PHENO- AND GENOTYPING OF HOPANOID PRODUCTION IN ACIDOBACTERIA: LIMITED CAPACITY FOR THE PRODUCTION OF METHYLATED HOPANOIDS

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Introduction

The biosynthesis of extended 2-methyl hopanoids was thought to be restricted to cyanobacteria and, consequently, their appearance in the geological record was used as evidence for the advent of an oxygenated atmosphere. However, extended 2-methyl hopanoids were subsequently also detected in a photosynthetic bacterium and an inventory of the presence of the gene (*hpnP*) encoding the methyl transferase enzyme responsible for methylation of hopanoids at the C-2 position revealed that the *hpnP* gene occurs even more widespread in the bacterial domain (Welander et al., 2010), including the *Acidobacteria*, a widespread and abundant bacterial phylum, occurring in peatlands, soil, and lake waters. Welander and Summons (2012) showed that the gene, *hpnR*, required for methylation of hopanoids at the C-3 position is not only found in genomes of methanotrophic and acetic acid bacteria but also in other bacteria such as members of the *Actinobacteria*, *Nitrospirae*, and *Acidobacteria*. These genetic surveys question the validity of the use of methylated hopanoids as specific biomarkers.

Results

We analyzed 38 different strains of seven subdivisions (SDs 1, 3, 4, 6, 8, 10, and 23) of the *Acidobacteria* for the presence of C_{30} hopenes and C_{35} bacteriohopane polyols (BHPs) using the Rohmer reaction (treatment with periodic acid/sodium borohydride to convert complex polyfunctionalized BHPs into hopanoid alcohols). Since it is known that the acidobacterial membrane lipids are difficult to extract with the commonly applied Bligh-Dyer protocol, the Rohmer degradation reaction was directly applied to lyophilized cells and not to the Bligh-Dyer extract as is commonly done. This resulted in a ten-fold increase in hopanoid yield, demonstrating that BHPs in acidobacteria, in addition to membrane-spanning lipids, are difficult to extract. BHPs and/or C_{30} hopenes were detected in all strains of SDs 1 and 3 but not in SDs 4 (excepting *Chloracidobacterium thermophilum*), 6, 8, 10 and 23.
This is in good agreement with a survey of genes required for hopanoid biosynthesis in the 30 available whole genomes of cultivated *Acidobacteria*. All genomes encode the enzymes involved in the non-mevalonate pathway ultimately leading to farnesyl diphosphate but only SDs 1 and 3 *Acidobacteria* and *C. thermophilum* encode all enzymes required for the synthesis of squalene (*hpnC*, *hpnD*, *hpnE*), its cyclization (*shc*), and addition of the extended side chain (*hpnG*, *hpnH*, *hpnI*, *hpnJ*, *hpnK)*. In almost all strains only tetrafunctionalized

BHPs were detected; three strains contained variable relative abundances (up to 45%) of pentafunctionalized BHPs but hexafunctionalized BHPs were absent. Only '*Ca.* Koribacter versatilis' Ellin245 contained methylated BHPs, although in low (<10%) and variable relative amounts. The most abundant methylated Rohmer degradation product was tentatively identified as 2,3-dimethyl bishomohopanol. This identification is consistent with the occurrence of the *hpnP* and *hpnR* genes in '*Ca.* K. versatilis'. These genes are not present in any other acidobacterium, consistent with the absence of methylated BHPs in the other examined strains. These data are in agreement with the scattered occurrence of methylated BHPs in other bacterial phyla such as the *Alpha-*, *Beta-* and other *proteobacteria* and the *Cyanobacteria*, which limits the biomarker potential of these specific lipids. Acidobacterial genomes derived from metagenomics sequencing of environmental samples were also examined for the presence of genes required for hopanoid biosynthesis. The complete pathway for BHP biosynthesis was only present in groups of *Acidobacteria* phylogenetically related to SD1 and SD3, in line with the limited occurrence of BHPs in acidobacterial cultures.

Conclusions

Since the *Acidobacteria* often form an important (i.e. up to 40%) fraction of the bacterial community (e.g. in soils), they could, despite the limited occurrence of BHP biosynthesis in a few SDs, still be considered potentially quantitatively important BHP producers in the environment. However, based on our results members of the phylum *Acidobacteria* unlikely play an important role in sourcing methylated BHPs in the environment.

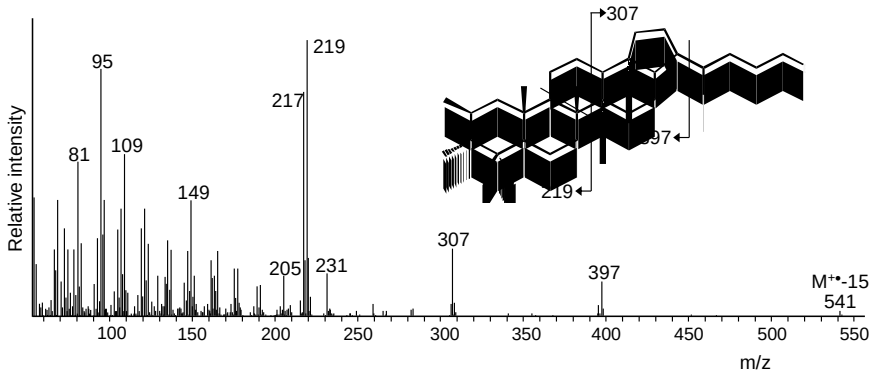

Figure 1 *Mass spectrum a putative dimethylated bishomohopanol (as TMS derivative) formed by Rohmer degradation of total cell material of '*Ca*. Koribacter versatilis' Ellin345. The methylation at position C-2 and C-3 is unprecedented but supported by the indicated mass spectral fragmentation and the presence of both the hpnP and hpnR genes in the genome of '*Ca*. K. versatilis'. The indicated stereochemistry of the additional methyl groups is hypothetical.*

References

Welander, P.V., and Summons, R.E., 2012. Discovery, taxonomic distribution, and phenotypic characterization of a gene required for 3-methylhopanoid production. Proceedings of the National Academy of Sciences USA 109, 12905-12910.

Welander, P.V., Coleman, M.L., Sessions, A.L., Summons, R.E., and Newman, D.K., 2010. Identification of a methylase required for 2-methylhopanoid production and implications for the interpretation of sedimentary hopanes. Proceedings of the National Academy of Sciences USA 107, 8537-8542.