Burkholderia glumae Competent Cells Preparation and Transformation

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[Abstract] *Bukholderia glumae* is a gram-negative bacterium which causes grain rot, seedling rot and panicle blight in rice and bacterial wilt in many field crops. This bacterium has been reported from major rice growing regions around the world and is now considered as an emerging major pathogen of rice (Tsushima *et al*., 1996; Jeong *et al*., 2003; Kim *et al*., 2010; Ham *et al*., 2011). Here we describe two methods for competent cells preparation and transformation of *B. glumae*. Using these methods, we have applied effector detector system (Sohn *et al*., 2007) to *B. glumae* (Sharma *et al*., 2013).

Materials and Reagents

1. *Burkholderia glumae* strain 106619 (National Institute of Agricultural Sciences Genebank, Tsukuba, Ibaraki, Japan)
2. pEDV5 based vectors (Fabro *et al*., 2011; Sharma *et al*., 2013)
3. Gentamycin
4. Tryptone
5. Yeast extract
6. Protease peptone no. 3
7. Ice
8. Lysogeny Broth (LB) medium (see Recipes)
9. King's Broth (KB) agar (see Recipes)
10. KB agar with 25 ng/ml gentamycin (see Recipes)
11. 10% glycerol (see Recipes)
12. 300 mM sucrose solution (see Recipes)

Equipment

1. Deep freezer
2. Autoclave
3. Clean bench
4. Petri plates

5. Sterile 1.5 ml tubes
6. Sterile 15 ml tubes
7. Sterile 50 ml tubes
8. Incubation Shaker
9. Aluminum foil
10. Spectrophotometer
11. Centrifuge
12. Toothpick
13. MicroPulser/Gene Pulser Cuvettes, 0.2 cm gap (Bio-Rad Laboratories, catalog number: 165-2089)
14. MicroPulser/Gene Pulser Cuvettes, 0.1 cm gap (Bio-Rad Laboratories, catalog number: 165-2086)
15. Gene Pulser Xcell™ Electroporation Systems (Bio-Rad Laboratories)
16. Minisart filters (pore size 0.2 µm) (Sigma-Aldrich, catalog number: 16534K)
17. Disposable Cell Spreaders

Procedure

Part I: Conventional method

A. Preparation of competent cells

1. 10 µl of glycerol stock of the *B. glumae* strain is inoculated to 20 ml of LB medium in a 50 ml tube and further incubated at 28 °C for 16-40 h with horizontal shaking at 200 rpm until OD_{600} = 0.8 is achieved.
2. The lid of the tube is opened for 30 sec under a clean bench.
3. The tube is incubated again at 28 °C for 4 h with horizontal shaking at 200 rpm.
4. The tube is centrifuged twice at 800 *x g* at 4 °C for 5 min.
5. Each time the pellet is dissolved in 20 ml of autoclaved cold 10% glycerol.
6. The pellet is dissolved in 200 µl of cold 10% glycerol and divided into 50 µl aliquots and stored at -80 °C for a further transformation step.

B. Transformation

1. Remove from -80 °C tubes containing 50 µl of electro-competent *B. glumae* cells.
2. Thaw the cells on ice.
3. Add ~1 µg of pEDV5 based vector into the *B. glumae* cells. Incubate on ice up to 3 min.
4. Transfer the mixture of cells + DNA to a cold electroporation cuvette (0.2 cm electrode gap). Make sure the suspension is at the bottom of the cuvette.

5. Set the Gene Pulser apparatus at 25 µF the volt at 2.5 kV. Set the Pulse resistance controller to 200 ohms.
6. Place the cold cuvette in the chamber slide (Cuvette notch facing away from you).
7. Push the slide into the chamber until the cuvette is seated between the contacts in the base of the chamber.
8. Electroporate by pushing the red button.
9. Remove the cuvette from the chamber and immediately add 1 ml of LB medium to the cuvette and quickly resuspend the cells by pipetting.
10. Transfer the cell suspension from the cuvette to 1.5 ml tubes and incubate on shaker (200 rpm) at 28 °C for 2 h to allow recovery and expression of the gentamycin resistance marker (Clean cuvettes successively with dH_2O, EtOH, sterile water, then wrap with aluminum foil then autoclave).
11. Pipette 200 µl of each transformation on KB agar plates containing 25 ng/ml gentamycin.
12. Spread the cells with cell spreaders. Place plates inverted at 28 °C for 2-3 days in the dark.

Part II. High competency method

A. Preparation of competent cells

1. A frozen glycerol stock of the *B. glumae* strain is picked with a toothpick and spread on a KB agar plate. Place plates inverted at 28 °C for 2-3 days in the dark.
2. Freshly grown *B. glumae* colony is inoculated to 5 ml of LB medium in a 15 ml tube and further incubated at 28 °C for 16 h with horizontal shaking at 200 rpm.
3. 1 ml aliquots is centrifuged twice at 3,500 *x g* at 4 °C for 5 min.
4. Each time the pellet is dissolved in 1 ml of filter sterilized and room temperature 300 mM sucrose solution.
5. The pellet is dissolved in 200 µl of 300 mM sucrose solution and divided into 100 µl aliquots and used the cells immediately for a further transformation step.

B. Transformation

1. Add ~1 µg of pEDV5 based vector into the *B. glumae* cells.
2. Transfer the mixture of cells + DNA to an electroporation cuvette (0.1 cm electrode gap). Make sure the suspension is at the bottom of the cuvette.
3. Set the Gene Pulser apparatus at 25 µF the volt at 1.8 kV. Set the Pulse resistance controller to 200 ohms.
4. Place the cuvette in the chamber slide (Cuvette notch facing away from you).
5. Push the slide into the chamber until the cuvette is seated between the contacts in the base of the chamber.

6. Electroporate by pushing the red button.
7. Remove the cuvette from the chamber and immediately add 1 ml of LB medium to the cuvette and quickly resuspend the cells by pipetting.
8. Transfer the cell suspension from the cuvette to 1.5 ml tubes and incubate on shaker (200 rpm) at 28 °C for 2 h to allow recovery and expression of the gentamycin resistance marker (Clean cuvettes successively with dH_2O, EtOH, sterile water, then wrap with aluminum foil then autoclave).
9. Pipette 200 µl of each transformation on KB agar plates containing 25 ng/ml gentamycin.
10. Spread the cells with cell spreaders. Place plates inverted at 28 °C for 2-3 days in the dark.

Recipes

1. LB medium
   Mix 5 g of tryptone
   2.5 g of yeast extract
   5 g of NaCl with 800 ml dH_2O
   Add 0.2 ml of 5 N NaOH
   Add dH_2O to 1,000 ml and autoclave it for 20 min
2. KB agar
   Mix 20 g of protease peptone no. 3
   10 g of glycerol
   1.5 g of K_2HPO_4
   1.5 g of $MgSO_4 \cdot 7H_2O$ with 800 ml dH_2O
   Adjust to pH7.2
   Add dH_2O to 1,000 ml, add 15 g of agar and autoclave it for 20 min
3. KB agar with 25 ng/ml gentamycin
   Cool down the autoclaved KB agar to 50 °C
   Add 1 ml of 25 mg/ml gentamycin
   Pour in 90 mm x 15 mm Perti dish
4. 10% glycerol
   Mix 100 g of glycerol with 900 ml dH_2O and autoclave it for 20 min
5. 300 mM sucrose solution
   Mix 10.27 g of sucrose with 80 ml
   Add dH_2O to 100 ml and filter (0.2 µm) sterilizes it

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