Heavy ion mutagenesis combined with triclosan screening provides a new strategy for improving the arachidonic acid yield in *Mortierella alpina*

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Abstract

Arachidonic acid (ARA),which is a ω-6 polyunsaturated fatty acid, has a wide range of biological activities and is an essential component of cellular membranes in some human tissues.*Mortierella alpina* is the best strain for industrial production of ARA. To increase its yield of arachidonic acid, heavy ion beam irradiation mutagenesis of *Mortierella alpina* was carried out in combination with triclosan and octyl gallate treatment.The obtained mutant strain F-23 ultimately achieved an ARA yield of 5.26 g L^{-1}, which is 3.24 times higher than that of the wild-type strain. In addition, quantitative real-time PCR confirmed that the expression levels of fatty acid synthase(FAS), Δ5-desaturase, Δ6-desaturase, and Δ9-desaturase were all significantly up-regulated in the mutant F-23 strain, especially Δ6- and Δ9-desaturase, which were up-regulated 3- and 2-fold, respectively.This study confirmed a feasible mutagenesis breeding strategy for improving ARA production and provided a mutant of *Mortierella alpina* with high ARA yield.

1. Schematics of the mutant screening experiment

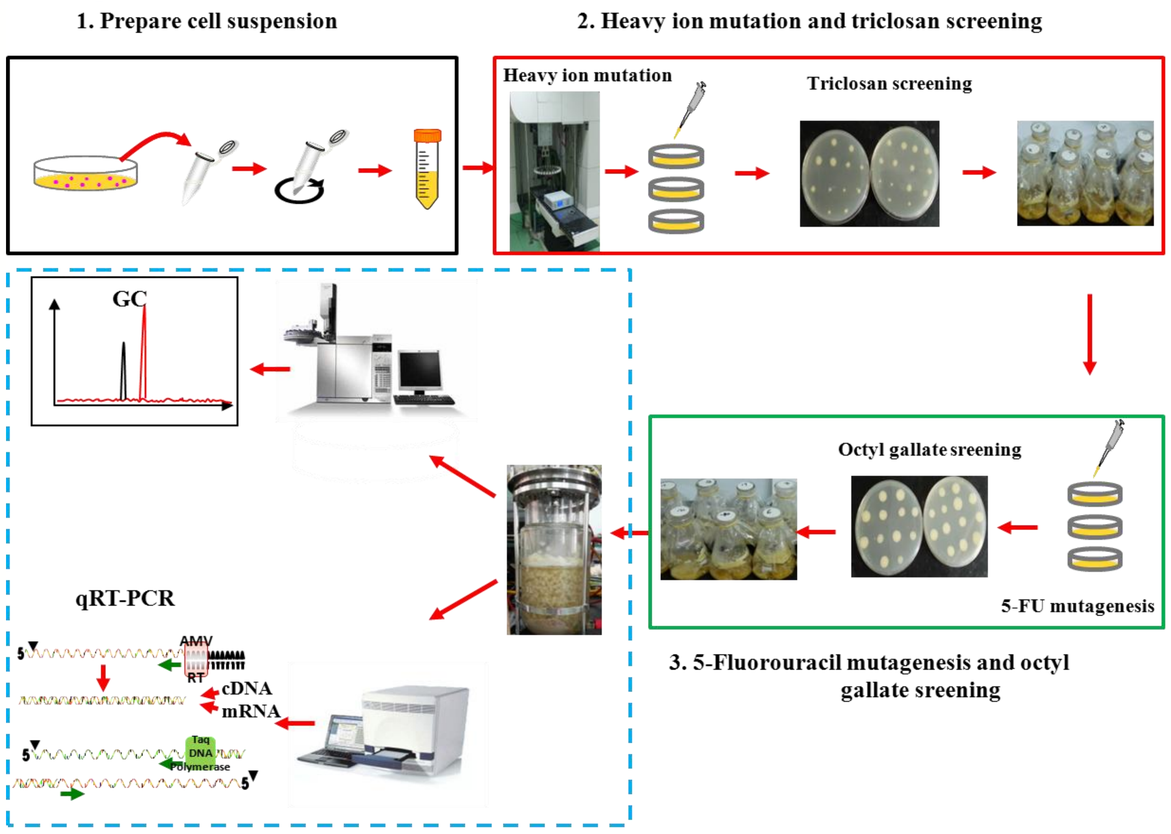


Fig.1 Shematics of the mutant screening experiment

2. Screening of high-yield ARA mutants with triclosan and octyl gallate

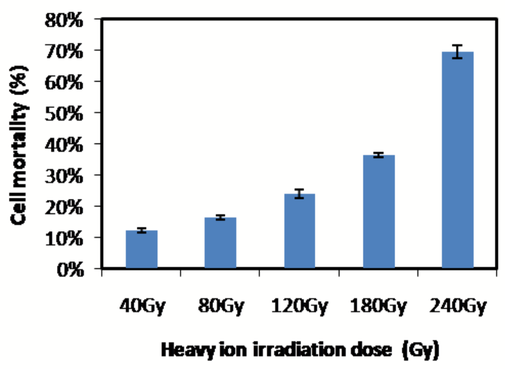


Fig.2 Mortality of *M.alpina*

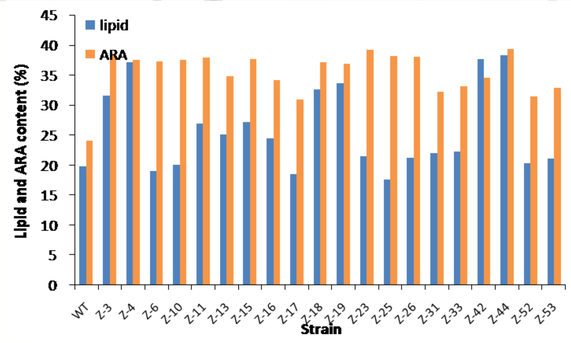
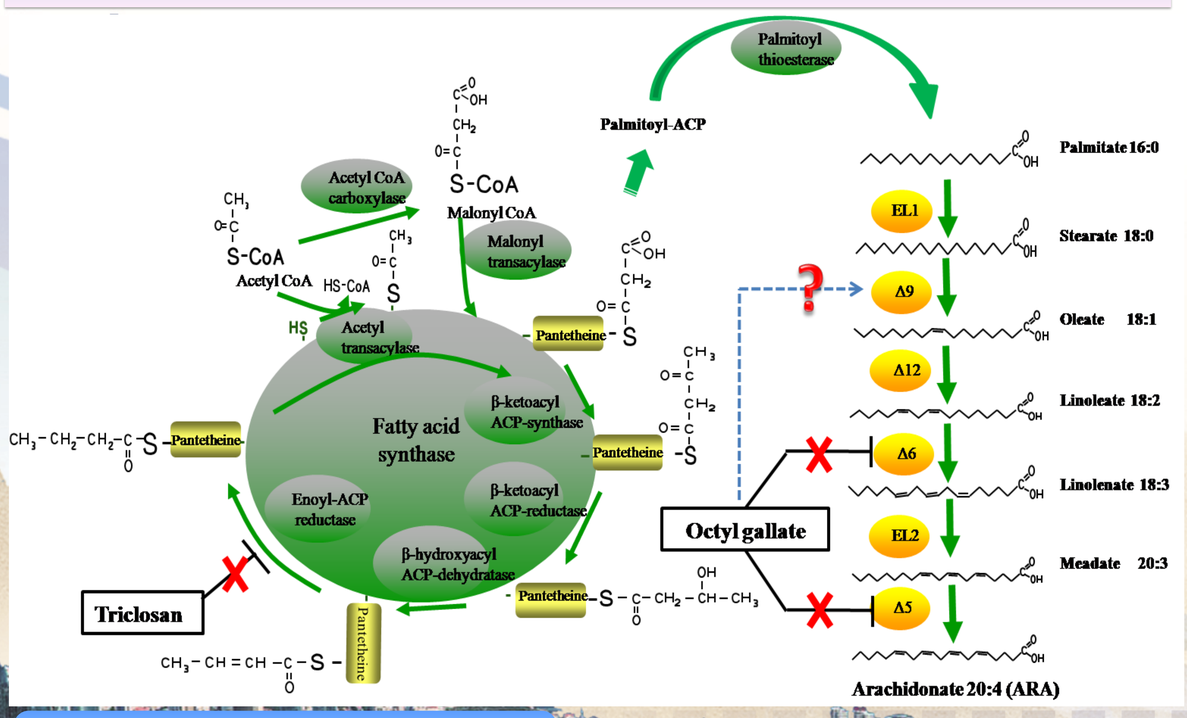


Fig.3 The total lipid and ARA contents of the isolated mutants in 250 mL shake flask

Table 1.Productivity of wild-type strain, mutant strain Z-44 and F-23

Strains	Biomass (g L^{-1})	Lipid content (%)	Lipid yield (g L^{-1})	ARA content (%)	ARA yield (g L^{-1})
WT	23.24±0.46	19.8±0.36	4.60±0.56	27.04±0.63	1.24±0.18
Z-44	25.2±0.93	38.3±0.97	9.65±0.26	39.37±0.28	3.80±0.65
F-23	28.2±0.36	38.0±0.25	10.72±0.41	49.08±0.17	5.26±0.48

3.The ARA biosynthesis pathway and the action sites of inhibitors in *Mortierella alpina*



4.Genetic stability and batch fermentation of mutant F-23

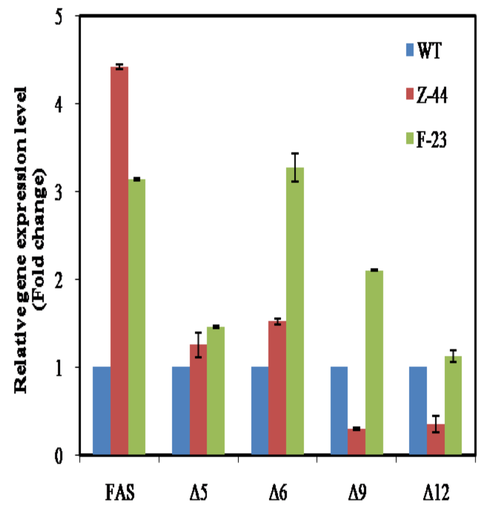


Fig. 5 Relative gene expression levels mutant Z-44 and F-23 strains.

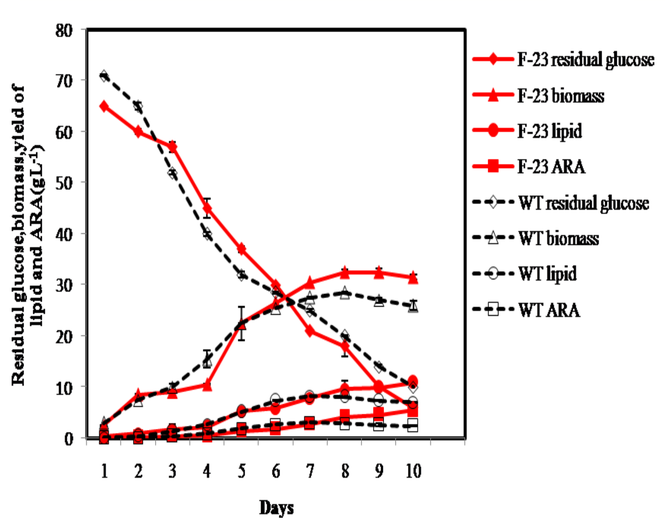


Fig. 6 Growth curves of the mutant F-23 and wild-type strains in 5-L fermenters

5.Conclusions

◆In this study, mutant F-23 was selected after heavy ion beam irradiation combined with triclosan and octyl gallate treatment. Compared with the wild-type strain, the total lipid and ARA yields were increased by 1.33 and 3.24 times, respectively.

◆These results provide a good method and strategy for screening microorganisms with high yield of unsaturated fatty acids.

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