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Metabolic profiling of a *Rhizopus oryzae* fumaric acid production mutant generated by femtosecond laser irradiation

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ABSTRACT

Femtosecond laser irradiation was employed to induce mutations in *Rhizopus oryzae*, leading to increases in fumaric acid production. Compared to the parental strain, mutant strain FM19 exhibited an increase in titer and yield of 56.3% and 36.6%, respectively, corresponding to a titer of 49.4 g/L and a yield of 0.56 g fumaric acid per g glucose. Metabolic profiling by gas chromatography–mass spectrometry revealed that higher levels of carbon (Embden–Meyerhof–Parnas and tricarboxylic acid cycle) and amino acid metabolism were operating in the high-yielding strain; particularly, 4-aminobutyric acid and 5-aminolevulinic acid were increased 10.33- and 7.22-fold, respectively, compared with parental strain during stationary phase. These findings provided new insights into metabolic characterization of high-yielding fumaric acid *R. oryzae*.

1. Introduction

Fumaric acid serves as an important intermediate for polymerization and esterification reactions (Roa Engel et al., 2008) and has been identified as one of the top 12 building block chemicals that can be produced from sugars via biological or chemical conversion in the 21st century by the US Department of Energy (Sauer et al., 2008). At present, fumaric acid is produced chemically from maleic anhydride; however, fermentative production is being explored (Liao et al., 2008; Xu et al., 2010), and a number of microorganisms (e.g. *Rhizopus* species) could be employed (Cao et al., 1996; Gangl et al., 1990; Zhou et al., 2011). Improvements in the fermentative yield of fumaric acid have been achieved by process optimization (Fu et al., 2009; Goldberg and Stieglitz, 1985) and strain improvement such as ultraviolet (UV) and γ-rays mutagenesis (Kang et al., 2010). However, despite these efforts, further strain improvements appear necessary and the mechanism behind increased fumaric acid production still has to be elucidated. Therefore, femtosecond laser irradiation was employed to generate mutants of *Rhizopus oryzae*. Femtosecond laser irradiation at a wavelength of 800 nm has previously been shown to cause changes in DNA and base structure of DNA (Botchway et al., 2010). Following exposure to the irradiation, survivors were screened for fumaric acid production, and following a second round of irradiation, a strain with improved fumaric acid production was obtained and its metabolic characteristics were examined using gas chromatography–mass spectrometry (GC–MS).

2. Methods

2.1. Microorganism, medium and culture conditions

Rhizopus oryzae Wild1.22 derived from *R. oryzae* (ATCC20344) was used as the parental strain. The fungus were maintained on potato-dextrose agar (PDA) slants at 35 °C for 7 days, and then

Abbreviations: 3PG, 3-phosphoglyceric acid; 5-ALA, 5-aminolevulinic acid; AcCoA, acetyl-CoA; ALA, alanine; ASN, asparagine; ASP, aspartic acid; ATP, adenosine triphosphate; CIT, citric acid; CYS, cysteine; DCW, dry cell weight; EMP, Embden–Meyerhof–Parnas; FUM, fumaric acid; G3P, glycerol-3-phosphate; GA3P, glyceraldehyde-3-phosphate; GABA, 4-aminobutyric acid; GC–MS, gas chromatography–mass spectrometry; GLC, glucose; GLE, glycerol; GLN, glutamine; GLU, glutamic acid; GLY, glycine; ILE, isoleucine; LAC, lactic acid; LEU, leucine; MAL, malic acid; NAD(H), nicotinamide adenine dinucleotide (reduced form); OAA, oxaloacetic acid; OKG, 2-oxoglutarate; ORN, ornithine; PC, pyruvate carboxylase; PCA, principal component analysis; PDA, potato-dextrose agar; PHE, phenylalanine; PRO, proline; PYR, pyruvic acid; SER, serine; SFA, saturated fatty acids; SFA16, palmitic acid; SFA17, heptadecanoic acid; SFA18, stearic acid; SFA20, arachidic acid; SPM, spermine; SUC, succinic acid; SUCCoA, succinyl-CoA; TCA, tricarboxylic acid; TFA, total fatty acid; THR, threonine; TYR, tyrosine; USFA, unsaturated fatty acids; USFA16, palmitoleic acid; USFA18, oleic acid; UV, ultraviolet; VAL, valine.

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