IDENTIFICATION OF THE RESIDUES THAT ARE RESPONSIBLE FOR IMPROVING THE ACTIVITIES OF CYANOBACTERIAL ENZYMES FOR HYDROCARBON BIOSYNTHESIS

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Cyanobacteria can produce hydrocarbons corresponding to diesel fuels via a reaction catalyzed by two enzymes, acyl-ACP reductase (AAR) and aldehyde deformylating oxygenase (ADO). Because *Escherichia coli* coexpressing these enzymes can produce and secrete hydrocarbons, both AAR and ADO are key enzymes for hydrocarbon biosynthesis. However, the activities of AAR and ADO are low. Therefore, construction of highly active mutants of AAR and ADO is necessary for industrial application of these enzymes for producing hydrocarbons. Our purpose in this study is to identify the residues that are responsible for improving the activities of AAR and ADO. First, we compared the activity of AARs from several cyanobacteria and detected a highly active AAR. Second, we introduced various single amino acid substitutions into a poorly active AAR, to make its sequence close to that of the highly active AAR. When we constructed and analyzed 40 mutants of AAR, we succeeded in identifying the residues that are important for high activity of AAR and those important for high expression level of soluble AAR (*Figure. 1*). Combination of single mutations greatly improved the aldehyde productivity. Similarly, we also identified the residues that are important for high activity of ADO and those important for high expression level of soluble ADO (*Figure. 2*). Mutational analysis of ADO revealed that high productivity of hydrocarbons can be achieved by increasing both the activity and amount of soluble ADO. Our data will be useful for producing higher amount of hydrocarbons using highly active mutants of AAR and ADO created by protein engineering.

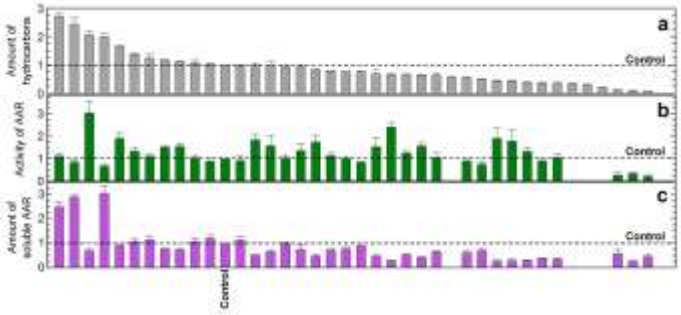

Figure 1. Characteristics of single mutants of AAR. a. Amount of hydrocarbons. b. Activity. c. Amount of soluble AAR.

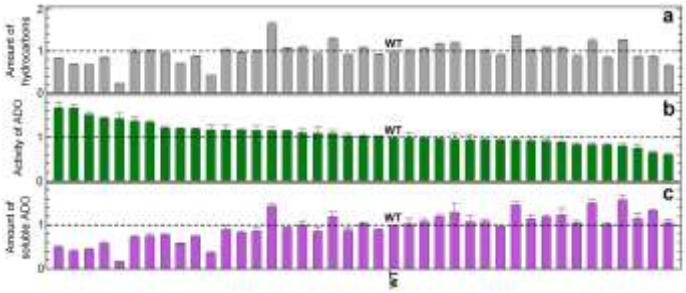

Figure 2. Characteristics of single mutants of ADO. a. Amount of hydrocarbons. b. Activity. c. Amount of soluble ADO.