Molecular characterization of microbial communities during ensiling conditions and biogas production in the GOBi project

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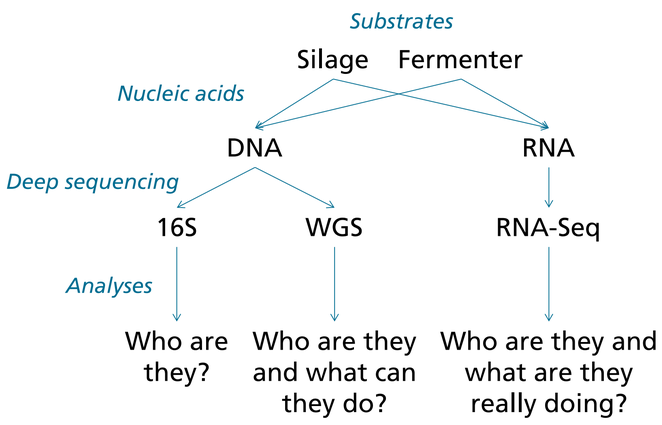
Scientists of Hohenheim University and Fraunhofer Institute for Interfacial Engineering and Biotechnology together with industrial partners focus on improvement of sustainability of biogas production. Therefore all single process steps, beginning with the plant production to the recycling of occurring residuals, are under investigation. Especially the knowledge of the spatial and temporal composition of microbial populations in ensiling and biogas processes and their targeted manipulation could provide innovative possibilities for the optimization of the processes to improve biogas yields. With the advent of Next-Generation Sequencing (NGS) technologies a comprehensive characterization of complex microbial communities has been facilitated. This also includes capturing of non-cultivable organisms which were previously impossible to identify using classical microbiology methods as they show poor or no growth *in vitro* - the so called microbial dark matter.
We plan to perform microbial characterization at three different levels: (i) sequencing of small subunit ribosomal RNA (SSU rRNA) to determine the population, (ii) sequencing of whole metagenomes to uncover gene coding potential and (iii) sequencing of whole metatranscriptomes to reveal transcriptional activity. All three levels significantly contribute to an improved understanding to further optimize biogas production in terms of controlling these microorganisms along the fermentation process. Here, we present the first insights into the molecular characterization of microbial populations during ensiling and biogas production.

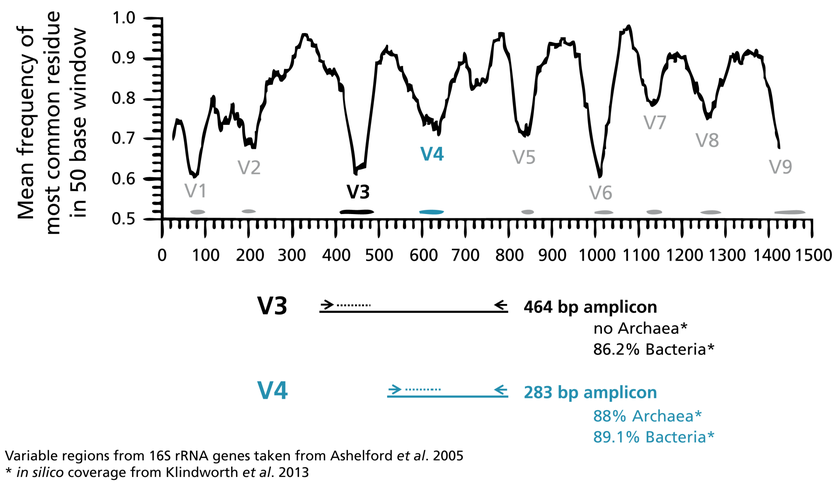
Key words: microbial communities, Next-Generation Sequencing (NGS), ensiling conditions, biogas production

New opportunities in characterization of microbial communities

The enormous advancements in sequencing technologies have opened up entirely new dimensions in nucleic acid analysis and revolutionized countless areas of research in Life Sciences like the characterization of complex, microbial communities.
In our project, we applied and compared three different ways to investigate microbiomes out of silages and biogas fermentation processes.



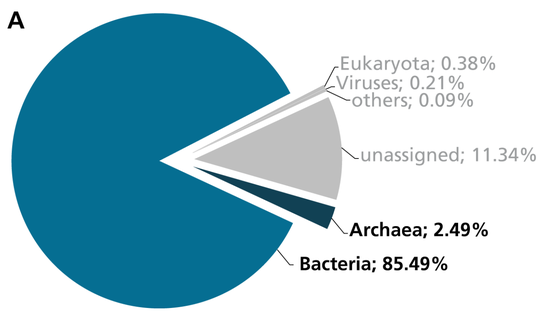
Evaluation of two primer pairs targeting 16S rRNA region



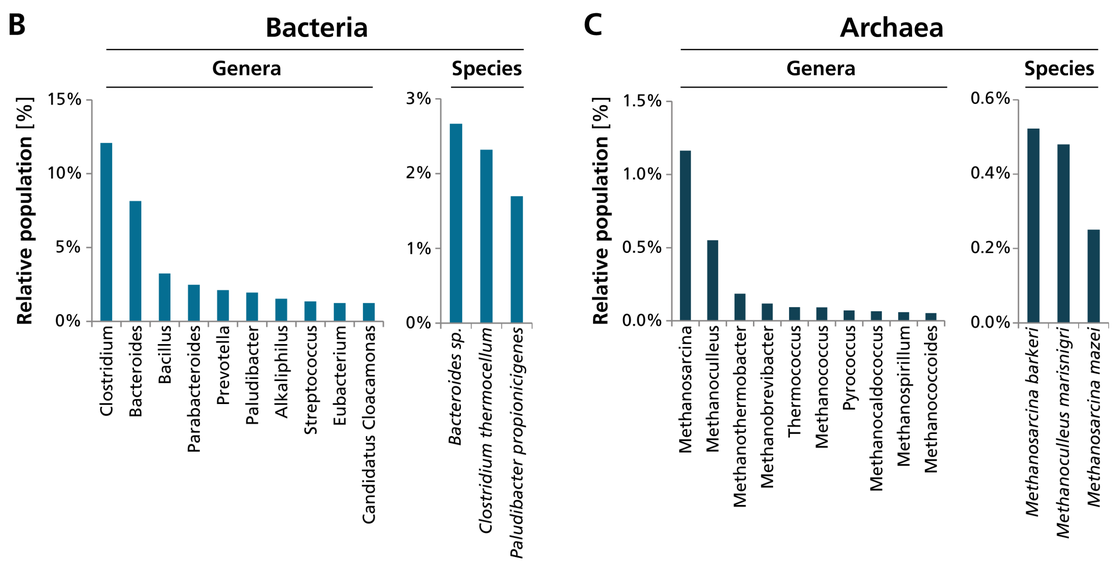
Variable regions from 16S rRNA genes taken from Ashelford *et al.* 2005
* *in silico* coverage from Klindworth *et al.* 2013

A fast and very cheap method for identification of Bacteria or Archaea makes use of the conserved 16S rRNA gene locus containing hypervariable regions. These can universally be amplified out of a complex population with distinct primer pairs, e. g. targeting V3 or V4. The resulting amplicons subsequently can then be sequenced.

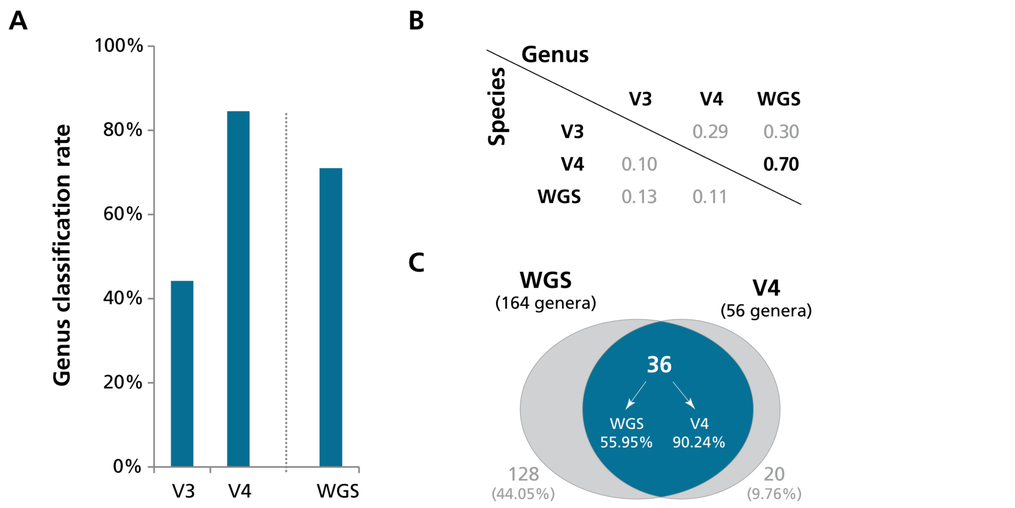
Whole genome shotgun sequencing (WGS) reveals stable microbial classification during fermentation



By sequencing whole genome shotgun libraries from fermentation process with 150 bases in length via HiSeq2500, we achieved a high determination rate of microbes (A). Reads were classified using the MG-RAST platform with default settings (16 out of 21 mio. reads). Both, the classification on the genus level as well as on the species level were robust (B, C). A significant amount of methane producing Archaea could also be detected (C).

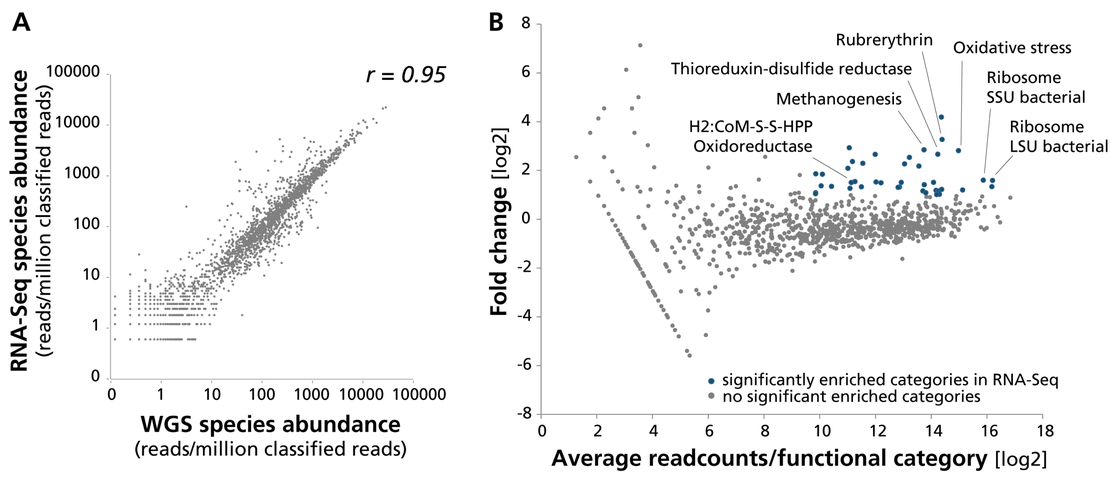


We evaluated the two most promising primer pairs with highest species coverage and equally highest species discrimination rates. Thus, two different 16S amplicon libraries (V3 and V4) were generated out of the identical sample used for WGS libraries. This was necessary to have an optimal basis for the comparisons by using WGS data as reference. In summary, the V4 primer had not only a higher classification rate than V3 (A), but showed also a higher Person correlation to WGS data (B). More than 90% of all classified reads in V4 were distributed among 36 genera which has also been determined in the WGS library (C). However, the correlation at species level significantly decreases and should be considered with caution.



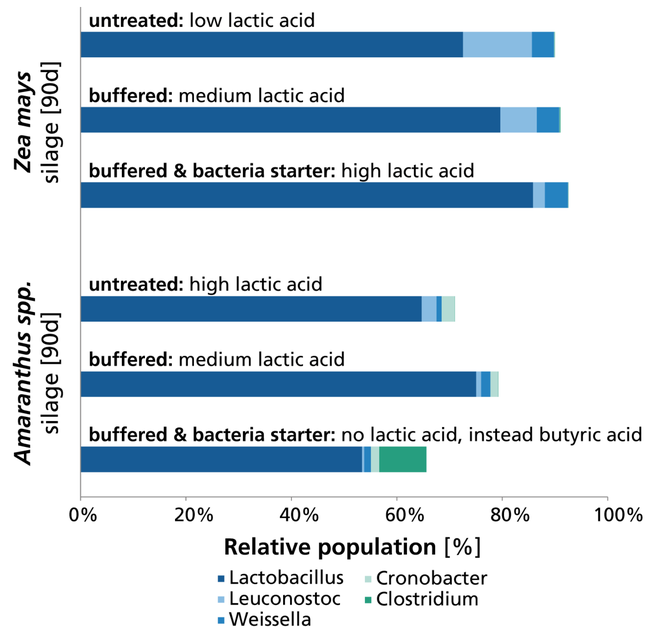
Metatranscriptomic data outruns WGS

Identical fermenter samples as used for WGS libraries were also prepared for RNA-Seq. Here, we sequenced 15 mio. reads with a sequencing length of 100 bases. Classification rates were highly comparable with the data from the WGS library (A, correlation factor $r = 0.95$). However, the comparison of readcount enrichments to distinct functional categories revealed a significant bias towards biogas related enzymes or processes (B).



Microbial composition differs during ensiling *Zea mays* and *Amaranthus spp.*

Here, we applied the established 16S amplicon library protocol targeting V4 region to describe microbial compositions of ten different ensiling conditions of *Zea mays* and *Amaranthus spec.* aiming at high lactic acid production rates. The nucleic acids were extracted from silage effluent after 90 d. The compsition in the maize silage varied relative to the treatment and correlated with produced lactic acid. In the amaranth silage we could detect even stronger variations dependent on the treatment as for example heavy clostridia growth was observed in the silage with buffer and bacteria starter population.



Outlook

In the next steps of the project, we are going to analyse the microbiomes during biogas production dependent of the previously ensiled substrates in a time series by WGS and by RNA-Seq. By identifying the key players and the key pathways, we hope to gain comprehensive insights which enable us to selectively manipulate fermentation steps and thus might contribute to an optimization of the whole process.

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