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Expanding the microbial color palette by mutagenesis of coral chromoproteins by site-directed mutagenesis

Abstract

GFP-like chromoproteins are abundant in the Anthozoa, especially among corals. These brightly colored proteins are believed to function as photoprotective pigments, protecting coral tissues from solar radiation (1). Mutations at specific sites in chromoproteins can result in significant changes in chromoprotein coloration. To take advantage of this phenomenon we used genetic techniques to induce mutations at these sites. Both random and site-directed mutagenesis can be utilized to generate novel chromoproteins for use in research, education, public engagement, and art.

Introduction

Corals produce a wide variety of fluorescent and chromogenic proteins (1). Purple chromogenic protein (AmilCP) is derived from the Scleractinian coral, *Acropora millepora.* AmilCP belongs to a family of GFP-like nonfluorescent chromoproteins that share structural similarity with green fluorescent protein (GFP) consisting of a β-barrel with an alpha helix containing the chromophore threaded through the interior of the barrel (2). Mutations within the chromophore and in regions of the barrel interacting with the chromophore have been demonstrated to produce changes in chromoprotein color (3). In AmilCP, the amino acid residue at position 62 appears to play an important role in chromophore structure and the subsequent color of the chromoprotein. To exploit this unique phenotype, we induced site-specific mutations at this position and observed the effects in recombinant *Escherichia coli* K-12.

Methods

Inverse PCR was utilized to generate site-specific mutations at codon 62 of the *amilCP* gene. PCR products were re-circularized by ligation and transformed into nonpathogenic strains of *Escherichia coli* K-12 . Chromogenic variants were also generated by random mutagenesis to generate a library of all possible combinations of sequences at codon 62 . Transformants were visually screened for novel color variation. DNA sequencing was utilized to identify the mutation(s) in each chromoprotein variant.

Results

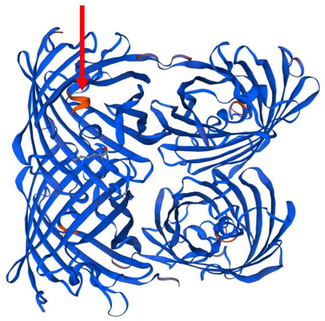
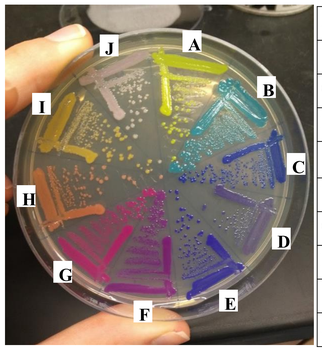
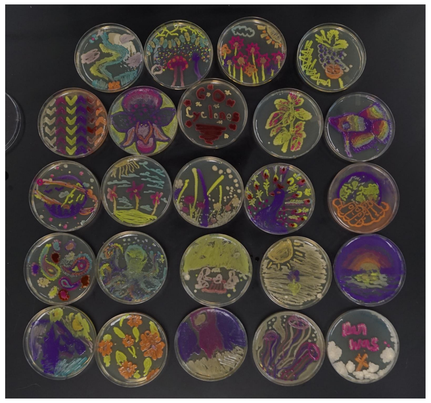


Figure 1. Predicted Structure of AmilCP. The structure of AmilCP has not yet been resolved by x-ray crystallography. The structure here is generated by predictive modeling based on data from similar fluorescent proteins (7, 8, 9, 10, 11). Computational models show a poorly resolved 3 amino acid region within the chromophore. GFP-like chromoproteins exist as a tetramer in vivo.



A		GFP
B		cyan
C		Aeblue
D		AmilCP periwinkle
E		AmilCP purple wild type
F		AmilCP fuchsia
G		AmilCP pink
H		AmilCP dragonfruit
I		RFP orange
J		AmilCP rose

Figure 2. Colorful chromoproteins
A variety of coral-derived natural and engineered chromoproteins expressed in *Escherichia coli* K12 **.**



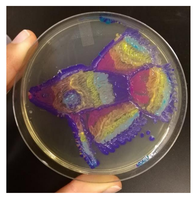
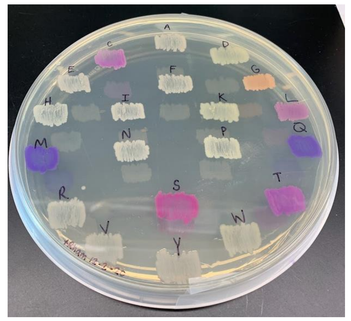


Figure 3. Applications of chromoproteins in art and public outreach. Artwork produced by students during the Spring 2019 Undergraduate Microbiology Club Agar Art Workshop. Subjects include ocean life, flowers, landscapes, and abstract designs. Scan the included QR code to access additional agar art images

Chromoprotein variants

A number of novel AmilCP variants were generated as well as several variants of cjBlue and RFP (Figure 2).



	Leucine	Hydrophobic
	Isoleucine	
	Alanine	
	Valine	
	Glycine	
	Methionine	
	Serine	Polar, uncharged
	Glutamine	
	Threonine	
	Cysteine	

Figure 4. Color variations in AmilCP purple chromoprotein as a result of targeted mutagenesis. Amino acid residue 62 is located within the chromophore on the interior of the beta-barrel (Fig. 1). Mutations at this site can result in a change in chromoprotein color, presumably by altering chromophore structure.

Conclusions

We have effectively used site directed mutagenesis to generate a color palette of living bacteria simply by altering the DNA sequence encoding the naturally-occurring glycine at position 62 of AmilCP . Since other positions of the protein are also important for formation of the chromophore (e.g., amino acid 157), random codon mutagenesis can potentially be used to generate additional color variants. Predictive structural modeling should be helpful to identify additional amino acids that influence chromophore structure and coloration. We anticipate that these AmilCP variants will have useful applications in research, STEM education, and biological art.

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