube. After centrifugation, the precipitated DNA was rinsed with 1ml of 70% (vol/vol) ethanol (- 20 °C), dried, and resuspended in double-distilled water (RNase, DNase free) and then stored at –20 °C.

**Polymerase Chain Reaction Amplification**

Each DNA fragment encoding 16S rRNA (corresponding to the positions 50-341 to 927-30 in the *Escherichia coli* sequence) was amplified using the eubacterial primer GM5F and the universal primer907R (18). A 40-base GC clamp was attached to the end-50 of the GM5F primer for DGGE analysis (18). The primer sequences were 50-CCTACGGAGAGGCAGCAG-30 for GM5F, 50-CCGTAATTCTTTRAGTTT-30 for 907R, and 50GCCCGCCGCGCCCGCCGCTCCGC CGCCCGCCGCCCTACGGAGAGGCAGCAG-30 for GC-GM5F. PCR amplifications were performed with a DNA Thermal cycler as described by Muyzer et al. (18). The template DNA solution for determination of the intensities of bands on a DGGE gel was diluted at adequate concentration with sterile water in order to minimize the formation of heteroduplex molecules (17). The PCR solution consisted of 76 ml of sterile water, 10 ml of 10x Ex Taq buffer with MgCl2, 25 pmol each of the primers, 10 ml of deoxynucleotide triphosphates mixture (2.5mM [each] dATP, dCTP, dGTP, and dTTP), and 1 ml of template DNA solution in a sterile 0.5 ml tube and overlaid with 2 drops of mineral oil. To minimize nonspecific annealing of the primers to non-target DNA, 2.5 U of Taq polymerase was added to the reaction mixture at 80 °C after an initial denaturing step of 94°C for 5 min. The temperature was subsequently cooled to 65°C for 1 min. This temperature was decreased by 1°C every second cycle until a touchdown of 55 °C, the temperature at which 10 cycles were carried out (18). Denaturation and primer extension were carried out at 94 °C for 1 min and at 72 °C for 3 min, respectively. Cycling was completed by a final extension at 72 °C for 10 min. The PCR products were analyzed by electrophoresis in 2 % (wt/vol) Nusieve 3:1 agarose gels containing ethidium bromide (1 mg/ml). DGGE analysis DGGE was performed as described by Muyzer et al. (18) using D-gene and D-code system with a 1.5mm gel width. Approximately, 500 ng of PCR products were applied directly onto 6% (wt/vol) polyacrylamide gels in 1 x TAE buffer (40mM Tris, 20mM acetic acid, 1mM EDTA, pH 8.3) with denaturing gradients which were formed with 6% (wt/vol) acrylamide stock solution containing 0% and 80% denaturant (5.6m Urea and 32% [vol/vol] deionized formamide). Electrophoresis was run in 1 x TAE buffer (40mM Tris, 20mM acetic acid, 1mM EDTA, pH 8.3) at a voltage of 200V and 60 °C for 4 h. After electrophoresis, the gels were incubated for 10 min in ethidium bromide (1.0 mg/l), rinsed for 10 min in distilled water, and then photographed using ATTO print graph. The intensities of bands on the digitized images were processed with the NIH Image software. DGGE bands were excised from the gels, and reamplified. The PCR products of second amplification were electrophoresed again in a DGGE gel to check the purity of the bands, and then purified using QiAquick TM PCR purification kit. The sequence of the band was determined for both strands by Cycle sequencing method with dye primer.

**Denaturing Gradient Gel Electrophoresis**

DGGE was performed with D-Gene system at 70 V and 60 °C for 720 minutes. Samples were loaded on a 6% (w / v) polyacrylamide gel (37.5: 1 acrylamide: N, N9-methylenebisacrylamide) in 0.5 × TAE buffer. In the denaturing gradient gel is formed by mixing two stock solutions, or 6%acrylamide denaturing agent containing 0% and 80% denaturing agent, respectively (5.6 M urea, 32% [v / v] formamide deionized with AG501-X8 resin) mixed bed. The separated DNA visualized by ethidium bromide spotted by the following procedure. First, the gel was removed from the glass plate. The gel was then
placed under a dish containing 250 ml of running buffer and 25 µL of 10 mg/ml ethidium bromide (50 µg/ml) for 5 min. After staining, the gel plate was transferred to buffer and destained for 15 minutes. The gel was placed on a UV transilluminator and it was photographed. Gel images were converted, normalized, and analyzed for Gel Compar II software package. DGGE profiles were compared using an independent band allocation method and a method based on the presence of band / absence. This is widely represented as the “factor r” in the regression and definition is shown in Eq. (1)

The Pearson product moment =

\[ r = \frac{\sum (X - \bar{X})(Y - \bar{Y})}{\sqrt{\sum (X - \bar{X})^2 \cdot \sum (Y - \bar{Y})^2}} \]  

(1)

where \(X, Y\) = variables, \(\bar{X}, \bar{Y}\) = means of variables, and \(N\) = total number of variables.

The Jaccard coefficient clustering is a similarity coefficient, and the definition is shown in Eq. (2)

The Jaccard coefficient = \(N_c / (N_q + N_t - N_c)\)  

(2)

Where \(N_q\) = the number of bands in the query line, \(N_t\) = the number of bands in the target line, \(N_c\) = the number of bands that are common to both the query and target lines (19). The dendrogram was constructed using unweighted pair group method with arithmetic means (UPGMA) tree building method (17). In band assignment, a 1% band position tolerance (relative to the total length of the gel) was applied, which indicates the maximal shift allowed for two bands indifferent DGGE tracks to be considered as identical. Multidimensional scaling (MDS) and principal components analysis (PCA) were performed with the Gel Compar II software package.

Phylogenetic Analysis

Sequences from DGGE bands were inserted into the BLAST programs of the National Center for Biotechnology Information (19) in order to identify phylogenetic relatives of what sequences were retrieved. Sequence alignments with 16S rRNA partial sequences of reference bacteria from Gen Bank and evolutionary distance were performed using CLASTAL W program (19). Phylogenetic trees were constructed from the evolutionary distance using Tree View software (19).

Cloning and Sequencing

DNA bands in the DGGE gel were cut with a razorblade and the DNA excised bands were eluted overnight at 4 °C in a 1.5 ml tube containing 100 ml or 13TAE buffer. The DNA was amplified by PCR with primers of both 341F without GC clamp and 907R. To obtain purified DNA, the cloning of the PCR product by the use of pGEM - T Vector System I and competent cells or ECOS 101, 9-5 was done as follows.

Five µL 2 × faster ligation buffer, 1 µL pGEM - T vector (50 ng), 2 µL PCR DNA product or control insert, 1 µL of T4 DNA Ligase and deionized water to a final volume of 10 µL were mixed by pipetting to establish ligation reactions. The solution was incubated overnight at 4 °C to obtain the maximum number of transformants. Frozen competent cells were thawed at room temperature to obtain a thawed state by about a third, and the ligation mixture was added immediately to obtain a mixture of 5% (v/v). This solution was vortexed for 1 second and incubated in a water bath at 42 °C for 45 sec. Then the mixing tube was vortexed again for 1 sec, and transferred on to chilled, dry selection medium and spread with 20 µL 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and 10 µL Isopropyl-β-D-thiogalactopyranoside using 4 or 5 glass beads for plating. Plates were incubated at 37 °C for 16 h, and the existence of transformed colonies was observed. After harvest, the white colonies of plates, colonies were incubated in 5 ml of LB broth for 24 h. The plasmid was derived from the cell using an UltraClean 6 Minute Mini Plasmid Prep Kit.
RESULTS AND DISCUSSION

Activated sludge adapted to each BTX 50 ppm for two week. Although adaptation periods were normally about a month, there was a report that the acclimatized microbial consortium may degrade toluene and benzene within a few days after the adjustment, as in an anaerobic (19) system. Because our sludge was obtained from a water treatment plant, and was therefore suitable wash water completely, we decided for the adjustment period two weeks. Resulting degradation profiles (Figure 1 and 2) showed that BTX were degraded within a few ppm, which confirmed the state of adaptation were reached. Each BTX was introduced as a sole carbon source for improve community change. The rates of degradation or BTX were measured after two weeks. As illustrated in Figure 1 and 2, the difference in degradation rate between the abiotic control and sludge culture was not distinct in the open system. For degradation of benzene, the disappearance in the mud culture was slower than the abiotic control. The reason was thought to be what dispersed sludge inhibited BTX by evaporation the reduction of the air contact surface, and delayed time volatilization in the culture of sludge. Furthermore, benzene had a time of delay time to the first peak, which was shown in the closed system. Benzene was not degraded first a few hours by the mud, and thus reduces the rate was slower than for the abiotic control. Toluene was no shift time and degradation rate was fast. Therefore, the rate of degradation of the sludge culture was faster than biotic control. As xylene degradation rate was between that of benzene and toluene in the concentration profiles between abiotic and biotic cultures outdoor system was almost similar. In the closed system, the toluene was degraded faster as benzene and o-xylene. This is a general phenomenon given the route of
The first aerobic degradation pathways step BTX is oxidation of the benzene ring attached to a hydroxyl group by mono and dioxygenase. This reaction has changed BTX the catechol-like chemicals, catechol-like metabolite catabolized yet to enter the Krebs cycle. Toluene, methyl, and slightly lateral group distort the electron cloud of the benzene ring to become responsive to enzymatic attack. No other group of long side as could give steric hindrance is the reaction. Besides that, benzene has no side chain, so that the ring conformation is perfectly stabilized by resonance. Therefore, the ring attack benzene enzyme is very difficult compared to toluene. Xylene has two side methyl groups and the side groups may give rise to the effect of steric hindrance (14,15). In the closed system, the degradation of benzene is the slower reaction between them. Degradation of toluene was completed after 5.5 h the third successive peak and xylene and benzene were completed after 6 and 8.5 h, respectively. Completion times were reduced as number of peaks substrate was increased for three compounds. Latency degradation of benzene was greatly reduced by the successive peak substrate. The accelerated by enzymes accumulated in the liquid the media was thought to be the main reason. Toluene and the xylene degradation also showed the same phenomena reducing latency. The nested PCR was used to obtain a large amount and concentration of DNA for DGGE. The whole area the 16S rRNA gene of the genomic DNA was amplified and small region PCR with GC-clamp using the primers16S rRNA as a template was performed. The nested-PCR product obtained easily large quantities of DNA and gave a clear band of DNA after amplification relative to direct-PCR from the chromosomal DNA. Eleven types of samples of DNA were extracted; original sludge (sludge), 50 ppm benzene adapted sludge(Benzene 50), 100 ppm benzene adapted sludge (benzene100), 250 ppm benzene adapted sludge (benzene250)50 ppm toluene adapted sludge (Toluene 50), 50 ppm sludge in xylene adapted (Xylene 50), 100 ppm xylene adapted sludge (Xylene 100), 250 ppm xylene adapted sludge (Xylene 250), 100 ppm benzene adapted sludge after 100 ppm toluene adaptation (TB 100), 250 ppm of benzene adapted sludge after 250 ppm adaptation toluene (TB 250), and 250 ppm xylen adapted after 250 ppm of toluene followed by adaptation 250 ppm adaptation benzene (TBX 250). As illustrated in Figure 3, many visible bands were observed. The arrows in figure 3 bands indicate excised identify species, the results are presented in Table 1. Clustering analyzes were performed to determine the correlation and the similarity between the strips. The coefficient binary analysis Jaccard and Dice are shown in Figure 4. In Jaccard analysis, three categories of DGGE bands have been set around a level of similarity50%. Although the level of 50% similarity was not so low in the bands of clustering, it was difficult to find the same or similar processing conditions for the communities within each category. According to the Dice outcome, the same groups were found, with a degree of similarity greater than 70%. As similar methods of analysis methods to measure similarity based on common and different bands, the resulting cluster groups were exactly the same. Some researchers said the product moment Pearson correlation coefficient analysis was affected much less tape-based similarity coefficients by the amount of PCR products loaded on the gel and was quick, objective method for comparing microbial community profiles (16,17). This was confirmed by this research that higher clusters of the similarity value and more reasonable were obtained using the Pearson correlation coefficient and the cosine correlation coefficient with respect to the cluster analyzes based on the absence or presence of the strip only, as the Jaccard and Dice coefficients. In the Pearson correlation coefficient in fig. 5, two different groups were revealed. One consisted mainly of communities that were suitable low concentration of BTX (50 ppm), and the other consisted of large communities (over 100 ppm) BT concentration. From the group of lower portion of Figure 5, the communities acclimated to benzene have profiles similarity values greater than 90%. From this analysis, microbial adapted communities directly benzene and the appropriate toluene to benzene after adaptation could be grouped. Overall, while benzene 50 was not so closely linked with others. Xylene adaptation of communities also showed similar high values of more than 85% similarity. The community profiles themselves in DGGE also given a different configuration of bands. The intense band in DGGE is digital dominance in the microbial community. Although digital domination does not necessarily reflect the catabolic activity domination (14), these intense bands probably represented catabolically active species in this study, since each community had been started in the same mud affected by different selection pressures.MDS and PCA are technical, design that produces two or three-dimensional plots in which the entries are divided according to their relatives. Using PCA and MDS analysis, the various data of the complex DGGE patterns of a sample could be reduced to a point in a three dimensional space. MDS is not analyzing all the original data, but the similarity matrix obtained by means of a similarity coefficient. The bars displayed MDS within Figure 6 were prepared according to the dendrogram Dice coefficient, because the similarity matrix coefficients used in MDS is the same as that used in the calculation dice. However, the relationships between each channel are different. Consortia benzene modifications were closely related to each other, except one acclimated to 250 ppm of benzene and those of the appropriate xylene were close in space, although they does not hold or share a position on a point in space. The slurry in toluene was slightly adapted to measure both benzene and xylenes, and the original sludge was located away from others. This analysis was difficult.
interpret because of its complexity characteristics. However, the relationship between each component was more precise with respect to the analysis of the dendrogram because dendrogram often simplifies the data available in a similarity matrix, and tends to produce overestimated hierarchies, and these characteristics were revealed in our study. An analysis of the APC is different from MDS, in that the data are directly analyzed. Fingerprint patterns cannot be treated generating a correspondence table of the first strip in PCA (4). The table is made of the information of the presence of the tape and absence, with a quantitative value as a band intensity, which has been done previously in the analysis of clustering in our case. As illustrated in Figure 7, APC result was similar to MDS result. The consortium sludge was located away from the other, which was the same result that the analysis of cluster and MDS. Toluene 50 was located far away from the others. Consortia were acclimated only in benzene at different concentrations were separate din a corner space. Furthermore, xylene 50, the xylene100 Xylene 250 and TBX 250 were intertwined long X axis (primary PCA axis), and tuberculosis and tuberculosis were 100 250also closely related and nearly 250 positions in benzene already grouped at Pearson analysis. All clustering analysis and design techniques made not show the same results for a picture. However, some common results could be drawn from these analyzes. A similar consortium could be obtained if the sludge tracks the same adjustment procedure, which has been obtained from100 TB and TB 250. We thought they could xylene be very different from those of benzene, but this conclusion has been difficult to derive because those xylene were not closely correlated and sometimes were located near benzene or toluene those. Benzene and 250,250 were TBX wave in the clarity of the band, which changed the positions in some, analyzes. DGGE data must be supplemented by phylogenetic analysis, as was done in this study. DNA sequencing from the excised band was performed by direct sequencing or cloning t-vector. The result of the band sequencing analysis is shown in Table 1. In the way of sludge, many bands were observed and two visible bands were sequenced as sludge and bacteria uncultured bacteria. After adaptation benzene, BTX 4 group that was closely linked to Dechloromonas sp. was dominant to 50 ppm and became vague in a high concentration. These two groups were also showed other benzene-related ways, but were less that a separate toluene. These Nitrosomonas sp. (BTX 6) and bacteria (BTX 7) may be consideredtoluene-degrading species and have the potential to degradebenzene or benzene metabolite. BTX 10 in benzene 10positioned at the same length of the migration was as BTX 7revealed as an alpha- proteobacteria. Whereas xylene linked channels, BTX 3 which has been closely related to Thauera sp. was dominantXylene 50 routes and could be seen on Xylene 100route. Since Thauera sp. was previously reported be capable of degrading toluene in terms denitrifying (19), this species may have the ability to degrade xylene. Xylene 250 Lane, two bacteria (BTX 8and the band corresponding to 7 BTX) were increasingly dominant and these species were also presented to the TBX250 channels. He was certain that these species were xylene degrading microbes, because they are not dominant microbes in the concentration of low xylene. The consortium change from 50 ppm to 250 ppm was obvious but abrupt, although the same bands were still observable in the TBX250 channels. The phylogenetic tree using the sequential analysis is shown in fig. 8. According to the general ecological principles more extreme environment should be less diversified. Accordingly, the number of groups is arranged to decrease after adapt to toxic chemicals, and produce similar and closely related sequences (micro diversity). However, these sequences can easily result in the heteroduplex formation (20, 21, 22). Several heteroduplexes were revealed in our experience and they were discarded. In this study, the bacterial communities are acclimatized differently by each BTX and probably acclimated to community similar when the same substrate was fed regardless of the concentration. Moreover communities having different ways to adapt to the same substrate showed a similar microbial consortium.

REFERENCES

electrophoresis (DGGE) and fluorescent in situ hybridization (FISH).


