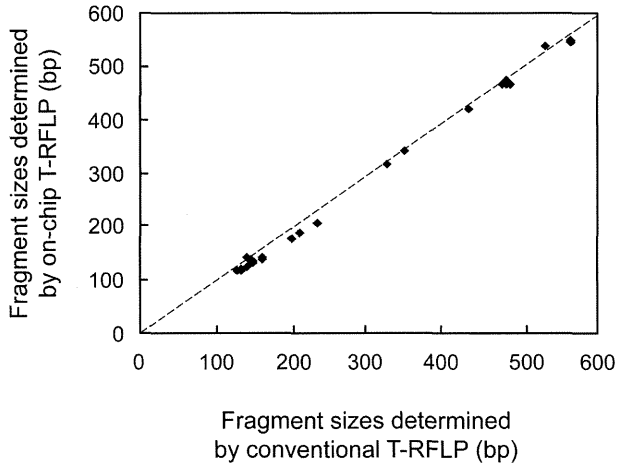

Fig. 3. Similarity of Fragment Sizes of Bacterial DNA in Freshwater (Spring Water, River Water, and Hydroponics Solution) Determined by Conventional and on-Chip T-RFLP

PCR products were digested with *Hha*I, *Mbo*I or *Msp*I. The dotted line represents linear regression.

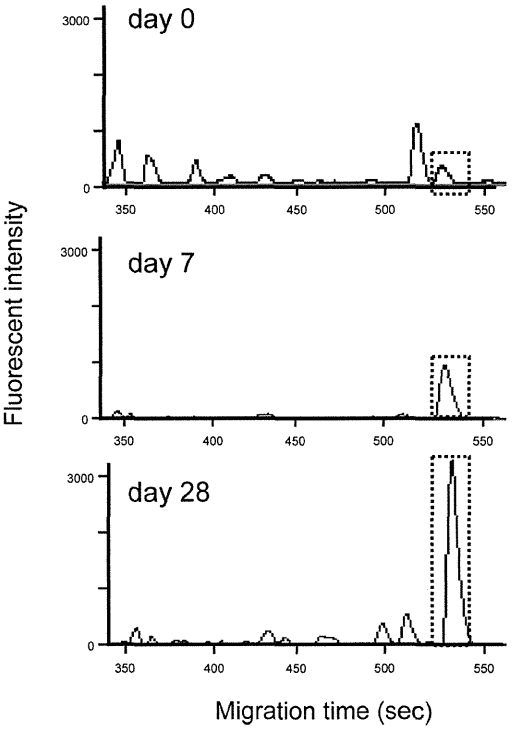

Fig. 4. Profiling of Temporal Variation in the Bacterial Community Structure of Hydroponics Solution by on-Chip T-RFLP Analysis

Dotted squares represent the most commonly observed peak among three samples.

solution during hydroponic culture. In addition, the appearance and disappearance of some peaks were observed by on-chip T-RFLP analysis. We identified the dominant bacterium shown in Fig. 4 by the database MiCA3[11] (http://mica.ibest.uidaho.edu/) and the result was "uncultured bacterium." More than 90% of bacteria in aquatic environments are difficult to culture under conventional conditions[1-3] and this result was convincing.

Furthermore, we analyzed T-RFLP profiles of nine hydroponics solution samples by MDS analysis (Fig. 5). MDS is a nonmetric procedure that is robust to outliers and preserves the rank orders of the relative distances among points in the higher dimensional data cloud. An important component of the plot is a measure of the goodness of fit of the final plot, termed the "stress." A stress less than 0.2 indicates a useful two-dimensional picture, while a stress greater than 0.2 indicates that the plot is close to random. MDS analysis showed similar results between the two T-RFLP analysis methods; large changes (Fig. 5; C to D and G to H) and stability (Fig. 5; E to F and H to I) in bacterial community structure during hydroponic culture were detected by both analyses. These results demonstrated that on-chip T-RFLP analysis could monitor changes in bacterial community structure, as well as conventional capillary T-RFLP analysis.

Several types of microchips have been developed for DNA extraction or PCR.[14] Microchip-based T-RFLP analysis examined in this study could be combined with these other microchip-based systems, and this integrated system will enable whole on-site bacterial monitoring. Total bacterial number of a targeted freshwater sample can be first determined by a microchip-based system for rapid quantification of bacteria in freshwater.[2,3] In addition, the bacterial community structure should be analyzed by a microchip-based system for DNA extraction, PCR, and on-chip T-RFLP to determine whether

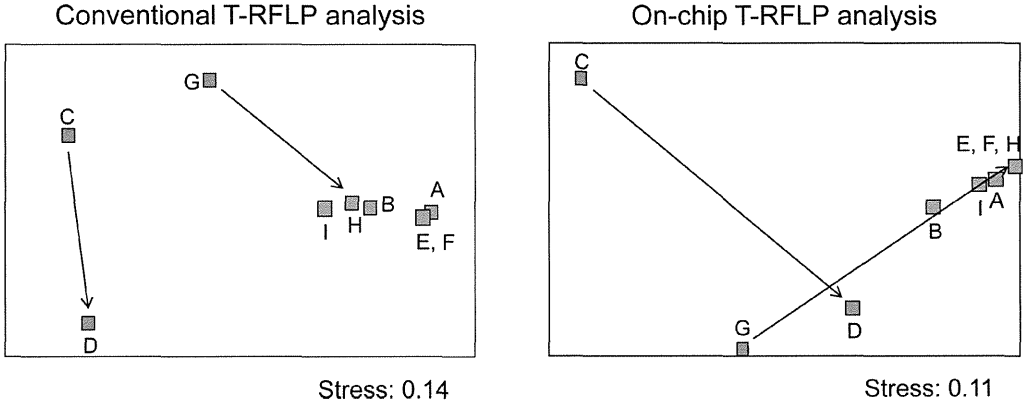

Fig. 5. MDS Analysis of T-RFLP Profiles of the Bacterial Community in Hydroponics Solution

A and B: control (without culture), C to D: 1-week-old culture (C: day 0, D: day 5), E to F: 2-week-old culture (E: day 0, F: day 14), and G to I: 4-week-old culture (G: day 0, H: day 7, I: day 28).

harmful bacteria are increasing when total bacterial number is increasing.

The present results indicate that on-chip T-RFLP analysis is an effective tool for "on-site" bacterial community profiling in freshwater environments, as well as freshwater used for medical[20,21] and industrial purposes.[1] Furthermore, people have activities in confined habitat such as Antarctic research bases and International Space Station. The importance of on-site monitoring, which means analysis at the sampling points, is now increasing especially in closed habitation systems. In microbiological monitoring, we usually take samples at the sampling points and bring the samples to a laboratory for the analysis. On the other hand, for microbiological monitoring in the International Space Station, astronauts have to bring samples to a laboratory on the ground for the analysis, while it takes more than a few weeks (sometimes more than several months). It is important to detect outbreaks of harmful bacteria as soon as possible for microbiological safety assurance and avoid infectious diseases among astronauts because of limited treatment and isolation possibilities and no immediate return to Earth. In addition, it is thought that diversity of bacterial community decreased in these confined environments where both air and water are regenerated and reused. Some harmful bacteria can sometimes easily increase in simplified microbial ecosystems.[19] "Real-time" and "on-site" microbiological monitoring is therefore more important in closed habitation systems than our usual living environments. On-chip T-RFLP system is small, portable and easy to maintain, and microchip is more robust than glass capillary. This rapid microbiological monitoring technique is very useful to assure microbiological safety in confined habitat where space and resources are limited.

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