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Use and limitations of genome-scale metabolic models in food microbiology

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Microbes are key to creating safe, edible and enriched fermented food products. This is largely achieved by their metabolism. Thus, the ability to understand the wiring of the complete cellular metabolism is critical to control the fermentation processes. Metabolic modelling is a useful tool for integration of large datasets to link genotype to phenotype. Here, we summarise how metabolic models are being used to address the challenges in safety, biotransformation and food enhancement in food-relevant settings. Finally, we discuss how metabolic modelling can be integrated to assess more complex scenarios such as microbial communities. Despite many remaining challenges, metabolic models hold a large potential for use in food microbiology.

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Introduction

In the beginning, food fermentation relied on spontaneous fermentation by natural microbial consortia and uncontrolled process conditions. With the start of backslopping and the understanding of microbes, the production got more controlled. Today people are understanding, optimizing and designing the fermentation process conditions and microbes at an industrial scale.

The objectives in food microbiology are often related to (i) shelf-life and safety of the food products, (ii) specific biotransformations and (iii) the enrichment of food with desired properties. The preservation of food includes rapid acidification to prevent growth of harmful microbes. Biotransformations include the preparation of indigestible food sources (e.g. coffee or cacao) but also the utilization of alternative dairy products (e.g. oat milk). The enrichment of food includes flavour compounds, organoleptic properties, the increase of nutrition value or strains with probiotic properties.

The tools to achieve these objectives have also advanced. Advancements started, and still continues, with the systematic collection and cataloguing of strains. The phenotyping focused on morphology, growth or acidification properties. Today, it includes whole genome sequences, and the prevalence and activity of strain in complex food matrices are routinely monitored by multi-omics tools.

Despite the advances in technology and data, bridging the gap from mere description to more mechanistic understanding has remained challenging. Here metabolic modelling, in particular through Genome Scale Metabolic Models (GSMM), has great potential. Such GSMMs organize an organism's metabolism as a set of gene – protein – metabolic reaction associations (reviewed in Ref. [1]). These models thus provide a metabolic context for integration of genomics and cell physiology data and can predict metabolic capabilities based on genomes (Figure 1). There is a wide variety of tools to help with each phase of the process (see Ref. [2]).

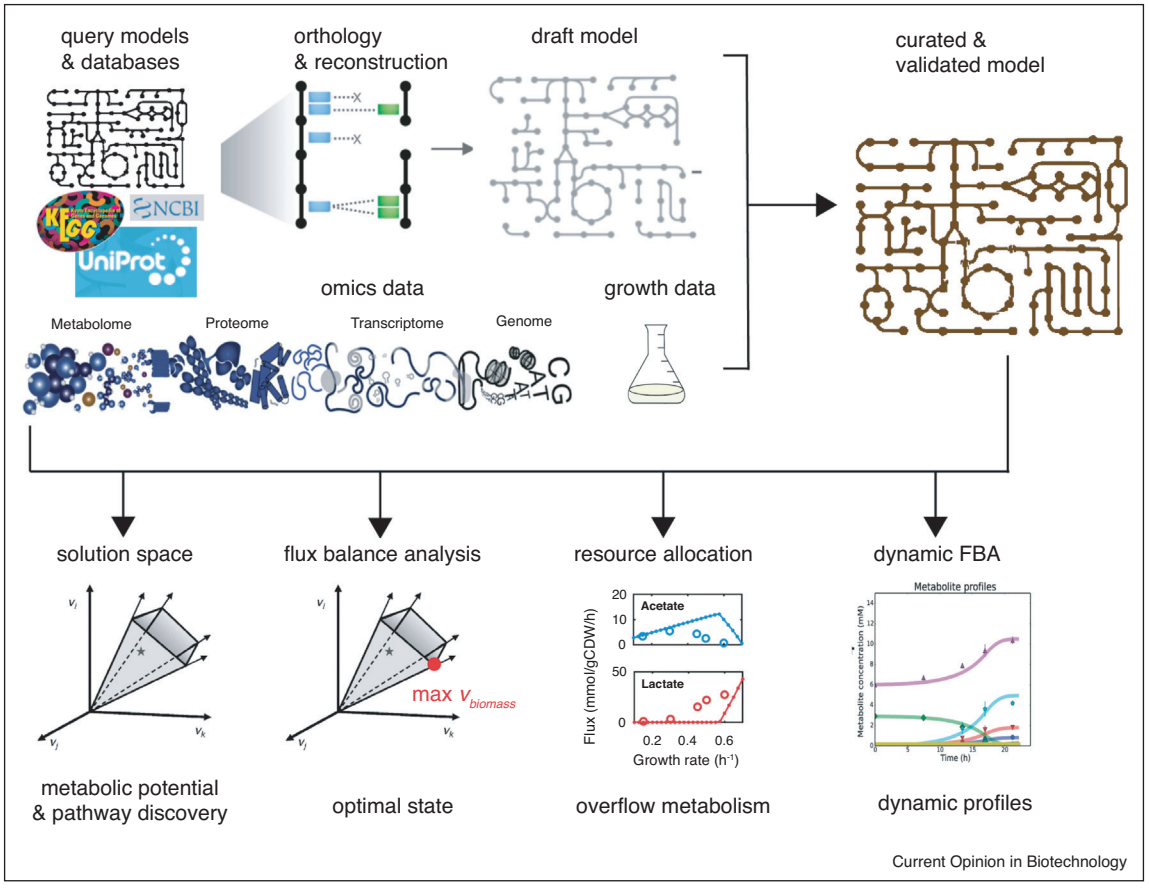
Here, we summarise how metabolic models have been used to address the key objectives of food microbiology (Figure 2). Throughout we will emphasize open questions and what the difficulties and limitations are. We also include relevant examples of modeling outside the field of food that we think could be promising; this includes recent developments in microbial community modelling, which we consider one of the most promising but also challenging next frontiers for food microbiology.

Chapters

Food production and safety

The production of safe-to-consume, stable and controllable products with enhanced shelf-lives is largely achieved by the metabolism of acting microorganisms. GSMMs have the potential to help understand the metabolism and control the fermentation processes. Such models have been developed for many food-related microorganisms,

Figure 1

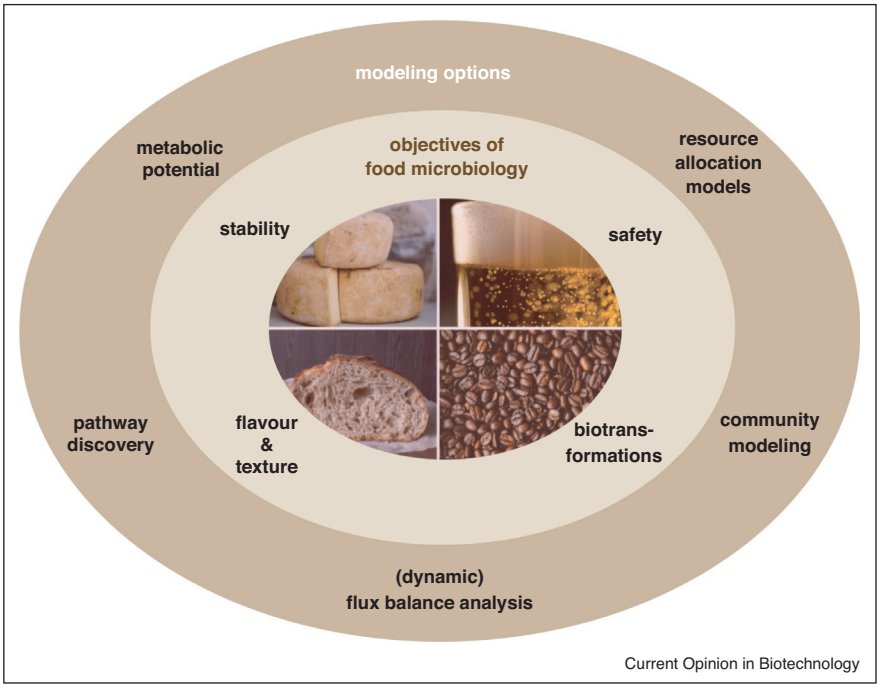


Steps for genome-scale metabolic model construction, curation and validation and illustration of the model analysis techniques discussed in this paper and their main outcomes. Exploring the steady-state solution space gives all possible flux distributions and corresponding pathways; subsequent optimization through flux balance analysis allows prediction of specific optimal states, for example, of maximal growth rate or maximal yield of a product. Adding resource allocation to FBA gives additional constraints that result in strategy switchers, such as overflow metabolism (i.e. lactate formation). Finally, dynamic FBA uses optimization in each time point to create dynamic growth profiles.

including baker's yeast [3,4], many lactic acid bacteria (LAB) [5] and recently acetobacteria [6]. By charting the complete metabolism, we can gain insights into connections between processes, such as the role of amino acid metabolism and redox balancing [5], or the regulation of arginine metabolism in energy metabolism [7•]. While in chemical biotechnology, such models have become powerful tools for prediction and control of metabolic engineering and cultivation strategies, in food biotechnology they are less used. Indeed, many complications arise in food that provide challenges to metabolic modeling. For one, foods are often solid, opaque, turbid or exist as a multi-phase system. An associated obstacle is cumbersome quantification of metabolites in complex media, critical to establish accurate exchange bounds in GSMMs. To monitor processes in such systems to generate data, alternative readouts, or accessible food-matrix models are needed, as recently developed for milk [8].

One key product that is used for food preservation is lactate (or ethanol). Ironically, traditional Flux Balance Analysis (FBA), the key modelling method for GSMMs, has trouble predicting it. Since GSMMs include no enzyme kinetics, only stoichiometry, they need uptake constraints to constrain the fluxes through the metabolic network [4]. FBA is an optimization problem: FBA maximizes an objective, which, in most cases, corresponds to maximal production of biomass components. As a result, FBA predicts the metabolic activities that produce the most biomass per limited substrate — high-yield strategies therefore. Without additional constraints to the FBA problem, it will always predict respiration over fermentation, or acetate formation (with 3 ATP/glucose) over lactate production (only 2 ATP/glucose) [9], even though inefficient overflow metabolism typically occurs under nutrient-rich conditions — in food.

Figure 2



Overview of some of the key objectives of food microbiology (inner circle) and different modeling options (outer circle) that could be applied to help reach those objectives.

Recent developments in modeling have improved the predictive capabilities of GSMMs under food-relevant conditions. By including resource allocation, that is, introducing additional constraints to capture the costs of implementing a metabolic pathway, it was shown that lactate formation costs the least protein per ATP produced. If glucose is abundant, protein cost (not metabolic yield) is the relevant fitness currency, shown recently for *Lactococcus lactis* [7•] and *Saccharomyces cerevisiae* [10]. However, optimal resource allocation assumes balanced growth in a constant environment. Recently, researchers identified metabolic routes which become active in oxidative stress and acid stress conditions, thus mechanistically explaining observed stress responses (e.g. deactivation of branched-chain amino acid biosynthesis under auxotrophy in oxidative stress) in *Escherichia coli*. This was achieved by integrating multi-omics (transcriptomics and quantitative proteomics) data into a whole-cell resource allocation model of *E. coli* [11,12].

New insights into microbial metabolism might also improve food safety. Following the work from well-studied classical food borne human pathogens (such as *Listeria monocytogenes* [13]), metabolic models can advance understanding of the metabolism of other pathogenic/undesired microbes. Recently, GSMMs were used to design minimal chemically defined media to cultivate two pathogenic bacteria, *Bordetella pertussis* [14] and *Campylobacter jejuni* [15•], and a reconstruction of *Lactobacillus vini* [16] identified nutrients assimilated into the biomass of this prominent contaminant in sugarcane fermentation.

Biotransformation to make use of inaccessible compounds

Further applications of fermentation in food sciences include the biotransformation of indigestible and untampered resources into edible food products. Traditional biotransformations include the fermentation of coffee and cacao beans for the removal of the mucilage layer [17]. Several models for cocoa-fermenting consortium members have been made recently. A metabolic model of *Acetobacter pasteurianus* [6] explained how *A. pasteurianus* first uses ethanol as carbon source, and subsequently consumes the off-flavours acetoin and acetate. Other metabolic models of bacteria and yeasts were created as a platform for the identification of species-specific reactions [18•]. Moreover such knowledge obtained from the metabolic models can be integrated with microbial dynamics and metabolite kinetics to create optimal fermentation models of for example, coffee [19] or cacao fermentation [20].

However, the biotransformation of many traditional foods is still largely based on spontaneous fermentation. One

option is to focus on a compound of interest, for example, reduction of citrulline during soy sauce fermentation with *Bacillus amyloliquefaciens* [21]. There are other examples outside food, where metabolic models are applied to identify for example, lignocellulosic biomass as a good resource for bioethanol production by *Clostridium thermocellum* [22].

Recently, biotransformation of plant-based protein sources, coined as precision fermentation, has received a lot of attention [23]. Currently the field is based on screening of strains with desired properties, but as data is accumulating GSMMs can help in crafting the relevant pathways, as is currently being done for flavour production.

Enhancement of food through fermentation

The creation of distinct flavours is a pivotal step for many fermented foods, but they are the result of relatively small fluxes of side reactions whose functions are poorly understood. Texture properties are often the result of polysaccharides whose pathways are subject to complex regulation. Therefore texture and flavour properties are difficult to predict quantitatively, and only the potential can be predicted.

It appears as if fast acidifiers are poor flavour producers, suggesting a trade-off that is not yet fully understood [24]. Flavour formation therefore occurs mostly by adjunct non-starter LABs. A GSMM of the heterolactic fermentative *Leuconostoc mesenteroides* subsp. *cremoris* was constructed which includes flavour compound formation [25]. The GSMM shows that citrate is used as a redox sink, allowing acetate rather than ethanol production. Flavour production from citrate can furthermore be steered by changing the environment.

Malolactic fermentation (MLF) is an essential step for deacidification of red wine and enhances organoleptic properties and flavour. The process is difficult to control, which may lead to stuck or sluggish fermentations. A GSMM was recently constructed for the malolactic organism *Oenococcus oeni* [26]. This GSMM was later used to investigate the response of *O. oeni* to ethanol content of the medium [27].

Metabolic models have been successfully used to improve production of single food-related compounds by genetically modified organism (GMO)-based biofactories. An example is the production of vanillin β-D-glucoside in *S. cerevisiae*. The model suggested multiple strategies, of which the partial reduction of flux through pyruvate decarboxylase (PDC) increased vanillin β-D-glucoside production the most [28]. New tools also have the potential to aid in the improvement of specific compound production in food. Although not applied to food, newer tools for *in silico* strain design showed engineering strategies to increase ethanol, succinate and 2,3-butanediol production in *S. cerevisiae* [29]. Other recent endeavours to use metabolic model-guided *in silico* strain design led to insights on how to increase production of naringenin in *Streptomyces albu*s [30] and pyrroloquinoline quinone in *Methylovorus* sp. [31]. The obvious limitation of metabolic engineering is that GMOs cannot be used in food processes in the European Union.

Increasing nutritional value of food by incorporation of probiotics and prebiotics has large industrial potential. Prebiotics enhance the nutritional value of the food itself, while probiotics are microorganisms that survive within the gut and confer a health benefit to the host [32]. The metabolic diversity between probiotic Bifidobacteria strains was investigated from semi-automatically generated genome-scale metabolic models [33]. This method, and others alike, could help in predicting the suitability of strains in different applications as *in silico* screening could save both time and resources.

Community modelling: a new frontier

Food fermentation often relies on the action of a community of microorganisms, rather than a single microbial strain. From a modelling perspective, moving from single GSMMs to community GSMMs raises several challenges. First, many approaches are based on optimisation of an objective, which is much more difficult to define – if at all possible – for a community. Community FBA solves this by analysing steady-state growth of all members in a community — whose growth rate must then be equal and is taken as the objective [34–36]. This analysis offers useful insights on the (optimal) structure of the community and its metabolite conversions, including otherwise poorly accessible *information*, such as potential cross-feeding fluxes, as applied to yogurt probiotics [37] and cacao fermentations [18•].

Another challenge is the inherent dynamic nature of (microbial) ecosystems. Dynamic FBA uses uptake kinetics to convert external metabolites into flux constraints for a subsequent FBA analysis. The FBA computes growth rates and uptake rates, which are subsequently used to update the environment [38]. This approach allows a natural extension to communities, as each species can aim to maximize its own growth rate, even in spatial structures: a powerful tool COMETS has been developed for this purpose [39••]. Such spatial effects were important to understand the population dynamics in kefir granules [40].

Dynamic FBA was used to analyse co-culture models for LAB present in cheese starter cultures [41•]. These models accurately predicted biomass compositions and concentration profiles of glucose and lactic acid. Moreover they showed that the cross-feeding of different amino acids depends on the interacting LABs.

Besides characterization of naturally occurring communities, another promising application of metabolic models is to use them for building synthetic consortia. Synthetic consortia can replicate or enhance the performances and the flavour profile of naturally occurring starter or ripening cultures [42], and allow the introduction of probiotic strains [37]. Metabolic models have already been used to screen for efficient syntrophic pairs for biotechnological applications. The resulting synthetic communities showed enhanced stability [43] and the ability to bypass community member-specific metabolic bottlenecks [44].

Conclusions

Many open questions in food sciences (Figure 2) relate to microbial metabolism, and metabolic modelling enables quantitative predictions of metabolic activity. Within chemical biotechnology metabolic models have proven to be powerful tools for metabolic engineering and cultivation strategies, and such strategies have the potential to be transferred to food sciences. However, we discussed specific challenges in food microbiology: the growth media are complex, the control knobs limited and not GMO-accessible, and many microbes interact.

A final complication over cell factories is that food microbiology exploits diversity of not only species but even strains. Moreover, the genes involved in important treats – often from secondary metabolism – may not be know yet. Pan-genome GSMMs have been used to study diversity, recently within the propionibacterium clade [47]. To address the need for strain-level resolution, we can benefit from methods that generate large collections of models [45,46], models for multiple strains of the same organism [48], and those that reconstruct genome scale metabolic models directly from metagenomes [49]. These models often require further manual curation (and a set of dedicated experiments!) to reach the desired level of confidence, which provides a major bottleneck to high-throughput generation of well curated models.

Within the food context, metabolic models are in particular relevant to better understand the physiology of microbes, as tools for advanced data integration, and for exploration of metabolic capabilities, rather than as predictive tools for direct steering towards better and safer foods. Overall, success stories over the years on fostering fundamental knowledge through metabolic modelling should encourage application of these methods in food-relevant settings.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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