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## Article 6-1: Metabolomics and Immunology Cultivation /新陈代谢组学与免疫力培养

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### Introduction

This article designs a novel method to analyze the isozyme electrophoretograms across different isozyme families, targeting the cultivation of environmental adaptiveness or immunology.

### Methods:

The same strain of microbes is divided into two samples for the bio-signal simulation:

1. There are two kinds of cultivation conditions simulated in Lab for microbe reproduction process: one is the 'comfortable' condition (Sample 1); the other is under UV-B radiation for cultivation (Sample 2). The microbe samples are collected after sufficient reproduction process (at least ten generations).

2. After sufficient reproduction process, the UV-B radiation simulation stops. Then both sample 1 and sample 2 are separately transferred into moisture simulation process: different moisture conditions of microbial cultivation are simulated in Lab, and labeled as T1, T2, ..., Tn.

3. Metabolomics tests are conducted after moisture simulation of T1, T2, ..., Tn respectively, resulting in different zymograms as: M1, M2, ..., Mn. The procedure of this test is presented in previous article of this journal[2].

4. Each isozyme family is labeled as 1, 2, 3..., and E; It is hypothesized that the bands at the same line across different isozyme families are the enzyme species at the same locus, named as enzyme 'species i' ( $i = 1, 2, \dots, I$ ), and each isozyme family has the same amount (I) of enzyme species (Please note: this is different from the identification of real enzyme species). Then there is a 3-dimension ( $I \times E \times N$ ) matrix presented in this research. I is the total amount of enzyme species within a isozyme family; E is the total amount of isozyme families; N is the total amount of zymograms among different simulated moisture conditions:

5. Then there is a 3-dimension ( $I \times E \times N$ ) matrix presented in this research. I is the total amount of enzyme species within a isozyme family; E is the total amount of

## Environmental Physiology/环境生理学

isozyme families; N is the total amount of zymograms among different simulated moisture conditions:

$$X = | X_{ien} | (i = 1, 2, \dots, I; e = 1, 2, \dots, E; n = 1, 2, \dots, N)$$

$X_{ien}$  is the occurrence of enzyme 'species  $i$ ' in the isozyme 'family  $e$ ' during simulated moisture condition  $T_n$ . The value of  $X_{ien}$  is one or zero. If the electrophoresis band occurs at this locus, the value is one; otherwise it is zero. The matrix  $X$  is below:

$$\begin{vmatrix} X_{111} & X_{112} & \dots & X_{11n} & X_{121} & X_{122} & \dots & X_{12n} & \dots & X_{1e1} & X_{1e2} & \dots & X_{1en} \\ X_{211} & X_{212} & \dots & X_{21n} & X_{221} & X_{222} & \dots & X_{22n} & \dots & X_{2e1} & X_{2e2} & \dots & X_{2en} \\ \dots & \dots & \dots & \dots & \dots & \dots & \dots & \dots & \dots & \dots & \dots & \dots & \dots \\ X_{i11} & X_{i12} & \dots & X_{i1n} & X_{i21} & X_{i22} & \dots & X_{i2n} & \dots & X_{ie1} & X_{ie2} & \dots & X_{ien} \end{vmatrix}$$

Matrix  $S_e = X_e \times (X_e)^T$ , where  $X_e = | X_{in} | (i = 1, 2, \dots, I; n = 1, 2, \dots, N)$ ;  $(X_e)^T$  is the transpose of the matrix  $X_e$ . The matrix  $X_e$  is below:

$$\begin{vmatrix} X_{11} & X_{12} & \dots & X_{1n} \\ X_{21} & X_{22} & \dots & X_{2n} \\ \dots & \dots & \dots & \dots \\ X_{i1} & X_{i2} & \dots & X_{in} \end{vmatrix}$$

The Principal Components Analysis (PCA) method of matrix  $X$  is specified [1]. However, the overall matrix  $X$  can be divided by sub-factors: PCA is firstly conducted on the basis of matrix  $S_e$ , revealing the biochemical dynamics of a isozyme 'family  $e$ ' among different simulated moisture conditions. In matrix  $S_e$ , it is hypothesized that the variable in PCA represents the biochemistry dynamics of each enzyme 'species  $i$ '.

$$\text{Matrix } S = \sum_E^1 S_e (e = 1, 2, \dots, E)$$

PCA is further conducted on the basis of matrix  $S$ , revealing the biochemical dynamics among different isozyme families over the whole simulated moisture conditions. In matrix  $S$ , it is hypothesized that the variable in PCA represents the biochemistry dynamics of each enzyme 'species  $i$ ' across all the isozyme families. Further application has been discussed in later articles of this journal.

6. However, for the comparison between sample 1 and sample 2, this article needs to present more procedures for subsequent analysis: in matrix Se, the biochemistry dynamics of the first three enzyme species, which contribute to the most variations by PCA in an isozyme family, are selected for comparison between sample 1 and sample 2; in matrix S, the biochemistry dynamics of the first three enzyme species, which contribute to the most variations by PCA across all the isozyme families, are selected for comparison between sample 1 and sample 2; the sum percentages of the first three enzyme species in an isozyme family (= the sum Variance Contribution Ratio (VCR) of the first three enzyme species in matrix Se), represents the total variation of an isozyme family over the whole simulated moisture conditions; the sum percentages of the first three enzyme species across all the isozyme families (= the sum Variance Contribution Ratio (VCR) of the first three enzyme species in matrix S), represents the variation of the total zymograms over the whole simulated moisture conditions. Finally, the sum VCP are compared between sample 1 and sample 2.

### Discussion

1. The higher variation in biochemical dynamics of enzyme expression, the better environmental adaptiveness or immunology. It is deduced that the biochemistry dynamics of the first three isozyme families (the sum VCR), which explain the highest variation by PCA, determines the conclusion of this comparison;

2. It is expected that sample 2 leads to higher variation in biochemical dynamics of enzyme expression, in terms of the higher sum VCR, which is also revealed by the higher adaptiveness during drought stress or higher immunology.

3. The findings of this article further support the theory proposed by other articles of this journal: the memory of cells can be 'trained' by the biophysical simulation in site, indicated by the zymograms in metabolomics test. **Consequently, the memory of cells, in terms of identifying the bio-signals of a specific environmental factor (can be biotic or abiotic) triggering the gene expression for environmental adaptiveness or immunology, can be trained and strengthened by the biophysical simulation of other environmental factors, because multiple gene traits of environmental adaptiveness or immunology should be located in the same linkage group of genome.** The next article (biophysical simulation for blood cell division) further supports above theories (please note: the theory, 'memory' of gene expression, is also applicable on cell division in an individual) by assessment of resistance or immunology in host cells.

## Environmental Physiology/环境生理学

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