Channelling enzymes for biofuel production enhancement

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Biofuels are a promising alternative to non-renewable fossil fuels but they require extensive areas of arable land. This problem would be solved with algae such as cyanobacteria. Cyanobacteria grow by photosynthesis, consuming CO_2 and producing O_2, just like plants, but grow faster and require the less nutrient input as some strains are able to fix Nitrogen from the atmosphere. Cyanobacteria do not produce biofuels naturally, but through genetic engineering technology they can synthesize the necessary enzymes to produce them.

Organisms, even simple ones like bacteria, have complex metabolic routes interconnected. The same molecule might be needed in different metabolic routes giving different final products; therefore enzymes have to compete for these molecules. Enzymes in the organism are designed to achieve a balance between different metabolic routes that are optimal for the organism's survival. In biotechnology this equilibrium needs to be tuned for production enhancement instead of survival.

Ethanol nowadays is one of the most important biofuel produced in the World. Pyruvate decarboxylase (Pdc) transforms pyruvate (produce by all living organisms) into acetaldehyde, and alcohol dehydrogenase (Adh) reduces acetaldehyde into ethanol. But acetaldehyde is toxic and usually organisms have different enzymes to degrade it and avoid accumulation of it in the cell. Let's imagine that Pdc is a soap bubble machine creating bubbles (acetaldehyde) that cats and dogs (Adh and other enzymes) are trying to catch. If the cats and dogs are all together they will catch around the same amount, more or less depending on how fast cats and dogs are. But, if we put all the cats beside the bubble machine, so the dogs can't get near it, then very few bubbles will reach the dogs, because the cats would have already popped most of the bubbles. In the same way, if we put Pdc and Adh together, most of the acetaldehyde produced by Pdc will be reduced to ethanol by Adh before reaching other enzymes.

This effect is called cluster mediated channelling and can be achieved by co-localization of the enzymes in different ways. In this study four different strategies of co-localization of Pdc and Adh were compared: the fusion of both enzymes in one bigger protein; the addition of domains that recognise each other, and adding domains that bind a specific DNA or RNA scaffold. Ethanol concentration by different methods of co-localization was compared, and some promising results were obtained, yet further study will be needed to reach consistent conclusions.

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