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Full Length Research Paper

Analysis of bacterial and fungal community structure in replant strawberry rhizosphere soil with denaturing gradient gel electrophoresis

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High quality DNA is the basis of analyzing bacterial and fungal community structure in replant strawberry rhizosphere soil with the method of denaturing gradient gel electrophoresis (DGGE). DNA of soil microorganisms was extracted from the rhizosphere soil of strawberries planted in different replanted years (0, two, six and seven), respectively, and crude DNA was purified after extraction. Three methods were established to evaluate the effects of cetyl trimethylammonium bromide (CTAB), polyvinylpolypyrolidone (PVPP), proteinase K and bacteriolytic enzymes on DNA extraction. DNA fragments above 23 kb in size were isolated well by method 1 (1% CTAB, proteinase K, no PVPP, no bacteriolytic enzyme) and method 3 (no CTAB, no proteinase K, 3% PVPP, bacteriolytic enzyme). Method 3 got the best yields 43.06 µg/g, and A260/A280 and A260/A230 were 1.1623 and 0.8135, respectively, which could ensure the veracity of subsequent DGGE analysis. Method 2 (3% CTAB, no proteinase K, no PVPP, no bacteriolytic enzyme) could not extract enough DNA to do the next PCR-DGGE analysis. F341/R534 and FR1/FF390 primers were used to amplify the 16S rDNA V3 region of bacteria and 18S rDNA of fungi, and the expected fragments of 230 bp 16S rDNA V3 region and 390 bp 18S rDNA were amplified. The results of DGGE analysis showed that there were common and specific bacterial and fungal communities in different replant soils of strawberry. There were 84 and 54% similarity of bacterial and fungal communities between different replant soils. The numbers of both bacterial and fungal communities increased in the replant strawberry soil, they were positively correlated with the replant years. As the number of replant years increased from two to seven years, while the ratio of bacteria/fungi was decreased from 2.29 to 1.46 in the rhizosphere soils planted with strawberries.

Key words: Rhizosphere soil, bacterial community, fungal community, replant strawberry, fruiting fields.

INTRODUCTION

China is one of the main countries for strawberry (*Fragaria ananassa* Duch.) production. About 2,000,000 tons of strawberries were produced in 2009, and cover 133, 300 ha in China (http://www.agri.gov.cn/). Both output and planting area are the biggest in the world. Strawberries are planted in Northern and Southern China, in the provinces of Hebei, Shandong, Liaoning and Jiangsu, with about 54.5% of the total strawberry cultivation area and 68.9% of the total production of strawberries in China.

Apart from China, strawberries are planted in the main fruit producing areas of other countries. For example in California, USA, strawberries are usually replaced after one season. Cropland in China is limited, and protected cultivation is widely used; so, it is very difficult to rotate in most strawberry planting areas. In China, strawberry plants are usually planted and replanted in the same field for many years. This situation leads to more serious

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Abbreviations: DGGE, Denaturing gradient gel electrophoresis; **CTAB,** cetyl trimethylammonium bromide; **PVPP,** polyvinylpolypyrolidone.

replant diseases in China than in other countries (Zhen et al., 2005b). Above ground symptoms of replant disease in strawberries (RDS) include: reduction in plant vigor, yield and quality of plant products. Typically, root systems of strawberry plants are small, with discolored feeder roots and few functional root hairs. Normally, RDS is more severe in protected cultivation. Cultivars differ widely in their resistance to RDS. Unfortunately, the most popular cultivars for the fruit production are susceptible to RDS.

The production of strawberries under protected cultivation is dependent on the intensive use of chemicals to control RDS. In the past, methyl bromide was the most effective control for RDS, and the use of 1,3-dichloropropene (1,3-D) and chloropicrin (Pic) have been extended in the last years (Sanchez-Moreno et al., 2010). Recently, chemical residues became a major issue, resulting in legislative actions to limit and regulate chemicals used in agriculture. Due to these regulatory constraints and public resistance to fumigant use, it is necessary to develop fumigant-free strawberry production systems. As a result, biological control agents (BCAs) are required in fruit production, to reduce chemical residues on fruits (Kokalis-Burelle et al., 2006; Porras et al., 2007; Moser et al., 2008). In BCAs research and development programs, researchers have mainly focused on the efficacy of these organisms against pests and diseases (Dhillon and Sharma, 2009; Felici et al., 2008). A crucial, but poorly investigated element in the development of a BCA is the analysis of the socio-economic environment in which the BCA will be applied. The enormous range of complexity in soil microbial communities has made it an incredibly challenging ecosystem to study (Torsvik et al., 2002).

A number of molecular biological approaches are now being used to gain a better understanding of the ecology of soil microbial communities (Nakatsu, 2004). It has enabled advancement beyond the traditional cultivation approaches that were able to capture only about 1% of the community in the past (Staley and Konopka, 1985). Molecular techniques have been useful in revealing the community structure of microbes, including microbes as yet unable to be cultured. Sequence analysis of the recovered DNA, usually of ribosomal RNA genes, allows researchers to infer the taxonomic position of microbes that exist in the sampled environment.

One such technique, the denaturing gradient gel electrophoresis (DGGE) has been used to analyze the genetic diversity of the rhizosphere microbial populations (Muyzer et al., 1993; Hoshino and Matsumoto, 2007). This technique provides novel insights and significant advances into research on soil complex microbial populations; because microbial cells may remain tightly bound to soil colloids, soils high in clay or organic matter in strawberry fields pose particular challenges to obtaining high yields of high molecular weight DNA for the DGGE analysis. Extraction of DNA from soils always results in co-extraction of humic matter, which interferes with DNA detection and measurement. This contamination can inhibit Taq DNA polymerase in PCR (Smalla et al., 1993; Smalla et al., 2001; Tsai and Olson, 1992), interfere with restriction enzyme digestion (Porteous and Armstrong, 1991), and reduce transformation efficiency (Tebbe and Vahjen, 1993) and DNA hybridization specificity (Steffan and Atlas, 1988). Since humic matter is difficult to remove, DNA purification is a critical step following direct extraction to obtain DNA of sufficient purity.

The objective of the present study was to develop a DNA extraction method on the subsequent DGGE analysis of soil microorganisms, and to analyze the bacterial and fungal community structure in the rhizosphere soil of replant strawberry fields with DGGE method.

MATERIALS AND METHODS

Soil samples

Four different soils from replant strawberry fields were used to evaluate the efficiency of DNA extraction and DGGE analysis of bacterial and fungal community structure. The soils were sampled from the greenhouses where the strawberry plants were replanted 0, two, six and seven years, respectively. For the 0 year, it means there is only the first year to plant strawberry in the greenhouse, and no replanting. The greenhouses belong to the Beijing TianYi Bioengineering Limited Company for strawberry production in ChangPing District in Beijing. Strawberry cultivar 'Benihoppe' (*Fragaria ananassa* Duch.) was used in these trials.

The soil samples from greenhouses replanted with strawberries for 0, two, six and seven years were designated as WL, LE, LL and LQ, respectively. There were Fusarium wilt and Verticilium wilt happening occasionally at the end of harvest during April, the poor growth and high disease index were reduced as the replant years increased; but there were no significant differences of the disease index between the treatments WL, LE, LL and LQ (data not published). A mass of organic fertilizer (poultry litter, Beijing Goldenway Bio-tech Company Limited) is used every year, and the replant diseases were controlled below the upper level allowed by organic farming practice. Soil samples were taken from each of the greenhouses, three months after planting. The WL, LE, LL and LQ samples each had five replicates of rhizosphere soil taken from soil adhering to the roots from 30 strawberry plants. The roots were shaken vigorously to separate soil. Soil samples for DNA extraction were kept frozen at -20**°C.**

Soil pre-treatment and DNA extraction

Three treatments were established to evaluate the effects of cetyltrimethyl-ammonium bromide (CTAB), polyvinylpolypyrrolidone (PVPP), proteinase K and bacteriolytic enzyme (ShangHai Biotechnology Limited) on soil pre-treatment and DNA extraction. Soil DNA extraction was combined by bead-beater and extraction solution in the three treatments.

Method 1 (1% CTAB, proteinase K, no PVPP, no bacteriolytic enzyme)

A) Soil pre-treatment

Soil samples 0.5 g were mixed with 1.35 ml DNA extraction buffer [100 mM Tris-HCl (pH 8.0), 100 mM sodium ethylenediaminetetraacetic acid (EDTA, pH 8.0), 100 mM sodium

phosphate (pH 8.0), 1.5 M NaCl (pH 8.0), 1% CTAB] and 15 µL proteinase K (10 mg/ml) in tubes, by horizontal shaking at 225 rpm, for 30 min at 37°C. After the shaking treatment, 160 µL 20% sodium dodecyl sulphate (SDS) was added, and the samples were incubated in 65°C water bath for 2 h with gentle end-over-end inversions every 20 min. The supernatants were collected after centrifugation at 6000 r/min for 10 min at room temperature and transferred into 50 ml new centrifuge tubes.

B) DNA extraction

Supernatants were mixed with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol). The aqueous phase was recovered by centrifugation and precipitated with 0.6 volume of isopropanol at 4°C for 12 h. The pellet of crude nucleic acids was obtained by centrifugation at 16000 r/min for 20 min, at room temperature, washed with cold 70% ethanol, and suspended again in sterile deionized water to give a final volume of 100 µL.

Method 2 (3% CTAB, no proteinase K, no PVPP, no bacteriolytic enzyme)

A) Soil pre-treatment

Soil samples 0.5 g were mixed with 1.35 ml DNA extraction buffer [100 mM Tris-HCl (pH 8.0), 100 mM sodium EDTA (pH 8.0), 100 mM sodium phosphate (pH 8.0), 1.5 M NaCl (pH 8.0), 3% CTAB]. 160 µL 20% SDS was added and the samples were incubated in 65°C water bath for 2 h with gentle end-over-end inversions every 20 min. The supernatants were collected after centrifugation at 6000 r/min for 10 min at room temperature and transferred into 50 ml new centrifuge tubes.

B) DNA extraction

The methods are same as Method 1 described above.

Method 3 (no CTAB, no proteinase K, 3% PVPP, bacteriolytic enzyme)

A) Soil pre-treatment

Soil samples 0.5 g were mixed with 3.0 ml TENP buffer (50 mmol/L Tris, 20 mmol/L EDTA, 100 mmol/L NaCl, 0.01 g/mL PVPP, pH 10), after volute shaking for 10 min, 10000 r/min centrifugation for 5 min at room temperature, the supernatants were remove. Washing was done twice as the above steps, then 3 mL PBS buffer (137 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L Na_2HPO_4, 2 mmol/L KH_2PO_4, pH 7.4) was added for once washing.

B) DNA extraction

0.3 g quartz sand and 2 mL DNA extraction buffer (100 mmol/L Tris, 100 mmol/L EDTA, 200 mmol/L NaCl, 3% PVPP, pH 9.0) was added and volute shaking done for 10 min. 500 µL bacteriolytic enzyme was added and volute shaking done for 5 min. The mixture was kept in 37°C water bath 30 min and volute shaking done for 5 min. 2 mL SDS buffer was added, reversely mixed to uniformity, and kept in 65°C water bath for 30 min, with 6000 r/min centrifugation for 15 min at room temperature and the supernatants were then collected. Supernatants were mixed with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol). The aqueous phase was recovered by centrifugation and mixed with 0.6 volume of 3 mol/L (pH 5.2) NaAc, precipitated with 0.6 volume of isopropanol at 4°C for 12 h. The pellet of crude nucleic acids was obtained by 6000 r/min centrifugation for 20 min at room temperature, washed with cold 70% ethanol, and suspended again in sterile deionized water to give a final volume of 100 µL.

Purification of DNA and evaluation of DNA quality

Glass ball DNA collection kit (ShangHai Biotechnology Limited) was used for the DNA purification, the DNA purification steps were according to the manufacturer's instructions. Spectrophotometric A260/A280 and A260/A230 ratios were determined to evaluate DNA quality.

Polymerase chain reaction (PCR) amplification of bacterial 16S rDNA V3 region fragments

The bacterial 16S rDNA V3 region fragments were amplified by PCR with the primer pair F341/R534 [5'-(GC)-CCTACGGGAGGCAGCAG-3'/5'-ATTACCGCGGCTGCTGG-3'] (Muyzer et al., 1993). A GC-rich sequence (CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGG G) (indicated as -GC) was attached to primer F341 to prevent complete melting of PCR products during separation in the denaturating gradient gel. Amplification was done using the 2× PCR Taq polymerase mixture (12.5 µL) as described by the manufacturers (Mylab Coperation Limited, Beijing) with 0.5 µL (25 pmol/µL) of each primer and 1.0 µL template DNA, adding ddH_2O to 25 µL. The bacterial 16S rDNA V3 region fragments were amplified by PCR methods described as the methods of Wang et al. (2006).

PCR amplification of fungal 18S rDNA fragments

The fungal 18S rDNA fragments was amplified by PCR with the primer pair FR1/FF390 [5'-(GC)AICCATTCAATCggTAIT-3'/5'-CgATAACgAACgAgACCT-3'] (Vainio and Hantula, 2000). A GC-rich sequence (same as above) was attached to primer FR1. Amplification was done using the 2× PCR Taq polymerase mixture (12.5 µL) as described by the manufacturers (Mylab Coperation Limited, Beijing) with 0.5 µL (25 pmol/µL) of each primer and 1.0 µL template DNA, adding ddH_2O to 25 µL. The 18S rDNA fragments were amplified by PCR methods described as the methods of Hoshino and Matsumoto (2007).

Denaturing gradient gel electrophoresis (DGGE) analysis

DGGE analysis was performed as previously described by Heuer et al. (1999, 2001) with a denaturing gradient of 30 to 55% and 30 to 60% denaturant for bacterial and fungal sample PCR, respectively. Aliquots of PCR samples (15 µL) were applied to the DGGE gel, and DGGE was performed in 1× Tris-acetate-EDTA buffer at 60°C at a constant voltage of 130 V for 4 h. After silver staining of the DGGE gels, they were air dried and scanned as described by Heuer et al. (2001). The Quantity One program (Bio-Rad) was used to analyze the bacterial and fungal community fingerprints of each denaturing gradient gel.

RESULTS

The effects of three methods of soil pre-treatment and DNA extraction

Four different soils (WL, LE, LL and LQ) were used to

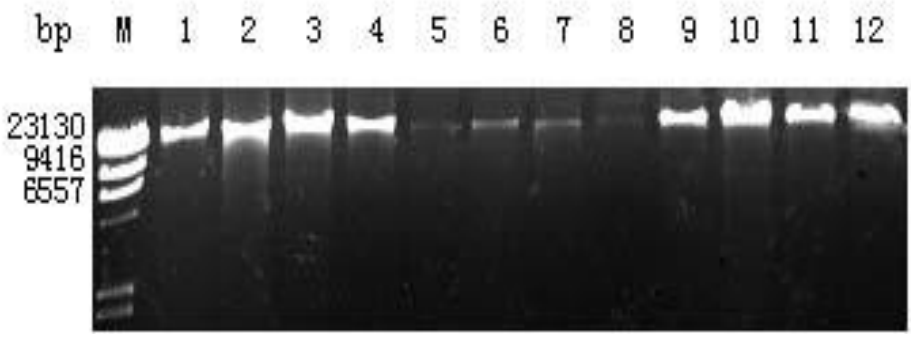

Figure 1. Electrophoresis of crude DNA extracted from soil microorganisms by three DNA extraction methods. M: λ DNA/Hind III marker; lane 1 to 4, DNA samples extracted from the soil WL, LE, LL, LQ using method 1; lane 5 to 8, DNA samples extracted from the soil WL, LE, LL and LQ using method 2; lane 9 to 12 DNA samples extracted from the soil WL, LE, LL and LQ using method 3.

Table 1. Yield and quality of crude DNA extracted by method 1 and 3.

No. of DNA bands	Sample	DNA yields [μg/g (dry wt) of soil]	A260/A280	A260/A230
Method 1	1	23.50	1.1088	0.6869
	2	40.25	1.1027	0.5725
	3	53.50	1.1463	0.5661
	4	14.50	1.1373	0.5349
Average		32.94	1.1238	0.5901
Method 3	1	37.50	1.1515	0.7726
	2	68.50	1.1935	0.8952
	3	37.50	1.1525	0.8610
	4	28.75	1.1515	0.7250
Average		43.06	1.1623	0.8135

DNA samples 1 to 4 were extracted from the soil WL, LE, LL, LQ, respectively.

evaluate the efficiency of soil pre-treatment and DNA extraction, using the three methods. The yield of extracted DNA was very low in all four soil samples when using method 2. Methods 1 and 3 could extract DNA from the four soil samples well and the DNA fragment extracted from the soil microorganisms is bigger than 23 Kb. There was no degradation of DNA fragments (Figure 1).

Purification of DNA and PCR amplification

The average DNA yield from method 3 was 43.06 μg/g, which was 1.3 times DNA yield of method 1. A260/A280 and 260/A230 of DNA extracted from method 3 were 1.1623 and 0.5901, respectively, all higher than the ratio of method 1. The results suggest that method 3 was more efficient at removing the protein and humic matter from the soil samples than method 1. Although, for both methods 1 and 3, the A260/A280 and 260/A230 of crude DNA extracted from four different soils were low (Table 1).

It appeared that method 3 could not amplify the bacterial 16S rDNA V3 region fragments and fungal 18S rDNA fragments, when the crude DNA was used in the PCR process. To resolve this problem, a DNA collection kit (Mylab Coperation Limited, Beijing) was used for the crude DNA purification. After purification, A260/A280 and 260/A230 of DNA extracted from method 3 were increased to 2.104 and 1.716. DNA quality was up to the demand of PCR amplification. A 230 bp fragment of the 16S rDNA V3 region was amplified with the primer pair F341/R534 (Figure 2), and a 390 bp fragment of the fungal 18S rDNA was amplified with the primer pair FR1/FF390 (Figure 3).

Comparing the results of the bacterial 16S rDNA V3 region amplicons, the fragments of the replant two, six and seven years soil samples (LE, LL and LQ) were stronger than that of the replant 0 year soil samples (WL). For the fungal 18S rDNA, the fragments also became stronger as the replant years increasing. The results suggest that the size of both bacterial and fungal

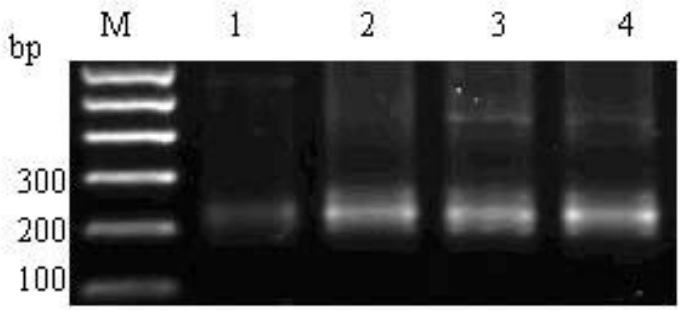

Figure 2. Bacterial 16S rDNA V3 region PCR amplified results from purified DNA samples M, DNA marker I; 1 to 4, bacterial 16S rDNA V3 region amplified fragments from the different soil samples of WL, LE, LL, LQ.

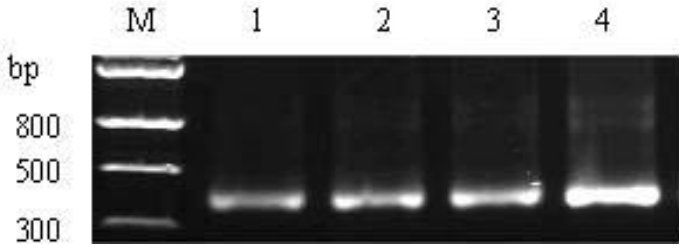

Figure 3. Fungal 18S rDNA PCR amplified results from purified DNA samples M, 100 bp DNA Ladder; 1 to 4, fungal 18S rDNA amplified fragments from the different soil samples of WL, LE, LL, LQ.

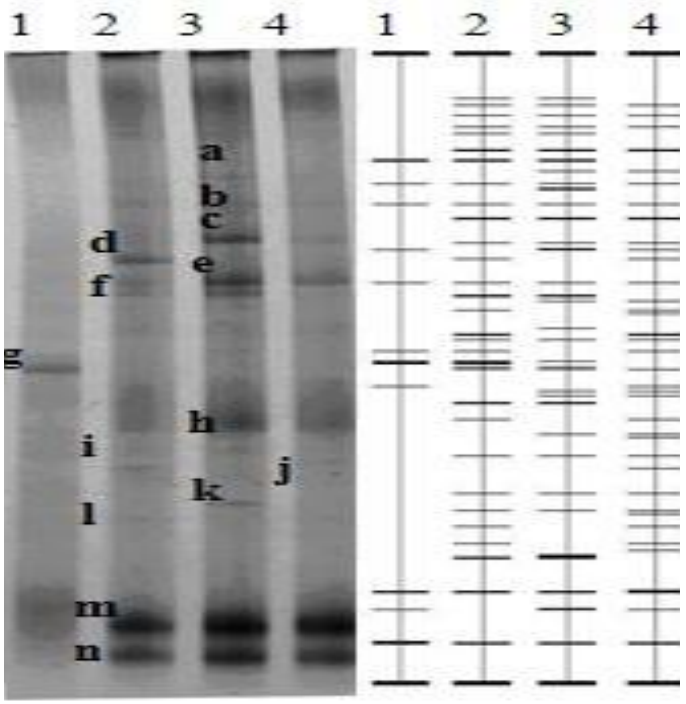

Figure 4. DGGE profile and sketch map of bacteria 16S rDNA V3 region in different replanted soil samples 1 to 4, the different bacterial soil DNA samples of WL, LE, LL, LQ.

communities increased in the replant soils, and that this is positively correlated with the year of replanting.

DGGE analysis of bacterial community structure of replant strawberry rhizosphere soil

The Quantity One program was used to analyze the DGGE fingerprints of bacteria 16S rDNA V3 region in different replant strawberry rhizosphere soils: WL, LE, LL and LQ, and transform the DGGE profile to the sketch map (Figure 4). There were two common bands *m* and *n* among the four soil samples which were stronger than others. This suggests that these two bacterial communities were larger in the soil. The 0 year replant soil WL showed weaker *m* and *n* banks than that of two, six and seven years replant soil samples LE, LL and LQ. According to the common bands in the sketch map, there was 84% similarity of the bacterial communities between different replant year soils. Both common bands and different bands were found in the four soils. For example, the common *e* and *h* bands were found in the LE, LL and LQ, the *d* and *l* band in LE, the *k* band in LL and the *j* band in LQ. These results showed that the strawberry rhizosphere soil in each different replant field had its own particular bacterial community.

The number of DNA bands for the bacterial community of each different replant soil sample is shown in Table 2. They were 13, 32, 34 and 38, respectively in the WL, LE, LL and LQ. There are big changes of the number of bacterial community between the replant year samples. As the number of replant years increases, the numbers of different bacterial communities present in the rhizosphere soil of strawberries also increases.

DGGE analysis of fungal community structure of strawberry replant rhizosphere soil

The DGGE fingerprints of fungi 18S rDNA in different soil WL, LE, LL and LQ and transformation of the DGGE profile to the sketch map is shown (Figure 5). There was a common band among the four soil samples and it was stronger than other bands. The number of DNA bands of fungi in different replant year soil samples is shown in Table 2. They were 12, 14, 24 and 26, respectively. According to the common bands in the sketch map, it showed 54% similarity of the fungal communities between LE, LL and LQ. As the number of replant years increased, the number of DNA bands of fungal communities also increased in the rhizosphere soil of strawberries.

The changes of bacterial and fungal communities in the rhizosphere soil of replant strawberries

Comparing the results of DGGE analysis, they were 1.08,

Table 2. DNA bands of bacteria and fungi in different replanted soil samples.

No. of DNA bands	WL	LE	LL	LQ
Bacteria	13	32	34	38
Bacteria increase ratio (BIR)	1	2.29(2.29)	2.62(0.33)	2.92(0.30)
Fungi	12	14	24	36
Fungi increase ratio (FIR)	1	1.17(1.17)	2.00(0.83)	2.17(0.17)
Bacteria/Fungi	1.08	2.29	1.42	1.46

Increase Ratio (IR) (%) = [Numbers of DNA band of each replanted soil samples (LE, LL or LQ)/Numbers of DNA band of no replanted soil sample (LQ)] × 100%. For example, in LL 2.62 = 34/13.The abbreviations FIR and BIR are referring to Bacteria increase ratio and Fungi increase ratio, respectively. Data in bracket are the current IR subtract anterior IR, for example, in LQ (0.30) = 2.92 - 2.62. They represent the increase speed of Bacteria increase ratio (BIR) or Fungi increase ratio (FIR).

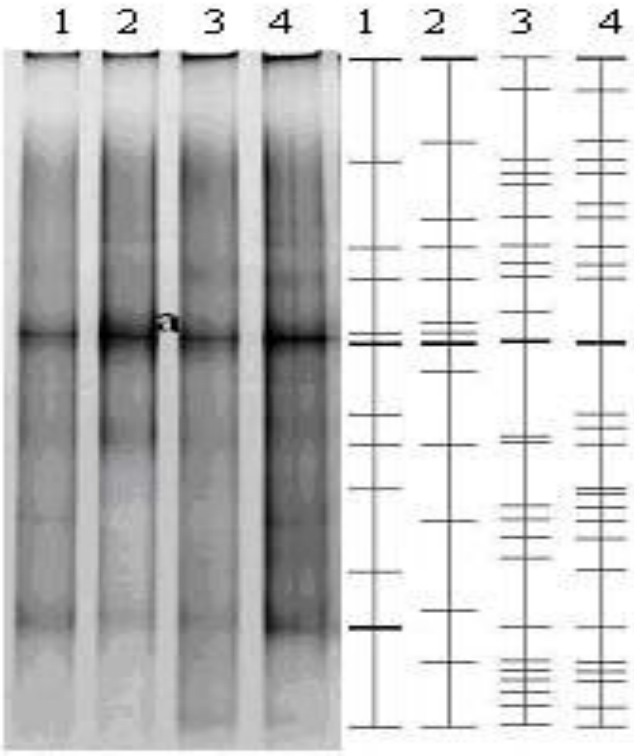

Figure 5. DGGE profile and sketch map of fungal 18S rDNA in different replanted soil samples 1 to 4, the different fungal soil DNA samples of WL, LE, LL and LQ.

2.29, 1.42 and 1.46, respectively, for the bacteria/fungí ratio in the different replant soils WL, LE, LL and LQ (Table 2). LE soil, replant 2 years, has the highest bacteria/fungi ratio of 2.29. It showed that there were larger numbers of bacterial communities in soil, early in the replanting sequence of strawberry production. For the 6th and 7th replant years, both bacterial and fungal communities have increased in numbers, with the number of fungal community increasing much more than that of the bacterial communities. These changes can be observed from the increased growth ratio of Bacteria increase ratio (BIR) or Fungi increase ratio (FIR) (Data in bracket). The increased growth ratio of FIR was 0.83 between the 2nd and 6th replant years, which is 0.50 higher than that of bacteria BIR (0.33). The different increasing degree or speed of the number of bacterial and fungal communities resulted in the decline of the bacteria/fungi ratio from 2.29 to 1.42, between 2 to 6 replant years.

DISCUSSION

DNA recovery from the diverse composition soils of strawberry fields

Isolation of soil microorganism DNA from crop fields has become a useful tool to detect bacteria or fungi that cannot be cultured (Liesack and Stackebrandt, 1992; Ward et al., 1990), to reveal the relationship of their genotypic diversity and the dynamic changes with the replant crop plants in soil ecosystems. Strawberry production soils are high in clay or organic matter, and the microbial cells may remain tightly bound to soil colloids that pose particular challenges to obtaining high yields of high quality DNA. To establish a simple, rapid method of DNA extraction for analysing soil bacteria or fungi was done with Denaturing Gradient Gel Electrophoresis (DGGE) method; three DNA extraction methods were studied to compare the extraction efficiency for the rhizosphere soils of different replant years of fields producing strawberry fruit. Method 3 (with Polyvinylpolypyrolidone (PVPP) and bacteriolytic enzyme) is most suitable to extract the total DNA of microorganisms in strawberry rhizosphere soils. Method 1 (with Cetyl trimethylammonium bromide (CTAB) and proteinase K) takes the second place. The DNA quantity extracted by Method 2 (with 3% CTAB, no proteinase K, no PVPP, no bacteriolytic enzyme) cannot meet the demand of next DGGE analysis. Most contents and steps are similar between Methods 1 and 2; the difference is that there is proteinase K in method 1, but not in method 2. Proteinase K can help to raise the DNA yields in the

extraction (Xing and Ren, 2006; Zhou et al., 1996). Comparing Methods 1 and 3, Method 3 uses the TENP and PBS buffer for the pre-treatment of soil samples, and instead, proteinase K with bacteriolytic enzyme was used to break cells of soil microorganisms. The colour of the removed supernatant liquid is brown after pre-treatment, which shows that some organic matter is washed out from the soils. The results that the components of TENP or PBS buffer and bacteriolytic enzyme can help to raise the DNA extraction quality in a complicated soil environment are consistent to the past reports. (LaMontagne et al., 2002). TENP and PBS buffer can availably remove the ions, inorganic or organic matter that influence the extraction efficiency, reduce the pollution of soluble inorganic or organic matter, especially to reduce the pollution of the humic matter. PVPP in TENP can remove part of the humic matter (Zhou et al., 1996). Bacteriolytic enzyme can not only recover the DNA yields and raise the level of purification, but also reduce the influences of humic matter.

To sum up, the problem of DNA recovery from strawberry production soils involves two component methods, that is, (i) cell lysis and extraction of crude DNA and; (ii) purification of crude DNA. Due to the fact that Method 3 in this paper has the advantages in combining cell lysis and purification methods for the different soils, it can remove the influence of soil organic matter, and yield the most DNA from strawberry rhizosphere soils.

DGGE analysis of bacterial and fungal community structure in the rhizosphere soil of replant strawberry fields

DGGE fingerprints of PCR-amplified 16S and 18S rDNA were used to study dominant bacterial and fungal populations in the rhizospheres of crop plants. In contrast to other recently published papers, the rhizosphere samples investigated in this study originated from the plants grown under field conditions in four strawberry replant greenhouses for fruit production. In general, the soils with poor fertility and replant diseases problems have a low ratio of bacteria/fungi (Zhen et al., 2005a). The health situation of replant strawberry fields can be indicated by the bacterial and fungal community structure in the rhizosphere soil, with DGGE analysis. When the bacteria/fungi ratios of the replanted soils are compared, soils replanted for the 2nd year show the highest bacteria/fungi ratio. As the number of replant years increase (6 and 7 years), the fungi communities increased much more than that of the bacteria communities, which leads to a decrease in the bacteria/fungi ratio. According to our investigation of the replant strawberry plants in the experiment fields, the poor growth and high disease index were reduced as the replant years increased. The experiment fields we conducted our trials in, were located on an organic farm. The replant diseases occurring in the fields were controlled below the allowable level limits. The farmers growing strawberries are conscious that the potential replant disease risk in strawberry crops increases as the number of replant years increase.

Although DGGE is not the most appropriate choice for a complete characterization of microbial communities or for measuring microbial diversity, DGGE is still ideal as an initial screen for comparing multiple samples and for identifying appropriate techniques for additional sequence-based sample analysis. DGGE analysis of the variation of bacteria/fungí ratio in the rhizosphere of replant strawberry provides a good way to indicate the increased risk of replant disease in strawberry plants. The analysis of bacteria/fungi ratio can be involved in a system for detecting, evaluating and for giving warning about the risk of outbreaks of strawberry replant disease. This is especially important in the huge areas of strawberry production in China.

We also detected a rhizosphere effect, namely an increased relative abundance of some populations in the vicinity of the roots for strawberry plants. Several bands observed in rhizosphere DGGE patterns were not detected in patterns of soil from the not replant greenhouse (0 year replanted) or were detected only as weak bands. The rhizosphere effects became more pronounced in the replant greenhouse (2, 6 and 7 year replanted). Our data provide further evidence for the assumption that, in different replant years, the strawberry plants show different bacterial communities in the proximity of their roots, and that these plant-specific “enrichments” can be increased by repeated cultivation of the same plant species in the same field.

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