Recombinant *Aspergillus japonicus* cysteine-S-conjugate β-lyase to releases thiol aroma compounds

張伸維 (5121)

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Outline

1. Introduction
2. Strain selection, activation, preservation and DNA extraction
3. Construct recombinant expression carrier
4. Protein expression, purification and analysis
5. Conclusion

Abstract

Volatile thiols 3-mercaptohexan-1-ol (3MH), 3-mercaptohexyl acetate (3MHA), 4-mercapto-4-methyl-pentan-2-one (4MMP) are powerful aromatic compounds, fungi rely on its metabolic versatility plays an important role in the food industry process, so the purpose of this study is to screen out the cysteine-S-conjugated β-lyase genes related to the release of volatile thiols from mold species, through recombinant protein technology allows it to be expressed in large quantities to generate the target protein, and explores the ability of this protein to produce sulfur-containing aroma compounds. Cysteine-S-conjugated β-lyase mainly exists in the form of tetramer, which is an enzyme containing pyridoxal 5′-phosphate, PLP cofactor, it can catalyze β-elimination reaction of cysteine-S-conjugated electron-withdrawing groups on sulfur, the final products are pyruvate, ammonium and sulfur-containing compounds, which contribute greatly to the aroma , in view of their high concentration and low threshold in food, but in the process of natural fermentation, the conversion rate of enzymes to convert odorless precursors into aroma compounds is very low, so it needs to be improved by transformation, through Prokaryotes produce a large number of target enzymes to elevate convertion efficiency. After using the protein database Uniprot and BLAST protein alignment tools for comparison, *Aspergillus japonicus* was selected as the gene source of this enzyme. After DNA molecular extraction and PCR amplification, which express in the *E. coli* C43 system, induce and purify the protein by using ÄKTA fast protein liquid chromatography, FPLC. Based on the above results, through subsequent quantitative, qualitative and enzyme activity analysis tests, the protein identity of the recombinant enzyme can be identified and compared, also can test its ability to produce sulfur compounds. Expecting to apply this technology in the spice market, providing a new option for the production of spices.

Bassi, D., Puglisi, E., & Cocconcelli, P. S. (2015). Comparing natural and selected starter cultures in meat and cheese fermentations. *Current Opinion in Food Science, 2*, 118-122

Campbell-Platt, G. (2014). FERMENTED FOODS| Origins and Applications.

Copetti, M. V. (2019). Yeasts and molds in fermented food production: an ancient bioprocess. *Current Opinion in Food Science, 25*, 57-61

Holt, S., Cordente, A. G., Williams, S. J., Capone, D. L., Jitjaroen, W., Menz, I. R., Curtin, C., & Anderson, P. A. (2011). Engineering Saccharomyces cerevisiae To Release 3-Mercaptohexan-1-ol during Fermentation through Overexpression of an S. cerevisiae Gene, STR3, for Improvement of Wine Aroma. *Applied and Environmental Microbiology, 77*(11), 3626-3632

Li, H. K., Chang, C. F., Lin, H. J., Lin, J. L., Lee, Y. T., Wu, Y. H., Liu, C. Y., Lin, T. C., Hsu, P. H., & Lin, H. T. V. (2021). Conversion of a Thiol Precursor into Aroma Compound 4-mercapto-4-methyl-2-pentanone Using Microbial Cell Extracts. *Fermentation-Basel, 7*(3), 129

Roland, A., Cavelier, F., & Schneider, R. (2012). How organic and analytical chemistry contribute to knowledge of the biogenesis of varietal thiols in wine. A review. *Flavour and Fragrance Journal, 27*(4), 266-272

Roland, A., Schneider, R., Razungles, A., & Cavelier, F. (2011). Varietal Thiols in Wine: Discovery, Analysis and Applications. *Chemical Reviews, 111*(11), 7355-7376

Roncoroni, M., Santiago, M., Hooks, D. O., Moroney, S., Harsch, M. J., Lee, S. A., Richards, K. D., Nicolau, L., & Gardner, R. C. (2011). The yeast *IRC7* gene encodes a beta-lyase responsible for production of the varietal thiol 4-mercapto-4-methylpentan-2-one in wine. *Food Microbiology, 28*(5), 926-935

Santiago, M., & Gardner, R. C. (2015). Yeast genes required for conversion of grape precursors to varietal thiols in wine. *FEMS Yeast Research, 15*(5)

Sasikumar, B., Swetha, V., Parvathy, V., & Sheeja, T. (2016). Advances in adulteration and authenticity testing of herbs and spices. In *Advances in food authenticity testing* (pp. 585-624): Elsevier.

Thibon, C., Bocker, C., Shinkaruk, S., Moine, V., Darriet, P., & Dubourdieu, D. (2016). Identification of S-3-(hexanal)-glutathione and its bisulfite adduct in grape juice from *Vitis vinifera* L. cv. *Sauvignon blanc* as new potential precursors of 3SH. *Food Chemistry, 199*, 711-719

Tominaga, T., Furrer, A., Henry, R., & Dubourdieu, D. (1998). Identification of new volatile thiols in the aroma of Vitis vinifera L. var. Sauvignon blanc wines. *Flavour and Fragrance Journal, 13*(3), 159-162