
ABSTRACTS (MASTER THESIS)

Functional analysis of terpene synthase from hop

**(Graduate School of Agriculture,
Laboratory of Plant Gene Expression, RISH, Kyoto University)**

Joji Kageyama

Hop (*Humulus lupulus* L.) is a perennial vine plant (Cannabaceae) that is cultivated worldwide as been essential for beer brewing. Female flowers of hops are the indispensable ingredient of beer to contribute its characteristic flavor and bitterness to beer taste since the medieval period. These green flowers develop many yellow glandular trichomes called ‘lupulins’, which are the tissue for the accumulation of various terpenoids, bitter acids (prenylated phloroglucinols) [1, 2] and prenylated flavonoids [3].

Hop cultivars can be roughly divided into two groups, aromatic and bitter cultivars. Linalool is one of the most prominent monoterpenes as a marker compound of aromatic hop varieties. Although most important terpene synthases have been already isolated from hops in USA and Canada [4], linalool synthase gene has not yet been reported. In this study, we have isolate and characterize the linalool synthase of hop.

Using EST analysis of lupulin-rich part of the female flowers of an aroma hop cultivar, a novel terpene synthase-like gene was found among various terpene synthase genes having motifs highly preserved in most terpene synthases requiring Mg^{2+} for the enzymatic function. Then, we have characterized the enzyme activity of the candidate gene with the recombinant protein prepared in *Nicotiana benthamiana* and in an expression system of *Escherichia coli*. Consequently, the candidate gene synthesized linalool in the presence of GPP, and nerolidol in the presence of FPP.

Furthermore, we have investigated the expression level of linalool synthase in each organs of a hop plant. We have also done a chemical analysis of volatile components of hop cone using GC-MS to find various volatile terpenes, such as myrcene and humulene, while linalool content was almost undetectable when fresh lot of hop cones were used. The reason should be further studied.

Acknowledgements

This study was supported by Dr. Tetsu Sugimura, Dr. Masaki Momose, Dr. Naoyuki Umemoto, Dr. Atsushi Murakami and Dr. Kazuaki Ohara of Kirin Co.; Dr. Ryosuke Munakata of RISH, Kyoto University; Dr. Kazutoshi Shindo of Ishikawa Prefecture University; Dr. Norihiko Misawa of Japan Women’s University; Dr. Tsuyoshi Nakagawa of Shimane University; Dr Hiroshi Kouchi of International Christian University; Dr. Shiro Suzuki of RISH, Kyoto University; and GC/MS provided by DASH/FBAS of RISH, Kyoto University.

References

- [1] Okada Y, Ito K. (2001). Cloning and analysis of valerophenone synthase gene expressed specifically in lupulin gland of hop (*Humulus lupulus* L.). *Biosci. Biotechnol. Biochem.* 65, 150-155.
- [2] Tsurumaru Y, Sasaki K, Miyawaki T, Uto Y, Momma T, Umemoto N, Momose M, Yazaki K. (2012). *HlPT-1*, a membrane-bound prenyltransferase responsible for the biosynthesis of bitter acids in hops. *Biochem. Biophys. Res. Commun.* 417, 393-398.
- [3] Nagel J, Culley LK, Lu Y, Liu E, Matthews PD, Stevenes JF, Page JF. (2008). EST analysis of hop glandular trichomes identifies an *O*-methyltransferase that catalyzes the biosynthesis of xanthohumol. *Plant Cell.* 20, 186-200.
- [4] Wang G, Tian L, Aziz N, Broun P, Dai X, He J, King A, Zhao PX, Dixon RA. (2008). Terpene biosynthesis in glandular trichomes of hop. *Plant Physiol.* 148, 1254-1266.