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Outline of Research Result

Ergothioneine production by methylotrophic bacteria

Akio Tani

Institute of Plant Science and Resources, Okayama University

Research aims

Methylobacterium species are methylotrophic bacteria belonging to Alphaproteobacteria, and have been studied in detail as a model for bacterial methylotrophy. Recent research studies have revealed that plants emit huge amounts of methanol and that these methylotrophic bacteria are one of the major bacterial species inhabiting the plant surface.[1] Furthermore, inoculation of the methylotrophic bacteria may promote plant growth.[2] During our research on the mechanism of plant growth promotion, we found through metabolome analysis that one of the species accumulated ergothioneine (EGT; Fig. 1). In this study, we aimed to investigate the physiological role of EGT accumulation in the species and the feasibility of fermentative production of EGT.

Methods

Methylobacterium aquaticum strain 22A was used as a model in this study.[3] The strain was isolated from the bryophyte *Racomitrium japonicum*, the complete genome of which has been sequenced.[4] A plasmid, pK18mobSacB, was used for generating a gene deletion mutant of this strain. HPLC was used for EGT quantification. Amino acids were analyzed with an amino acid analyzer.

Results

In the metabolome analysis of strain 22A cells grown on methanol, EGT was found as a most prominent peak by capillary electrophoresis-mass spectrometry. A quantification method based on HPLC analysis was established. Methanol was found to be the best substrate for EGT production among other carbon sources, such as ethanol, succinate, and glucose. EGT was the most abundant amino acid accumulated in the cell compared with other amino acids (Fig. 2). We also found that most *Methylobacterium* species could synthesize EGT, with strain 22A being one of the best strains among them. The cultivation condition was optimized by changing the concentration of the nitrogen sources, iron, lanthanoid, and amino acids. As a result, the productivity reached up to 1 mg/100 mL culture medium (1.2 mg/g wet weight cells, 6.3 mg/g dry weight cells) using 2% methanol as a carbon source in one month of cultivation. The productivity overwhelmed that of *Pleurotus osteatus* (oyster mushroom).[5]

EGT biosynthesis genes were first identified as a gene cluster (*egtABCDE*) in *Mycobacterium* species.[6] We found the homologous genes to be scattered among the genome of strain 22A, except for *egtB* and *egtD* that were clustered. A deletion mutant of *egtBD* was generated, which completely abolished the productivity of EGT. The mutant (Δ*egtBD*) showed more severe susceptibility to heat shock and UV irradiation and higher resistance to hydrogen peroxide than the wild type. We also found that EGT could be degraded by UV irradiation, and the intracellular EGT content decreased upon UV irradiation of the mutant cells. In addition, the mutant was more susceptible to sunlight irradiation. However, the mutant could grow on methanol, suggesting that EGT is not involved in methylotrophy.

Conclusion

Taken together, we showed that it is possible to produce EGT from *Methylobacterium* species utilizing methanol as a carbon source. High-cell density cultivation using the

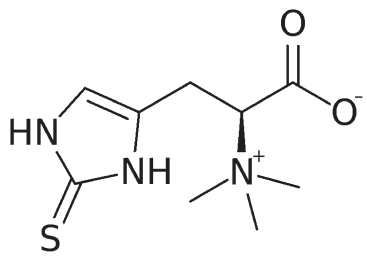

Fig. 1. Ergothioneine

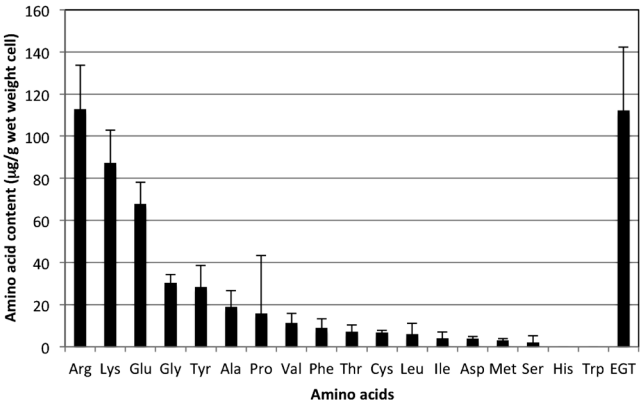

Fig. 2. Intracellular amino acids in strain 22A.

genus is possible, with a yield of 100 g dry weight cells/liter.[7] We are still optimizing the production by examining the high-cell density cultivation, screening microorganisms with higher productivity, optimizing the cultivation conditions, and promoting the gene expression. In this study, we found that EGT accumulates in high amounts when the strain is grown on methanol, but EGT is not involved in methylotrophy. Since the mutant showed higher susceptibility to sunlight, EGT would appear to be an important amino acid for the genus to inhabit the plant surface.

References

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