Isolation and Preliminary Identification of Aluminium-resistant Microorganisms from the Rhizophric Soil of Tea Plant[1]

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Abstract

Microorganism isolated from rhizospheric soil of tea plant was inoculated in culture medium containing aluminium 5-20 mmol/L and an aluminium-resistant fungus ALF-1 was isolated. The ALF-1 fungus possesses aluminium-resistant ability. It could grow normally in the medium containing 20 mmol/L of aluminium and decreased the aluminium content in medium and faded the haematocylin colour of the medium. The fungus was identified as a species of *Neurospora* sp.

Keywords: Tea plant, Rhizosphere soil, Fungus, *Neurospora* sp., Aluminium resistance,

1 Introduction

The aluminium toxicity of soil is a worldwide severe problem in agricultural production [1], the crop production on 40% cultivated land is affected [2], especially in Brazil,Andes distract of South America,China,Central Africa,East America and North Europe. Nowadays, in the practice of crop production on acid soil, the lime application is the main measure to elevate soil pH value, but it is only a temporary means, and with high cost also [3,4]. Whereas, by means of the absorption and absorption of the active aluminium by aluminium-resistant microorganisms and also the chelating of the active aluminium by the secretion of aluminium-resistant microorganisms in acid soil, a prolonged effect with lower cost could be gotten. In the 1970's, an aluminium-consuming bacterium was found on the vehicles, such as steamship by Japanese [5]. Through yearly researches Konishi *et al* [5] found aluminium-tolerant bacterium ST-3991 in severely acidified soil in 1994, and was identified as a new bacterium species. Under the culture condition of pH 3.4 and 100 mg/L aluminium, the pH value of the culture medium was elevated by the bacterium to 4.4 after 5 days, and the aluminium concentration was decreased to 50% of the original. This result brought us a new hope on the improvement of acid soil.

We isolated 2 species of aluminium-resistant microorganism from rhizosphere in acid soil of tea garden in 1996, one as bacterium, being classified to *Xanthomonas* genus, another as fungius ALF-1. Both these two microorganisms could decease the aluminium concentration and increase the pH value of the culture medium, and the later is more effective [6]. Therefore, the further isolation and identification of ALF-1 were carried out.

2 Materials and methods

2.1 Sampling of rhizospheric soil of tea plant

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5 soil samples were sampled from tea garden in Panban Experimental Tea Plantation of Zhejiang University, with 30 years old tea plants of Fuding Dabai cultivar. The sampling method was as follows: to dig the soil at the distance of 20-30 cm from the rhizome of tea plant and collected the soil samples at soil layer of 10-20 cm depth, 1000 g soil was picked for each sample. The pH value of soil samples is 3.8-4.1.

2.2 Preparation of liquid culture medium

200 g neutral soil (pH 7.2) was sampled from Huajiachi Experimental Mulberry Garden of Zhejiang University, sterilized 30 min under 121℃, added 2 L distilled water and shook 30 min, filtrated with No.102 filter paper, and then filtered through the sieve film of 0.45 *u*m. The filtrate was used to prepare S-LB liquid culture medium. The S-LB liquid culture medium contains 0.5 g/L peptone, 0.25 g/L yeast extracts and 10 g/L NaCl. After 30 min's sterilization under 121℃, $Al_2(SO_4)_3$ was added to get 3.7 mmol/L of aluminium concentration, and also to adjust the acidity of liquid culture medium to pH 3.7.

2.3 Preparation of solid culture medium

Fetch 200 g peeled fresh potato, add 1 L of filtrate of the above neutral soil and heat it to boil, at last turn it to mild-boiling temperature for 20 min. The upper liquid was filtered with absorbent cotton, and the filtrate was used to prepare solid culture medium. The solid culture medium contains 0.5 g/L peptone, 0.25 g/L yeast extracts and 20 g/L agar powder. After adjusting pH value to 3.7, the solid culture medium was distributed into 250 ml triangle flasks, each flask with 50 ml. After 30 min's sterilization under 121℃, different amounts of $Al_2(SO_4)_3$ were added under ultraclean condition to adjust aluminium concentration as 5, 10, 15 and 20 mmol/L respectively, at the same time a small amounts of hematoxylin stain was added. The preparation was cooled and ready for use.

2.4 Isolation of aluminium-resistant microorganism

10 ml of S-LB liquid culture medium (pH=3.7) which containing 3.7 mmol/L Al^{3+} , was added into 1 g soil sample (sampled in rhizospheric soil of tea plant), shook and kept overnight under (28±1) ℃. The bacteria that were fetched from the upper liquid were inoculated on the solid culture medium, culture under (28±1) ℃, and the observation of once a day was carried out. After the colonies were grown up, each colony was transplanted respectively on the solid culture medium, which contains higher aluminium concentration for further culture centration for further culture, purification and identification.

2.5 The morphological observation and taxonomy identification of aluminium-resistant fungi

The preparation of wet-chamber and the methods of melting, inoculation, culture and microscope observation etc refers to the method of Chen Shengming etc [7]. The observation and microscopeic observation were carried out 24 h after the inoculation. After that the observation

was carried out once every 8 h, and the photographs were taken in proper field of vision. The taxonomic identification was taken according to Zhou Deqing's method [8].

3 Results and analysis

Having been cultured overnight on S-LB liquid culture medium the microorganism was inoculated on the solid culture medium in which 5 mmol/L Al^{3+} and hematoxylin staining agent contained. Cultured for one day, colonies appeared, some colonies reached 0.5-1.0 cm after 3 days, and several configuration of mycelium could be observed. The mycelium showed white and gray color, in some colonies the pink hematoxylin color in culture medium was faded (Fig.1). Due to that the aluminium is the mordant of hematoxylin stain, the fading of hematoxylin indicated the aluminium concentration around the colonies had been lowed, showing that the growth of these microorganisms possessed the effect of lowering aluminium concentration. So, we selected 3 colonies that could fad hematoxylin stain in culture medium most obviously to be transferred on the solid culture medium which containing higher aluminium concentration, so as to be further screened and purified. Through culturing in different culture media with different aluminium concentrations (10 mmol/L, 15mmol/L and 20 mmol/L), a colony that could normally grow and develop in the culture medium with aluminium concentration of 20 mmol/L was selected and propagated. This colony was used for further studies on morphological observation and aluminium-resistant character, and was named as ALF-1.

After cultured for 24 h under (28±1) ℃in wet-chamber, the ALF-1 mycelium grew, branched and formed a radial colony (Fig.2). Under optical microscope, the colony showed a loose and hairly structure with a rough and long mycelium. The mycelium septum, every cell possesses 1 or 2 nucleus, diameter of mycelium is 6.6-9.2 *u*m (Fig.3). As prolonging of the culture time the cell wall and septum showed more evidence. The cell nucleus is disappeared gradually in aged mycelium (Fig.4).

Two days after the inoculation the conidium of ALF-1 fungus began to appear. Under the microscope, the conidium was located on the erected and branched conidiophere, growing in chain mode. The conidium is long oval shaped, in orange-yellow color and with the dimension of (2.4－3.2 *u*m) × (3.9－5.3 *u*m) (Fig.5), resembling to that of *Neurospora crossa*[8]. The main propagation mode of ALF-1 is asexual propagation, while sometime the sexual reproduction also could be found during the culture. Ascus and ascospores were produced during the sexual reproduction. The ascus located in perithecium was round, smooth and in brown color.

During the culture process the vegetative mycelium grown up constantly, the diameter of colony is varied. At the place contacting with culture medium the mycelium differentiated and formed the rhizomorphous structure, which is called rhizoid. The colony showed pink color gradually, which was caused by the colored secretion by mycelium or the conidium that were dispersed on the culture medium.

Cultured in liquid medium for 3 d under shaken condition, ALF-1 fungus formed a mycelium ball (Fig.6), the mycelium gathered together tightly to form a granular structure and suspended even on the culture liquid. The components of liquid culture medium, especially aluminium, showed an important effect on the formation of mycelium ball. The fungus accelerated its growth in aluminium containing medium and began to form mycelium ball 2 d late. Cultured for 12 h in

the liquid culture medium which no aluminium contained in, then been divided into 2 groups, in one group $Al_2(SO_4)_3$ was added to provide the cultural liquid with 5 mmol/L of aluminium concentration, in another group no $Al_2(SO_4)_3$ was added and was taken as the control. It was found that in aluminium treated mediuml more mycelium was grown up and formed mycelium ball after 6 h of the incubation, at same time the culture liquid became more dense and adhesive, showing pink color. While in control group, no mycelium ball and no color change were found. It is indicated by above test that aluminium could improve the growth and development of the fungus.

Based on above experimental results, ALF-1 fungus is an aluminium-resistant fungus isolated from the rhizospheric soil of tea plant, its growth and development could be improved by aluminium. As the growth of the mycelium hematoxylin stain in the aluminium contained culture medium was faded, so it is possible that this fungus could eliminate the aluminium in environment. According to morphological character and as well as referring to Ainsworth classification system[8], ALF-1 fungus could be classified as a species of *Neuropora* genus. According to the classification nomenclature, a new microorganism that has not been nominated should be denoted as sp., so, we nominated the fungus as *Neurospora* sp..

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