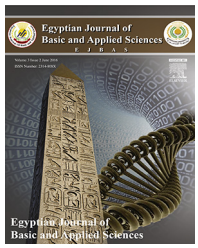
HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/ejbas/default.asp



Full Length Article

Effect of soil disinfection with chemical and biological methods on bacterial communities

Md Rokunuzzaman [a], *Ayumi Hayakawa* [b], *Shinzo Yamane* [b], *Sota Tanaka* [c], *Kouhei Ohnishi* [d,*]

[a] *The United Graduate School of Agricultural Sciences, Ehime University, Matsuyama, Ehime, Japan*
[b] *Faculty of Agriculture, Kochi University, Nankoku, Kochi, Japan*
[c] *Graduate School of Kuroshio Science, Kochi University, Nankoku, Kochi, Japan*
[d] *Research Institute of Molecular Genetics, Kochi University, Nankoku, Kochi, Japan*

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 13 January 2016
Accepted 20 January 2016
Available online 11 February 2016

Keywords:
Pyrosequencing
Chloropicrin
Mustard greens
Disinfection

ABSTRACT

Little is known about the effect of soil disinfection on bacterial communities. Soils were treated with an effective chemical fumigant chloropicrin and biofumigant mustard greens (*Brassica juncea*). While mustard greens did not affect the soil bacterial community structures very much, chloropicrin greatly reduced soil biomass and bacterial species richness. Chloropicrin also influenced the bacterial community structure, making the phylum *Firmicutes* dominant by occupying about 75%. In more than two months, the proportion of *Firmicutes* was reduced to the basal level, and the phyla *Bacteroidetes* and *Proteobacteria* became dominant. Since mustard greens worked as carbon sources for soil reduction, soils were treated with wheat bran and a low concentration of ethanol. Soil reduction with wheat bran and ethanol did not influence the soil bacterial community structures. Beta diversity analyzed by Principal Coordinate Analysis showed that bacterial communities in the soils except chloropicrin-applied soils formed a cluster. All together, biofumigant mustard greens, a probable substitute for chloropicrin, were demonstrated to cause much less damage on soil bacterial community than chemical chloropicrin.

1. Introduction

Soil bacteria play important roles for the maintenance of the soil ecosystem by regulating several significant soil processes, such as decomposition of organic materials, nutrient recycling and mineralization, and inducing pollutant degradation. Many studies have already revealed that agronomic and crop protection practices significantly influence both function and structure of soil microbial communities [1–3].

Soil disinfection with chemical methods, such as pesticides, herbicides and fumigants, has been applied to control weeds, plant diseases and soil borne toxic pathogens all over the world [4]. Some of these chemicals are known to damage

* *Corresponding author.* Tel.: +81 888645213.
E-mail address: kouheio@kochi-u.ac.jp (K. Ohnishi).
http://dx.doi.org/10.1016/j.ejbas.2016.01.003

the environment, be toxic to human, and have some negative effects on soil microorganisms [5–7]. Methyl bromide, one of the highly effective fumigants, is banned in several developed countries because it damages the ozone layer [8]. Chloropicrin, which is widely used in green houses, is also banned by EU as a pesticide for agricultural purposes due to its carcinogenic effects [9]. Chloropicrin is generally regarded as effective on fungal pests, but less effective on nematodes and weeds than Methyl bromide [10].

For the promotion of environment friendly agriculture, the use of organic compounds and green materials such as rice bran [11], oil cake [12], charcoal and ashes are now increasingly attempted in controlling weeds and soil borne pathogens. For the search of alternatives of chemical fumigants, studies are initiated on various aspects to find out the suitable biofumigants. Biofumigation is the agronomic practice of using volatile chemicals (allelochemicals) released from decomposing plant tissues to suppress pests [13,14]. Most of the studies have been done to search for biofumigants with *Brassica* families [15] plants containing isothiocyanate, which has the biocidal effects to nematodes, bacteria, fungi, insects and germinating seeds of weeds. However, very few information is available for the effects of biofumigants on whole soil bacterial communities. On the basis of these consequences, the main objective of this study was to figure out the effects of biofumigants and chemical fumigant chloropicrin application on the bacterial community structures along with soil reduction treatments. *Brassica* family plants for biofumigation are used as carbon sources for soil reduction. Wheat bran and a low concentration of ethanol were used for soil reduction.

Next-generation DNA sequencing technology, in particular pyrosequencing using the Roche/454 platform, has been applied to studies in microbial ecology [16–18]. In this study, we surveyed the bacterial community composition by using barcoded 16S rRNA gene 454-pyrosequencing technology. We found that the chloropicrin fumigants greatly affect the natural soil bacterial composition, whereas the application of biofumigant and reductive treatment did not affect the natural soil bacterial communities too much.

2. Materials and methods

2.1. Soil sampling, preparation and physicochemical properties

2.1.1. Soil preparation and soil sample collection

Plastic containers containing approximately 45 kg of soil were used for the experiment. The soil was sampled from the farm of Education and Research Center for Subtropical Field Science, Kochi University. Each treatment was conducted in five replicates. 1.4 kg of small pieces of shoots and 100 g of roots from two-month-old mustard greens (*Brassica juncea*) were mixed with soil (mustard greens). Soils were mixed with 0.24 kg of wheat bran (wheat bran) and 2% ethanol (ethanol). Containers were covered by plastic sheets and submerged in water for about one month for soil reduction disinfection. Soils were treated with 4 ml of chloropicrin in two holes and covered by plastic sheets for 10 days (chloropicrin). Two tomato seedlings were planted in each container after the first treatment. Soil samples were periodically collected at 5 cm depth from the surface. Time schedule of soil treatment and soil sampling was summarized (Fig. 1).

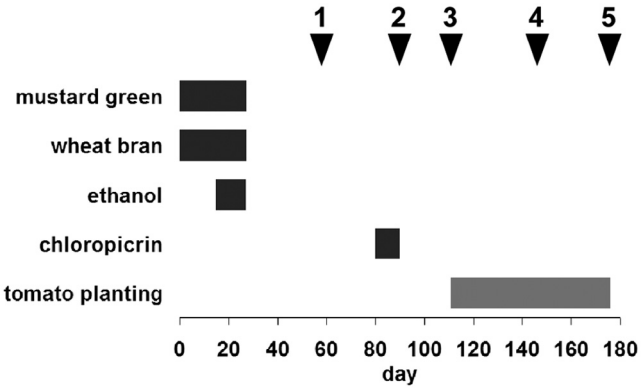


Fig. 1 – Time schedule of soil treatments and soil sampling. Soil was treated with materials shown on the left throughout time indicated by black bars. Tomato plants were grown in the soil during time shown by a gray bar. Soils were collected on the day shown by arrowheads.

2.1.2. Analysis of soil physicochemical properties

Physicochemical properties of soil were analyzed with the following methods. Soil samples were air-dried and passed through a sieve with 2 mm mesh. Soil particle size was determined by the pipette method with sodium hexametaphosphate as dispersing agent. Soil pH was determined in water in a soil solution ratio of 1:5 using the glass electrode. Total carbon and nitrogen contents were analyzed using an NC analyzer (JM1000CN, J-Science). After fresh soil samples were passed through a sieve with 4 mm mesh, soil organic carbon was extracted with a 0.5 M K_2SO_4 solution in a soil to solution ratio of 1:5 and the C concentration was determined by a TOC meter (TOC-VCPH, Shimadzu) [19].

2.2. 454 pyrosequencing and data analysis

DNA was extracted from soil using ISOIL for Beads Beating (Nippon Gene). 0.5 g of soil was disrupted at 5500 rpm for 45 seconds using a Micro Smash MS-100 (Tomy Seiko). The extracted DNA was diluted, sonicated for 5 min, and used as PCR template. The hyper variable V4- and V5-region of 16S rRNA gene was PCR-amplified. The forward primer F563-LXA contained a sequence (CCATCTCATCCCTGCGTGTCTCCGAC) in its 5′ end and a key sequence (TCAG), followed by titanium adaptor (MID1 to MID6, Roche) and specific sequence (AYTGGGYDTAAAGNG). The reverse primer was BSR926-LB (5′-CCTATCCCCTGTGTGCC TTGGCAGTCTCAGCCGTCAATTYYTTTRAGTTT-3′). The PCR product (about 450-bp in size) was purified by Agencourt AMPure XP using sizing buffer (7% PEG6000 and 1 M NaCl). Emulsion PCR was done with Lib-L kit (Roche) and amplicons were analyzed on GS Junior 454 system (Roche).

Raw sequence data were processed and analyzed using QIIME 1.8 [20] through OTUMAMi 3.13 [21]. RDP was used in classifying the sequences into phylum/class and clustering the sequences into operational taxonomic units (OTUs). Representative sequences were selected from each OTU and used