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Localization of alternative oxidase in mitochondria of citric acid-producing *Aspergillus niger*.

A filamentous fungus *Aspergillus niger* is industrially used for the production of citric acid. However, it remains unclear why citric acid is overproduced by *A. niger*. The cyanide-insensitive respiratory pathway, catalyzed by an alternative oxidase (AOX) has been shown to contribute to the production of a large amount of the acid. As the first step to analyze the mechanism underlying the overproduction of citric acid, a fusion gene, *aox1-egfp*, encoding AOX and enhanced green fluorescent protein (EGFP) were constructed and introduced into *A. niger*.

In *A. niger* transformant, the fusion protein AOX-EGFP was confirmed to occur in mitochondria through the comparison of the sites of the green fluorescence by AOX-EGFP (middle panel) with those of the red fluorescence stained with Mito Tracker Red CMXRos (bottom panel), suggesting the relation between citric acid production and AOX in *A. niger*.

Related article: Kirimura, K., Ogawa, S., Hattori, T., and Kino, K., "Expression analysis of alternative oxidase gene (*aox1*) with enhanced green fluorescent protein as marker in citric acid-producing *Aspergillus niger*", **J. Biosci. Bioeng.**, vol. 102, 210-214 (2006).

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