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Protocol preparation of bacterial cells

In 1 collection

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Abstract

This protocol details the preparation of bacterial cells to set up LSBA.

Materials

- MOKA rich medium

For 1 liter:

	A	B
	Yeast extract	4 g
	Casamino acids	8 g
	K2HPO4	2 g
	MgSO4.7H2O, pH 7.3	0.3 g
	Autoclaved at 120°C for 20 min	

- 1 mM $MgCl_2$.
- LB rich medium

For 1 liter:

	A	B
	Tryptone	10 g
	Yeast extract	5 g
	NaCl, pH 7.2	5 g
	Autoclaved at 120°C for 20 min	

- LM medium

For 1 liter:

	A	B
	Yeast extract	0.2 g
	Peptone	0.1 g
	HEPES, pH 7.4	10 mM
	NaHCO3	10 mM
	Tween 20, pH 7.2	0.1%
	Filtered using a 0.22 micron filter	

Biological material

- Xcc strain 8004::GUS*-GFP* (carrying the point mutations inactivating the catalytic sites of the β-glucuronidase and GFP proteins, made in a 8004::GUS-GFP background [3,4].
- Xcc strain 8004::GUS-GFP deleted for both the fliC flagellin and the pilA and pilE pillins [4].
- Shewanella oneidensis strain MR1-R [2].

For *Xanthomonas campestris* pv. *campestris* (Xcc)

8h 20m

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Note

Estimated preparation time of the bacterial suspension is around one hour excluding an overnight growth step.

We collect bacteria in exponential phase. (OD_{600nm} between 1 and 2). For our experiments, 400 mL of washed bacteria at an OD_{600nm} of 0.1 (1×10^8 cfu/ml) are required for the lower receiver of each of the LSBAs.

2 For 1 LSBAs, inoculate 100 mL of liquid MOKA medium with appropriate antibiotics (here Rifampicin 50 µg/ml) using Xcc and grow Overnight at 28 °C under agitation at 200 rpm.

8h

3 Centrifuge the overnight growth for 10 minutes at 6,000 rpm and wash twice with [M] 1 millimolar (mM) $MgCl_2$.

Note

1 mM $MgCl_2$ is the common physiological serum used for different *Xanthomonas* species including Xcc.

3.1 Centrifuge the overnight growth for 6000 rpm, 00:10:00 and wash twice with [M] 1 millimolar (mM) $MgCl_2$ (1/2).

10m

3.2 Centrifuge the overnight growth for 6000 rpm, 00:10:00 and wash twice with [M] 1 millimolar (mM) $MgCl_2$ (2/2).

10m

4 Resuspend the final pellet in 5 mL of [M] 1 millimolar (mM) $MgCl_2$ and measure the OD_{600nm}.

5 Add 1 mL of bacterial suspension at a concentration of 4 x 10^{10} cells/ml to a syringe with a 25G needle.

For *Schewanella oneidensis* (So) 20m

6 We collect bacteria in exponential phase. (OD_{600nm} between 3 and 4). For our experiments, 400 ml of washed bacteria at an OD_{600nm} of 0.1 (1 × 10^8 cfu/ml) are required for the lower receiver of each of the LSBAs.

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For *Schewanella oneidensis* (So) 20m

8 For 1 LSBAs, inoculate 100 mL of liquid LB medium with appropriate antibiotics (here Rifampicin 50 µg/ml) using So and grow Overnight at 28 °C under agitation at 200 rpm.

9 Centrifuge the overnight growth for 10 minutes at 3,500 rpm and wash twice with LM medium.

9.1 Centrifuge the overnight growth for 3500 rpm, 00:10:00 and wash twice with LM medium. (1/2) 10m

9.2 Centrifuge the overnight growth for 3500 rpm, 00:10:00 and wash twice with LM medium. (2/2) 10m

10 Resuspend the final pellet in 5 mL LM medium and measure the OD_{600nm}.

11 Add 1 mL of bacterial suspension at a concentration of 4 x 10^{10} cells/ml to a syringe with a 25G needle.

Protocol references

1. Blanvillain S, Meyer D, Boulanger A, Lautier M, Guynet C, Denancé N, et al. Plant carbohydrate scavenging through tonB-dependent receptors: a feature shared by phytopathogenic and aquatic bacteria. PloS One. 2007;2: e224. doi:10.1371/journal.pone.0000224

2. Boyeldieu A, Poli J-P, Ali Chaouche A, Fierobe H-P, Giudici-Orticoni M-T, Méjean V, et al. Multiple detection of both attractants and repellents by the dCache-chemoreceptor SO_1056 of Shewanella oneidensis. FEBS J. 2022;289: 6752–6766. doi:10.1111/febs.16548

3. Cerutti A, Jauneau A, Auriac M-C, Lauber E, Martinez Y, Chiarenza S, et al. Immunity at Cauliflower Hydathodes Controls Systemic Infection by Xanthomonas campestris pv campestris. Plant Physiol. 2017;174: 700–716. doi:10.1104/pp.16.01852

4. Luneau JS, Baudin M, Quiroz Monnens T, Carrère S, Bouchez O, Jardinaud M-F, et al. Genome-wide identification of fitness determinants in the Xanthomonas campestris bacterial pathogen during early stages of plant infection. New Phytol. 2022. doi:10.1111/nph.18313