Figure S1. Supernatant of freshly harvested *Enterobacter* C6-6 was digested 4 h at 55-60°C with 100 µg/ml Proteinase K (Qiagen) after which any residual Proteinase K was removed by using a 20-kDa centrifugal filtration unit (Amicon). Undigested supernatant was also heated 4 h and passed through a filtration unit. *Flavobacterium psychrophilum* (strain CSF 259-93) was cultured in 100 µl 2X TYES with 100 µl of filter-sterilized supernatant from CSF 259-93 (no supernatant control), digested supernatant from *Enterobacter* C6-6 (+digested supernatant), or undigested supernatant (+undigested supernatant) from *Enterobacter* C6-6. Optical density (600 nm) was recorded for the cultures. Technical replicates were included, but no biological replicates were conducted. Data from this figure was used to justify efforts to isolate an inhibitory protein.

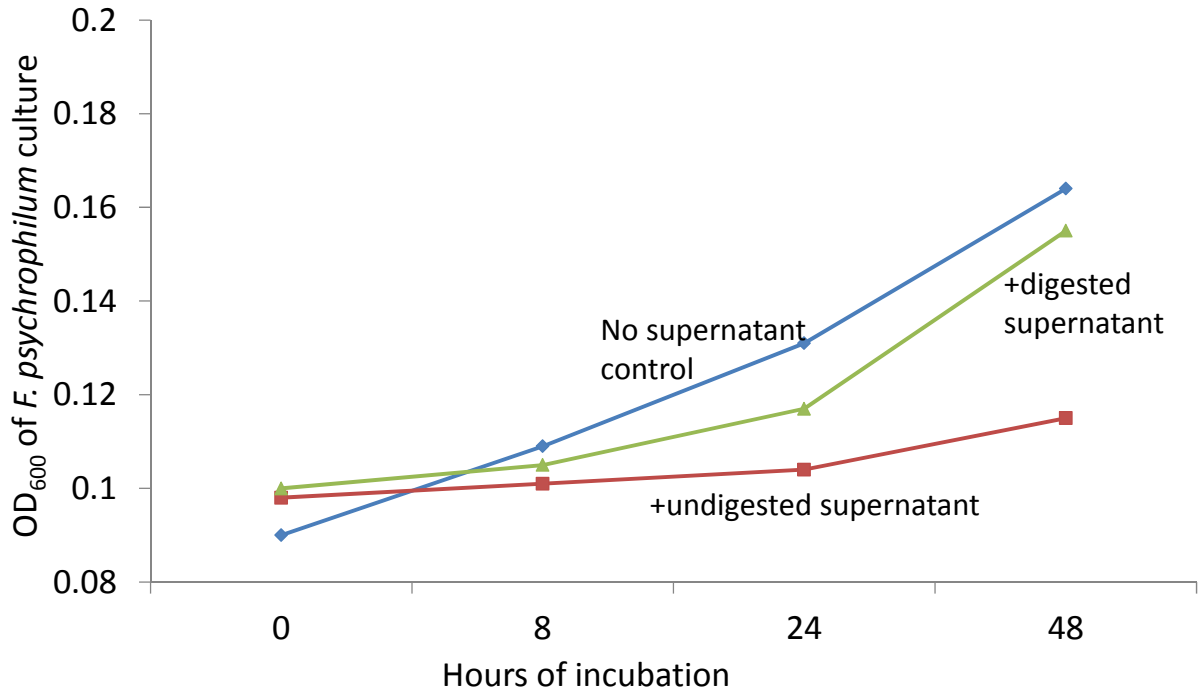
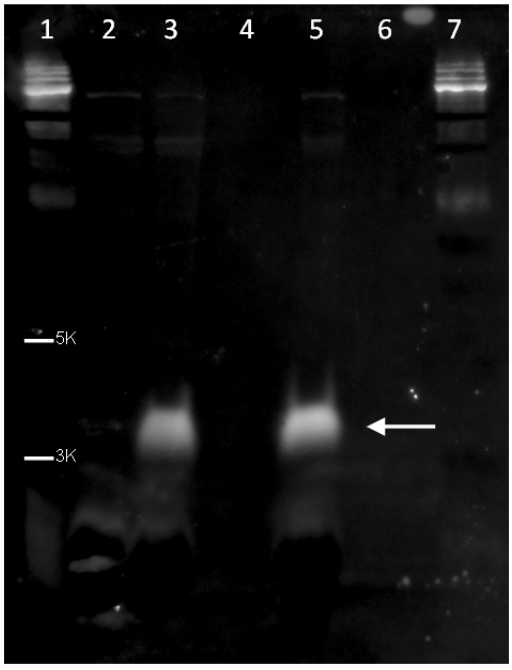


Figure S2. Western blot showing detection of a His-tagged protein approximately 3-3.5 kDa in mass (white arrow) for both IPTG induced and uninduced culture. The predicted mass for EcnB is ~3.1 kDa.

Enterobacter C6-6 and *Enterobacter* C6-6Δ*ecnAB*<pET101::*ecnAB*> were grown in 15 ml LB with ampicillin (37°C with shaking). Overnight culture (1 ml) was pelleted (1,600 x g, 5 min) and the supernatant discarded. Overnight culture of *Enterobacter* C6-6Δ*ecnAB*<pET101::*ecnAB*> (150 µl) was seeded into 15 ml of LB with ampicillin and grown to optical density (OD_{600}) 0.6. IPTG (1 M) was added and culture was continued for 2 h. An aliquot of culture (1 ml) was pelleted (1,600 x g, 5 min)and the supernatant was discarded. Tricine sample buffer (100 µl) (Bio-Rad) was added to the pellet. Cells were lysed for 10 min at 85°C and then the samples were centrifuged briefly (1,600 x g, 30 s). Precision Plus Protein™ Dual Xtra Standards (3 µl; Bio-Rad) and 19 µl of each sample of cell lysate were separated using a 16.5% Tris-Tricine precast SDS-page gel (100 volts, 2 h, in 1X Tris/Tricine/SDS buffer; Bio-Rad). The gel was then transferred to Hybond LFP PVDF membrane (GE Healthcare) using a Trans-Blot® Turbo™ Transfer system (Bio-Rad). After transfer the PVDF membrane was washed in PBST (PBS with 0.5% Tween® 20; Fisher Scientific) for 5 min on a platform shaker, then the PBST was replaced by 25 ml PBST with 2.5% non-fat dry milk (Bio-Rad) and shaken for 30 min before being washed in PBST again for 5 min. His-Tag monoclonal antibody (3 µl; Qiagen) and 10 ml of fresh PBST with 2.5% non-fat dry milk was added to the PVDF membrane and shaken for 2 h. The membrane was washed 3 times in PBST for 5 min each. Goat Anti-Mouse IgG Dylight 488 conjugated (3 µl; Thermo Scientific) was added with 10 ml of fresh PBST containing 2.5% non-fat dry milk and shaken for 1 h. The membrane was washed again 3 times in PBST for 5 min each and imaged using a ChemiDoc™ MP (Bio-Rad).



Lanes:
1 = Mass ladder
2 = *Enterobacter* C6-6
3 = *Enterobacter* C6-6Δ*ecnAB*<pET101::*ecnAB*>, no IPTG
4 = Blank lane
5 = *Enterobacter* C6-6Δ*ecnAB*<pET101::*ecnAB*>, induced with 1 M IPTG
6 = Blank lane
7 = Mass ladder

Figure S3. Inhibitory effect of size fractions from *Enterobacter* C6-6 (C6-6) and *Escherichia coli* K12 (K12) on growth of *Flavobacterium columnare* (ATCC 23463). The methods used were similar to those used for Fig. 1 from the manuscript except the negative control was TYES broth rather than spent media from *F. columnare*. Unfractionated supernatant (SN) and the <3 kDa fraction from both *Enterobacter* C6-6 and *E. coli* K12 inhibited growth of *F. columnare* whereas the larger fraction (>3 kDa) had no effect on growth. Asterisks indicate statistical differences from the TYES control (one-way ANOVA followed by Tukey's multiple-comparison test, $P<0.05$).

Inset: Zone of clearance for *F. columnare* in the presence of *Enterobacter* C6-6 supernatant. The methods used were similar to the manuscript except as follows: *F. columnare* was grown in 20 ml of TYES broth at 17°C for 3-4 days prior to the experiment and adjusted to an optical density (OD) of approximately 0.2 at 600 nm in a 50 ml conical tube. This culture (75 µl) was spread evenly onto a TYES plate using glass beads. Plates were then allowed to briefly dry at 17°C and a sterile biopsy punch with diameter 4 mm was used to create wells into the agar. *Enterobacter* C6-6 was inoculated in 20 ml of TYES broth 2 days prior at 37°C and pelleted at 1,600 x g for 10 min at 17°C. The supernatant was decanted and 100 µl was added to the well. A zone of growth inhibition is visible in the well "C" (C6-6 SN) while there is no such zone in the well "B" (broth) which was filled with 100 µl TYES broth as a control.

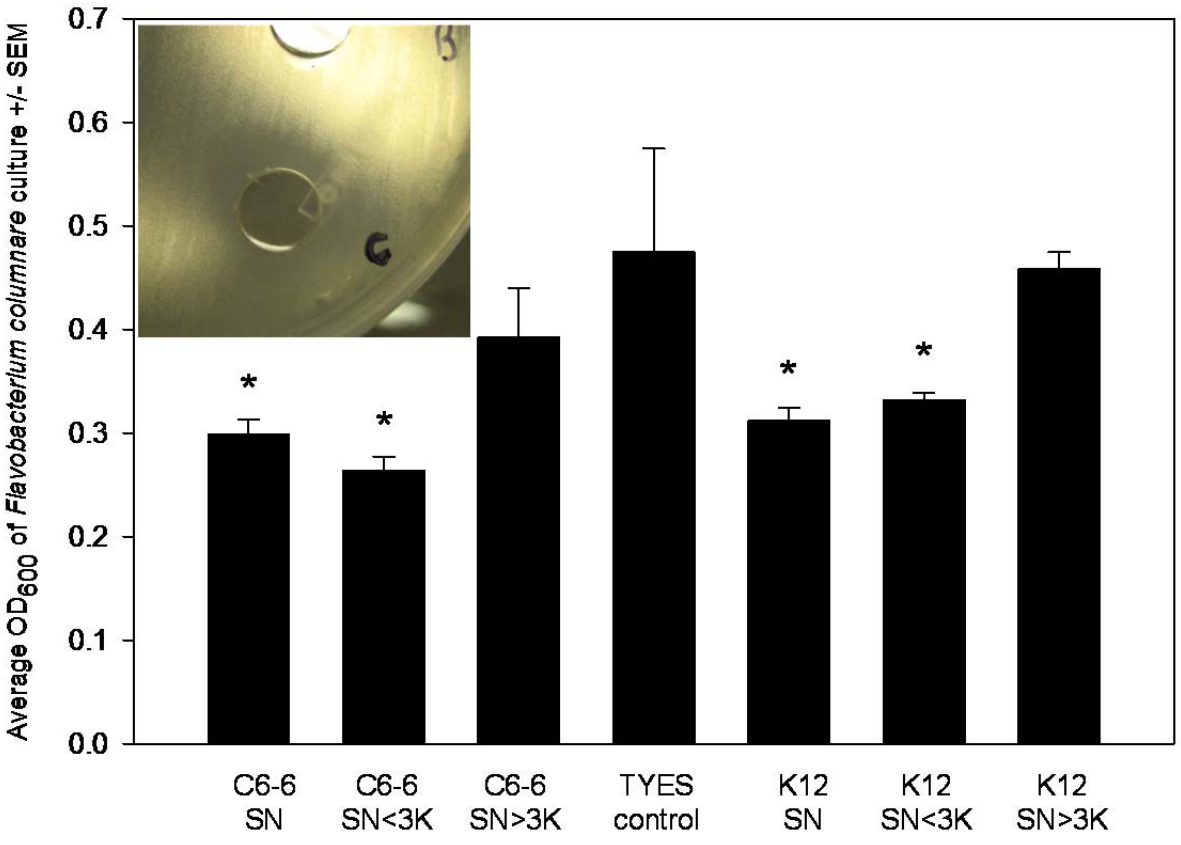


Figure S4. Amino acid alignment for EcnB found in select bacterial species. Percent amino acid similarity values (%Similarity) were calculated by comparing the amino acid sequences of a subset of bacteria from Proteobacteria against *Enterobacter* C6-6 EcnB using Vector NTI Advance 11. No EcnB were annotated in Delta-, Epsilon- or Zetaproteobacteria.