

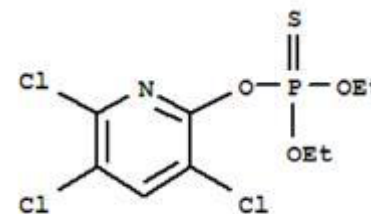


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Soil bacterial and fungal community successions under the stress of chlorpyrifos application and molecular characterization of chlorpyrifos-degrading isolates using ERIC-PCR

Key words: Denaturing gradient gel electrophoresis (DGGE), Bacterial community, Fungal community, Chlorpyrifosdegrading isolates, Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR)

- Chlorpyrifos is a broad-spectrum moderately toxic organophosphorus insecticide.
- Interactions between chlorpyrifos and soil microorganisms, including microbial biomass carbon and nitrogen, microbial populations, microbial functional diversity, microbial respiration, enzymatic activities, and nitrogen cycling, have been widely studied
- However, the successions of soil bacterial and fungal community structures, which can truly reveal dynamic changes of microbes, have remained unknown. Furthermore, different chlorpyrifos-degrading isolates may exist in different soil environments.



In this study, we aimed to reveal the response of soil bacterial and fungal community structures to chlorpyrifos applications, furthermore, to molecular characterize chlorpyrifos-degrading strains isolated from chlorpyrifos-contaminated soil by ERIC-PCR.

RESULTS

Table 1 Kinetics data of chlorpyrifos in soil

Chlorpyrifos concentration (mg/kg)	Dynamic function	DT ₅₀ ^a (d)	R ²
5	$y=5.252e^{-0.09x}$	8.25	0.910
20	$y=19.41e^{-0.08x}$	8.29	0.908

^aDT₅₀ stands for the half-lives of chlorpyrifos in this experiment.

RESULTS

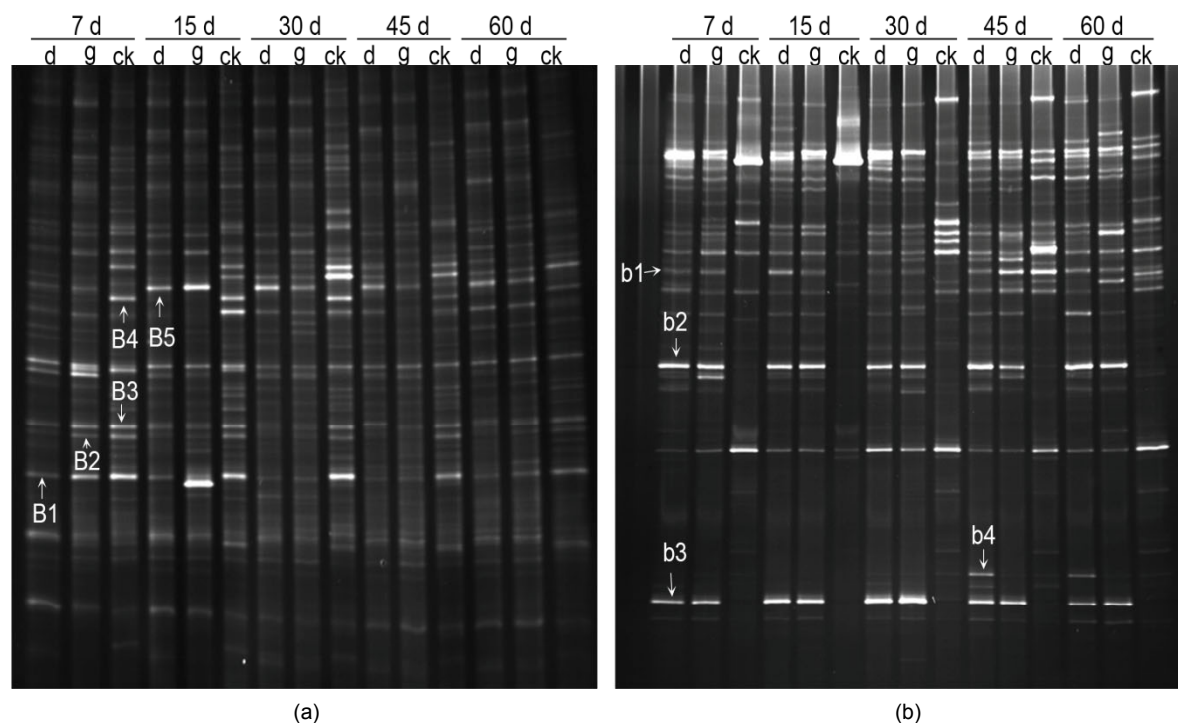


Fig. 1 DGGE community fingerprints of soil total bacteria (a) and fungi (b) in soil samples amended with chlorpyrifos at different sampling times

Lanes d, g, and ck stand for soil contaminated by chlorpyrifos of 5, 20, and 0 mg/kg, respectively

RESULTS

Table 2 Shannon-Wiener diversity indices of total bacterial and fungal community under stress of chlorpyrifos

Sample ^a	Shannon index (<i>H</i>)	
	Bacterial community	Fungal community
d7	3.68 ± 0.12 ^{cd}	3.35 ± 0.03 ^d
g7	3.56 ± 0.04 ^b	3.30 ± 0.04 ^c
ck7	3.69 ± 0.03 ^{cd}	3.06 ± 0.02 ^b
d15	3.66 ± 0.05 ^{cd}	3.42 ± 0.03 ^{fg}
g15	3.48 ± 0.04 ^a	3.39 ± 0.02 ^{ef}
ck15	3.62 ± 0.02 ^{bc}	2.68 ± 0.02 ^a
d30	3.68 ± 0.04 ^{cd}	3.43 ± 0.02 ^g
g30	3.88 ± 0.06 ^e	3.29 ± 0.02 ^c
ck30	3.67 ± 0.02 ^{cd}	3.53 ± 0.02 ⁱ
d45	3.96 ± 0.01 ^f	3.54 ± 0.02 ⁱ
g45	4.02 ± 0.05 ^f	3.34 ± 0.01 ^d
ck45	3.72 ± 0.02 ^d	3.37 ± 0.05 ^{de}
d60	3.82 ± 0.02 ^e	3.56 ± 0.02 ⁱ
g60	3.87 ± 0.02 ^e	3.44 ± 0.02 ^{gh}
ck60	3.83 ± 0.02 ^e	3.47 ± 0.02 ^h

^a d, g and ck stand for soil contaminated by chlorpyrifos of 5, 20, and 0 mg/kg, respectively; 7, 15, 30, 45, 60 stand for sampling time after chlorpyrifos application. Values are expressed as mean ± SD of triplicate samples, means followed by the same letter within a column are not significantly different according to LSD's multiple range test ($P < 0.05$)

RESULTS

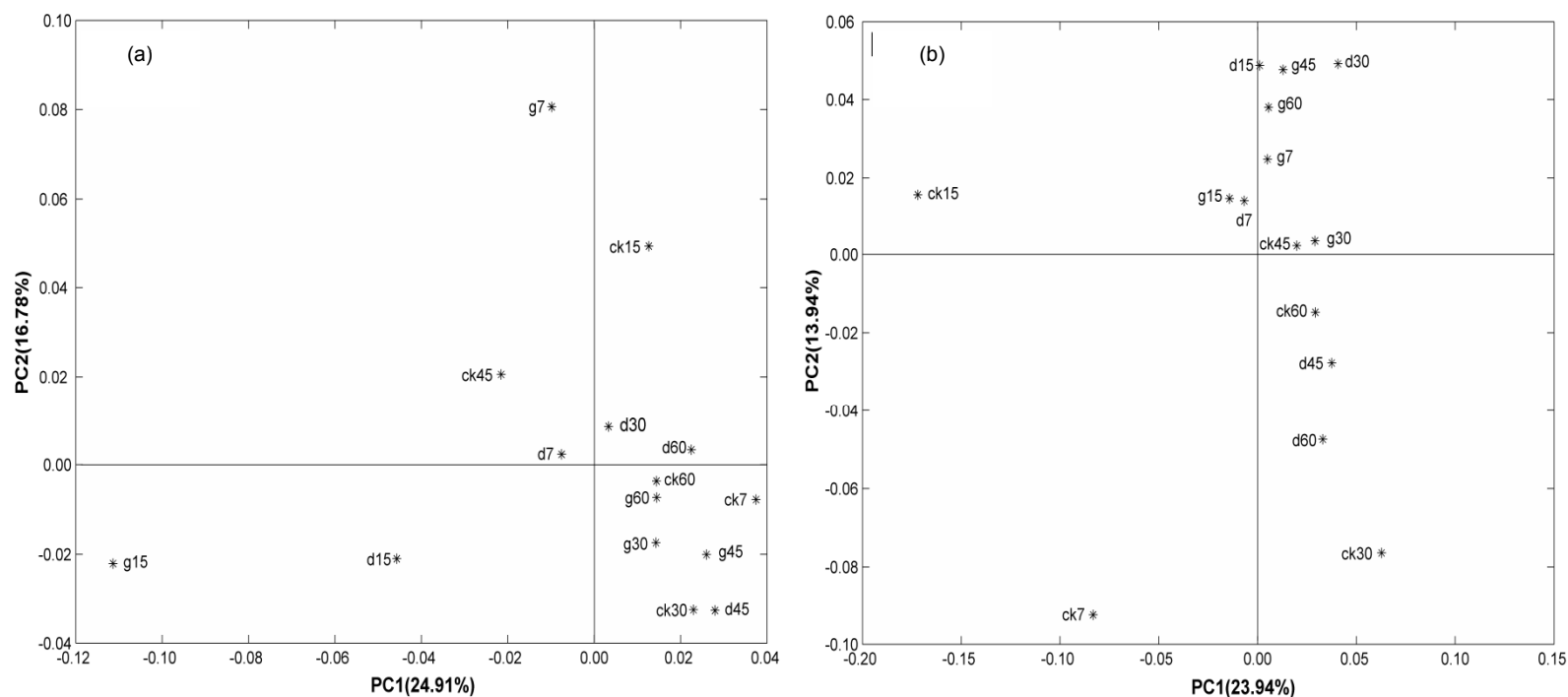


Fig. 2 PCA analysis of the soil total bacteria (a) and fungi (b) in soil samples amended with chlorpyrifos

d, g and ck stand for soil contaminated by chlorpyrifos of 5, 20, and 0 mg/kg, respectively. 7, 15, 30, 45, and 60 stand for sampling days after chlorpyrifos treatment. The numbers following the PC1 and PC2 in the axis stand for percentage of the first and second principal component to all components.

RESULTS

Table 3 Species of clones with ITS sequence for fungi and 16S rDNA sequence for bacteria in NCBI GenBank database most similar to the nine ERIC types of chlorpyrifos-degrading strains

ERIC type	Clone No.	Closest match from Genbank		Genbank accession No.
		Match	Sequence similarity by BLAST (%)	
I	LR4, LR5	<i>Bacillus cereus</i>	99%	JX966388
II	CR10	<i>Fusarium</i> sp.	100%	JX915255
III	CR9	<i>Fusarium oxysporum</i>	100%	JX915246
IV	GR4, CR13	<i>Fusarium</i> sp.	100%	JX915247
V	CR11	<i>Gibberella moniliformis</i>	99%	JX915252
VI	CR12, GR2	<i>Dipodascaceae</i> sp.	95%	JX915245
VII	GR3, GR1	<i>Gibberella moniliformis</i>	100%	JX915251
VIII	CR4	<i>Gibberella moniliformis</i>	100%	JX915250
IX	CR1, CR14	<i>Chaetomium globosum</i>	100%	JX915254

CONCLUSIONS

- Under the experimental conditions that were applied, degradation of chlorpyrifos in soil was interpreted with first-order kinetics, and the half-lives of chlorpyrifos at 5 and 20 mg/kg doses were calculated to be 8.25 and 8.29 d, respectively.
- DGGE fingerprint and principal component analysis (PCA) indicated that the composition of the fungal community was obviously changed with the chlorpyrifos treatment, and that samples of chlorpyrifos treatment were significantly separated from those of the control from the beginning to end. While for the bacterial community, chlorpyrifos-treated soil samples were apparently different in the first 30 d and recovered to a similar level of controls up until 60 d, and the distance in the PCA between chlorpyrifos-treated samples and controls was getting shorter through time and was finally clustered into one group. **Together, our results demonstrated that the application of chlorpyrifos could affect the fungal community structure in a quick and lasting way, while only affecting the bacterial community in a temporary way.**
- **Nine typical ERIC types of chlorpyrifos-degrading isolates were screened.**