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# Construction of a Compact Array of Microplasma Jet Devices and its Application for Random Mutagenesis of Rhodosporidium toruloides

## Background/Objective

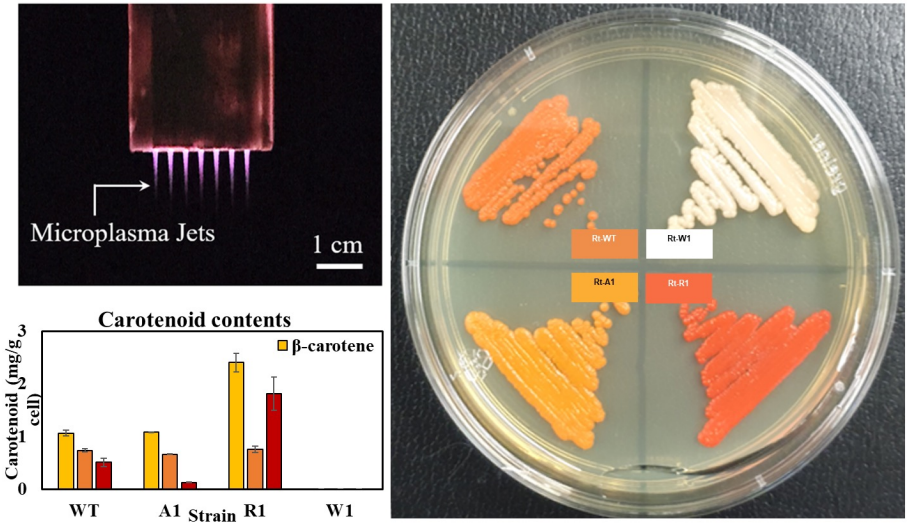
Random mutagenesis introduces mutations into microorganisms where genetic engineering tools are physically limited or undeveloped. A plasma-based method, where the microorganism is exposed to ionized gas that disrupts its DNA, could produce broader and more evenly distributed mutations compared to traditional methods and be advantageous in cost, work safety, and speed. This study explored the results of random mutation, caused by an array of microscale plasma jet devices, in the yeast *Rhodosporidium toruloides,* selected to use its color as a physical indicator of gene mutations.

## Approach

A compact, DNA mutation-inducing machine was built using a row of seven microplasma jet devices, and its physical properties of plasma volume, concentration, stability, and uniformity were examined. After confirming it effectively alters DNA in vitro, *R. toruloides* IFO0880, selected for its natural orange-like color that originates from carotenoid synthesis, was treated with different conditions. Effectiveness of mutation generation was observed by color, a physical indicator of gene mutations in its carotenoid synthesis pathway, by produced reactive oxygen and nitrogen species. Mutants with varied carotenoid content were further characterized.

## Results

The length of microplasma channels affected the plasma volume and concentration of the treatment. Most treated cells (94.8%) were killed, and 0.44% of surviving cells showed different colony colors compared to their parental colony. The reduced carotenoid levels in these mutants proved the potential of the microplasma jet device to cause heritable mutations at random positions in the genome.




**Microplasma jet streams generated with a new device, and the resulting *R. toruloides* mutants.**

## Significance/Impacts

This microplasma-based DNA mutation device can be an efficient and safer alternative for inducing mutations compared to conventional methods and has potential for further application in random mutagenesis of industrial microorganisms.

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