Title
Phylogenetic Analysis of Shewanella Strains by DNA Relatedness Derived from Whole Genome Microarray DNA-DNA Hybridization and Comparison with Other Methods

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Phylogenetic analyses were done for the Shewanella strains isolated from Baltic Sea (38 strains), US DOE Hanford Uranium bioaccumulation site (Hanford Reach of the Columbia River (HRCR), 14 strains), Pacific Ocean and Hawaiian sediments (8 strains), and strains from other resources (16 strains) with three out group strains. Rhodopseudomonas palustris, Clostridium cellulolyticum, and Thermotoga maritima ethanolicus X514, using DNA relatedness derived from WCGA-based DNA-DNA hybridizations, sequence similarities of 16S rRNA gene and gyrB gene, and sequence similarities of loci of Shewanella genome selected from a shared gene list of the Shewanella strains with whole genome sequenced based on the average nucleotide identity of them (ANI). The phylogenetic trees based on 16S rRNA and gyrB gene sequences, and DNA relatedness derived from WCGA hybridizations of the tested Shewanella strains share exactly the same sub-clusters with very few exceptions, in which the strains were basically grouped by species. However, the phylogenetic analysis based on DNA relatedness derived from WCGA hybridizations dramatically increased the differentiation resolution at species and strain levels within Shewanella genus. When the tree based on DNA relatedness derived from WCGA hybridizations was compared to the tree based on the combined sequences of the selected functional genes (8 loci), we found that the resolutions of both methods are similar, but the clustering of the tree based on DNA relatedness derived from WCGA hybridizations was clearer. These results indicate that WCGA-based DNA-DNA hybridization is an idea alternative of conventional DNA-DNA hybridization methods and it is superior to the phylogenetics methods based on sequence similarities of single genes. Detailed analysis is being performed for the re-classification of the strains examined.

Keywords: Phylogenetic analysis, Shewanella, whole genome Microarray

METHODS AND STRAINS

1. Strains tested (table 1);
2. DNA isolation: The genomic DNAs were isolated from pure cultures using phenol/chloroform method.
3. 16S rRNA and gyrB amplification and phylogenetic tree construction. 16S rRNA and gyrB were amplified from genomic DNAs, Sequenced at Oklahoma Medical Research Center. Phylogenetic tree were constructed using MEGA software with NJ method.
4. Microarray Construction, Labeling, and Hybridization. Genomic DNAs were printed on glass slides at concentrations of 500 ng/µl. Genomic DNA from collected Shewanella spp., as well as several strains not belonging to Shewanella (Thermotoga maritima ethanolicus X514, Rhodopseudomonas palustris, and Paracoccus denitrificans) as outsid groups were printed on the arrays. Whole genomic DNA of target strain was fluorescently labeled using the random priming method, and Microarray hybridization was carried out on Tecan and performed as described by Wu, L et al (2004).
5. Microarray Scanning and Data Analysis. A ScanArray 5000 Microarray Analysis System was used for scanning microarrays at a resolution of 5 µm with 75% laser power and 65% PMT gain. Scanned image displays were analyzed using ImageJ version 4.0. A grid of individual circles defining the location of each DNA spot on the array was superimposed on the image to designate each fluorescent spot to be quantified. Mean signal intensity was determined for each spot. For decreasing the variations of DNA amount from printing, the corresponding signals of Syto 61 of the same strains stained after completely washed were used for normalization. 6. Phylogenetic analysis. A distant matrix of DNA relatedness was constructed according to mean signal intensity among tested strains, and clustered using MEGA software. Phylogenetic analyses were done for sequence similarities of 16S rRNA gyrB gene, and multiple loci using the same methods.