# Study on the Genetic Variation of *Physalospore piricola* in Appleby Using RAPD Technique[1]

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**ABSTRACT In order to clarify the phenomenon of genetic variation in 4 different disease symptoms of apple disease, caused by *Physalospore piriola*, at molecular level, and the degree of genetic variation in 4 isolates of pathogenetic bacteria, the DNA fingerprint analysis of 26 related bacterial isolates of *Ph. piricola* from three major productive regions of apple in China was made by using RAPD technique. The results indicated that (1) Obvious genetic differences at the level of genomic DNA were found in different pathogenic bacterial isolate, and fingerprint of 4 of 11 polymorphic primers of amplification could distinguish the above mentioned 4 bacterial isolates from each other, among which the genetic distance was revealed by 0.1314~0.2541 on the basis of calculation of analytic data; (2) High genetic similarity (88.4~100%) between the identical symptom types from different sources of pathogenic bacteria were shown. It thus indicates the insignificant relation between the genetic differentiation and geographic difference. Above results of research are of importance in theoretic demonstration about the heredity and evolution of the disease *Ph. piricola* in apple fruit as well as in practice in improvement of identification and control technique for the disease.**

**Keywords: apple fruit, *Physalospore piricola* diseased, genetic differentiation, RAPD analysis**

## 1. INTRODUCTION

Disease *Physalospore piricola* in apple fruit causes the serious disease of fruit rotin apple. It was found in recent investigation and survey that, in three main production regions of Apple in China, Bohai apple production region (Including Hebei, Liaoning, Shandong, Beijing and Tianjin), Jiang-Huai apple production regions (Jiangsu, Anhui, West Shandong and shangqiu District of East Henan) and hill-apple-production region of west China (including major parts of Henan, Shanxi, Shanxi, Gansu and Ningxia Hui Nat. AR). Besides the typical epidemic D-type of *Ph. pricola* spreading around, stronger pathogenic types occurred and spread, types A, B, and F. More identification of this disease was made through laboratory and field inoculation by Zhu Shihong, *et al*. and the results proved that the above three pathogenic types revealed constant pathogenicity to apple. It indicated that there exists the phenomenon of genetic differentiation in different pathogenic bacteria. The present study was conducted for the purpose of examination of the polymorphism of DNA of the isolates in different pathogenic bacterial types originated from one pathogenic bacteria and the isolates in identical pathogenic type originated from different pathogenic bacteria, and thus for further clarifying the degree of genetic variation shown in the above research results and that in different pathogenic bacteria, in order to provide theoretical basis for improvement and creation of identification of *Ph. piricola* disease in apple fruit and techniques of disease control.

## 2. MATERIALS AND METHODS

### 2.1. Pathogenic bacteria strains

The 26 tested bacteria isolates of *Ph. piricola* were sampled from the mature fruits of Fuji apple variety, cultivated in three major apple production regions and were used in experiment after isolation and purification which involved 7 isolates for each of type A, B and D and 5 isolates for type F (see **Table 1**).

**Table 1** Isolates used in research and the pathogenic bacterial types (T) of symptom

| No. | Isolates | T | Sources | No. | Isolates | T | Sources |
|---|---|---|---|---|---|---|---|
| 1 | RSFA | A | Rushan, Shandong | 14 | TGFB | B | Tongguan, Shanxi |
| 2 | FXFA | A | Feng xian, Jiangsu | 15 | RSFD | D | Rushan, Shandong |
| 3 | TGFA | A | Tongguan, Shanxi | 16 | FXFD | D | Feng xian, Jiangsu |
| 4 | BXFA | A | Bing xian, Shanxi | 17 | YCFD | D | Yuncheng, Shanxi |
| 5 | TAFA | A | Tai'an, Shandong | 18 | SZFD | D | Suizhing Liaoning |
| 6 | TXFA | A | Tong xian, Beijing | 19 | TSFD | D | Tianshui, Gansu |
| 7 | TSFA | B | Tangshan, Hebei | 20 | TXFD | D | Tong xian, Beijing |
| 8 | GLGB | B | Dalian, Liaoning | 21 | TSFD | D | Tangshan, Hebei |
| 9 | RSFB | B | Rushan, Shandong | 22 | DLFF | F | Dalian Liaoning |
| 10 | YCFB | B | Yuncheng, Shanxi | 23 | HRFF | F | Huairou, Beijing |
| 11 | XZFB | B | Suizhong, Liaoning | 24 | TXFF | F | Tong xian, Beijing |
| 12 | TXFB | B | Tong xian, Beijing | 25 | TSFF | F | Tangshan, Hebei |
| 13 | TSFB | B | Tangshan, Hebei | 26 | JXFF | F | Ji xian, Tianjin |

### 2.2. Extraction of DNA from pathogenic bacterium

Inoculation of tested bacteria isolates took place in PDA medium, the cultured isolates were cultivated in shaking condition at 28℃ for 10~12 days; then, the cultures underwent centrifugation of 5000 ×g for 10 min to collect mycelia which were used for DNA extracting following the methods by Chou Zeng-ming*et al*[2]. and Wang Jian-rong[3].

### 2.3. Primers and reagents

The primers (deca-base random primer), used in experiment, were bought from Operon Co., the agarose was from Serva Co., Taq DNA polymerase was bought from Ding Guo Biotechnology Co.

### 2.4. PCR amplification

Following the techniques reported previously, the reaction of amplification and observation were carried out[4].

### 2.5. Processing of data and analysis of calculation

The DNA fingerprint obtained from electronphoresis: amplification band was marked as "1" or "+", the absence of amplification band on the same migration position was marked as "0" or "–", depending on the requirement of analysis. The amplification bands were numbered in order of fingerprint position. The amplification band, displayed after the primer repeating amplification reaction twice, was used for analysis of results. According to Nei-Li method, the genetic similarity (GS) and genetic distance (GD) were calculated: GS = 2 Nij / (Ni + Nj). Nij represented the number of common amplification band for isolates "i" and "j, Ni + Nj represented the total of the amplification band number. GD = 1– GS.

## 3. RESULTS AND ANALYSIS

### 2.1. Genetic similarity between isolates of identical symptom type derived from different pathogenic bacteria

PAPD analysis of 26 pathogenic isolates derived from different apple production regions, which were grouped as A, B, C and F symptom types, were made separately. The results indicated that the genetic similarity was revealed in different isolates derived from the pathogenic bacterial type of identical symptom at the level of genomic DNA. In 7 isolates of symptom type A which were collected separately from Shandong, Jiangsu, Shanxi, Hebei and Beijing, and were amplified with

**Table 2** Analyses on genetic similarity of 7 different isolates of type A amplified with 9 RAPD Primers

| Primers | RSFA/ FXFA | RSFA/ TGFA | RSFA/ BXFA | RSFA/ TAFA | RSFA/ TXFA | RSFA/ TSFA | FXFA/ TGFA |
|---|---|---|---|---|---|---|---|
| OPA1 | 12/13 | 12/13 | 12/13 | 10/11 | 10/12 | 12/13 | 12/13 |
| OPA 3 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 |
| OPG7 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 |
| OPG10 | 16/16 | 16/16 | 16/16 | 16/16 | 16/16 | 16/16 | 16/16 |
| OPD7 | 8/8 | 8/8 | 8/8 | 8/8 | 8/8 | 8/10 | 8/10 |
| OPD8 | 26/28 | 28/29 | 28/29 | 30/30 | 26/28 | 24/27 | 26/27 |
| OPD9 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 |
| OPD12 | 20/21 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/21 |
| OPO2 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 |
| Mean values | 97.8 | 98.7 | 98.7 | 99.0 | 97.3 | 95.7 | 96.0 |

(continue)

| Primers | FXFA/ BXFA | FXFA/ TAFA | FXFA/ TXFA | FXFA/ TSFA | TGFA/ BXFA | TGFA/ TAFA | TGFA/ TXFA |
|---|---|---|---|---|---|---|---|
| OPA1 | 14/14 | 10/12 | 12/13 | 14/14 | 14/14 | 10/12 | 12/13 |
| OPA 3 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 |
| OPG7 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 |
| OPG10 | 16/16 | 16/16 | 16/16 | 16/16 | 16/16 | 16/16 | 16/16 |
| OPD7 | 8/8 | 8/8 | 8/8 | 8/10 | 8/8 | 8/8 | 8/8 |
| OPD8 | 24/26 | 26/28 | 26/26 | 24/25 | 28/28 | 28/29 | 26/27 |
| OPD9 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 |
| OPD12 | 20/21 | 20/21 | 20/21 | 20/21 | 20/20 | 20/20 | 20/20 |
| OPO2 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 |
| Mean values | 98.6 | 96.5 | 97.8 | 96.8 | 100 | 97.7 | 98.7 |

(continue)

| Primers | TGFA/ TSFA | BXFA/ TAFA | BXFA/ TXFA | BXFA/ TSFA | TAFA/ TXFA | TAFA/ TSFA | TXFA/ TSFA | Mean values |
|---|---|---|---|---|---|---|---|---|
| OPA1 | 14/14 | 10/12 | 12/13 | 14/14 | 10/11 | 10/12 | 14/14 | |
| OPA 3 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | |
| OPG7 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | |
| OPG10 | 16/16 | 16/16 | 16/16 | 16/16 | 16/16 | 16/16 | 16/16 | |
| OPD7 | 8/10 | 8/8 | 8/8 | 8/10 | 8/8 | 8/10 | 8/8 | |
| OPD8 | 24/26 | 28/29 | 26/27 | 24/26 | 26/28 | 24/27 | 24/25 | |
| OPD9 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | |
| OPD12 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | 20/20 | |
| OPO2 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | |
| Mean values | 96.9 | 97.4 | 98.7 | 96.9 | 98.2 | 94.9 | 99.6 | 97.7(%) |

9 primers, the amplification fingerprint of 5 primer among them showed no difference from each other (**Table 2**) and the amplification fingerprint of other 4 primers (OPD 12, OPD 7, OPA 1 and OPD 8) displayed 1, 2, 2 and 4 polymorphic bands, respectively, making 11.39 % of the total number of amplification bands (79). The calculation of analysis results indicated that the mean

genetic similarity among the 7 pathogenic isolates of pathogenic type A is 97.7% in range of 94.9～100% (**Table 2**). Among them the lowest similarity was shown between TAFA (Tai'an, Shangdong) and TSFA (Tangshan, Hebei), the highest was shown between FGFA (Tongguan, Shanxi) and BXFA (Bing Xian, Shanxi), and amplification fingers of 9 primers are completely identical to each other. These results demonstrate that there was no obvious genetic variation among the above 7 isolates of type A.

The details of the results of RAPD analysis of other isolates (of Types B, D and F) than above-mentioned 7 isolates of type A could be seen in the **Table 3**. The major results were similar to those of the above-mentioned 7 isolates of A type. Their main genetic similarity values are 90.0%, 91.6% and 96.9%,respectively.

**Table 3** RAPD analytical results for the isolates of different sources of the same symptom

| Pathogenic type | No. of isolate | No. of primer | No. of amplificated bands | No. of polymorphic bands | Genetic similarity (%) | |
|---|---|---|---|---|---|---|
| | | | | | Mean value | Range |
| A | 7 | 9 | 79 | 9 | 97.7 | 94.9~100 |
| B | 7 | 7 | 66 | 8 | 96.0 | 90.1~98.7 |
| D | 7 | 11 | 83 | 14 | 91.6 | 88.4~99.0 |
| F | 5 | 7 | 59 | 5 | 96.9 | 93.2~100 |

To sum up, the results of analysis indicate that there is no obvious genetic variation among the isolates of the identical symptom type derived from different sources.

**2.2. Polymorphism of the genome DNA from different pathogenic isolate**

RAPD analysis for the isolates,TSFA,TSFB, TSFD and TSFF of pathogenic types A,B, D and F, respectively, were carried out. A total of 17 random primers were used in the experiment. Among them, polymorphism in fingerprint of amplification was shown in 11 primers (**Table 4**) and, besides, completely different figures were shown in amplification fingerprint of 4 primers ($OPA_2$, $OPA_5$, $OPA_7$ and $OPA_{16}$) among different pathogenic isolates (**Fig.1** and **Table 5**). In aspects of polymorphism, the total of 99 bands were amplified by 11 primers, and 34 of them were polymorphic bands and covered 34.3% of the total of the amplified band.

**Table 4** Numbers of amplified DNA band with 11 PAPD primers among 4 different isolates in symptom

| Primers | Sequence (5'→3') | Amplified bands | Polymorphic bands |
|---|---|---|---|
| OPA1 | CAGGCCCTTC | 7 | 3 |
| OPA2 | TGCCGAGCTG | 12 | 4 |
| OPA3 | AGTCAGCCAC | 10 | 2 |
| OPA5 | AGGGGTCTTG | 9 | 4 |
| OPA7 | GAAACGGGTG | 15 | 5 |
| OPA9 | GGGTAACGCC | 9 | 7 |
| OPA10 | GTGATCGCAG | 8 | 3 |
| OPA02 | ACGTAGCGCTC | 6 | 2 |
| OPA04 | AAGTCCGCTC | 11 | 4 |
| OPA05 | CCCAGTCACT | 6 | 2 |
| OPA16 | AGGGCGTAAG | 6 | 5 |
| Total | | 99 | 34 |

These results indicate that there is obvious genetic heterogeneity among 4 different pathogenic isolate types. Accordingly, the further calculation for the genetic distance among all the pathogenic isolate types on the basis of DNA amplification patterns. The calculation revealed higher GD value between the isolates of A and B types and those of D type, and between the

isolate of B type and that ofF type, by 0.2487, 0.254 and 0.2418, respectively. Lower GD value between the pathogenic isolate of type A and B was shown as 0.1314 (**Table 5**). The measure of values of the above genetic distance were arranged in successive order: B, D > A, D > B, F > A, F >B, > F A, B.

**Table 5** RAPD Polymorphism of different isolates in symptom using 4 primers

| Primers | Code No. of DNA bands | Isolates | | | |
|---|---|---|---|---|---|
| | | Type A | Type B | Type D | Type F |
| OPA7 | 1 | + | + | - | - |
| | 2 | + | + | - | + |
| | 3 | + | + | + | - |
| | 4 | + | + | - | - |
| | 5 | – | + | – | – |
| | 6 | + | + | + | + |
| | 7 | + | + | + | + |
| | 8 | + | + | + | - |
| | 9 | + | + | + | + |
| | 10 | - | + | - | - |
| | 11 | + | + | - | - |
| | 12 | + | + | + | + |
| | 13 | + | + | - | + |
| | 14 | + | - | + | + |
| | 15 | + | + | + | + |
| OPA2 | 1 | + | - | + | + |
| | 2 | + | - | + | + |
| | 3 | + | + | + | + |
| | 4 | + | + | + | + |
| | 5 | - | - | + | - |
| | 6 | + | + | + | + |
| | 7 | - | - | + | + |
| | 8 | + | + | - | - |
| | 9 | - | - | + | - |
| | 10 | + | + | + | + |
| | 11 | + | + | + | + |
| | 12 | + | + | + | - |
| OPA5 | 1 | + | + | + | + |
| | 2 | - | + | - | - |
| | 3 | + | + | - | - |
| | 4 | + | - | - | + |
| | 5 | + | + | + | + |
| | 6 | + | + | + | + |
| | 7 | - | + | + | + |
| | 8 | + | – | – | – |
| | 9 | + | + | + | + |
| OPD16 | 1 | + | + | - | + |
| | 2 | - | + | + | + |
| | 3 | + | - | - | - |
| | 4 | + | - | + | + |
| | 5 | + | - | + | + |
| | 6 | - | + | - | + |

Note: “+”shows presence of amplified band; “-”shows absence of amplified band.

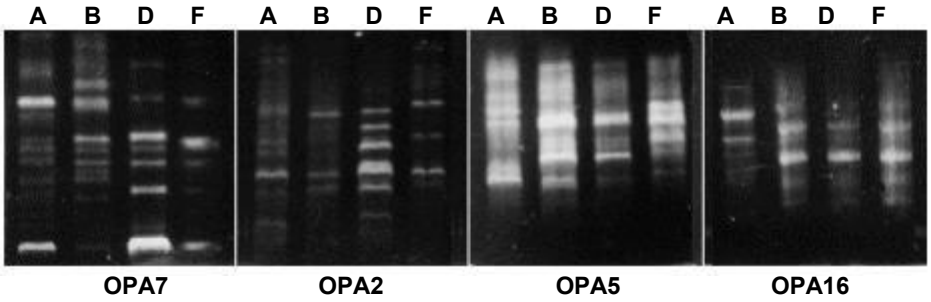


**Fig. 1** Comparison of RAPD patterns of 4 different pathogenic bacterial types

**Table 6** Genetic distance among 4 different isolates in symptom

| | A | B | D | F |
|---|---|---|---|---|
| A | | | | |
| B | 0.1314 | | | |
| D | 0.2487 | 0.2541 | | |
| F | 0.1936 | 0.2418 | 0.1908 | |

## 3. DISCUSSION

To collect isolates of all the 4 pathogenic isolate types in same host which was cultivated in same region for experiment is very difficult, only the isolates, derived from the 4 pathogenic bacterial types (A, B, D and F) originated from Tangshan region, were used for the analysis of DNA fingerprint in present experiment. Nevertheless, the analytic results is of certain reliability depending on that the 4 pathogenic isolates were derived from one apple variety, Fuji, which is cultivated in Tangshan apple production region, thus excluding differences caused by geological and environmental and host factors in present experiment.

The results of present research indicated that there is no difference at genomic DNA level among pathogenic isolates of identical symptom, which were derived from different geological sources. It means that there is no obvious relationship between the genetic differentiation in disease bacteria (*Ph. piricola*) in apple fruit and geological and environmental differences. This inference shows identical views held in earlier researches by other authors[5,6,7].

This paper for the first time presents the results of preliminary research by using RAPD technique on the genetic variation of the pathogenic bacteria *Ph. piricola* in apple fruit, which is cultivated in three major apple-production regions. And, these are of theoretical and practical significance in further demonstration on the law of heredity and evolution of the pathogenic bacteria and in the detection and identification of apple fruit disease *Ph. piricola* as well as the improvement of its control technique.

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