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Research Article

Meristem culture of *Aloe vera* Linn. and its acclimatization

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Abstract

Live, attenuated *Salmonella enterica* serovar Typhi strain Ty21a, a licensed oral typhoid fever vaccine, has also been employed for use as a vector to deliver protective antigens of *Shigella* and other pathogens. Importantly, lipopolysaccharide (LPS) alone has been shown to be a potent antigen for specific protection against shigellosis. We reported previously the plasmid cloning of heterologous LPS biosynthetic genes and the expression in Ty21a of either *S. sonnei* or of *S. dysenteriae* 1 LPS's. The resulting plasmids encoding *Shigella* LPS's were reasonably stable for >50 generations of growth in nonselective media, but still contained an antibiotic resistance marker that is objectionable to vaccine regulatory authorities. Deletion of this antibiotic-resistance marker inexplicably resulted in significant plasmid instability. Thus, we sought a method to insert the large ~12 kb *S. sonnei* LPS gene region into the chromosome, that would allow for subsequent removal of a selectable marker and would result in 100% genetic stability. Toward this objective, we optimized an existing recombination method to mediate the insertion of a ~12 kb region encoding the *S. sonnei* LPS genes into the Ty21a genome in a region that is nonfunctional due to mutation. The resulting strain Ty21a-Ss simultaneously expresses both homologous Ty21a and heterologous *S. sonnei* O-antigens. This chromosomal insert was shown to be 100% genetically stable in vitro and in vivo. Moreover, Ty21a-Ss elicited strong dual anti-LPS serum immune responses and 100% protection in mice against a virulent *S. sonnei* challenge. This new vaccine candidate, absolutely stable for vaccine manufacture, should provide combined protection against enteric fevers due to *Salmonella* serovar Typhi as shown previously (and some Paratyphi infections) and against shigellosis due to *S. sonnei*.

Introduction

Aloe vera Linn. (crocodile tongue) is included into Liliaceae family. It is an ornamental plant and planted in pot or in house yard.

The leaves, root and flower have been used for recover headache, diabetic, constipation, malnutrition, pertussis. They contain Aloin, barbaloin, isobarbaloin, aloe-emodin, aloenin, aloesin [8]. Figure 1 shows an *Aloe vera* plant.

It is necessary to propagate the *Aloe vera*, because of their advantages, Therefore, in this study, meristem of *Aloe vera* was cultured. Meristem culture has benefits as follow [1-7]

1. The plants will be pathogen free because donor plant do not contain vascular bundle. Vascular bundle is able to carry the pathogen.

2. The plants will be genetically stable because meristem is a differentiated tissue.
3. The plants will not be callusing. Therefore, it will reduce somaclonal variation.

The purpose of this study was to propagate the *Aloe vera* so that pathogen free, genetically stable and reduce somaclonal variation.

Materials and Methods

Meristem culture and multiplication

1. The explant (shoot) was surface sterilized by 96% alcohol for 30 second and 20% chlorox for 3 minutes. Then, the explants was washed by sterile water 4 times.

2. The scale of the shoot was opened under stereomicroscopy until 0.2-0.5 mm shoot meristem was obtained.
3. The shoot meristem was cultured on initiation media MS containing 1 g/l active charcoal for 2 weeks.
4. Thereafter, the shoot was subcultured on MS media supplemented with 0.5 mg/l BAP and 0.025 mg/l NAA for multiplication.

Acclimatization

After plantlet developing, the plants were acclimatized by covering them with plastic bag.

Results and Discussion



Figure 1. Aloe vera Linn.

Based on the method above, plantlets of Aloe vera developed. Figure 2 shows the plantlets of Aloe vera. Its

appearance is similar as plant of Aloe vera in the yard.



Figure 2. Plantlet of Aloe vera Linn.

Aloe vera contains vitamin A, B, B2, B3, B12, C, E, choline, inositol and folic acid. Besides, it also contains macro and micronutrients, namely Ca, Mg, K, Na, Fe, Zn and Cr. In addition, several kind of enzymes were found in Aloe vera, such as amylase, catalase, carboxypeptidase, bradykinase.

Those nutrient, vitamins and enzymes could act as natural antioxidant. Therefore, people who consume Aloe vera

could avoid disease like cancer disease. The antioxidants good for supporting immunity of our body [9] (<http://tarmediadi.blogspot.com/2011/08/cara-menanam-lidah-buaya-dan-manfaatnya.html>). Consequently, Aloe vera could cure cancer disease naturally.

The *Aloe vera* during acclimatization is shown in figure 3 and 4.



Figure 3. One plant of Aloe vera during acclimatization



Figure 4. Six plants of Aloe vera during acclimatization

According to information in the following website <http://www.slideshare.net/guest800e180/plant-tissue-culture-of-aloe-vera>, Aloe vera is a fairly well known herbal preparation with a long history of use. It is widely used in modern herbal practice and is often available in proprietary herbal preparations.

It has two distinct types of medicinal use. The clear gel contained within the leaf makes an excellent treatment for wounds, burns and other skin disorders, placing a protective coat over the affected area. This action is in part due to the presence of aloectin B, which stimulates the immune system

The second use comes from the yellow sap at the base of the leaf. The leaves are cut transversally at their base and the liquid that exudes from this cut is dried. It is called bitter aloes and contains anthraquinones which are a useful digestive stimulant .

Aloe is used in many skin care products because of its ability to stimulate healthy cell growth and repair damaged tissues. Most people think of using Aloe only on their skin because that is all they know about

Used for the treatment of chronic constipation

Aloe extract may be useful in the treatment of diabetes. Contains numerous vitamins and enzymes which have anti-microbial active

Uses in cosmetics :

It is beneficial for the cosmetic products such as make up, anti-wrinkle creams, facial masks, skin conditioners and lipsticks.

The leaf extracts are used in skin-care cosmetic products. Based on the result and discussion, we concluded that the *Aloe vera* Linn. could be meristem culture method.

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