

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.

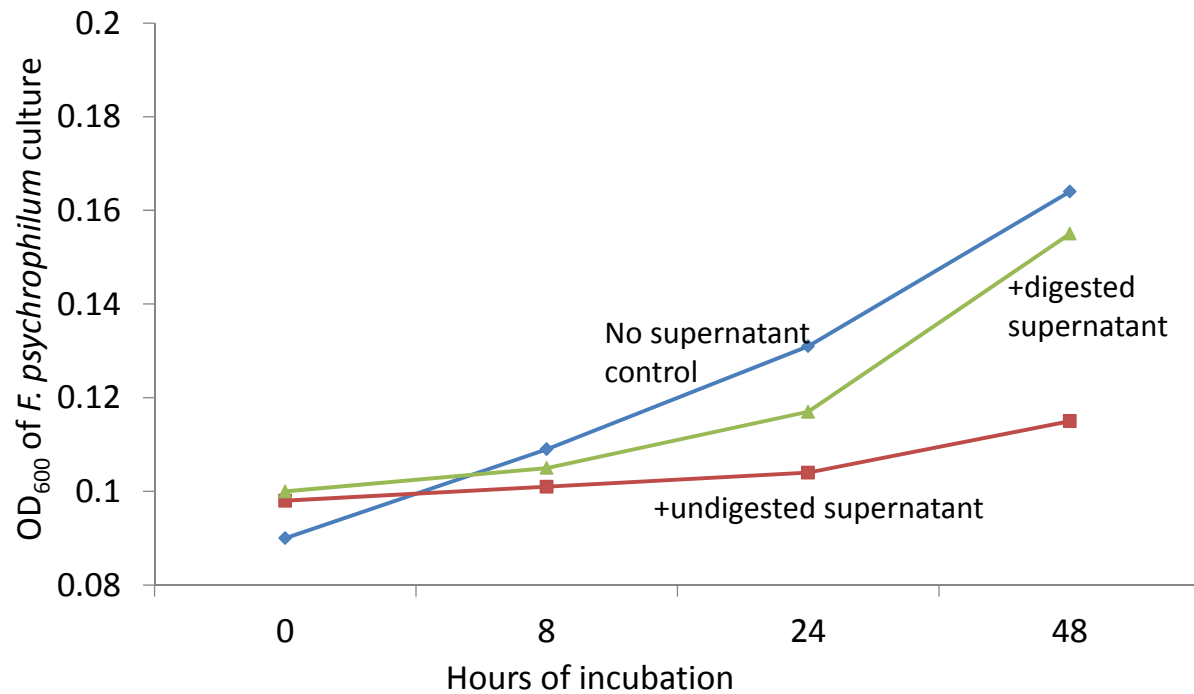
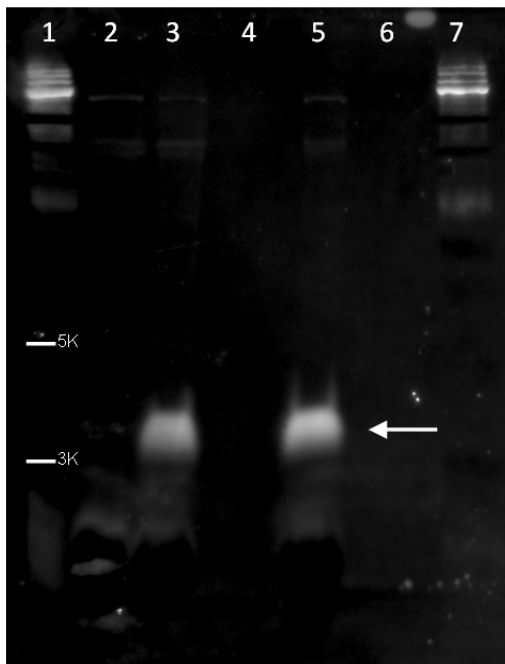


Figure S2. Western blot showing detection of a His-tagged protein approximately 3-3.5 kDa in mass (white arrow) for both IPTG induced and uninduced culture. The predicted mass for EcnB is ~3.1 kDa.

Enterobacter C6-6 and *Enterobacter* C6-6 Δ 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* C6-6 Δ ecnAB<pET101::ecnAB> (150 μ l) was seeded into 15 ml of LB with ampicillin and grown to optical density (OD₆₀₀) 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 μ l) (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 μ l; Bio-Rad) and 19 μ l 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 μ l; 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 μ l; 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* C6-6 Δ ecnAB<pET101::ecnAB>, no IPTG
- 4 = Blank lane
- 5 = *Enterobacter* C6-6 Δ 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.

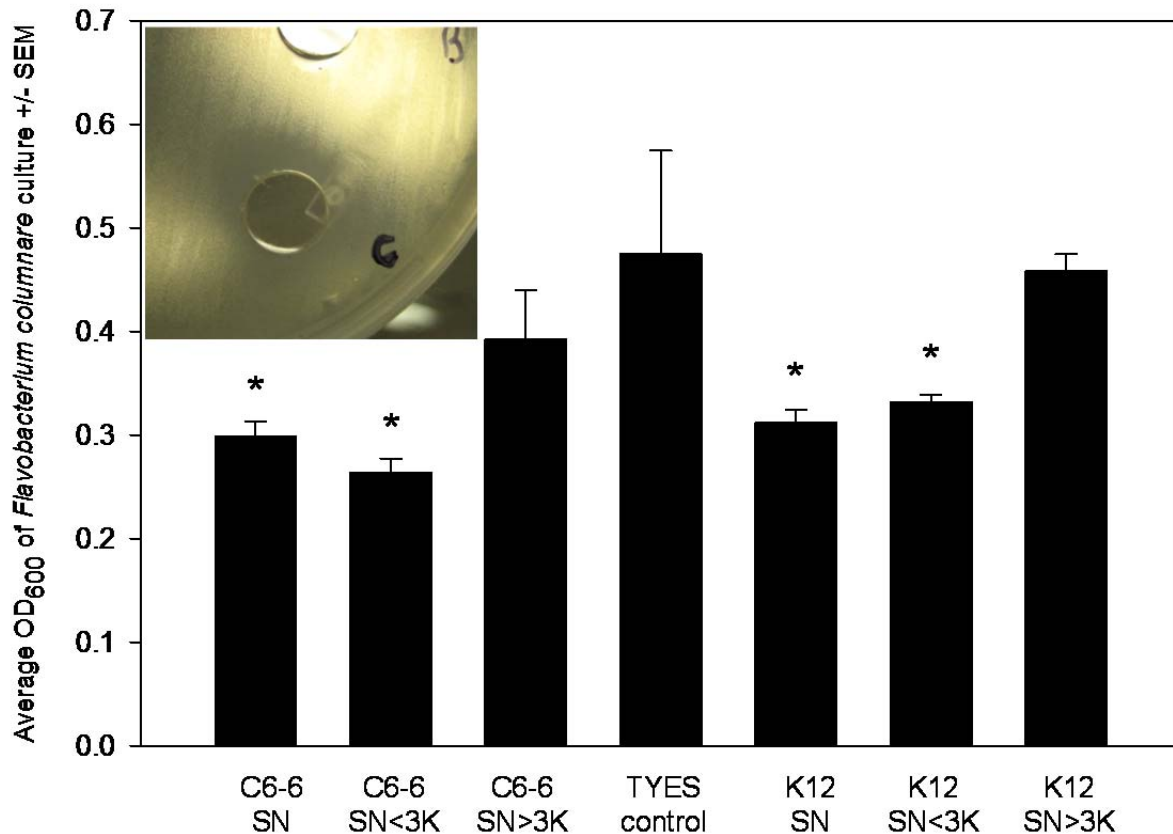


Figure S4. Amino acid alignment for EcnB found in select bacterial species. Percent amino acid similarity values (%Similarity) were calculated by comparing the amino acid sequences of a subset of bacteria from Proteobacteria against *Enterobacter* C6-6 EcnB using Vector NTI Advance 11. No EcnB were annotated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

	(1)	1	10	20	30	40	54	Genbank#	%Similarity
Alpha	<i>Azospirillum lipoferum</i>	(1)	MTTVKKFALLAVLGTLLGTTLAACNTMEGAGQDVQAGGRAIERGADNVQKKM--	NC_016622	50				
	<i>Rhizobium etli</i>	(1)	---MTTIAKIAAAFMVLLALSSCGNIRGMGKDTANAVNATQDAGRSVDRAAKK	NC_010994	47				
	<i>Sinorhizobium meliloti</i>	(1)	-MTTKSIATIGAVLIALLAALSSCANTIRGAGQDTANAVNATQCGAGCRVAKAAN-	NC_003047	46				
Beta	<i>Achromobacter piechaudii</i>	(1)	----MRGKIVLTAFFVVFGEVLAGCNTVAGMGRDMSRAGNAITNAADK-----	ADMS01000052	60				
	<i>Bordetella avium</i>	(1)	----MRSKIVLAAFFVVFGEVLAGCNTMAGMGKDVSRAGNAITNAAEK-----	NC_010645	63				
	<i>Bordetella bronchiseptica</i>	(1)	----MRSKIVLTAFFVVFGEVLAGCNTVAGMGKDMSDAGSAITHAAEK-----	HE983628	65				
	<i>Bordetella pertussis</i>	(1)	----MRSKIVLTAFFVVFGEVLAGCNTVAGMGKDMSDAGSAITHAAEK-----	NC_018518	65				
	<i>Burkholderia pseudomallei</i>	(1)	-----MNRATAAALLILTAALAGCNTIAGVGCQDISKGGQAI SNTAEKAK-----	CP000573	64				
Gamma	<i>Acinetobacter baumannii</i>	(1)	-----MMKKVIVASVMVAFVLTGCNTFRGFGQDVSKAGDAVTINTAÇKTENKM--	NC_017162	60				
	<i>Enterobacter cloacae</i>	(1)	--MVKKTIAAIFSVLVLSVLTACNTRGVGQDISEGGSAISGAASKAQC-----	NC_016514	98				
	<i>Enterobacter</i> sp.	(1)	--MVKKTIAAIFSVLVLSVLTACNTRGVGQDISEGGSAISGAATKAQC-----	NZ_AZUA00000000	98				
	<i>Erwinia toletana</i>	(1)	--MLKKSIAAIFSVLILSSLLSACNTRGVGEDVEAGGQAIÇKSAD-----	NZ_AOCZ00000000	86				
	<i>Escherichia albertii</i>	(1)	--MVKKTIAAIFSVLVLSVLTACNTRGVGEDISEGGSAISGAATKAQC-----	BBMY01000124	98				
	<i>Escherichia coli</i>	(1)	--MVKKTIAAIFSVLVLSVLTACNTRGVGEDISDGGNAISGAATKAQC-----	NC_013364	98				
	<i>Klebsiella pneumoniae</i>	(1)	--MVKKTIAAIFSVLVLSVLTACNTRGVGQDISEGGSAISGAATKAQC-----	NC_011283	94				
	<i>Pantoea ananatis</i>	(1)	MCMLKKSIVAFISVMMFSALLSGCNTTRGVGEDVEAGGHAIÇRSAÇ-----	NC_013956	80				
	<i>Salmonella enterica</i>	(1)	-MKFFKKVIRVRKTEKRGSEFVÇVTNSKVVFYIHLQLIKDNILSVVICYT-----	WP_023254843	23				
	<i>Salmonella enterica</i> .	(1)	-MKFFKKVIRVRKSEKRGSEFVÇVTNSKVVFYIHLQLIKDNILSVVICYT-----	NC_017623	23				
	<i>Serratia liquefaciens</i>	(1)	--MLKKSIAAIFSLMILTS--LTACNTRGVGEDIQAGGKAIÇRSAE-----	NC_021741	84				
	<i>Yersinia enterocolitica</i>	(1)	--MLKKSIAAIFSLIVISS--LSACNTRGVGKDVQSAGSAIERSAE-----	NC_008800	84				
	<i>Enterobacter C6-6</i>	(1)	--MVKKTIAAIFSVLVLSVLTACNTRGVGEDISDGGSAISGAATKAQN-----	KM407562	Reference				
Consensus	(1)	VKKSI I SVLVLSVLTACNTRGVG DIS GG AIS AA K							