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Article 1-2. Classification of Bacteria by DNA Genetic Marker and Its Theory/DNA 遗传标记与细菌分类以及相关理论

Author: Liu Huan(1983 -), Master of Science (First Class Honours, 2009), The University of Auckland.

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Abstract

This article designs new methodology to systematically classify both genotype and phenotype of bacteria, and the relevant theories are discussed in detail. Especially, the methods of weighted average evaluation on enzyme activity is proposed.

Key words: Classification of Bacteria, Genotype, Phenotype, DNA Genetic Marker, Weighted Average of Enzyme Activity.

Methods

In addition to the classification of genotype, a supplementary metabolomics test is advised for further classification of bacteria phenotype, and the detailed methods of metabolomics test is presented in my another article this journal [5], resulting in more specific classification of bacteria families related to the incidence of pathological characters. In principal, the more enzyme species variation between pathogenic bacteria families, the higher pathogenicity against the epidemiological receptors due to the higher environmental adaptiveness of pathogens. Consequently, this methodology is listed below:

Step 1. Each bacterium is isolated from bacteria samples, and cultivated separately in situ forming a bacterium stream. Then each bacterium stream is named as stream 1, stream 2, ... , stream n.

Step 2. The cytoplasm sample is abstracted in each bacterium stream labeled for subsequent step 8, and the abstracting procedure and storage of isozyme is listed in page 47 of isozyme chapter [1]. Then the genome sample of each bacterium stream labeled is prepared for step 3.

Step 3. The molecular cytogenetic karyotype of each bacterium stream labeled is analyzed by fluorescence in situ hybridization (FISH) technique [2] using transmission electron microscopy;

Step 4. These bacterium streams are classified by multivariate cluster analysis and genetic distance analysis on the basis of molecular cytogenetic karyotype, preliminarily leading to different families of bacteria;

Step 5. The optima sampling units of each preliminary bacteria family, which can well represent the genetic diversity of each bacteria family, is examined and determined as pointed out by Liu et al.,(2015) [3] for further classification based on DNA molecular marker (SSR or AFLP);

Step 6. Classification of preliminary bacteria families is further conducted on the basis of DNA molecular marker, analyzed by multivariate cluster analysis of UPGMA (unweighted pair group method with arithmetic averages) and genetic distance analysis;

Step 7. Gene recombination and gene mutation rate is analyzed by the inconsistency of classification between molecular cytogenetic karyotype and DNA molecular marker;

Step 8. Bacteria family 'F' is identified by cluster analysis and genetic distance analysis based on DNA molecular markers (genotype), which results in apparent incidence of pathological characters when exposure dose to bacteria family 'F' increases significantly (phenotype);

Step 9. Biochemical samples are abstracted from streams of Bacteria family 'F,' leading to various zymogram calculated as the average similarity coefficient across different isozyme families, which is listed [5]. A novel matrix of Matrix X_{sum} is designed for this classification across different bacteria bio-samples [7].

Step 10. Bacteria family 'F' is further classified into different sub-families by UPGMA (unweighted pair group method with arithmetic averages) method on the basis of the average similarity coefficient across different isozyme families between any two streams. The UPGMA calculation is listed in page 63 of isozyme chapter [1].

Step 11. Sub-Bacteria families, named as F1, F2 .... Fn, should be more specific in terms of correlation to the incidence of pathological characters.

Discussion

Note: the hypotheses and discussion about virus in my another article [6] are also applicable on the bacteria ecosystem. This designed methodology is also applicable on other more advanced creatures (such as plants), and step 3 to step 6 may be replaced by molecular structure biology method designed by my another article [8] to facilitate and improve the DNA classification, if it is necessary. The calculation of

similarity coefficient between zymogram of different bacteria families is performed within one isozyme family[1]. However, this method is performed on the basis of unweighted average. Hence this article advises the steps of analyzing the zymograms with weighted average in future research:

There are two different kinds of method to evaluate the weight average of each enzyme species designed in my research:

1. There is only one isozyme family tested between different bio-samples. If the electrophoresis pipe is the vertical one, then the horizontal bands in a pipe represent various enzyme species in an isozyme family. The bands at the same horizontal line between different pipes represent the same enzyme species between different bio-samples, and the clearness of bands indicates activity of enzyme species (the more clearness, the higher activity of enzyme). Please note: the reproduction rate of microbial streams varies among different environmental cultivation conditions. Consequently, the density of microbial samples should be counted, ensuring the uniform concentration of microbial samples for the enzyme activity observation. Then the weight of each enzyme species can be conveniently evaluated according to the clearness (activity) of electrophoresis bands.

2.The whole environmental conditions (such as temperature) are simulated in situ from T1 to Tn (T1,T2,……,Tn). Within the environmental range [T1, Tn], the range of [T2, Ta] is the environmental range triggering the gene expression of enzyme species A, and the range of [T3, Tb] is the environmental range triggering the gene expression of enzyme species B,….. etc. Consequently, the weight of enzyme species A is the ratio of range [T2,Ta] to the total range [T1, Tn], and the weight of enzyme species B is the ratio of range [T2, Tb] to the total range [T1,Tn],… etc.

Then the similarity coefficient in one isozyme family between zymogram of different bacteria sub-families is calculated as: similarity coefficient = $2*\sum(\text{enzyme i} * \text{weight i}) / \{\sum(\text{enzyme j} * \text{weight j}) + \sum(\text{enzyme k} * \text{weight k})\}$. In this equation, enzyme j is the enzyme species in bacteria sub-family 1 and weight j is the weight of enzyme species j; enzyme k is the enzyme species in bacteria sub-family 2 and weight k is the weight of enzyme species k; enzyme i is the common (or same) enzyme species between sub-family 1 and sub-family 2.

In principle, the gene expression of enzyme species A should start at the environmental condition T2 with increasing activity along the environmental gradient, and the activity should decrease after the peak value, until gene expression ceases at environmental condition Ta, which can be observed by the ‘smooth’ comparison of one bacteria sub-family’s zymograms between different bacteria cultivation conditions. Of course, this weighted average calculation is also applicable on the cells of other more advanced creatures (such as plants).

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