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From wild strain to domesticated strain: the philosophy of microbial diversity in foods

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In context with "the different and contrasting roles of microorganisms in food quality, shelf life, and safety" (Guerzoni, 2010), the knowledge on how wild strains become domesticated represents a common research priority for food microbiologists. The diversity of microbial communities and their ecological and metabolic functions have the potential for remarkable scientific, social, and economic impact. However, microbiological diversity remains largely undiscovered and the knowledge of its global distribution and temporal variability remain elusive. The biosphere is estimated to contain between 10^{30} and 10^{31} different microbial genomes; however, we likely only know a minority of them at present (Whitman et al., 1998). Sequencing surveys of amplified regions of small subunit ribosomal RNA (SSU rRNA) genes have revealed that microbial diversity is much greater than the 5,000 microbial species described using phenotypic features in Bergey's taxonomic outline (Staley, 2006), and that microbial communities are far more complex than initially thought. Hence the application of molecular phylogenetic methods to study natural microbial ecosystems has resulted in the unexpected discovery of many evolutionary lineages. In addition, the recent surge of research in molecular microbial ecology produced compelling evidence for the existence of many novel types of microorganisms in the environment – both regarding abundance and diversity – that dwarf those of the comparatively few microorganisms amenable to laboratory cultivation. Collectively, the genomes of the total microbiota found in nature, termed the metagenome (Sebat et al., 2003; Handelsman, 2004), contain vastly more genetic information than is contained in the culturable subset. Comparative genomics, gaining a better understanding of how species have evolved, has shown the power to determine the function of genes and non-coding regions of the genome. The comparative genomics of the lactic acid bacteria reported by Makarova et al. (2006), for instance, demonstrated the phylogenetic and functional diversity of these bacteria. The reconstruction of ancestral gene sets revealed a combination of gene loss and gain during coevolution of lactic acid bacteria with animals and the foods they consumed. The study proposed that the origin of *Lactobacillales* involved extensive loss of ancestral genes (600–1200 genes) during their transition to life in a nutritionally rich medium, which allowed a reduction in catabolic capacity and an increased stress resistance.

A powerful approach for studying microbial diversity in a complex environment such as food is the direct cloning of DNA from environmental samples. Genomic fragments that are >100-kb long can be obtained, and they provide functional and taxonomic information about the organisms, which they were derived from. Such metagenomic libraries have been used to identify microorganisms or enzymes that are responsible for significant processes. Functional analysis has identified novel antibiotics, degradative enzymes, bioactive compounds, etc. (Henne et al., 2000). Metabolomics that focuses on high-throughput characterization of small molecule metabolites in biological matrices has also great relevance to food science and technology as it is suitable to identify and highlight the microbial biodiversity in food systems not only at the level of different species but also among strains.

The analysis of a microorganism in different environments and the quantification of metabolic fluxes can help to better understand its role in food and also optimize starter strain performance. In fact the evaluation of strain diversity provides a great chance to increase the knowledge of metabolic functions and regulation and to speed up the process of targeted strain improvement. Phenotypic investigations enable us to compare the different strains with similar overall metabolic behaviors and to select a certain strain as most promising for further targeted optimization (Wittmann and Heinzle, 2002). Moreover, the microbial activity impacts on the composition and nutritional status, which can be valorized by the introduction of specific health beneficial attributes. Despite the profound differences in the microbial consortia involved in the fermentation of different foods there is a striking similarity in the health attributes that can be delivered along the chain from fermented food to gut microbiota through three encompassing phases: microbial ecosystem, health impacting molecules, and the possibility to modulate the gut ecosystem (Van Hylckama Vlieg et al., 2011).

In contrast to other habitats, foods are generally characterized by a not relevant number of microbial species. The factors affecting this peculiar characteristic are multiple and depend on the origin of raw materials and the processes used to stabilize the foods and to improve their sensorial and heath quality. Microorganisms living in foods are constantly exposed to fluctuating environmental conditions and many of these conditions are potentially detrimental and negatively affect the physiological state and growth rate of resident microorganisms. Nevertheless, their multiple regulatory networks of stress response systems allow them to withstand harsh conditions and sudden changes in environmental conditions (Van de Guchte et al., 2002). Hence the complex network of such responses, involving several metabolic activities, can reflect upon the composition and organoleptic properties of the growth medium. Even if autochthonous bacteria are adapted and competitive, the food system must be considered as a stressful environment for them. Moreover, mutations may favor generation of strain variants that are better adapted to survive under these stress conditions. In addition, DNA instability might result in a so-called mutator phenotype, where sharply elevated spontaneous mutation rates (transiently) enhance a strain's ability to adapt to radical changes in the environment (Machielsen

et al., 2010). So the species present in a food are always intrinsically differentiated into many populations of strains belonging to the same species. Among these strains and species environment and stress parameters highlight their biodiversity, from strain to strain.

Many microorganisms involved in food processing are considered "domesticated"; the best known case is that of *Saccharomyces cerevisiae*, which is predominantly found in association with anthropogenic processes, particularly the production of bread, beer, and fermented beverages. To date, *S. cerevisiae* includes both wild and domesticated forma species, where the domestication event that resulted in grape wine yeasts likely occurred approximately 2,700 years ago (Mortimer and Polsinelli, 1999; Fay and Benavides, 2005). The wine strains of *S. cerevisiae* are highly diverse; populations fermenting grape are usually polyclonic and the clones can differ significantly in enological performance and genotype. The extent of genetic differences ranges from single-nucleotide substitutions to whole-genome duplication (Sipiczki, 2011). Similar genotypic diversity can be expected in other microorganisms associated with food habitats. For instance, phylogenetic analyses, comparisons of gene content across groups, and reconstruction of ancestral gene sets indicate a combination of extensive gene loss and key gene acquisition events via horizontal gene transfer during the coevolution of lactic acid bacteria and their habitats (Makarova et al., 2006). This coevolution is particularly evident in *Oenococcus oeni* and *Lactobacillus sanfranciscensis*, both of which can be considered highly specialized microorganisms because they uniquely occupy very narrow ecological niches: wine and sourdough, respectively. *O. oeni* plays an important role in the elaboration of wine, where it is often added as a starter culture to carry out the malolactic conversion. While the taxonomic structure of this species was believed to be quite homogeneous, recently, however, multilocus sequence typing (MLST) of strains revealed a high level of allelic diversity in *O. oeni* resulting in a panmictic population structure where lines of clonal descent are difficult to define (de Las Rivas et al., 2004). Panmictic populations are often characterized by high levels of horizontal transfer and recombination among strains. The hypermutable status in the genus, due to the absence of the mismatch repair genes *mutS* and *mutL* results in the accumulation of spontaneous errors in DNA replication and in facilitating the generation of isolates with beneficial mutations, resulting in increased fitness for the environment (Prunier and Leclercq, 2005; Bon et al., 2009). It is likely that this factor contributed to the unique adaptation of *O. oeni* to acidic and alcoholic environments that made it an ideal organism for the malolactic fermentation during the production of wine.

Lactobacillus sanfranciscensis represents a good example of specialized adaptation to dough environment, since it preferentially ferments the maltose (the main sugar of the dough) as an energy source and uses fructose (present in the flour, too) as an additional electron acceptor in order to gain an extra mole of ATP via acetate kinase thereby improving its growth rate and competition. A new pathway proposed by Ganesan et al. (2006) and Serrazanetti et al. (2011) to convert leucine to 2-metylbutanoic acid, maybe useful in the production and use of intermediates and cofactors resulting in energy (3 mol ATP) and reducing equivalents. This novel pathway may contribute to the microorganisms' survival during cellular stress and limitation of carbon source.

Taken together, it appears that selective pressure from many environmental parameters and processing conditions during time rather than the geographical area have driven the domestication process leading to specialized strains in species with a high genetic diversity. It appears therefore, that genetic engineering, directed evolution or even the creation of entirely synthetic genomes could serve to overcome and fast-forward the series of mutagenic events that is usually driven by the environment to create biodiversity. This approach of reaching "maximum utility based on a minimum of knowledge" appears to be the new paradigm of applied sciences reaffirming Jacques Monod's statement that "the first scientific postulate is the objectivity of nature. Nature does not have any intention or goal."

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