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The Best Chassis Depends on the Molecule: A Comparative Decision Model for Rebuilding Plant Natural-Product Pathways in Yeast, Bacteria, and Plant Cells

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ABSTRACT

Reconstructing plant natural-product pathways outside their native organisms is often framed as a choice among established production hosts, yet pathway success depends on biochemical requirements that differ substantially among molecules. Yeast, bacterial, and plant-cell systems offer distinct combinations of membrane organization, redox physiology, precursor metabolism, transport capacity, compartmentation, tolerance, and engineering accessibility. Consequently, a chassis that performs well for one pathway may impose avoidable engineering burdens on another. This article develops a proposed molecule-centered comparative decision model for selecting among yeast, bacterial, plant-cell, and, where appropriate, hybrid production architectures. The analysis distinguishes intrinsic or readily available host compatibility from engineering flexibility and argues that chassis selection should begin with the pathway's molecular dependencies rather than host familiarity or platform popularity. Decision-relevant features include membrane-bound tailoring chemistry, cofactor and redox-partner requirements, nucleotide-sugar demand, intracellular localization, transport, product toxicity, pathway topology, side-reaction risk, and the extent to which pathway modules can be separated. Recent reconstructions show that chemically demanding pathways can be realized in more than one chassis, but the engineering burden is redistributed rather than eliminated. The proposed model therefore treats chassis choice as conditional and multidimensional, permits deliberate use of incomplete cellular reconstructions and hybrid architectures, and separates biosynthetic feasibility from manufacturing readiness. Its principal limitation is that existing evidence is dominated by pathway-specific demonstrations rather than standardized prospective cross-host comparisons. The framework should therefore be considered a testable decision architecture, not a validated universal ranking of host systems.

Keywords: Metabolic engineering, Plant natural products, Synthetic biology, Microbial chassis, Nicotiana benthamiana, Pathway reconstruction

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Introduction

Synthetic biology has made increasingly complex natural-product pathways accessible to reconstruction through coordinated enzyme discovery, pathway assembly, host engineering, and metabolic control. Contemporary work spans microbial and plant systems and increasingly couples pathway elucidation to deliberate modification of the cellular environment in which a pathway must function. Chassis choice is therefore not merely a practical decision made after a biosynthetic route is known; it is part of pathway design itself because the host determines which biochemical functions are already available, which must be rebuilt, and which incompatibilities may emerge during production [1–3]. Plant natural-product reconstruction in microbes illustrates this dependence particularly

clearly: success can require adaptation of heterologous enzymes, precursor supply, redox metabolism, transport, and cellular organization rather than simple transfer of a set of coding sequences [4].

The usual question—whether yeast, a bacterium, or a plant cell is the "best" host—therefore begins at the wrong level of abstraction. Chassis have characteristic advantages, but those advantages matter only in relation to a molecule and the pathway required to reach it. A eukaryotic host may simplify membrane-associated oxidation while complicating precursor redistribution; a bacterial host may permit rapid refactoring yet demand extensive reconstruction of a eukaryotic redox system; a plant chassis may preserve useful cellular context while introducing endogenous metabolism that obscures or diverts the intended pathway. These are not mutually exclusive observations. They imply that performance cannot be reduced to an intrinsic host hierarchy independent of chemistry.

This article develops a proposed molecule-centered chassis decision model for rebuilding plant natural-product pathways in yeast, bacteria, and plant cells. Its purpose is not to rank hosts universally or infer prospective production performance from retrospective success stories. Instead, it organizes the decision around the biochemical obligations imposed by the target pathway: enzyme topology, redox and cofactor dependence, precursor and activated-donor requirements, transport, compartmentation, cytotoxicity, pathway length and branching, and the feasibility of separating incompatible modules. The central distinction is between compatibility, meaning functions a host supplies with limited reconstruction, and engineering flexibility, meaning functions that can be deliberately introduced or reconfigured. Those dimensions can converge, but they are not equivalent.

The resulting argument also permits a third outcome beyond choosing a single chassis. When a pathway contains modules with irreconcilable cellular requirements, completing every step in one organism may be unnecessary. Cell-free finishing, enzyme cascades, sequential processing, microbial consortia, or cross-species modularization can instead become rational architectures. Such alternatives should not be assumed superior: each introduces new interfaces and validation requirements. The proposed model is therefore a comparative decision structure for locating engineering burden, not a formula that predicts the winning chassis. Its usefulness ultimately depends on prospective testing against molecules and pathways not used to construct the reasoning.

Why chassis selection cannot be universal

The strongest argument against universal chassis selection is that pathway complexity is qualitative as well as quantitative. The engineered production of QS-21 in yeast required an unusually extensive heterologous reconstruction that incorporated numerous biosynthetic functions and recreated aspects of plant-like spatial organization, demonstrating that successful use of a powerful chassis can depend on rebuilding capabilities that the host does not naturally provide [5]. A long gene list is not itself a universal measure of difficulty; what matters is whether those genes collectively impose membrane, redox, transport, precursor, compartmental, or toxicity requirements that align poorly with the host.

Equally important, the existence of a successful reconstruction does not establish that the host used is uniquely suitable. Withanolide biosynthetic functions have been interrogated through both yeast and *Nicotiana benthamiana*, while verazine has been reconstructed separately in a plant chassis and in engineered *Saccharomyces cerevisiae* [6–8]. These examples are not standardized head-to-head productivity comparisons, so they cannot support claims of host superiority. They instead show something more useful for decision theory: a biosynthetic objective may be achievable in multiple cellular environments, but the set of interventions required to make it work can differ substantially.

Cellular architecture provides another reason why host rankings do not transfer cleanly between molecules. Yeast organelles can be exploited as metabolically distinct reaction spaces, altering local enzyme concentration, metabolite exposure, cofactor availability, and separation from competing reactions [9]. Yet localization also introduces transport requirements and can create new bottlenecks. Compartmentation is therefore neither an inherent advantage nor a liability; its value depends on whether the pathway benefits from spatial separation and whether substrates and intermediates can cross the resulting boundaries efficiently.

A universal chassis rule would consequently collapse several different questions: Can the enzymes function? Can the host supply their substrates and redox partners? Can intermediates reach the right location? Can the cell tolerate the product? Can competing native reactions be suppressed? And can the resulting architecture be engineered without creating greater problems elsewhere? The proposed model treats these as separable sources of engineering

burden. A chassis is suitable not because it is generally powerful, but because the liabilities created by a particular molecule and pathway remain acceptable, compensable, or strategically avoidable in that host.

What different host systems offer

Plant cells offer a cellular environment closer to the origin of many specialized metabolic pathways. *Nicotiana benthamiana* has become especially useful for rapid heterologous reconstruction because transient expression can combine plant enzymes within plant membranes, organelles, redox systems, and metabolite-transport contexts [10]. That compatibility is not equivalent to a neutral background. Endogenous plant metabolism can consume precursors, generate competing products, or obscure pathway interpretation, and experimental redesign of *N. benthamiana* has shown that the chassis itself may need genetic modification before it becomes a cleaner production platform [11]. Plant context can therefore reduce some reconstruction burdens while creating others.

Bacterial hosts provide a different form of leverage: rapid genetic manipulation, modular pathway assembly, and substantial physiological diversity. That diversity means that "bacterial chassis" should not be treated as a single decision category. *Pseudomonas putida*, nontraditional bacterial hosts, and *Corynebacterium glutamicum* illustrate distinct combinations of redox capacity, stress tolerance, precursor metabolism, and engineering characteristics that can favor different chemical objectives [12–14]. A molecule-centered comparison should therefore first ask what bacterial physiology is required and only then ask which species best approximates it.

Even *Escherichia coli*, despite lacking native eukaryotic architecture, can accommodate plant-derived biosynthetic chemistry when the pathway is appropriately engineered. Production of the furanocoumarin intermediate marmesin demonstrates that a bacterial environment can support selected plant pathway steps [15]. The harder cases reveal the cost of that flexibility. Plant cytochrome P450 systems depend on membrane organization and electron transfer relationships that differ from ordinary bacterial physiology; architectural redesign of eukaryotic P450–reductase organization in *E. coli* can restore and improve function, but the need for such redesign is itself evidence of an initial compatibility gap [16].

Yeast occupies an intermediate position in this comparison. It offers eukaryotic membranes, organelles, trafficking, and established metabolic-engineering tools, making it attractive for pathways rich in membrane-bound enzymes or requiring spatial organization. At the same time, yeast is not a surrogate plant cell: donor metabolism, transport, endogenous side reactions, localization, and tolerance still require pathway-specific intervention. Its advantage is therefore not that it is generically "plant-like," but that certain eukaryotic functions can be easier to adapt than to construct de novo.

The relevant comparison is consequently functional rather than taxonomic. Plant hosts offer greater continuity with plant cellular context, bacterial hosts offer strong refactoring potential across diverse physiologies, and yeast combines eukaryotic organization with microbial engineering. None of these properties determines the preferred chassis in isolation. Their value emerges only when matched against the molecular liabilities and dependencies of the target pathway.

Molecular features that change chassis suitability

The first decision-relevant molecular feature is dependence on activated donors that the host may not naturally supply. Glycosylated natural products can require nucleotide sugars absent from, or poorly produced by, the chosen chassis. Engineering yeast to generate non-native nucleotide sugars demonstrates that donor metabolism can itself become a substantial design problem independent of the glycosyltransferase that ultimately uses the donor [17]. A host that expresses the catalytic enzyme successfully may still be poorly matched to the pathway if the required activated substrate must be reconstructed at comparable engineering cost.

Spatial dependence is a second feature. In engineered yeast producing benzylisoquinoline alkaloids, deliberate subcellular organization formed part of the strategy for improving a complex multistep pathway [18]. The implication is broader than the specific pathway: enzymes that require particular membranes, local redox environments, protected intermediates, or restricted exposure to competing reactions create a spatial burden that should be assessed before host selection. Sequence compatibility alone cannot capture that constraint.

Third, the molecule itself may damage the chassis. Sanguinarine biosynthesis in yeast required dynamic control that separated growth from production, using temperature-responsive regulation to manage cytotoxicity [19]. Toxicity therefore changes the optimization problem. A host with otherwise excellent enzyme compatibility may become unattractive if the product or an intermediate suppresses growth before sufficient biomass is established.

Conversely, a less intrinsically compatible host may remain competitive if it offers more effective tolerance, sequestration, export, or temporal control.

Finally, pathway architecture can make monolithic reconstruction unnecessary. A modular *E. coli* platform for halogenated tryptophan-derived products distributed biosynthetic functions across engineered populations, allowing modules to be combined rather than forcing every reaction into one strain [20]. Partitioning, however, is not free: metabolites must cross cellular or process interfaces, population balance becomes relevant, and intermediates may be lost or transformed between modules.

These observations motivate four proposed screening dimensions before any host is ranked: molecular dependency, covering cofactors, donors, membranes, and precursor chemistry; spatial dependency, covering localization and transport; physiological burden, covering toxicity and competing metabolism; and partitionability, covering whether problematic pathway modules can be separated without destroying flux or control. Additional pathway-specific features may be required, but these dimensions prevent a common error: choosing a familiar chassis first and discovering its most consequential incompatibilities only after substantial pathway construction. The next section converts these molecular constraints into the proposed comparative decision model.

A molecule-centered chassis decision model

A molecule-centered decision should begin by translating a target pathway into biochemical obligations before assigning a host. Plant reconstruction of the early paclitaxel pathway demonstrates the value of a cellular context capable of supporting a difficult, P450-rich pathway while allowing functional gene combinations to be resolved experimentally [21]. The proposed model therefore starts with a dependency profile rather than a preferred chassis: which enzymes require membranes or dedicated redox partners, which substrates and activated donors must be supplied, whether intermediates must be spatially separated, and whether the target molecule or its precursors impose toxicity, transport, or competing-reaction burdens.

The second step is to classify pathway topology. Reconstitution of the early paclitaxel pathway revealed a network of products and side reactions rather than a simple linear sequence, showing why counting pathway steps alone can underestimate reconstruction difficulty [22]. In the proposed model, branching, enzyme promiscuity, reversible transformations, and competing intermediate fates increase the value of a host that either supplies appropriate cellular organization or allows those interactions to be measured and redesigned.

The third step is to estimate routing burden. Complete tropane-alkaloid biosynthesis in yeast required not only heterologous catalysis but also transport and intracellular pathway organization [23]. The model therefore treats movement of substrates, intermediates, redox equivalents, and products as a chassis cost rather than an afterthought. A host may be enzymatically compatible yet become inefficient if multiple transport interfaces must be created to connect the relevant compartments or protect unstable intermediates.

The fourth step is to decide where cellular reconstruction should stop. The microbial supply chain for vinblastine demonstrates that very long plant pathways can be rebuilt extensively in yeast while still using a separate finishing operation rather than requiring every transformation to occur in one living chassis [24]. Accordingly, the proposed model does not define complete single-cell reconstruction as the default optimum. If a late transformation is unusually toxic, chemically simpler outside the cell, or poorly matched to the chosen host, intentional pathway termination followed by enzymatic, chemical, or second-host completion can be a rational design state.

These four steps generate a comparative rather than absolute decision. A candidate host is favored when its existing cellular functions reduce high-consequence pathway liabilities and when the remaining mismatches are tractable with available engineering. It is disfavored when several critical dependencies must be rebuilt simultaneously, especially when those interventions create new transport, toxicity, or regulatory interfaces. Importantly, the model proposes no universal numerical score. The same liability can have different consequences depending on enzyme identity, expression level, pathway context, and process objective, so the decision should remain evidence-bounded and revisable as pathway-specific data accumulate.

Decision status should also remain reversible. Early enzyme tests may favor a bacterium because precursor supply and cloning are convenient, whereas later evidence of membrane dependence or toxic intermediate accumulation may shift the preferred architecture toward yeast, a plant system, or a partitioned process. Conversely, apparently plant-specific requirements may prove engineerable once their biochemical basis is isolated. The model therefore treats chassis selection as an iterative hypothesis updated by pathway evidence, not a one-time taxonomic assignment.

The integrated logic is shown in **Figure 1**, where evidence-supported chassis constraints feed a proposed compatibility–flexibility decision field and can branch toward single-host or hybrid reconstruction while remaining inside an explicit validation boundary.

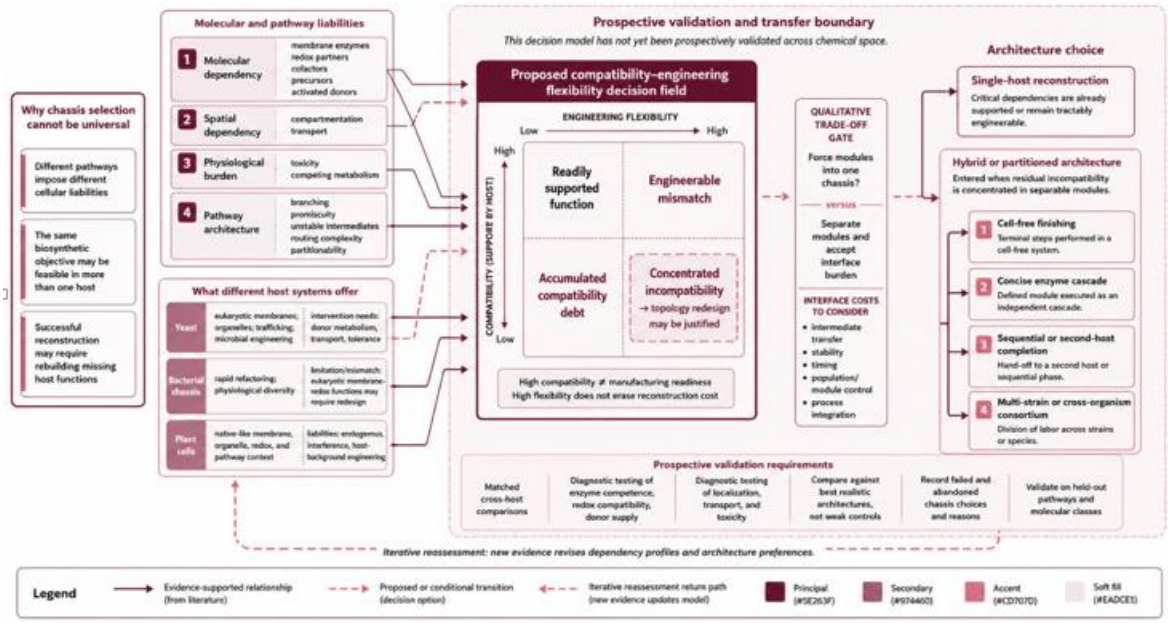

Figure 1. From molecular liabilities to chassis architecture: a proposed decision model for plant natural-product reconstruction

Table 1 condenses the decision logic into qualitative states that distinguish native or readily available compatibility from engineerable mismatch and from conditions that should trigger consideration of pathway partitioning.

Table 1. Molecule-centered decision states for selecting a pathway chassis

Decision dimension	Primary question	Favorable state	Warning state	Proposed decision implication
Catalytic context	Can required enzymes function in the host?	Native-like or readily supported	Membrane/redox reconstruction required	Compare compatibility cost
Metabolic supply	Are precursors, cofactors, and donors available?	Endogenous or easily expanded	Multiple missing supply routes	Penalize hidden pathway burden
Spatial routing	Must intermediates cross compartments or membranes?	Short, controllable routing	Repeated transport interfaces	Reconsider host or topology
Physiological burden	Are product and intermediates tolerated?	Growth-compatible	Toxicity or competing metabolism	Add control, export, or separation
Network behavior	Is pathway behavior predictable after transfer?	Limited branching	Promiscuity or unstable intermediates	Favor diagnostic context
Partitionability	Can difficult modules be separated?	Clean modular interfaces	Strong intermodule dependence	Consider hybrid architecture cautiously

Trade-offs between compatibility and engineering flexibility

Compatibility is valuable because every function supplied by the chassis is one fewer function that must be reconstructed, tuned, and validated. Plant transient-expression systems can preserve cellular features relevant to specialized metabolism while supporting pathway interrogation and metabolite characterization; reconstruction of *Calendula* triterpenoid biosynthesis illustrates how a plant context can connect enzyme function with product formation and downstream functional testing [25]. That advantage should not be confused with manufacturing

superiority. Native-like context may simplify some biochemical questions while leaving transformation stability, growth, process control, or scale-up unresolved.

Engineering flexibility addresses the opposite problem: how far a host can be pushed when intrinsic compatibility is poor. A redox-proficient *E. coli* platform designed for cytochrome P450 work shows that host physiology can be deliberately altered to support catalytic demands that bacteria do not naturally meet well [26]. Such cases make a simple compatibility ranking misleading. Low initial compatibility can sometimes be overcome, but the required intervention consumes engineering effort and may create new dependencies.

The proposed model therefore treats compatibility and flexibility as orthogonal dimensions. The extensive QS-21 reconstruction shows that yeast can support chemically demanding plant biosynthesis after substantial cellular and pathway engineering [5]. Architectural redesign of eukaryotic P450 systems in *E. coli* shows that a major incompatibility can be made tractable through targeted refactoring [16]. Conversely, plant reconstruction of paclitaxel chemistry shows the value of retaining a cellular environment in which difficult plant enzymes and pathway interactions can be interrogated directly [21]. None of these examples establishes a universal winner; they show different ways of paying the cost of incompatibility.

This distinction also clarifies when apparently "easy to engineer" is an insufficient chassis criterion. Engineering speed matters most when mismatches are modular and diagnosable. It matters less when success depends on many interacting cellular properties that must be rebuilt simultaneously. Conversely, high compatibility is most valuable when the preserved context is mechanistically relevant to the pathway rather than merely taxonomically familiar. Chassis selection should therefore compare the expected burden of preserving useful biology with the expected burden of replacing it. The preferred design is the one in which high-consequence liabilities are either already accommodated or can be altered without generating a larger set of secondary constraints.

This comparison can be framed as compatibility debt: every missing high-consequence function creates an obligation that must be paid through host engineering, pathway redesign, or process separation. The most attractive chassis is therefore not necessarily the one requiring the fewest edits; it is the one in which the remaining edits address well-defined liabilities without destabilizing other essential pathway functions.

When hybrid production strategies become attractive

A hybrid strategy becomes attractive when the strongest available chassis for one pathway segment is a poor environment for another. Cell-free synthetic biology makes this trade-off explicit because removing the living cell eliminates growth, membrane, and some toxicity constraints while introducing different limitations in enzyme lifetime, cofactor regeneration, cost, and process scale [27]. The relevant question is therefore not whether cell-free production is intrinsically more advanced, but whether avoiding cellular incompatibility is worth the new burdens created outside the cell.

Enzyme cascades offer one version of this logic. A concise cascade for protoberberine alkaloids demonstrated that an artificial sequence can manufacture natural and halogenated products without reproducing the full cellular route from which the chemistry originated [28]. In the proposed model, such pathway shortening becomes attractive when difficult cellular steps can be replaced by a smaller set of robust biocatalytic transformations. This is a redesign of the production topology, not evidence that purified-enzyme systems generally outperform living chassis.

A second option is to distribute pathway modules among living populations. Complete anthocyanin biosynthesis using an *E. coli* polyculture showed that a long pathway can be divided among specialized strains, distributing metabolic burden and allowing modules to be adjusted separately [29]. Partitioning can be particularly appealing when one segment is toxic, precursor-intensive, or incompatible with the regulatory state needed by another. Yet division also creates interface costs: intermediates must leave one module and reach the next, population ratios may shift, and control that was intracellular becomes ecological or process-level.

More recent block-building work extends modular reasoning across organisms. Yeast-based reconstruction of glabridin biosynthesis was combined conceptually and experimentally with broader modular strategies, including a yeast–*E. coli* consortium used for equol production [30]. This distinction matters: the consortium does not establish hybrid glabridin production. It instead demonstrates that organism-level modularity can be engineered when pathway blocks have different host requirements.

The proposed decision rule is consequently conservative. Hybridization should be considered when the predicted penalty of forcing incompatible modules into one chassis exceeds the added interface burden of separating them. That judgment requires evidence on intermediate stability, transport, module productivity, population control,

downstream purification, and process integration. Hybrid architectures are therefore an option for resolving concentrated incompatibility, not an automatic destination for increasingly complex pathways.

Hybridization also changes what must be validated. A successful module in isolation does not establish successful transfer between modules, and high activity in a cell-free step does not establish compatibility with upstream fermentation or downstream purification. Interface performance, intermediate recovery, timing, and control become part of the production mechanism. These interfaces should be treated as explicit engineering objects rather than hidden costs added after the pathway has been divided.

Future chassis design priorities

The central validation need is prospective comparison. Existing reconstructions show that related biosynthetic objectives can function in more than one host, including withanolide work spanning yeast and *N. benthamiana* [6]. Plant reconstruction of verazine establishes one feasible host context [7]. Independent yeast reconstruction establishes another [8]. Because these studies were not standardized head-to-head chassis trials, they cannot reveal whether the proposed decision variables predict the lower engineering burden. Future benchmarks should therefore hold pathway identity as constant as biologically possible while comparing host-specific intervention requirements, not merely final titers obtained under unrelated optimization regimes.

A second priority is diagnostic measurement before full pathway construction. P450 architecture and redox compatibility can be tested directly rather than inferred from host taxonomy [16]. Required nucleotide-sugar metabolism can likewise be measured before downstream glycosylation is optimized [17]. Spatial dependence can be probed through alternative localization designs before a complete strain is committed [18]. A prospective chassis workflow should combine such assays with measurements of precursor availability, transport, product toxicity, side-product formation, and host growth. The purpose is not to generate a universal score, but to identify which predicted liabilities actually dominate for a particular molecule.

Third, future work should compare architectures, not just organisms. Plant systems can preserve useful biosynthetic context but still require engineering and process qualification [25]. Cell-free routes remove cellular constraints while creating their own stability and scale boundaries [27]. Cross-organism modularity can distribute pathway functions, but consortium evidence remains pathway-specific and must be compared against strong single-host controls [30]. Benchmarking should therefore include the best realistic architecture for each strategy rather than a poorly optimized representative chosen for convenience.

Finally, failure data deserve greater analytical value. A nonfunctional P450, an accumulating intermediate, a toxic branch, or a transport bottleneck can reveal more about chassis suitability than a final successful titer after extensive optimization. Prospective validation of the proposed model should record the interventions required to reach function, the new liabilities created by those interventions, and cases in which an initially favored chassis is abandoned. Held-out pathways with different enzyme topologies and chemical classes would provide the strongest test of whether molecule-centered reasoning transfers beyond the examples from which it was derived. Until such comparisons exist, the model should guide explicit hypothesis formation rather than claim predictive accuracy.

Conclusion

The central claim of this comparative decision model is simple but consequential: a production chassis should be selected in relation to the molecule and pathway it must support, not from a universal hierarchy of yeast, bacterial, and plant platforms. Those hosts differ in membrane organization, compartmentation, redox physiology, precursor and donor metabolism, transport, tolerance, endogenous chemistry, and engineering accessibility.

The proposed framework therefore begins with molecular and pathway liabilities, separates compatibility from engineering flexibility, and asks where the resulting engineering burden is most tractable. A host is attractive when it already supplies high-consequence functions or when its missing functions can be reconstructed without producing larger secondary constraints. The model also rejects complete single-cell reconstruction as an obligatory endpoint. When incompatibility is concentrated in separable modules, cell-free finishing, enzyme cascades, consortia, or other hybrid architectures may be more coherent design choices, although they introduce their own transport, control, stability, and process interfaces.

The framework remains deliberately evidence-bounded. Current literature contains sophisticated demonstrations of pathway reconstruction, but relatively few standardized comparisons that hold molecular objectives constant

across chassis. Consequently, the decision dimensions proposed here should not be interpreted as validated predictors, numerical ranking criteria, or guarantees of production performance. Their value is to make hidden assumptions visible: which biochemical functions are expected from a host, which must be engineered, which mismatches matter most, and when a different architecture should be considered.

A useful future chassis-selection discipline would therefore judge success not only by the product ultimately obtained, but also by the number and severity of incompatibilities that had to be solved to obtain it. Prospective cross-host testing, diagnostic measurements before full pathway assembly, and systematic reporting of abandoned as well as successful designs are needed to determine whether molecule-centered selection can reduce avoidable engineering effort. The best chassis, in this view, is not a fixed organism. It is the architecture whose biological context and engineering burden best match the chemistry that must be rebuilt.

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