Protocol for Biotin Bioassay-based Cross Feeding

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[Abstract] Biotin bioassay-based cross-feeding experiments were performed to elucidate the effect on biotin production by *bioR*bme expression in *Agrobacterium tumefaciens* (*A. tumefaciens*) (Feng *et al.*, 2013). The indicator strain used here is the biotin auxotrophic strain of *Escherichia coli* (*E. coli*), ER90 (Δ*bioF bioC bioD*), which was cross-fed by *A. tumefaciens* species (Feng *et al.*, 2013a). The biotin-free M9 minimal medium plates were formulated as described by others and our research groups (Feng *et al.*, 2013b; Lin *et al.*, 2010; del *et al.*, 1979). Of note, 0.01% (w/v) the redox indicator 2, 3, 5-triphenyl tetrazolium chloride (TTC) was supplemented into the above media. Consequently, biotin generation/production was observed via the reduction of TTC to the insoluble red formazan, which is due to the ER90 growth fed by *A. tumefaciens* strains (Feng *et al.*, 2014). Detailed procedures are described as follows.

Materials and Reagents

1. Four *A. tumefaciens* strains [NTL4 (WT), FYJ283 (Δ*bioBFDA*), FYJ212 (Δ*bioR*at) and FYJ341 (Δ*bioR::*Km+*bioR*bme)]
2. Biotin auxotrophic strain of *E. coli*, ER90
3. 1 nM biotin (Sigma-Aldrich, catalog number: B4510)
4. 0.01% (w/v) 2, 3, 5-triphenyl tetrazolium chloride (TTC) (AMRESCO, catalog number: 0765)
5. 0.1% vitamin-free casamino acids hydrolysate (Sigma-Aldrich, catalog number: C7710)
6. $MgSO_4$
7. Glucose
8. M9 minimal medium (see Recipes)

Equipment

1. Centrifuge
2. Petri dishes Petri dishes (90 mm) (Thermo Fisher Scientific, catalog number: 502VF)
3. Sterile paper disks (6 mm, BBL)

Procedure

A. Preparation of biotin bioassay plates

1. The biotin assay plates were prepared as previously described with few changes, one of which referred to the thinner thickness of the plate agar where we dropped paper discs (Feng *et al.*, 2013b; Lin *et al.*, 2010).
2. Overnight cultures of strain ER90 (grown in 6 ml of defined M9 minimal medium with 1 nM biotin at 30 °C) were collected by centrifugation (3,600 rpm, 16 min), washed three times with the same volume (6 ml) of M9 medium, and were cultivated at 37 °C to 0.8 OD_{600} in 200 ml of M9 minimal medium containing 1 nM biotin.
3. To remove excess of biotin, all the bacterial cells from 200 ml culture were washed twice in M9 media and sub-cultured into 1 L of M9 minimal medium at 37 °C for 5 h to de-repress expression of *bio* operon by starvation for biotin.
4. The bacteria were harvested by centrifugation (3,600 rpm, 16 min), washed three times with M9 medium, re-suspended in 1 ml of the same medium and mixed into 150 ml of the defined M9 agar media supplemented with 0.01% (w/v) TTC as a redox indicator.
5. Finally, the mixture (5 ml per sector) was poured into Petri dishes sectored with plastic walls to avoid cross-feeding and a sterile paper disk (6 mm, BBL) was centered on the agar top of each sector.

B. Preparation of cross-feeder strains

1. In total, four feeder strains of *A. tumefaciens* corresponded to NTL4 (WT), FYJ283. (Δ*bioBFDA*), FYJ212 (Δ*bioR*at) and FYJ341 (Δ*bioR::*Km+*bioR*bme).
2. The biotin auxotroph strain FYJ283 was cultivated in 5 ml of M9 medium supplement ed with 1 nM biotin, whereas the other three strains were cultivated in 5 ml of biotin-free M9 minimal media overnight.
3. Overnight cultures were collected by centrifugation (3,000 rpm, 10 min), washed thre e times using the M9 liquid medium, and transferred into 100 ml of biotin-free M9 media for 6 more hours of growth at 30 °C to deplete trace amounts of intrac ellular biotin in the biotin auxotroph strain FYJ283.
4. Following three rounds of washing with same media, bacteria were resuspended in M

9 media and their optical densities at 600 nM (OD_{600}) were adjusted to 1.5. 20 µl of *A. tumefaciens* culture (OD_{600} = 1.0) was spotted on the paper disc, and incubated over night at 30 °C.

5. Finally, the red deposit of formazan (Lin *et al.*, 2010; del *et al.*, 1979; Feng *et al.*, 2014) suggests that the indicator strain ER90 is fed by the *A. tumefaciens* strains (seen in Figure 1), and the area size (square centimeters) of formazan represents the level of biotin pool produced by the different feeder strains.

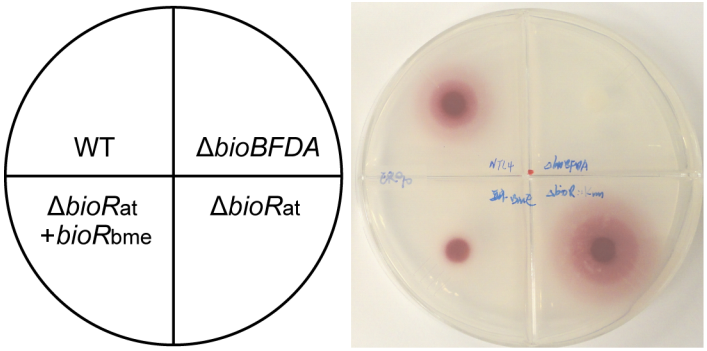

Figure 1. A representative photograph illustrating the relevance of BioR-mediated regulation to biotin synthesis. It was fully adapted from Feng *et al*. (2013a). Four *A. tumefaciens* strains cross-feed *E. coli* strain ER90 with deletion of full *bio* operon, which are NTL4 (WT), FYJ283 (Δ*bioBFDA*), FYJ212 (Δ*bioR*at), and FYJ 341 (Δ*bioR*at+*bioR*bme), respectively.

Recipes

1. M9 minimal medium
   6 g of Na_2PO_4, 3 g of KH_2PO_4, 0.5 g of NaCl and 1 g of NH_4Cl per liter

Acknowledgments

This protocol was adapted/modified from previous works seen in del Campillo-Campbell *et al.* (1079); Feng *et al.* (2014); Feng *et al.* (2013a); Feng *et al.* (2013b) and Lin *et al.* (2010).

References

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