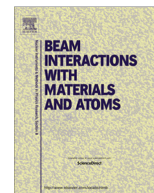
Nuclear Instruments and Methods in Physics Research B 326 (2014) 209–213

Contents lists available at ScienceDirect

Nuclear Instruments and Methods in Physics Research B

journal homepage: www.elsevier.com/locate/nimb



A novel ion-beam-mutation effect application in identification of gene involved in bacterial antagonism to fungal infection of ornamental crops

S. Mahadtanapuk [a], W. Teraarusiri [b], W. Nanakorn [c], L.D. Yu [d,e,*], P. Thongkumkoon [d], S. Anuntalabhochai [f,*]

[a] Faculty of Agriculture and Natural Resources, University of Phayao, Maeka, Muang, Phayao 56000, Thailand
[b] Central Laboratory, University of Phayao, Maeka, Muang, Phayao 56000, Thailand
[c] The Crown Property Bureau, 173 Nakhonratchasrima Road, Dusit, Bangkok 10300, Thailand
[d] Plasma and Beam Physics Research Facility, Department of Physics and Materials Science, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[e] Thailand Center of Excellence in Physics, Commission on Higher Education, 328 Si Ayutthaya Road, Bangkok 10400, Thailand
[f] Molecular Biology Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form 15 October 2013
Accepted 22 October 2013
Available online 22 January 2014

Keywords:
Ion beam
Mutation
Bacterium
Bacillus licheniformis
Fungus
Antagonism

ABSTRACT

This work is on a novel application of ion beam effect on biological mutation. *Bacillus licheniformis* (*B. licheniformis*) is a common soil bacterium with an antagonistic effect on *Curcuma alismatifolia* Gagnep. and *Chrysanthemum indicum* Linn. In an attempt to control fungal diseases of local crops by utilizing *B. licheniformis*, we carried out gene analysis of the bacterium to understand the bacterial antagonistic mechanism. The bacterial cells were bombarded to induce mutations using nitrogen ion beam. After ion bombardment, DNA analysis revealed that the modified polymorphism fragment present in the wild type was missing in a bacterial mutant which lost the antifungal activity. The fragments conserved in the wild type but lost in the mutant bacteria was identified to code for the thioredoxin reductase (*TrxR*) gene. The gene analysis showed that the *TrxR* gene from *B. licheniformis* had the expression of the antagonism to fungi in a synchronous time evolution with the fungus inhibition when the bacteria were co-cultivated with the fungi. The collective results indicate the *TrxR* gene responsible for the antagonism of bacteria *B. licheniformis* to fungal infection.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Low-energy ion beams have been developed for biotechnology applications [1]. The ion beam biotechnology is the use of energetic ions to produce radiation damage in biological matter. The ion-beam-induced radiation damage can act as a new mutagenic source for genetic modification of organisms. It has been reported that low-energy ion beams can produce biological effects as significant as using high-energy ion beams, and therefore, research into low-energy ion beam biotechnology has recently shown rapid growth [1]. One important application of ion beam biotechnology is the induction of mutations in biological objects including rice, flowers and bacteria. Ion beam bombardment can induce a broad spectrum of mutations and treat a large number of biological targets simultaneously. The intended effect of the induced mutations is generally to create improved biological samples. However, in this work, we utilized ion-beam-induced mutation for a novel application in gene identification.

Colletotrichum is a large genus of Ascomycete fungi containing species that are amongst the most successful plant pathogenic fungi. These fungi can cause significant economic damage to crops in tropical, subtropical and temperate regions [2]. The current method to protect against this disease has been through the application of synthetic fungicides, but alternative methods to combat this disease could potentially be less harmful to human health and the environment. One such method has already achieved considerable success utilizing antagonism [3], in which naturally occurring nonpathogenic microorganisms reduce the activity of plant pathogens and thereby suppress diseases. *Bacillus* spp., a potential antagonistic bacterium, has been applied to control anthracnose in many plants [4]. Being pervasive in most soils, *Bacillus* spp. is highly thermally tolerant, capable of rapid growing in liquid culture and easy forming resistant spores, hence it is considered to be a safe biological agent. Therefore, the use of this bacterial species shows great potential in being as a biocontrol agent. However, the mechanism by which it can suppress plant pathogens has not been extensively investigated. In this study, we developed a novel way to identify gene involved in the bacterial

* Corresponding authors. Address: Plasma and Beam Physics Research Facility, Department of Physics and Materials Science, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand. Tel.: +66 53 942464; fax: +66 53 222776.
E-mail addresses: yuld@thep-center.org (L.D. Yu), soanu.1@gmail.com (S. Anuntalabhochai).

0168-583X/$ - see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.nimb.2013.10.036

antagonism to the fungal infection of ornamental crops by using low-energy ion beam bombardment of *Bacillus* spp. bacteria to induce mutation and screening useful phenotypes which could be used for gene selection and cloning in order to investigate the antagonistic property of *Bacillus licheniformis* on Anthracnose, a disease caused by *Colletotrichum musae* (*C. musae*) in *Curcuma alismatifolia* Gagnep. [5].

2. Materials and methods

2.1. Media and culture conditions

The plant-disease fungi, *C. musae*, initially isolated from *C. alismatifolia* Gagnep. bract (Fig. 1A and B), were routinely subcultured onto potato dextrose agar (PDA) [5]. The fungal isolation was following the standard fungi isolation procedures [6]. The *B. licheniformis* strain with highly antagonistic activity against fungal diseases on *C. alismatifolia* Gagnep. and *Chrysanthemum indicum* Linn. and thermo-tolerance was isolated from local hot springs in Sankampaeng, Chiang Mai, Thailand. Typical colonies of the isolates were streaked on lysogeny broth (LB) medium and selected for storage at 4 °C. The bacteria were tested to demonstrate the antagonistic activity in dual culture [7] (Fig. 2A) and on plants as well (Fig. 1C and D). The inhibition percentage of the growth of the fungi was obtained using the formula:

$$100 \times (R1 - R2)/R1[or, = 100 \times (1 - R2/R1)], \quad (1)$$

where $R1$ was the furthest radial growth distance of the fungus in control (or without the antagonistic bacteria) and $R2$ was the radial growth distance of the fungus in dual culture with the antagonistic bacteria [8].

2.2. Ion beam bombardment and median lethal dose (LD50) determination

For the median lethal dose (LD50) determination, one loop of cells of *B. licheniformis* was transferred into 100 ml of LB medium in a 500-ml flask. The cells of *B. licheniformis* were centrifuged to

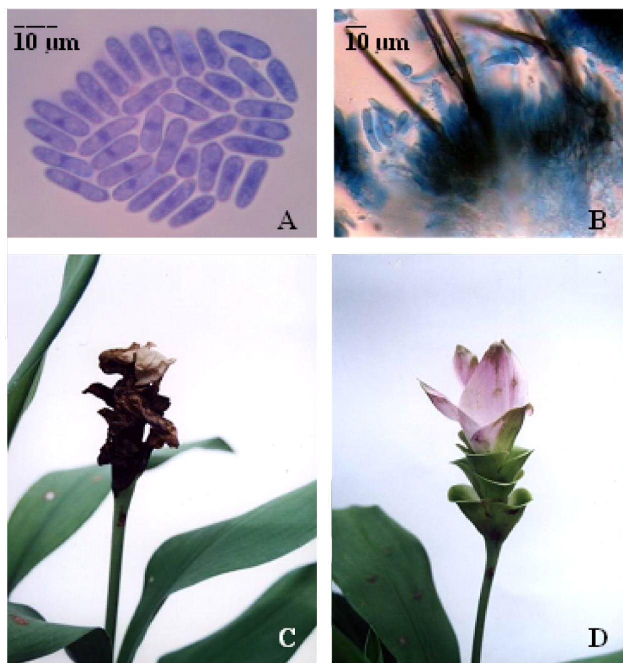


Fig. 1. Anthacnose of curcuma: (A) conidia and (B) acervulus of *C. musae*, (C) diseased flower and (D) diseased flower controlled by *B. licheniformis*. Scale bar: 10 μm.

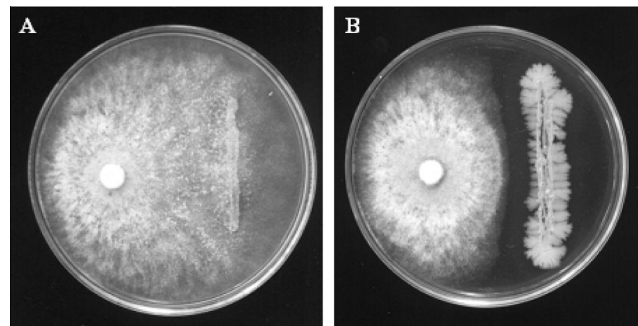


Fig. 2. A dual culture test of the bacterial antagonistic activity. (A) Wild type of *B. licheniformis*. (B) Mutant of *B. licheniformis*. In each dish, on the left side are the bacteria while on the right are the fungi *C. musae*. (B) can be taken as the control for a comparison with the bacterial antagonism shown in (A).

precipitate the cells and then spread as a single-cell layer on a sterile adhesive tape which was attached to a Petri dish and then placed inside a sample holder, which also held a vacuum control. Ion beam bombardment was carried out using the vertical bioengineering ion beam facility at Chiang Mai University. In the experiment, nitrogen (N) ions were used for ion irradiation with energy of 30 keV and fluences in the range of 10^{14}–10^{16} ions/cm^2 at a normal ion flux of an order of 10^{13} ions/cm^2/sec. This flux level was demonstrated to be low enough to maintain the cells survival [1].

After ion beam bombardment, the samples were separately washed down with 10-ml LB solution and centrifuged for 1 min at 9000 rpm. The precipitates were resuspended in 5 ml of LB medium and incubated for 30 min at 37 °C on a rotating shaker at a speed of 220 rpm until the optical density at 600 nm (OD_{600}) reached 0.3–0.6. These cell suspensions were subsequently 10-fold diluted to form samples at concentrations ranging from 10^{-1} to 10^{-11} and grown on solid LB medium. All culture plates were then incubated for 1 day at 37 °C to ensure cell viability. Bacterial mutants were screened for the phenotype which lost their antagonistic ability against the fungi to be further tested. The mutant screening was operated using the dual culture method as mentioned above (Fig. 2).

2.3. DNA fingerprint determination

Genetic alteration in terms of the fingerprint of the ion-bombarded bacteria was detected by the high annealing temperature-random amplified polymorphic DNA (HAT-RAPD) [9]. More than 30 arbitrary RAPD primers (QIAGEN OPERON, USA) were used in PCR amplification. The PCR was performed in a total volume of 20 μl containing 10 × PCR buffer (Tris–HCl 100 mM, pH 8.3, KCl 500 mM), 100 μM each dNTP (dATP, dTTP, dGTP, dCTP), 2.5 mM $MgCl_2$, 0.2–0.4 μM primer, 0.5 unit of Taq DNA polymerase, and 20 ng of DNA template. Reaction tubes were placed in Perkin-Elmer thermal cycle (Gene Amp PCR system 2400) and the following cycling profile was used: 95 °C for 2 min, then 95 °C for 30 s for denaturation, 46–48 °C for 30 s for annealing, 72 °C for 45 s for extension, totally 35 cycles, and finally, 72 °C for 5 min.

2.4. Cloning and sequencing of the gene

From the HAT-RAPD fingerprint, the polymorphic fragment, which was missing in the mutant with an absence of DNA bands in a comparison between the mutant bacteria and wild type bacteria, was reamplified by PCR. The PCR product was electrophoretically resolved on a 1% (w/v) agarose gel, and the appropriate DNA fragment recovered with an Agarose Gel DNA Extraction Kit (Roche, Germany). The purified DNA fragment was ligated into a

Download English Version:

https://daneshyari.com/en/article/1681052

Download Persian Version:

https://daneshyari.com/article/1681052

Daneshyari.com