A Fishing Experiment

Isolating Proteins that Neutralize the Adaptive Immune System in Bacteria

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Hypothesis

Using affinity chromatography, a purified Strep-tagged Csy complex bound to a Strep-Tactin column can be used as a “hook” to “fish out” novel, virally encoded anti-CRISPR proteins that bind the Csy complex.

Abstract

Bacteria have an adaptive immune system which evades invasive bacteriophage DNA by utilizing clustered regularly interspaced short palindromic repeats (CRISPR) separated by copies of small segments of viral DNA, which are transcribed and combine with proteins to form a surveillance complex (Figure 1). This ribonucleoprotein surveys the cell to identify viral DNA with which a base-pair interaction can be formed, subsequently degrading the viral genome. Bacteriophage have evolved a defense mechanism against the CRISPR-Cas immune system through proteins that bind to, and thereby inactivate the CRISPR-Cas system. Here we demonstrate that anti-CRISPR (ACR) proteins produced by bacteriophages isolated from environmental samples can be eluted from a Step-Tactin column in tandem with a Strep-tagged Csy complex due to binding of the ACR proteins to the complex. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the eluted protein fractions shows all four subunits of the Csy complex, along with a band of approximately 11kDa, consistent with ACR F1 protein.

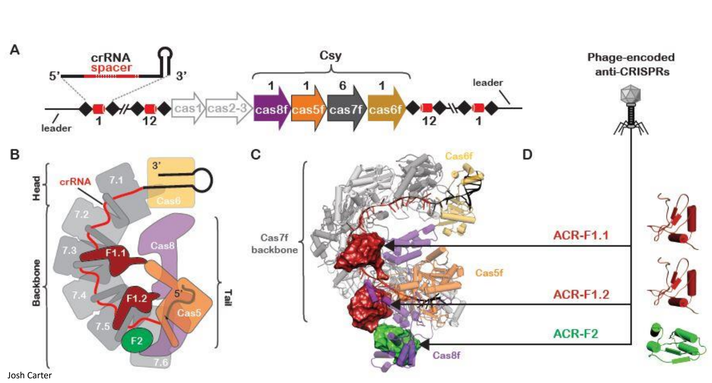


Figure 1: A) The CRISPR locus of a bacterial genome. B) Protein composition of the Csy complex, illustrating where the ACR F1 and both ACR F2 bind. C) Protein structure of the Csy complex along with ACR F1 and F2 proteins.

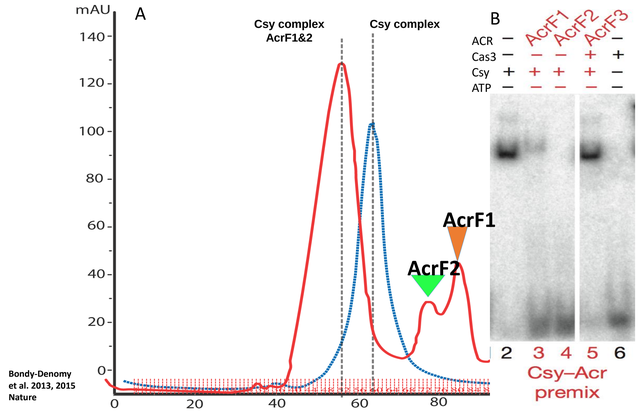
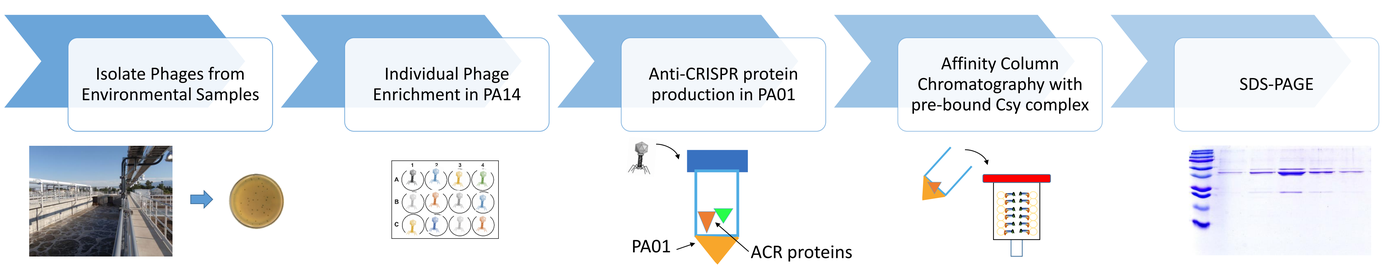


Figure 2: A) Overlay of size exclusion chromatography with Csy complex alone or in the presence of ACR F1 and F2. B) EMSA illustrating dsDNA binding of Cas3 alone, the Csy complex alone and in the presence of ACR F1, F2, and F3.

Methods



Phage isolates from plaque assays conducted with environmental samples from the Bozeman Water Treatment Plant were enriched overnight in *P. aeruginosa* strain PA14, then 5 samples were pooled and inoculated into *P. aeruginosa* strain PA01 for one hour to produce free anti-CRISPR proteins. Samples were chemically lysed and sonicated, then the lysate was applied to a Strep-Tactin column pre-bound with a strep-tagged Csy complex, and eluted over eight fractions. SDS-PAGE was used to visualize the proteins.

Results

The binding affinity between ACR F1 and ACR F2 to the type I-F Csy surveillance complex in *P. aeruginosa* (Figure 2) was utilized in an attempt to isolate novel ACR proteins. Through binding a strep-tagged Csy complex to a Strep-Tactin Column, ACR proteins were eluted together with the Csy complex and were visualized using SDS-PAGE.

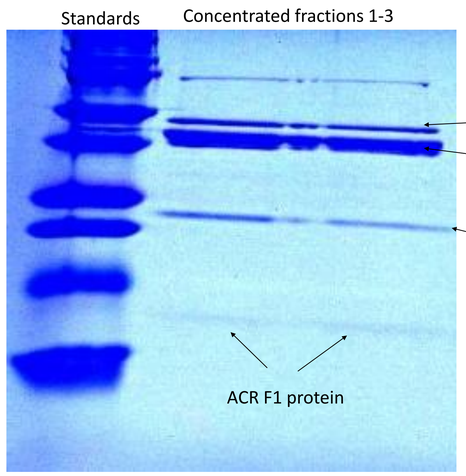


Figure 3: Coomassie stained 15% SDS polyacrylamide gel from positive control of experiment using phage DMS3m:35 which produces the ACR F1 protein[3] with molecular weight of 11kDa[2]. Lane 1 is BioRad Kaleidoscope Ladder. Lane 2 and 3 are concentrated elution fractions 1-3; lane 2 is from the pellet post incubation in PA01, lane 3 is from the supernatant.

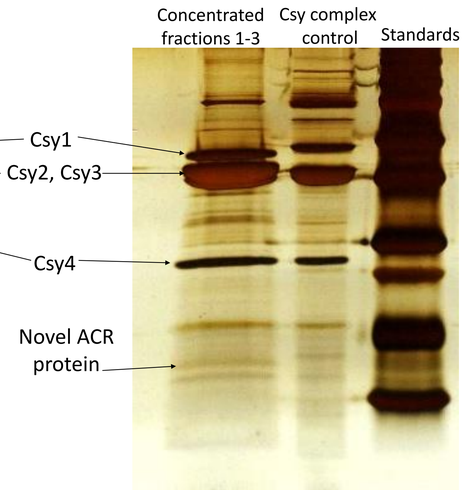


Figure 4: Silver stained 15% SDS polyacrylamide gel containing concentrated elution fractions 1-3 from experiment done with 5 pooled phage isolates. BioRad Kaleidoscope Marker was used for the standards lane. Csy complex control lane contained His-tagged Csy complex (1T-4).

This data confirms the hypothesis that the technique described can be used to isolate novel ACR proteins.

References

1. Rollins MF, Schuman JT, Paulus K, Bukhari HST, Wiedenheft B. Mechanism of foreign DNA recognition by a CRISPR RNA-guided surveillance complex from*Pseudomonas aeruginosa*. *Nucleic Acids Research*. 2015;43(4):2216-2222. doi:10.1093/nar/gkv094.
2. Bondy-Denomy J, Garcia B, Strum S, *et al*. Multiple mechanisms for CRISPR-Cas inhibition by anti-CRISPR proteins. *Nature*. 2015;526:136-139. doi:10.1038/nature15254.
3. Bondy-Denomy J, Pawluk A, Maxwell K, Davidson A. Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system. *Nature*. 2013;429-432. doi:10.1038/nature11723

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