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Genomics and metabolism of chloromethanedegrading methylotrophic bacteria

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Chloromethane (CH₃Cl) is a halogenated carbon compound abundantly present in the atmosphere, and is responsible for

significant chlorine-dependent ozone destruction. Although chloromethane is produced industrially, larger amounts are emitted naturally in terrestrial ecosystems through specific biological activities of plants and the degradation of plant organic matter in soils. Nonetheless, the magnitudes of chloromethane production and of its microbial degradation are still poorly characterized. To evaluate chloromethane biodegradation in the environment, we have been studying the key enzymes and regulation elements involved in halogenated methane microbial degradation.

Under laboratory conditions, chloromethane-degrading bacteria isolated from leaves and soils harbor the *cmu* pathway (for <u>chloromethane utilization</u>), the only known microbial pathway for utilization of chloromethane. Among the methylotrophic microorganisms able to utilize C₁ compounds, only a few are able to grow with chloromethane provided as the sole source of carbon and energy. The *cmu* pathway involves tetrahydrofolate and vitamin B₁₂ as essential cofactors of chloromethane dehalogenase and has been characterized in detail for *Methylorubrum extorquens* CM4. Key players of the bacterial response to growth with chloromethane were identified using random mini-transposon Tn5 mutagenesis, comparative genomics, proteomics, and transcriptomics, as well as experimental evolution approaches.

Bacteria associated with chloromethane degradation in soils and plant leaves were identified by stable isotope probing (SIP) using incubation with ¹³C-labelled CH₃Cl. High throughput sequencing of the heavy DNA targeted taxonomical and *cmu* genes, as well as metagenomes. In most experiments, detected taxa did not correspond to bacteria known to contain *cmu*.

Future research is needed to grasp the diversity of chloromethane-degradingassociated metabolisms and taxa of the microbial chloromethane sink. High variations in chloromethane flux emissions have been reported which suggests that low and high affinity chloromethane-degrading populations may be found in the environment, as is the case for methane.

