# Transformation of Agrobacterium by cry1Ac gene from *Bacillus thuringiensis*

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**Abstract**

Biotech crops express novel traits derived from other organisms, such as increased insect, herbicide, virus, and drought resistance. Among insect-resistant varieties, Bacillus thuringiensis (Bt) crops are most often used. Bt crops are engineered to produce Cry toxins from the soil bacterium Bt that compromise the gut integrity of members of specific insect families, making them highly selective insecticides. One such toxin is Cry1Ac (Cry), effective against the larvae of certain lepidopteran insects. Biotech crops express novel traits derived from other organisms, such as increased insect, herbicide, virus, and drought resistance. Among insect-resistant varieties, Bacillus thuringiensis (Bt) crops are most often used. Bt crops are engineered to produce Cry toxins from the soil bacterium Bt that compromise the gut integrity of members of specific insect families, making them highly selective insecticides. One such toxin is Cry1Ac (Cry), effective against the larvae of certain lepidopteran insects. The Cry1 gene (3537 bp) was loaded on a clone vector (PICS11052 T-DNA binary vector 4875 bp) to form a hybrid DNA. The hybrid DNA was introduced into Agrobacterium, the process of transformation into E. coli BL21(DE3), the bacteria were used to monitor the transformation process and the culture of the transformed bacteria on a medium containing the ampicillin.

**Keywords:** *Agrobacterium, cry1Ac, Bt, Transformation, E. coli.*

## Introduction

Agrobacterium-mediated plant transformation has become the most commonly used method to introduce foreign genes into plant cells. The molecular mechanism underlying this transformation consists of different steps, in which both Agrobacterium and plant host proteins are involved (Gelvin SB., 2010), (Pitzschke A and Hirt H., 2010).

After attachment of Agrobacterium to its host, the T-DNA strand and several VIR proteins are transferred to the plant cell. Subsequently, the T-DNA strand migrates towards the nucleus, where It becomes double stranded (Tzfira T and Citovsky V., 2006).

The strain of Agrobacterium tumefaciens includes the pAL4404 plasmid, which only contains the T-DNA vir region LBA4404 has

an octoprine-type Ti plasmid pAL4404 without self-transport function, (genes responsible for vir gene induction and T-DNA transfer). LBA4404 is a widely used strain for plant transformation. This product contains competent cells for transformation by used CaCl2. Using A. tumefaciens and the binary vector method, you can transform various plants for infection (transfection) experiments.

Bacillus thuringiensis, a Gram-positive bacterium, is widely known for the production of parasporal crystals in mother cells (Pardo-Lopez L et al. 2009). Because of the insecticidal properties of the crystal proteins, B. thuringiensis has been commercially used for many years as a pesticide in biocontrol applications ((Abdulla Hussan E., 2018). The parasporal crystal consists mainly of proteins encoded by cry genes that are classified according to amino acid sequence identity among their respective Cry proteins (Crickmore N et al. 1998). Cry1Ac protoxin is a crystal protein produced by the gram-positive bacterium , Bacillus thuringiensis (Bt) during sporulation.

Cry1Ac is one of the delta endotoxins produced by this bacterium which act as insecticides. Because of this, the genes for these have been introduced into commercially important crops by genetic engineering (such as cotton, tomato and corn) in order to confer pest. resistance on those plants (McLean M ., 2011).

By wounding of plant tissue it will induces the production of numerous defense compounds, including some phenolics that can, along with neutral and acidic sugars, trigger a two-component sensory response system (VirA–VirG) that induces Agrobacterium vir gene expression ( Safaa A. Hadie .,2016). Among the induced Vir proteins are VirD1, a helicase, and VirD2, a nuclease (Herrera-Estella A.,1990). Together, they nick the T-DNA region of Ti plasmids, usually between bases 3 and 4 of nearly identical 25-bp left border (LB) and right border (RB) repeat sequences.

Cry1Ac provides protection from feeding damage by target lepidopteran pests (Zahra M. Al-Khafaji et al., 2011). Characterization studies have confirmed that the genetic modification in MON 87701 contains a single insert with the intended sequence, and that the insert is stable over multiple generations. Their primary action is to lyse midgut epithelial cells by inserting into the target membrane and forming pores. Among this group of proteins, members of the 3-Domain Cry family are used worldwide for insect control, and their mode of action has been characterized in some detail. (Figure -1).

## Material and methods

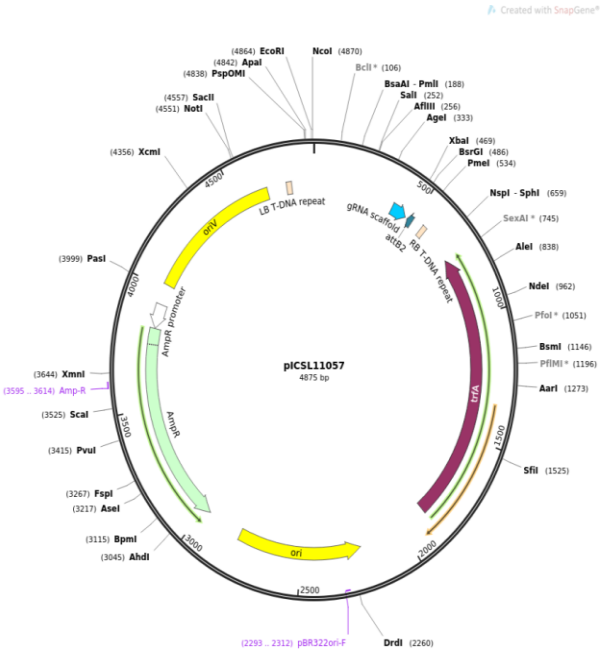
### Agrobacterium tumefaciens strain LBA4404

The Agrobacterium tumefaciens strain LBA4404 ,obtained as a gift from professor: Hooykaas, P.J.J. (Paul) /Leiden University/Netherlands. A. tumefaciens (LBA4404 strain ) ,used for transfer T-DNA (transfer DNA), which is part of its own Ti plasmid, into host plant cells and insert this DNA into the plant chromosomal DNA. E. coli BL21(DE3) strain (NEB, USA-C2527H) obtained as a gift from Dr. Almuthana K. Hameed.

### T-DNA binary vector(PICS11052) and CRY1AC gene recobination

The synthesized cry1ac gene size (3537 bp) and the vector PICS11052 (4875 bp)(figure 1) and cloning the gene on the vector obtained from Genscript company/ USA depending order. (GenScript Biotech Corporation (Stock Code: 1548.HK) New Jersey, USA.

**(Figure -1) PICS11052 T-DNA binary vector Map.**




## 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.

First described in 1951 by Giuseppe Bertani, a 1-liter medium consists of 10 grams of tryptone, 5 grams of yeast extract, and 10 grams of sodium chloride16 mg agar.containing rifampicin (100 mg/L) ,streptomycin (50 mg/L) for 2–3 days (Fig3-1)). One colony was isolated and grown in 50 mL LB broth at 28 °C under constant shaking (200 rpm) (figur:3-2)

## Bacterial Transformation

Transformation of Agrobacterium strain LBA4044 and E. coli BL21 with the ligation products (cry1ac gene with vector) was done by used heat shock and CaCl2 to production the competent cells (Sambrook and Russell, 2006).

## Extraction of Plasmid (LBA4044 +Cry1ac)

PureYield™ Plasmid Miniprep System protocol (Promega, 2021) was used for extracting recombinant vector (PICS4404 and DNA insert) from transformants of A. tumefacies and E. coli BL21. A. tumefaciens and E. coli BL21( used as a positive control) with the (PICS11052+Cry1ac) was done, by used

## DNA Loading and Electrophoresis

Two microliters of loading dye were mixed with 3µl of DNA. After carefully loading the samples into the gel wells, the electrical power is turned on at 70 volts/cm for 60 min. Using a UV transilluminator set to 254 nm, the Ethidium Bromide stained the DNA band in the gel, which was subsequently photographed in a gel document device (Sambrook and Russell, 2001).

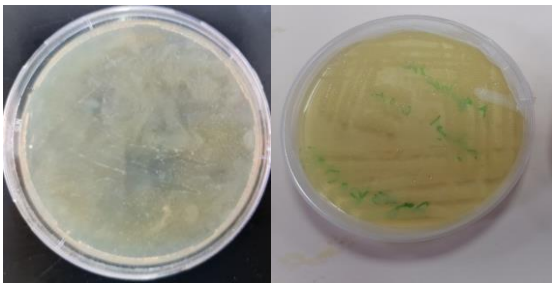
## Results

Agrobacterium containing the construct T-DNA binary vector (PICS11052) contain ampicillin gene resistance + cry1Ac gene) and also E. coli 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 E. coli LB media broth at 37 C. The A. tumefacie and E. coli grow in LB agar(1% tryptone, 0.5% yeast extract, 1% NaCl, 1.6% agar) contain same antibiotic for 24 h . The

result provet only recombination plasmid (contain gene ampcilin resistance ) can grow in this media (Figure-3).

Transformed agrobacterium placed on petri dish contain selection medium (MS containing Gamborg's B5 vitamins, 50 mg/l ,sucrose30g/1, 1 g/l IAA, g 0.5 mg/l of 2,4-D supplemented with 100 mg/l kanamycin ) (Figure:- 4).

**(Figure-3) (right) Recombination Agrobacterium grow on ager contains ampicillin antibiotic(resistance) (left) Non recombination agrobacterium not grow on agar contain ampicillin antibiotic(sensitive)**

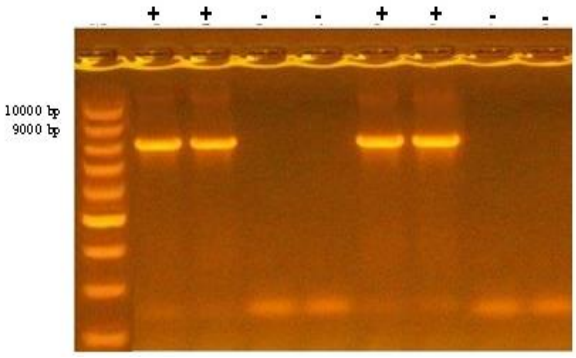


**(Figure - 4) The right and left transformation agrobacterium and Ecoli cell Grow on LB agar contain ampicillin (resistance) because both bacteria become they have T-DNA binary vector +cry1ac that resistance ampicillin.**

**Electrophoresis of recombination plasmid**

The result when electrophoresis using ladr size 10000 pb to a migrate the plasmid extracted from agrbacteriium ( Cry1ac size 3537 pb +T-DNA binary vector PCS11057 size 4875 pb = 8412 pb ), it was found that the transformation process occurred (figure-5).

**(Figure- 5) electrophoresis of transfer extraction plasmid (PICS11052 + cry1ac gene size 8412 pb) from agrobacterium cell transformation positive (+) with agrobacterium non-transformation negative (-). With 1Kbp DNA marker.**




**Discussion**

Agrobacterium-mediated plant transformation has become the most used method to introduce foreign genes to obtain a desired phenotype in a variety of crops, the fundamental knowledge underlying the molecular mechanism of Agrobacterium-mediated plant transformation has been a hot topic since many years. The important events including the bacterial attachment to the plant cell, vir gene activation, T-DNA processing, nuclear targeting and T-DNA integration have been quite well studied. Cry 1ac genes are widely used in crop genetic engineering and the resulting plants, including cotton and maize, comprise a huge portion of total transgenic crop production (Sanahuja et al. 2011). Bt crops are used for eco-friendly control of insect pests on plants and have been reported to be safe to vertebrates and natural enemies of insect pests (Ferré et al. 2008)although the role of the host cellular proteins involved in the transformation process remains largely obscure and is still under extensive investigation. A better

understanding of all molecular events in the process as well as the plant proteins involved could be exploited for the further improvement of Agrobacterium-mediated plant transformation. In addition, the knowledge on the factors that influence the transformation efficiency is also crucial. The cry1ac gene is very important in (G.M) of Agricultural crops, because it have been introduced into commercially important crops by genetic engineering (such as cotton, tomato and corn) in order to confer pest resistance on those plants. (McLean M (2011).

We recommendation to using transformation agrobacterium ( T-DNA PICS11057+cry1ac gene) to infection crop agreculture and record the results

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