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MEDIA FACTOR USES FOR ISOLATION TRANSFORMATION AGROBACTERIUM STRAIN LBA4404 FROM OTHER BACTERIA

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Abstract

Growing Agrobacterium for commercial generation of recombinant proteins via transient expression has not been thoroughly studied in the literature. The effects of media composition on Agrobacterium growth for transient expression were examined in this work. Lysogeny broth (LB) media (also known as Luria-Bertani media or Luria Broth) 10 and yeast extract-peptone (YEP) media are extensively utilized in studies for Agrobacterium growth. Benthamiana leaves infiltrated with Agrobacterium in chemically specified AB media outperformed leaves infiltrated with Agrobacterium in LB media in terms of transient expression level and post-incubation leaf health.

Key words: *Agrobacterium*, (LB) media, (YEP) media

Introduction

Agrobacterium tumefaciens belongs to the Rhizobiaceae family of -Proteobacteria. It is a soil microorganism and a facultative phytopathogen capable of infecting plants with crown gall disease. A. tumefaciens is closely linked to nitrogen-fixing rhizobia symbiotic species. Its pathogenicity is dependent on the presence of a plasmid called the Ti (tumor-inducing)plasmid. For a thorough examination of Agrobacterium biology (Tzfira and Citovsky., 2008), growth mediums for Agrobacterium?Complex media such as lysogeny broth (LB) media (alternatively known as Luria-Bertani media or Luria Broth) 10 and yeast extract-peptone (YEP) media are commonly used Supplemented with 50 mg/l Kanamycin and 25 mg/l Rifampicin for Agrobacterium growth in research (Shamloul and Trusa et al). One milliliter of this overnight developed Agrobacterium tumefaciens culture was pelleted at 4000 rpm for 5 minutes (Akiyoshi et al., 1984). The presence of two genetic components situated on the bacterial Ti-plasmid is required for the A. tumefaciens-mediated plant genetic transformation process. The first critical component is T-DNA, which is defined by border sequences, which are conserved 25-base pair imperfect repeats at the extremities of the T-region (Jeon et al., 1998). The second is the virulence (*vir*) region, which is composed of at least seven major loci (*virA, virB, virC, virD, virE, virF,* and *virG*) encoding components of the bacterial protein machinery mediating T-DNA processing and transfer (Gelvin SB ., 2000) . The VirA and VirG proteins are two-component regulators that activate the expression of other *vir* genes on the Ti-plasmid. The VirB, VirC, VirD,

VirE and perhaps VirF are involved in the processing, transfer, and integration of the T-DNA from *A. tumefaciens* into a plant cell. Figure 1 shows the major steps of the *Agrobacterium*-mediated plant transformation process.

Agrobacterium tumefaciens causes crown gall disease of a wide range of plants, especially members of the rose family such as apple, pear, peach, cherry, almond, raspberry and roses. The discovery of the bacterial origin of crown gall disease (Smith and Townsend, 1907) sparked a number of studies with understanding the mechanisms of oncogenesis in general and applied it to study of cancer disease in animals and humans as objectives. The elegant work of Binns and Thomashaw (1988) which revealed that A. tumefaciens is capable of transferring a particular DNA segment Transfer (T)-DNA of the tumour-inducing (Ti) plasmid into the nucleus of infected cells where it is subsequently integrated into the host genome, changed the objectives of research on A. tumefaciens to transformation of plants (Reem Waleed Yonis., 2012). Early realization of this goal was brighten with the report that the T-DNA contains two types of genes: the oncogenic genes, encoding for enzymes involved in the synthesis of auxins and cytokinins and responsible for tumour formation; and the genes encoding for the synthesis of opines, a product resulted from condensation between amino acids and sugars, which are produced and excreted by the crown gall cells and consume by A. tumefaciens as carbon and nitrogen sources (Norrya Ali3 *et al.,* 2017*).* Outside the T-DNA, are located the genes for the opine catabolism, the genes involved in the process of T-DNA transfer from the bacterium to the plant cell and for the bacterium-bacterium plasmid conjugative transfer genes (Zupan and Zambrysky, 1995).

Material and method

MacConkey agar Examnation

Suspend the components, dehydrated powder, in water (49.53 grams in 1000 ml of purified / distilled water). The medium is boiled for a few seconds until the ingredients are completely dissolved. Sterilize by autoclaving at 15 lbs (121 ° C) pressure for 15 minutes. Cool to 47 ° C, mix well before pouring into sterile Petri dishes .

Preparation of MacConkey agar

Suspend 49.53 grams of the dehydrated medium in 1000 ml purified/distilled water.

1. Heat to boiling to dissolve the medium completely.
2. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes i.e. validated cycle.
3. Cool to 45-50°C.
4. Mix well before pouring into sterile Petri plates.
5. Leave for drying.

Gram positive vs gram negative.

Gram positive bacteria are surrounded by a single thick peptidoglycan cell wall and are therefore termed monoderms. Gram negative bacteria have a much thinner peptidoglycan cell wall, but in addition they have an outer membrane containing lipopolysaccharides surrounding the cell and are consequently termed diderms (Gram, H.C., 1884).

Culture Media.

E. coli was grown in Luria Bertani (LB) broth and agar in all of the experiments, Table 3-4. For LB broth, dissolve 10g/L Sodium chloride, 10g/L Tryptone, and 5g/L Yeast extract in distilled water, then adjust the pH to 7.3 with 1N NaOH. For LB agar, 15g/L agar was added to the broth.The culture media were sterilized in an autoclave at 121 ºC (15 Ib/In2) for 20 min., following the manufacturer's recommendations.Then leave the media to cool at room temperature and add Ampicillin solution (100 mg/ml) to the media, some of the media were poured into Petri dishes, then incubated at 37 ºC for 24 hr. and then kept at 4ºC until use.

Source of Genes.

PICS11052 vector contains the *CRY1AC* gene, which encodes for the production of crystal protein. This study sample was obtained (synthrsis)from Genscript compony USA. Gene synthesis is biology method used to engineer artificial genes within a laboratory setting. This method has allowed us, at Synbio Technologies, to go from a sequence in text format to physical copy of the genetic sequence itself with ease and extreme accuracy

Agrobacterium tumefaciens strain LBA4404 .

Agrobacterium tumefaciens strain LBA4404 obtained from Leiden University in the Netherlands and engineered by prof: Hooykaas.P.j.j.(Paul) , that has become disarmed.Transformation of Agrobacterium.tumefaciens and *E. coli* BL21(as apositive control) with the (PICS11052+Cry1ac) was done. $CaCl_2$ is used in the production of competent cells (Sambrook and Russell, 2006).

Results

MacConkey agar examed.

MacConkey agar only grows gram-negative *bacteria* , and those bacteria will appear differently based on their lactose fermenting ability as well as the rate of fermentation and the presence of a capsule or no.Lactose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. (Figure 4-3)

Figure 4-3) *Agrobacterium* gram nagative grown on McConkey agar with lactose fermentation (left)and macConkey media nonformentation lactose(right)

3 Gram-positive and Gram-negative Examnation.

Gram-positive and Gram-negative are two distinct types of bacteria. People categorize these types based on their structures and their appearance after Gram staining. Gram staining is a process of dying bacteria and then viewing them beneath a microscope. Gram positive bacteria have a distinctive purple appearance when observed under a light microscope following Gram staining. This is due to retention of the purple crystal violet stain in the thick peptidoglycan layer of the cell wall (Akamal Mohammed and Z Mohsen Ahmed., 2021) (Figure 4-5).

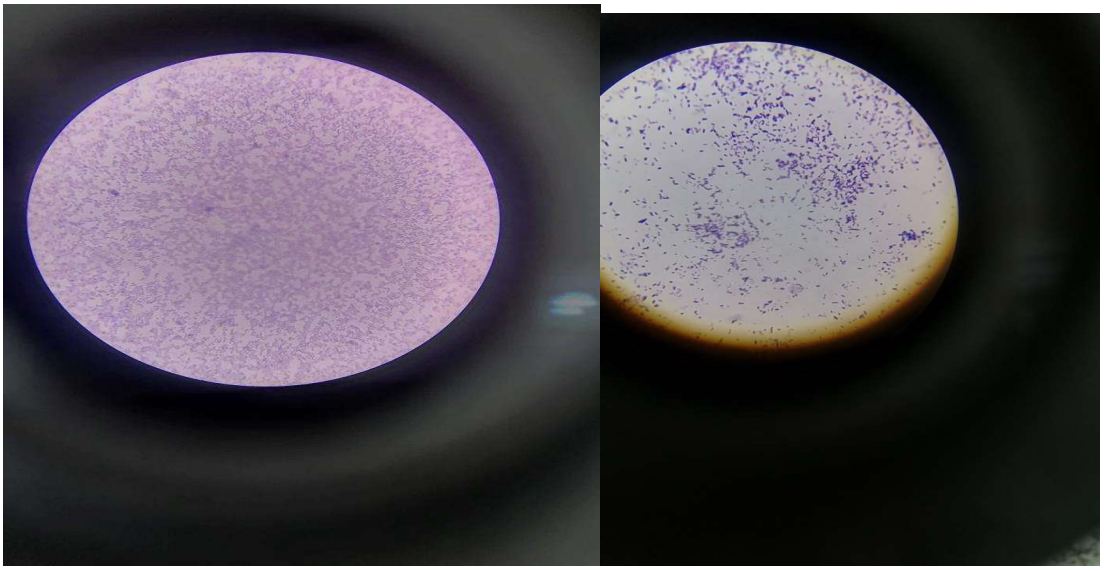

Figure 4-5) Agrobacterium gram negative Bacteria bacillus thuringiensis (control) gram Positive
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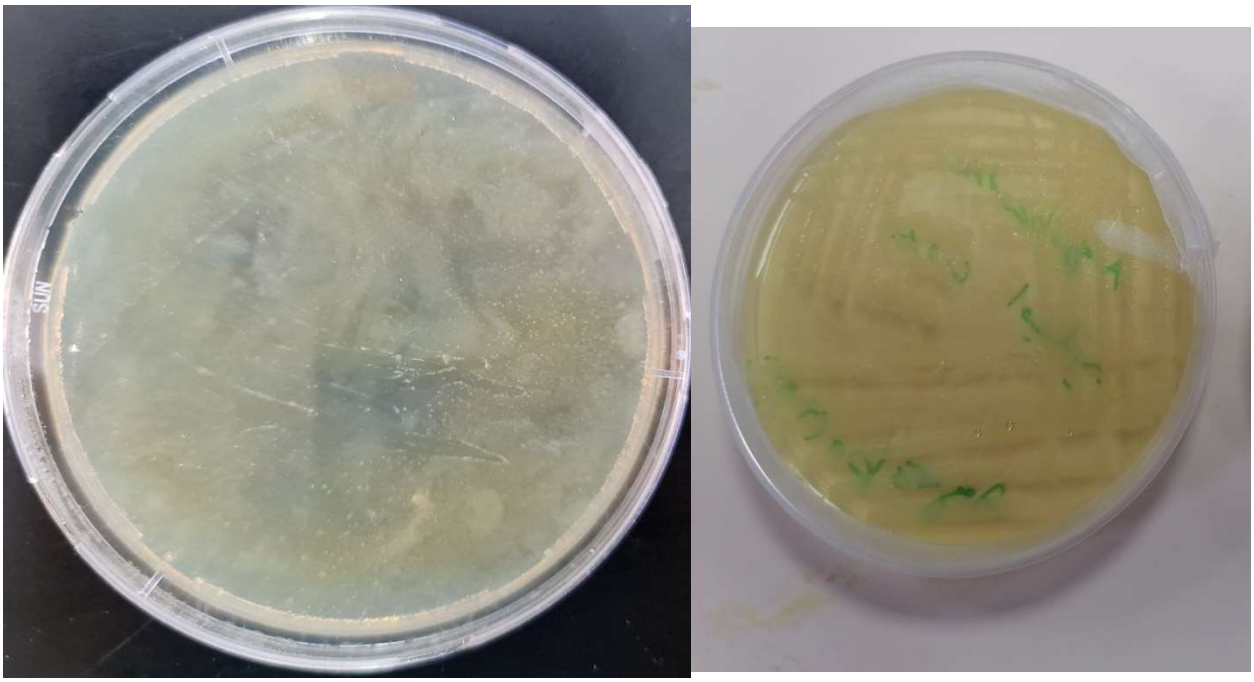
Transformation of tomato plant with recombination vector.

Agrobacterium containing the construct (PICS11052) contain ambicilin *gene* resistance + *cry1Ac gene*) and also Ecoli bacteria contain same recombination vector was grown for 24 h at 28°C, with shaking at 200 rpm in Luria broth LB, containing kanamycin (100 mg/l) , rifampicin (100 mg/l), ampciline(100mg/l) for A. tumefacie and only ampciline(100mg/l) for Ecoli LB media broth at 37 C. The A.tumefacie and Ecoli grow in LB agar(1% tryptone, 0.5% yeast extract, 1% NaCl, 1.6% agar) contain seme antibiotic for 24 h. (Figur:4-8).



Figur:4-8) (right) Recombination Agrobacterium grow on ager contains ampicillin antibiotic(resistance) (left) Non recombination agrobacterium not grow on agar contain ampicillin antibiotic(sensitive).

Discussion

Efficiency of Agrobacterium-mediated in vitro plant transformation is influenced by bacterial factors such as the culture density and the ability of the used strain to attach to the host (Shen et al., 1993) and by plant factors, such as the secretion of molecules inducing the Agrobacterium T-DNA transfer system and references therein, the plant cell's competence for transformation, the cell cycle stage and regeneration. In addition, several environmental factors, such as low temperatures (between20 and 25 °c depending on the transformed plant species), low pH, several sugars and continuous light, have a positive effect on transient or stable transformation efficiencies (Zambre et al., 2003).

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