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The Tier System: A Host Development Framework for Bioengineering

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Abstract

Development of microorganisms into mature bioproduction host strains has typically been a slow and circuitous process, wherein multiple groups apply disparate approaches with minimal coordination over decades. To help organize and streamline host development efforts, we introduce the *Tier System for Host Development*, a conceptual model and guide for developing microbial hosts that can ultimately lead to a systematic, standardized, less expensive, and more rapid workflow. The *Tier System* is made up of three Tiers, each consisting of a unique set of strain development Targets including experimental tools, strain properties, experimental information, and process models. By introducing the *Tier System*, we hope to improve host development activities through standardization and systematization pertaining to non-traditional chassis organisms.

1. Introduction

Model host organisms for biomanufacturing are strains where the necessary experimental tools and fundamental data to direct metabolism via genetic manipulation have been developed, enabling rational metabolic engineering. However, interest in commercial deployment of non-model microbes is increasing [1-4]. By domesticating organisms that are innately more suitable to industrial conditions, synthetic biology efforts can be more narrowly focused on optimizing substrate utilization, product titers/rates/yields, and cell growth/maintenance [5-10]. Expanding the scope of domesticated hosts for biomanufacturing is also critical for expanding the repertoire of feedstocks and products accessible for bio-based manufacturing [3,11-14]. Experimental validation of the utility of novel hosts has been demonstrated through improved rates of production, unique biochemistries, production under non-standard conditions, and utilization of unique feedstocks [4,8,15-19]. Furthermore, the design, creation, and cultivation of physiologically diverse strains that remain genetically stable and retain performance metrics over time in the presence of diverse feedstocks and products will reduce the costs involved in the development and scaling of biological production [5,8,15,16,18-21].

DNA-delivery and gene editing approaches that can target a wide range of undomesticated microorganisms are rapidly being developed to reduce the time and resources that would otherwise be required for organism-specific procedures [5,9,14,22-28]. These approaches have launched an unprecedented wave of efforts to engineer metabolic pathways in metabolically, physiologically, and phylogenetically diverse emerging hosts [4,7,13,17-19,22,26,29-33]. Notwithstanding these transformative advances, relatively few organisms have been developed, or “domesticated”, to the point that they could be considered established

industrial platforms – where rational metabolic engineering could be applied rapidly towards a bioproduction goal [2,5,15].

Organisms often share traits that have led to their evolution into canonical bioproduction chassis strains, including: rapid growth, ease of use in the laboratory (safe, genetic tractability, etc.), genetic stability, performance at scale, etc. [15,21]. Another common thread amongst the progression of microorganisms into established hosts is that their development was undertaken by a multitude of groups, often using disparate approaches, for a myriad of purposes. Thus, accessing comprehensive and current information regarding the developmental status of different hosts for industrial applications is time consuming and requires access to many sources of information [15]. Although eventually successful with a handful of strains, a haphazard, slow approach cannot support the needs of a burgeoning biomanufacturing industry (Figure 1A).

Another critical gap in the pipeline for developing non-traditional hosts is access to a portal for tracking strain development. However, there are few resources designed to support host strain selection and development. The ChassiDex database (https://chassidex.github.io/) was built as a project for the International Genetically Engineered Machine (iGEM) Competition in 2017 and provides information such as available strains, growth media, BioBrick parts, metabolic models, key references, transformation and other protocols, vectors, and genome information for 23 hosts, including bacteria and fungi [34] . More recently in 2023, the Cultivarium portal (https://www.cultivarium.org/) was developed and provides a wealth of information on 128 microbial strains with the goal of providing "open-source tools and assays designed for use in multiple microbes to minimize trial-and-error time in new species". The portal emphasizes culture, molecular, and sequencing information, and is particularly useful for early-stage development of non-model hosts. Finally, the MCF2Chem knowledge base,

published in 2023, provides information on product formation data, culturing and fermentation conditions, and genetic methods [12]. It can be queried to recommend hosts suitable for synthesizing specific compounds. These types of portals will be critical for coordination of community-based chassis development efforts.

We propose that systematic, intentional, coordinated approaches can be applied to greatly improve the speed and efficiency of host selection and development (Figure 1B). Here, we introduce the *Tier System for Host Development (Tier System)*, which has been created to aid in organizing, standardizing (to the extent possible), and communicating host development activities. We outline the objective and structure of the *Tier System*, list and briefly describe the underlying capabilities/datasets/knowledge that comprise the *Tier System* and describe how the *Tier System* can benefit the wider bioproduction community.

2. Tier System

2.1 Background & Framework. The primary objectives of the *Tier System* are to: 1) identify key technologies and knowledge needed for a new bioprocessing host, 2) guide prioritization of research toward technologies and knowledge based on complexity and level of need, 3) provide a level of standardization for host development for the biomanufacturing community, 4) facilitate communication for host development activity, and 5) guide and track the development of hosts over time. The overall conceptualization of the *Tier System* is illustrated in Figure 2. Each Tier consists of a set of "*Targets*" – experimental tools, strain properties, and experimental information critical for host development (Box 1). The Targets are ranked into different Tiers based on the importance of that tool or knowledge for the Design-Build-Test-Learn (DBTL) cycle, as well as the level of effort (time/cost) needed to develop the capability or generate the data for each host (Figure 2). Hosts advance up Tier levels as synthetic biology tools are successfully developed/applied and as strain-specific knowledge/data is collected and analyzed – a process we call "*Tier elevation*" (Box 1). Tiers 1 and 2 contain commonplace, yet essential tools and knowledge/data sets that form the foundation for the development of more advanced technologies and analytics in Tier 3. Novel or uncommon hosts will initially have met few of the Targets laid out in the *Tier System*; therefore, they require development of basic transformation and genome editing tools, as well as baseline characterization of strain properties (e.g., growth requirements, genome information, antibiotic

Box 1

Targets: tools, information, and strain properties useful for host development.

***Onboarding*:** the act of meeting the minimum set of Targets that a new organism would need to be used constructively in DBTL (completion of Tier 1).

***Tier Elevation*:** the act of further developing a strain for bioproduction

susceptibility, etc.) to complete Tier 1 before they are considered "*Onboarded*" and equipped for any rational DBTL cycle (Box 1, Figure 2).

The *Tier System* is flexible in that individual Targets may be deprioritized (or ignored altogether) for a given host depending on the level of effort (time/cost) needed to attain that Target and the importance of that Target (tool/data) for the DBTL cycle using that organism. For example, replicating plasmids are possible for fungi, but can be far more difficult to develop than for prokaryotes [[35]; thus, while plasmid vectors are a normal first step in the development of bacterial hosts, other types of vector systems may be prioritized for fungal strains. An additional level of adaptability has been built into the *Tier System* to accommodate the rapid pace of biotechnological innovation, whereby Targets can be added, removed, or reassigned to different Tier levels over time. Undoubtedly, some tools currently situated in Tier 3 will be advanced to the point that they will be better classified as Tier 2 or even Tier 1 tools in the future, and future technologies will be added to the appropriate Tier as they are developed.

2.2 Structure of the Tiers. Tier 1 is defined as the minimum set of Targets that an organism would typically need developed for it to be used constructively in the DBTL cycle (Table 1). To be classified as a Tier 1 host, or "Onboarded", 100% of the Targets in Tier 1 must be met. This Tier consists of the fundamental information and tools required to support reliable growth and basic genetic engineering of a candidate host strain. DNA-delivery methods are typically the most critically needed advancement, as rational metabolic engineering and DBTL methods are impossible without this ability. Other fundamental tools required for basic genetic editing and manipulation might include the availability of selection and counter-selection markers, genome integration systems, basic promoter, ribosome binding site, terminator parts, and a complete

(bacteria) or high quality, draft (fungal) genome sequence. Baseline knowledge required to support robust, consistent growth of host organisms include media composition and growth conditions, basic understanding of growth kinetics, antibiotic resistance properties, and availability of simple growth models (e.g., Malthusian, Monod).

Biosafety should be considered at the outset of onboarding a new organism [19,36-40]. Biomanufacturing hosts will typically be limited to Bio Safety Level 1 (BSL1) or Risk Group 1 organisms that are preferably labelled GRAS (generally recognized as safe) by the United States Food and Drug Administration or equivalent classifications in other countries. Organisms classified as BSL2 or Risk Group 2 (e.g., *Aspergillus niger*) can be considered on a case-by-case basis, but their potential for use in commercial-scale production is likely limited. ASTM standard E3214 – 19, Standard Classification for Industrial Microorganisms, can be used to classify hosts with regards to genotype class (native strains, strains expressing a) native or b) non-native DNA, or those producing chemicals not observed in nature), biosafety, mode/intent of use, and the extent of DNA sequence information for a given industrial microbial strain [41].

The Targets described in Tier 2 provide additional functionality and a broader set of tools/information for host strains, but unlike the Targets in Tier 1, Tier 2 Targets are not absolutely required to perform strain engineering. Targets in Tier 2 represent the research and tool gaps that are often needed for a partially developed strain to become a fully operational biomanufacturing host. Tier 2 Targets consist of the tools and knowledge needed to facilitate rapid and robust DBTL cycles [9], empower greater control over metabolic pathways, and improve host performance in lab-scale experiments (Table 1). Phenotypic- and cultivation-related Targets in this Tier include thorough substrate utilization and inhibitor tolerance panels, small scale (< 2L) bioreactor growth capabilities, and adaptive laboratory evolution

techniques/results. Tier 2 genetic tools include chromosomal landing pads, regulated gene expression systems, biosensors, and CRISPR editing techniques. Along with the genetic tools, knowledge from omics datasets and genome scale metabolic models are key Tier 2 Targets that enhance DBTL cycle efficiency and lab-scale experimentation. Finally, genetic stability, defined as consistency of strain phenotype and genotype over multiple generations and/or rounds of experiments, should be demonstrated in Tier 2.

Targets within Tier 3 represent more advanced tools, datasets, and models that can be applied for strain optimization for industrial use and to overcome unique bottlenecks (Table 1). Accomplishing the individual Targets in this Tier are frequently more costly, time intensive, and complex compared with those in Tiers 1 – 2, requiring greater expertise, larger datasets, advanced computation, and/or expensive instrumentation. Oftentimes, Targets in Tier 3 can be critical to generate the understanding needed during scale-up. Ideally, preliminary testing of at-scale performance and strain robustness for industrial conditions would be performed at Tier 2, but informative, standard methods are not yet available.

In addition to guiding development, the Tier System can also facilitate communication about the state of available tools and knowledge about a strain, with the Tier level giving a researcher a quick point of reference on how developed a strain is. However, some Targets in Tiers 2 – 3 can be contingent upon developments in lower Tiers, so the Tiers should not be interpreted in a strictly hierarchical manner. Host development will often not proceed linearly through the *Tier System*, and hosts under development will likely meet Targets across multiple Tiers. As part of the built-in flexibility of the *Tier System* framework, advancement of hosts to "Tier 2" status, or beyond, can proceed via different tracks. If 100% of the Targets have been met for Tier 2, the host is classified at that Tier level. Alternatively, if at least 80% of the Targets

for Tier 2 and at least 20% of the Targets for Tier 3 have been met, the host can be considered to have achieved "Tier 2" status. If a Target is not applicable for a certain host or there is a strong, justifiable reason that the Target should not or cannot be met, that Target can be excluded and not "counted" toward the overall status of the host. The aim is that the Tier System serves as a useful, living, flexible framework that can be applied to a broad taxonomic range of microbial hosts and stand up to the challenges of a rapidly evolving field.

3. Concluding Remarks

Here, we have introduced the *Tier System for Host Development* as a living document to improve host development activities through thoughtful prioritization of the most critical research needs for a new target organism. The Tier System differentiates itself from other host development tools in that it quantifiably tracks the developmental status of host organisms for biomanufacturing – from initial onboarding to industry-ready status. The *Tier System* has been integrated into the Agile Biofoundry's Host Onboarding Tool (HObT) website (https://hobt.agilebiofoundry.org), where it serves as a publicly available, easy-to-use guide for evaluating the readiness level of non-traditional hosts for bioproduction. Using this framework, we hope to encourage the coordination of effort and tool development to reduce the time needed for bioprocess development and facilitate the growth of a functional and profitable bioeconomy that can significantly lessen our reliance on fossil-derived carbon.

Data Availability

No data were used for the research described in the article.

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Figure Captions

Figure 1. *Contrast between traditional approaches for host development and the Tier System.* **A)** Traditionally, multiple groups have studied the same host with disparate approaches and minimal coordination. This haphazard model leads to slow progress and increased cost**. B)** The *Tier System* provides a framework for groups to prioritize and coordinate efforts when working with the same host, leading to a systematic, standardized, and quicker workflow. Insights from one organism can also enhance and accelerate progress on other organisms.

Figure 2. *Tier System for Host Development.* Candidate wild type hosts can be selected based upon a suite of useful traits, including physiological properties (e.g., metabolic flexibility, toxicity tolerance, or flux to a particular metabolic node), and Targets (genetic tools, physiological data and models) are developed to facilitate rational metabolic engineering. Tiers 1 – 3 within the host development framework organize these Targets and proceed from basic, yet essential, tools and knowledge (Tier 1) to more advanced technologies and analytics (Tiers 2 and 3) that are typically more costly and time intensive. Microbes must achieve the basic Targets in Tier 1 to be considered "*onboarded*". Hosts achieving the minimum Targets for onboarding can be paired with an appropriate bio-product and developed further in DBTL cycles. Thereafter, a host is further improved and elevated in the *Tier System* (Tiers 2 – 3) to meet the demands of the DBTL process until a production-ready chassis is generated.

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