Trends in Microbiology

Opinion

Ecological Insights into the Dynamics of Plant Biomass-Degrading Microbial Consortia

Diego Javier Jiménez,[1,*,@] Francisco Dini-Andreote,[1]
Kristen M. DeAngelis,[2] Steven W. Singer,[3,4]
Joana Falcão Salles,[1] and Jan Dirk van Elsas[1]

Plant biomass (PB) is an important resource for biofuel production. However, the frequent lack of efficiency of PB saccharification is still an industrial bottleneck. The use of enzyme cocktails produced from PB-degrading microbial consortia (PB-dmc) is a promising approach to optimize this process. Nevertheless, the proper use and manipulation of PB-dmc depends on a sound understanding of the ecological processes and mechanisms that exist in these communities. This Opinion article provides an overview of arguments as to how spatiotemporal nutritional fluxes influence the successional dynamics and ecological interactions (synergism versus competition) between populations in PB-dmc. The themes of niche occupancy, 'sugar cheaters', minimal effective consortium, and the Black Queen Hypothesis are raised as key subjects that foster our appraisal of such systems. Here we provide a conceptual framework that describes the critical topics underpinning the ecological basis of PB-dmc, giving a solid foundation upon which further prospective experimentation can be developed.

Trends

Throughout the enrichment process for the development of plant biomass-degrading microbial consortia (PB-dmc), the inoculum source and status, substrate type, temperature, and oxygen availability determine consortial richness, diversity, and structural shifts.

Spatiotemporal nutritional fluxes and microbial interactions are key factors that drive the successional dynamics in the development of PB-dmc.

The dynamics of microbial interactions (synergism versus competition) that occur within PB-dmc are mostly influenced by the spatiotemporal variation in nutritional resources.

The ecological understanding of PB-dmc is fundamental for the development of high-efficiency synthetic consortia that can help to maximize biological plant biomass saccharification processes.

Plant Biomass-Degrading Microbial Consortia (PB-dmc)

In the last decade, the production of renewable bio-based compounds (e.g., biofuels and/or commodity chemicals) from plant biomass (PB)-derived sugars has increased exponentially [1]. A key bottleneck in these 'bioprocesses' is an often-inefficient **saccharification** (see Glossary) rate that is limited in temperature and pH range, and which is largely caused by the recalcitrant nature of the PB [2]. Recently, the application of meta-omics techniques on PB-dmc has been proposed as a promising avenue to uncover new **lignocellulolytic** enzymes that can enhance PB saccharification [3–6]. Approach-wise, the development of PB-dmc is often achieved by the 'dilution-to-stimulation' method [7]. Briefly, a microbial community (inoculum) taken from nature is set to grow on agricultural residues (e.g., wheat straw, corn stover, or switchgrass) in liquid batch cultures that are sequentially diluted across several transfers [8,9], the so-called 'enrichment process' (Figure 1A). Along this process, the inoculum gradually changes in composition, yielding specialized PB-dmc with progressively reduced **richness** (from thousands to hundreds of microbial species), constrained presumably by the limited number of ecological **niches** available in the cultures (Box 1). We specifically assume that these consortia are shaped by the force of selection towards more efficient degraders, that is, those that produce the highest cell biomass per unit of lignocellulose consumed. Whereas this is a general principle in enrichment cultures, here the physical and chemical complexities of the PB substrate are key features that drive the development of the resulting consortia in a unique manner. Throughout

[1]Microbial Ecology Cluster, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747AG, Groningen, The Netherlands
[2]Department of Microbiology, University of Massachusetts, 639 North Pleasant Street, Amherst, MA 01003-9298, USA
[3]Joint BioEnergy Institute,5885 Hollis Street, Emeryville, CA 94608, USA
[4]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road,

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Box 1. The Concept of 'Ecological Niche' in Plant Biomass-Degrading Microbial Consortia

According to classical ecological theory, the number of species in a community is defined by the number of ecological niches (spatially-/temporally-defined) [10]. The competitive exclusion principle (primarily proposed by Gause, 1934) [11] states, 'the niche that is occupied by one organismal type is very unlikely to be also occupied by another one'. Hence, those taxa in the inoculum with capacities to rapidly occupy the available niches are presumably key to the success of niche occupancy, on a 'first come, first served' basis. In PB-dmc, competitive organisms are expected to survive by growth ('survival of the fittest'), in accordance with the niches they occupy. Different members of a consortium will theoretically occupy different niches that are defined by the availability and accessibility of (limiting) carbon (energy) sources, spatiotemporally nutritional fluxes, and shifting physicochemical conditions. In the case of PB-dmc, it is reasonable to assume that the high complexity of carbon sources, as well as spatiotemporal variations in the system, determines consortial richness. For instance, Gladden *et al*. [12] reported an estimated richness of 176–180 bacterial OTUs (operational taxonomic units) in a switchgrass-adapted thermophilic consortium derived from compost. This richness decreased, when the consortium was further cultivated on pretreated switchgrass, to 120–141 OTUs, and also decreased when cultivated on microcrystalline cellulose (88–111 OTUs), the latter being the result of a chemically simpler substrate. However, Eichorst *et al*. [13] found that pretreatment of switchgrass by ammonia fiber expansion and ionic liquids does not affect the microbial richness as compared with that obtained with untreated PB (ca. 68 OTUs). Thus, OTU richness values may reflect the number of niches occurring in the PB substrate. Furthermore, there is evidence that the structure of PB-dmc is strongly driven by substrate type (pretreated or untreated PB, or the use of different agricultural residues) [9,14,15]. Moreover, in the simplest scenario of PB deconstruction, a number of parallel selective events can be envisioned, yielding a final community richness that is a result of the substrate complexity. However, this simplified theory bypasses the fact that organisms may start to interact with each other, developing one-sided or mutual connections with respect to the utilization of sugars released during PB degradation. In this case, **niche partitioning** and social interactions across taxa may interplay, thus enhancing the organismal connectivity in the system.

Berkeley, CA 94720, USA
@Twitter: @Jimenez_ADJ

*Correspondence:
d.j.jimenez.avella@rug.nl,
djimenez1909@gmail.com
(D.J. Jiménez).

the enrichment process, the dynamic conditions present in the system are presumed to directly drive the diversity and metabolic potential of PB-dmc. These include the inoculum status and the sectional constraints imposed on it (e.g., type of substrate, temperature, pH, and oxygen availability) [14–21]. In this article, we posit that the understanding of **successional dynamics**, ecological mechanisms, and interactions within PB-dmc members are key to the development and manipulation of high-efficiency synthetic consortia that will help to maximize PB saccharification processes.

Successional Dynamics within PB-dmc

Throughout the development of PB-dmc, faster structural stabilization is commonly achieved by the use of an inoculum that is already preadapted to deconstruct PB (e.g., microbial communities from compost, forest soil, decayed wood, or the cow rumen) [17]. Thus, we here posit that the initial community assembly of PB-dmc could be influenced by the **historical contingency** of the community used as the inoculum [22,23], as similarly reported in wood-decaying fungal communities [24]. Although the primary order of species arrival is expected to be stochastic, the high similarity between biological replicates for most PB-dmc suggests a strong selective force in community assembly. However, irrespective of the processes that drive the initial assembly of PB-dmc, relatively little is known with respect to the ecological mechanisms that further determine the outcome of PB-dmc. For instance, the early establishment of a species in the system can significantly modify the availability and chemistry of subsequent available niches, which may drive communities towards different states. To address this issue, a dynamic manipulation of the species diversity/composition in the system needs be tested. Indeed, having a minimal effective consortium (Box 2) at hand can greatly aid our comprehension of ecological mechanisms within the PB-dmc [23].

Two types of successional dynamics likely occur in PB-dmc. The first one is the succession along the sequential transfers through the course of the enrichment process. Here, microbial communities are often tracked at the final growth stage, being compared against previous batches (on a weekly basis) [31]. The second type of succession occurs within the relatively 'stable' consortia, where microbial communities are evaluated within a unique culture batch (on a daily basis) (Figure 1B). On the basis of a previously developed conceptual model [32], we

Box 2. Towards the Minimal Effective Plant Biomass-Degrading Microbial Consortium

The overall growth yield (microbial biomass produced per unit of lignocellulose consumed) in PB-dmc can be directly correlated with the rate of PB degradation. However, the extent to which the rate of degradation and the microbial diversity correlates in PB-dmc is still underexplored. It is expected that this relationship is positive, that is, the PB degradation rate is predicted to be higher in communities with more species (operational taxonomic units, OTUs), where the metabolic diversity and niche-partitioning events tend to be higher. Experimental support for this idea is provided by Evans *et al*. [25]. These authors synthetically assembled microbial consortia with different levels of community richness and showed that there exists a positive relationship between diversity and microbial productivity (CO_2 production by consumption of wheat straw as a unique carbon source). In addition, they suggested that one of the metabolic traits that increased this productivity is the ability of particular species to degrade β-glucan. In PB-dmc, it is tempting to speculate that microbial diversity correlates with PB degradation rate values only up to a certain 'optimal' level of species richness. At this optimal stage, a core set of organisms (that is, a minimal effective consortium) is reached, indicating a high, close-to-optimal efficiency of PB degradation. Above such a level, increments in species richness can be either negative to the system (for instance, by enhancing competition) or neutral (maintaining the system efficiency at a stable level).

In PB-dmc, a high number of species and their network of interactions could confer **functional stability**, redundancy, and robustness, thus providing a potential buffer against external disturbance [26,27,28]. To study the degree at which species richness affects PB-degradation rate, the 'dilution-to-extinction' approach can be used. [7]. Here, the selective removal of microbial populations by dilution, and their reinoculation on a fresh medium, provides a way to unravel the minimal effective consortium with the optimized PB degradation rate; a similar approach has been carried out to unveil a minimal effective hydrogen-producing microbial consortium derived from cow rumen communities [29]. Moreover, the construction of a synthetic microbial consortium using a different number of functionally diverse isolates (phylogenetically related or not) derived from stable PB-dmc can provide insights into the diversity–functionality relationship. For biotechnological purposes, the finding of minimal effective PB-dmc, and the understanding of their relevant metabolic traits, can lead to a significant improvement in the PB saccharification processes [25,30].

assume that PB-dmc align with the '**endogenous heterotrophic succession**' scenario. Here, organic carbon supplies are derived from a single initial input contained within the substrate itself. Thus, microbial populations affect the quantity and quality of the available carbon supplies throughout the succession. A characteristic feature of endogenous heterotrophic succession is the rapid appearance of pioneer populations that initiate the deconstruction of the PB and the relative decline of these populations as species that outcompete the pioneer population in sugar uptake proliferate. Along the endogenous heterotrophic succession, we hypothesized that the abundance of PB-dmc members fluctuates in time, mostly depending on (i) changes in **substrate structure** (e.g., shifts in **cellulose** crystallinity) [33], (ii) **spatiotemporal nutritional fluxes** (e.g., due to the continuous release and consumption of oligo- and/or monosaccharides), (iii) microbial interactions, (iv) dynamics in the local physicochemical conditions (e.g., changes in pH or redox potential), and (v) metabolic versatility of the key taxa.

Biological Interactions: Conceptual Scenarios Underlying the Dynamics of PB-dmc

In liquid PB-driven enrichment cultures, a strong selection for nutrient use takes place. In these systems, microbial interactions are key for the efficient PB bioconversion process. For instance, **synergism** (i.e., division of labor), competition (resulting in species displacement), and commensalism can play fundamental roles [34,35]. These mechanisms are not mutually exclusive as there are cases of synergism–competition and synergism–commensalism occurring across the same population, depending on spatiotemporal nutritional fluxes [36]. One key mechanism lies in the types of enzyme produced, which may be complementary to each other (i.e., enzyme–enzyme synergism). Thus, the producer organisms may be interdependent (e.g., endoglucanase from species A, endoxylanase from species B, and alpha-arabinofuranosidase from species C) [37,38]. It is worth mentioning that complete degradation of PB requires the synergistic interaction of a set of enzymes that can act on the main backbone (e.g., endoglucanases and endoxylanases), in the side linkages (e.g., cellobiohydrolases, α-arabinofuranosidases, and β-galactosidases), and in the sugar dimers (e.g., β-glucosidases and

Glossary

Cellulose: plant polysaccharide that consists of **β**-1,4-linked d-glucose units that form linear polymeric chains of about 8000–12 000 glucose units. In its crystalline form, cellulose consists of chains that are packed together by hydrogen bonds.

Economy and trade: concept developed to explain the reward (resources) for the microbial populations in relation to their contribution for plant biomass degradation.

Endogenous dynamics: change in community composition or gene-expression patterns over time. It is caused by interactions among species and occurs even under constant environmental conditions (that is, without exogenous perturbations).

Endogenous heterotrophic succession: systematic change in the prevalence of microbial species mediated by the differential use of organic carbon, derived from the substrate itself, for growth.

Functional redundancy: the situation in which two or more taxa within a microbial community are able to perform the same function.

Functional stability: the ability of microbial communities to keep functioning during a disturbance event.

Glycosyl hydrolases: enzymes that assist in the breakdown of glycosidic bonds in complex carbohydrates.

Historical contingency: the order in which different species arrive/stabilize within a microbial community.

Lignocellulolytic: enzymes or functions destructive to polymers that comprise the plant biomass (cellulose, hemicellulose, and lignin).

Niche: an *n* dimensional space coupled to the resources (e.g., sugars) that are to be exploited within a community.

Niche partitioning: co-occurrence of species that avoid competition and exploits different resources available in the same environment.

Public goods: the nutrients (often sugars) released during PB degradation. Both the contributors and sugar cheaters can benefit from its production.

Richness: number of microbial species in a given community.

β-xylosidases) of the (hemi)cellulose fraction [39]. In such a synergistic scenario, combinations (or pairs) of organisms efficiently degrade PB moieties and then often compete for the released sugars, depending on their niche occupancy. Thus, one species can take advantage of the abundant sugars, affecting (or not) other populations (commensalism or ecological cheating). The enzyme–enzyme synergism often differs between PB-dmc cultivated under aerobic versus anaerobic conditions. In brief, the lignocellulolytic enzymatic machinery in anaerobic bacteria is commonly assembled as a large multienzyme complex, called the cellulosome [40], whereas in aerobic bacteria, PB degradation generally occurs by the secretion of freely diffusible single catalytic proteins. Moreover, based on the experimental findings of Deng and Wang [41,42], we posit at least two main conceptualizations for PB-dmc: (i) the complexity of the PB carbon sources directly relates to the degree of synergism, and (ii) the combination of particular species influences synergistic interactions. In this respect, the nature of the microbial interaction is potentially linked to **functional redundancy** and niche overlap (competition) in the system.

In the development of PB-dmc, the substrate nourishes the consortium members only if the latter show some degree of collaboration. In the theoretically simplest case, synergism occurs between pairs of organisms, here coined 'collaborative pairs'. The interaction is not confined to pairs, as it may involve more partners (thus forming collaborative groups). For the sake of simplicity, we will limit our discussion to collaborative pairs. These, by definition, perform better jointly than each one alone, and so the final outcome of synergism may be that (i) the two individual members enhance a particular function, as compared to the state of that function when either one of them is acting alone (complementarity between species), and/or (ii) one organism, performing a particular function, may be able to catalyze and 'kick start' an additional relevant function in the second organism, for instance by modifying the local substrate or habitat. In both cases, the outcome is similar, as the forces of selection for efficient growth maintain the synergistic action of the collaborating organisms. However, the nature of microbial interactions is dynamic [35,36], and so it is possible that an organism that is part of a collaborative group switches from synergism to competition or commensalism when local nutritional conditions shift in space and/or time [28]. As an example, different polymers of PB, for example, **xylan**, **xyloglucan**, and cellulose, can be utilized at the same time, at different locations, by different collaborating organisms. Thus, different classes of oligo- and/or monosaccharides are generated (e.g., xylotriose, xylose, and/or glucose). In a time-resolved scenario, the conversion of the complex substrate, for example, cellulose to cellobiose, can be divided over two to several collaborative organisms, which are active in the process in a sequential manner (cellulose via cellobiose to glucose). This cascade of metabolic activities makes the (PB)-derived sugars available to support the overall growth of the consortium. During this process, the composition of PB-dmc can be continually in flux, and directed by **endogenous dynamics** imposed by the network of interactions between collaborative groups and the PB complexity. Such mechanisms can produce either negative or positive effects on specific populations. In addition, they constitute key factors for the persistence of low-abundance populations and/or sugar 'cheaters' in PB-dmc [28].

Sugar Cheaters: An Important, Yet Overlooked, Ecological Strategy in PB-dmc

Some opportunistic organisms (termed 'sugar cheaters') can be selected throughout the enrichment cultures in PB-dmc. These organisms profit from the **public goods** made available by the other community members, without effectively contributing to the PB degradation process. In effective PB-dmc, a balance must be struck between effective degraders (contributors) and sugar cheaters, as the high prevalence of the latter will lower degradation effectiveness. In PB-dmc, species that release public goods (contributors) can, for instance, gain preferential access to the goods, thereby avoiding the emergence of a '**tragedy of the commons**' situation [43–45]. Here, we examine this issue using an '**economy and trade**'

Saccharification: depolymerization of (hemi)cellulose moieties into monosaccharide components.
Spatiotemporal nutritional fluxes: dynamics in the abundances and fluxes of nutrients in the system due to the continual release and consumption of oligo- and/or monosaccharides in time and/or space. This term is also related to the quality and quantity of available carbon supplies at different successional stages.
Substrate structure: related to structural conformation of plant polysacharides. For instance, the type of glycosidic and hydrogen bonds, coiling, branching, number and type of monosaccharides, and degree of crystallinity.
Successional dynamics: systematic changes in species composition, functions, and nutritional resources through time and space within a given microbial community.
Synergism: the combination of activities of organisms that produce an effect greater than the sum of their individual actions.
Tragedy of the commons: when each microbial population tries to reap the greatest benefit from a given resource, demand for the resource overwhelms the supply, resulting in populations harming each other (competition), spoiling the resource in the process.
Xylan: the major component of the hemicellulose fraction in plant biomass. Xylan is composed of β-1,4-linked d-xylose units, which may be substituted by different side groups, such as d-galactose, l-arabinose, glucuronic acid, acetyl, feruloyl, and *p*-coumaroyl residues.
Xyloglucan: plant polysaccharide that consists of a β-1,4-linked d-glucose backbone together with d-xylose side linkages. In addition, l-arabinose and d-galactose residues can be attached to the xylose residues.

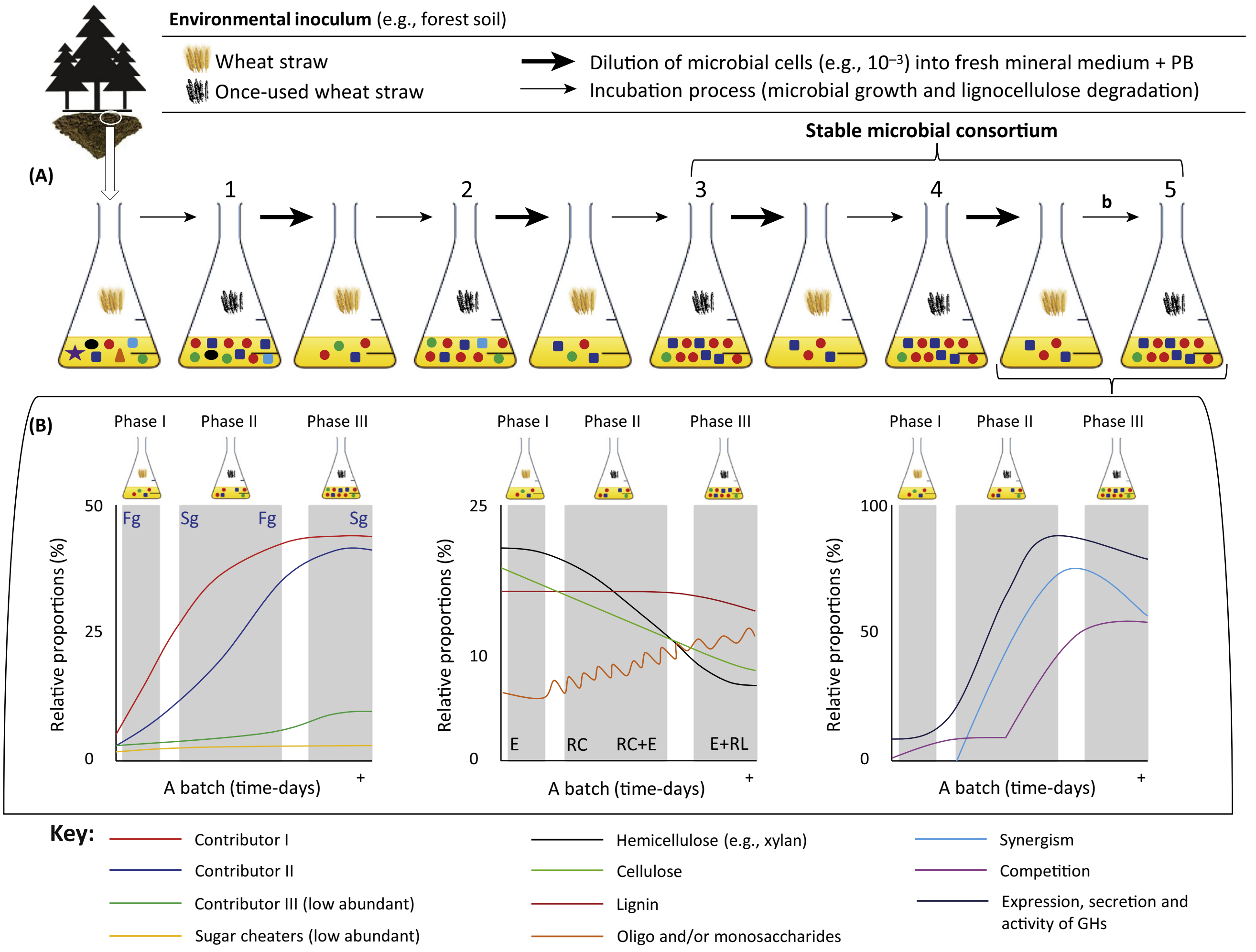

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Figure 1. Successional Dynamics of Plant Biomass-Degrading Microbial Consortia (PB-dmc). (A) Schematic representation of the dilution-to-stimulation approach used to enrich PB-dmc. The basis of the approach is that consortia containing microorganisms with superior capacities to grow on (pretreated or untreated) plant biomass (PB) as the sole energy and carbon source, in the presence of inorganic sources of nitrogen, sulfur, iron, and phosphorus, are progressively selected through the transfers. A state of relative stability is finally reached when the consortium starts to reveal similar community structures after a determined number of batches. The shapes (circles, triangles, stars, and squares) inside the flasks represent different microbial species, and the numbers (on top) depict the sequential transfers. The letter ‘b’ indicates the last growth batch (‘stable’ consortium), upon which most of the contextualization and models were based. (B) Conceptual model showing the PB-dmc behaviour and degradation process in one batch of cultivation. *Left:* Relative proportions of the most important contributors and ecological strategy of growth (Fg: fast-growing but inefficient and Sg: slow-growing but efficient) for the contributor II. *Center:* Relative proportions of lignin, hemicellulose, cellulose, and oligo and/or monosaccharides. The letters represent the quantities and qualities of the available carbon supplies (E: easy-to-consume carbon substrates such as oligo- and/or monosaccharides; RC: complex carbon substrates, such as cellulose, xylan, xyloglucan, and galacto(gluco)mannan; RL: recalcitrant and limiting carbon substrates such as lignin and cellulose/xylan, respectively). *Right:* Relative proportions of the main microbial interactions, that is, synergism versus competition and expression, secretion and activity glycosyl hydrolases (GHs).

framework. First, we posit that the abundant organisms present in PB-dmc contain a high metabolic capacity to degrade (hemi)cellulose, indicating that they contribute to the release of public goods [46]. However, prominent consortial members expressing only low numbers of lignocellulolytic enzymes can be considered as ‘sugar cheaters’ in this system. This is the case with *Thermus thermophilus*, which lacks the metabolic potential for polysaccharide hydrolysis, in a switchgrass-degrading consortium [47]. Theoretically, different consortial members contribute in distinct ways and proportions to the PB degradation process, as governed by laws of input (investment) and reward (resources). In a hypothetical case, that describes microbial

Box 3. The Black Queen Hypothesis in the Context of Plant Biomass-Degrading Microbial Consortia

The Black Queen Hypothesis (BQH) states that if one organism provides a service (e.g., degradation of plant biomass (PB) and release of sugars), then another organism may exploit those services with the concomitant loss of the genetic capacity to perform those services itself [36]. This assumption provides a theoretical interpretation for the evolution of dependencies through adaptive gene loss in an interactive community context [49]. In PB-dmc, sugars become commonly available. Thus, interactions between contributors and sugar cheaters constitute a case of indirect symbiosis in which the flux of nutritional resources is the driver. Within the community, contributors tend to transform their vicinities into a stable and homogeneous environment (by continuous release of oligo- and/or monosaccharides), allowing beneficiary mutant forms to evolve in accordance with the proposition of the BQH. Indeed, in the BQH, a ‘passive’ interaction (production and consumption of a public good) is at the basis of the emergence of tighter metabolic interdependencies between organismal types [50]. However, in PB-dmc this conceptualization has never yet been applied, probably because of the short-term nature of the experiments. Nevertheless, the maintenance of stable batch cultures for longer periods could potentially lead to the strengthening of the interactions and, as such, to the evolution of interdependent organismal types. In a hypothetical case, we can assume gene loss to occur in both contributors and sugar cheaters. For instance, a contributor can lose the capacity to metabolize specific sugars (e.g., organism X glucose, and organism Y xylose), but they can survive in the system by occupying a different niche (e.g., organism X consumes xylose, and organism Y consumes glucose). In summary, in a system where the majority of the species are competing for nutrients, the emergence of a beneficial dependency between two or more partners (through resource trade or public good use) is likely to represent an adaptive advantage. In a more dynamic scenario, the contributor in one stage of the degradation processes may behave as (or even evolve to become) a sugar cheater in another, losing the ability to produce **glycosyl hydrolases** (GHs), for example. However, in another scenario, organisms can benefit from ensuring the status of contributors by acquiring traits (e.g., by horizontal transfer of genes encoding lignocellulolytic enzymes) that enhance the production of the public goods [50].

‘justice’, organisms that contributed to only 10% of the lignocellulose degradation may take advantage of their fair share, that is, the less-abundant sugars (e.g., fucose or galactose), whereas members that contributed up to 80% to the substrate transformation may profit from the most abundant sugars (e.g., glucose or xylose). Based on this conceptualization, the important contributors to the degradation process might indeed be the abundant ones that, on a population basis, invest strongly and subsequently consume the largest proportion of public goods. Moreover, the functional role of low-abundance populations in PB-dmc is still underexplored. For instance, these could be involved in feedback regulation, cross-feeding, maintenance of functional stability, and inhibition of metabolite repression. In addition, some low-abundance populations may also have the ability to produce stimulatory compounds (signaling or quorum sensing, or other [nutritional, e.g., vitamins] molecules) that assist in coordinating interactions among coexisting organisms in the system [48]. As such, the presence of low-abundance populations in the system is an essential factor for cooperative and eco-evolutionary processes (Box 3) and thus it also may modulate the efficiency of PB degradation [28,51,52].

A Simplified Conceptual Model of the Dynamics of Microbial Consortia in Plant Biomass Degradation

There is considerable support for the tenet of an inverse relationship between growth rate (biomass produced per unit time) and yield in heterotrophic microbial metabolism. This trade-off tends to create two divergent ecological strategies, that is, the fast-growing but inefficient versus the slow-growing but efficient [53,54]. Regarding PB-dmc selected in liquid fed-batch enrichment cultures, for the abundant organisms the relative importance of one strategy over the other depends mostly on the spatiotemporal nutritional fluxes [55]. In contrast, we assume that in most cases the low-abundance populations display a slow-growing strategy. Here, we propose a simple model that describes the behavior of PB-dmc in one batch culture (Figure 1B). The model comprises three phases. In phase I (initial 6 to 12–48 h), the source microbial community undergoes an acclimation process in which growth starts at the expense of soluble oligo- and/or monosaccharides (e.g., cellobiose, glucose, and/or xylose) that are present in low proportions in the PB substrate [56]. For the high-abundance populations, the conditions in this initial stage favor fast-growing organisms, based on low competition pressure in a low-cell-density environment [57]. In this stage, enzymes acting on the backbones of cellulose and hemicellulose become progressively more relevant (e.g., endoglucanases and endoxylanases). The second stage (phase II; between 2 and 5–8 days) is characterized by the consumption of nonlignified tissues, such as parenchyma and collenchyma, in which cellulose and hemicellulose become available. Organisms expressing enzymes that utilize easily accessible polysaccharides, such as extractable (hemi)celluloses, starches, and pectins, are expected to dominate at this stage. In this phase, enzymes acting in the backbones and mainly in the side linkages of cellulose (e.g., CAZy families GH1, GH3, GH5, GH6, GH9, GH16, and GH48) and hemicellulose (e.g., GH2, GH8, GH10, GH11, GH30, GH39, GH43, GH51, GH67, and GH92) fractions can be highly expressed and secreted [46,56,58,59]. The degradation of cellulose, xylan, and xyloglucan increases progressively over time, thus shifting the substrate structure with a concomitant increasing growth of the major contributors. At this stage, the production of sugars (by polymer degradation), and their consumption, occurs simultaneously, and so the sugar levels fluctuate rapidly. Here, synergistic relationships may be selected across the consortial members, as this drives a more efficient degradation of the substrate. After this stage, overall growth may increase, which reflects – as a result of previous processes – the enhanced availability of accessible carbon supplies. Here, the environment becomes relatively homogeneous, resulting in the high level of competition. Thus, the release of easily assimilable carbon sources (e.g., glucose and xylose) is expected to enhance ecological competition across the existing taxa [41,42]. Finally, at phase III (ca. days 7 to 10), with the depletion of cellulose and hemicellulose, the most recalcitrant part of the PB (lignin) starts to be progressively converted, as similarly reported in composting processes [60]. At this stage, the major contributors to the PB degradation process may have entered the stationary phase of growth, which is reflected in a decrease in the expression of glycosyl hydrolases (GHs). Overall, the cellulose and hemicellulose degradation, coupled with the production and consumption of released sugars, decreases gradually. It is expected that, at the final stage of cultivation, conditions will favor the slow-growing strategy due to the lowered resource flux, which is related to the high recalcitrance of lignin. After phase III, a major fraction of the PB substrate is degraded, and it does not support microbial growth. At this point, not only carbon sources but also other essential nutrients, such as nitrogen, can become progressively limiting.

Concluding Remarks and Future Perspectives

In this Opinion article we have provided arguments for the contention that niche availability and the successional dynamics of PB-dmc are largely influenced by the spatiotemporal dynamics of nutritional fluxes, the quantity and quality of available carbon substrates, and ecological interactions (synergism and/or competition). In addition, we propose that the deconstruction of PB and the systematic release of sugars in these consortia are directly controlled by the activity of key microbes. These activities are linked to their numbers (species and cell biomass) and metabolic traits, and also to the level of interaction among pairs or groups. However, prospective experiments (e.g., assembly of synthetic microbial consortia) need to be set up in order to understand the ecological mechanisms underpinning these interactions, and, most important, to link the ecological basis of the system with the PB degradation process. Moreover, long-term and time-series experiments that measure kinetic responses (e.g., the abundance of key members, gene expression profiles, enzymatic activities, sugar fluxes, growth and degradation rates) are fundamental to elucidate the eco-evolutionary processes in PB-dmc (see Outstanding Questions). We advocate that an understanding of the ecological mechanisms and population dynamics that govern PB-dmc will help to maximize the PB saccharification processes and the production of building blocks for biorefining. Indeed, this ecological comprehension is key to the further development and manipulation of high-efficiency synthetic consortia [28,30,36]. Our opinion aligns with the idea that improvements in our understanding of temporal and spatial dynamics will ultimately assist in the engineering of PB-dmc with outputs that can be predicted and, ultimately, controlled (e.g., high expression and

Outstanding Questions

To what extent does microbial diversity (taxonomic and functional) influence plant biomass (PB) degradation rates and dynamics? What are the most important populations traits for efficient PB degradation? Does the dilution-to-extinction approach and the use of synthetic consortia help in the investigation of the relationship between diversity and PB degradation rates?

What is the minimal set of organisms, genes and/or functions in PB-degrading microbial consortia (PB-dmc) that is required for an optimal PB degradation? Does a stable PB-dmc perform better than the sum of each individual (the most abundant ones) organism?

Can ^{13}C stable isotope probing (SIP), using labeled plant material (e.g., ^{13}C-cellulose, ^{13}C-xylan, and ^{13}C-PB) and/or sugars (e.g., ^{13}C-glucose and ^{13}C-xylose) improve our understanding of how PB-derived substrates affect the diversity, metabolic activity, and the interactions within PB-dmc? In addition, to what extent does the lignin fraction affect successional dynamics in PB-dmc?

How can conceptual models about successional dynamics be constructed and tested in PB-dmc? What is the best approach to quantify the flux of sugars in these systems, as well as the metabolic activity, cell biomass production, and enzyme secretion? How does the delineation of interactive modes between consortial members facilitate the engineering of the system towards high-efficiency synthetic PB-dmc? Finally, how can we foster the production of enzyme cocktails for PB saccharification using an established minimal effective consortium?

secretion of lignocellulolytic enzymes). Finally, we here provide the basis for further experimental designs, which, at a broader scale, aim to improve our engineering capability of PB-dmc towards higher metabolic efficiency of PB saccharification in biorefining settings.

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