In vitro Chitin Binding Assay

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[Abstract] Chitin is polymer of N-acetylglucosamine (GlcNAc) found in the exoskeleton of arthropods and the fungal cell wall. GlcNAc is also implicated in bacterial development, adherence, and signal transduction but can also be used as a carbon source. *In vitro* chitin binding assay is performed to determine the affinity of a purified protein to the chitin molecule. The principle is based on the co-sedimentation of chitin-binding proteins together with chitin-coated beads.

Materials and Reagents

1. Purified protein with chitin binding affinity
   Note: We used histidine-tagged chitin binding protein CbpD. The protein was purified by affinity chromatography onto a 5-ml HisTrap nickel column (Pharmacia) on an Äkta system (Amersham Biosciences). The complete purification protocol is described in details in Cadoret et al. (2014).
2. Chitin beads (New England Biolabs, catalog number: S6651)
3. Tris Base
4. EDTA
5. NaCl
6. Tween 100
7. Freshly made solution of chitin binding buffer (see Recipes)

Equipment

1. Laboratory vortex adapted to Eppendorf tubes
2. Laboratory rotating wheel adapted to Eppendorf tubes
3. Cold room or refrigerated incubator (4 °C)

Procedure

A. Wash the chitin beads as follows

1. Vortex the chitin beads before pipetting.
2. Wash the desired amount of beads solution (100 µl per experiment) twice with 5 volumes of chitin binding buffer. This washing step is performed by gravity flow by leaving the sample 5 min at room temperature.

B. Binding assay

1. Prepare a 200 µl solution of chitin-binding buffer containing the purified CbpD protein at 60 µg/ml (total fraction). For that, 1.5 µl of purified CbpD at 8 mg/ml in 50 mM Tris pH 8, 300 mM NaCl, 250 mM imidazole was mixed with 198. 5 µl of chitin binding buffer.
2. Add 100 µl of pre-washed chitin beads in chitin-binding buffer to the purified protein solution.
3. Incubate the sample on a laboratory rotating wheel for 90 min at 4 °C.
4. After gravity flow separation, collect the supernatant (unbound fraction).
5. Wash the beads (bound fraction) twice with 200 µl of chitin binding buffer by gravity flow.
6. Analyse 15 µl of Total (equivalent to 0.9 µg), Unbound and Bound fractions by SDS-PAGE followed by Coomassie blue staining and/or immunoblotting (see Figure 1).

Representative data

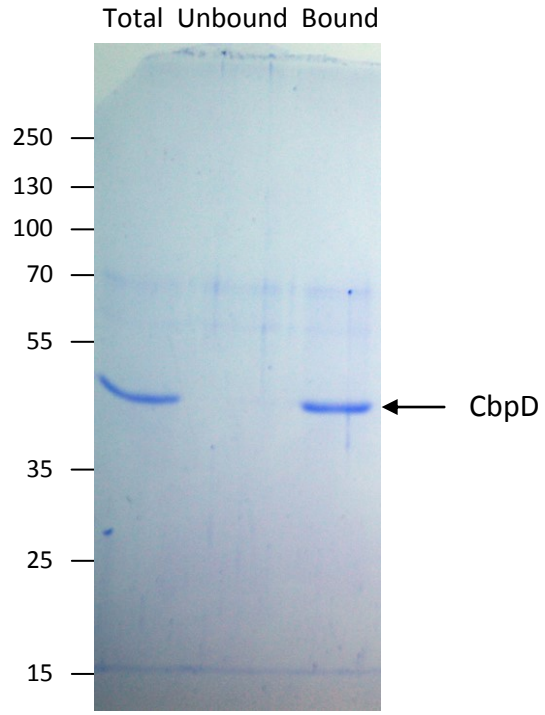

Figure 1. Chitin-binding affinity of CbpD. To testthe chitin-binding affinity of CbpD, a solution of purified CbpD at 200 µg/ml in chitin binding buffer (Total) was incubated with chitin beads according to

the present protocol. 15 µl samples of Total, Unbound and Bound fractions were analyzed by SDS-PAGE followed by Coomassie blue staining. Molecular mass markers (in kDa) are indicated on the left.

Notes

1. The chitin binding buffer and the protein solution in chitin binding buffer must be freshly prepared.
2. A negative control can consist of the same experiment where the chitin beads solution is replaced by chitin binding buffer.
3. Additionally, purified proteins without chitin binding affinity such as Elastase (LasB) and exotoxin A (ToxA) can be used as negative control [see Figure 5B in Cadoret *et al*. (2014)].

Recipes

1. Freshly made solution of chitin binding buffer
   Chitin binding buffer:
   50 mM Tris HCl (pH 8)
   1 mM EDTA (pH 8)
   500 mM NaCl
   0.1% Tween 100
   The chitin binding buffer is prepared from stock solutions:
   Tris HCl 1 M (pH 8)
   EDTA 0.5 M (pH 8)
   NaCl 5 M

Acknowledgments

This protocol adapted from Shutinoski *et al.* (2010) was used in Cadoret *et al*. (2014). The work is funded by a Ph.D. grant from the French government.

References

1. Cadoret, F., Ball, G., Douzi, B. and Voulhoux, R. (2014). Txc, a new type II secretion system of *Pseudomonas aeruginosa* strain PA7, is regulated by the TtsS/TtsR two-component system and directs specific secretion of the CbpE chitin-binding protein. *J Bacteriol* 196(13): 2376-2386.

2. Shutinoski, B., Schmidt, M. A. and Heusipp, G. (2010). Transcriptional regulation of the Yts1 type II secretion system of *Yersinia enterocolitica* and identification of secretion substrates. *Mol Microbiol* 75(3): 676-691.