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Plant Pathways Do Not Become Microbial Pathways by Gene Transfer Alone: A Compatibility Model for Cofactors, Compartmentation, Toxicity, Flux, and Product Export

James O'Leary[1]*, Aisling Dunne[1], Sean O'Brien[2]

[1] Department of Pathway Compatibility and Heterologous Expression, School of Pharmacy, Trinity College Dublin, Dublin, Ireland.
[2] Department of Cofactor and Product Export Modeling, School of Pharmacy, University College Cork, Cork, Ireland.

***E-mail** ✉ james.oleary@tcd.ie

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ABSTRACT

Transferring plant biosynthetic genes into a microbial chassis can reproduce enzyme inventories without reproducing the cellular conditions that made the pathway functional in its native organism. This distinction becomes increasingly consequential as heterologous routes incorporate membrane-associated enzymes, redox-intensive reactions, spatially separated intermediates, toxic metabolites, competing precursor demands, and products that require active removal. This article develops an original pathway-compatibility model for interpreting these problems before they are treated as isolated optimization failures. Evidence from microbial production of plant natural products is integrated across five recurrent domains: catalytic and cofactor context, compartmentation and metabolite trafficking, precursor and flux accommodation, toxicity and metabolic burden, and product export. The analysis argues that compatibility is not an intrinsic property of either a pathway or a chassis. It is a conditional relationship between the demands imposed by a particular biosynthetic architecture and the capacities of a particular host under defined operating conditions. The proposed model therefore separates observed production failure from its possible causes, treats compatibility dimensions as potentially interacting rather than automatically additive, and links diagnosis to chassis selection and engineering response. The model is intended as a qualitative decision framework, not a validated score or universal host ranking. Its main limitations are pathway-specific enzyme behavior, incomplete measurement of intracellular states, context-dependent transport and toxicity, and the possibility that process conditions alter the dominant constraint. Prospective cross-host reconstruction and factorial perturbation will be required before predictive claims are justified.

Keywords: Heterologous biosynthesis, Plant natural products, Microbial chassis, Metabolic engineering, Compartmentation, Pathway compatibility

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Introduction

Microbial production of plant natural products has advanced from expression of individual enzymes toward reconstruction of long, chemically diverse pathways. Yet the successes themselves show why a plant pathway cannot be treated as a portable DNA cassette. Reviews of microbial and yeast-based natural-product biosynthesis repeatedly describe successful reconstruction as a combination of enzyme selection, expression control, precursor engineering, subcellular organization, transport, and process optimization rather than gene transfer alone [1–3]. The practical implication is not that every transferred pathway will fail, but that genetic completeness and functional completeness are different states. A microbial cell may contain all nominal catalytic steps while still lacking the redox environment, spatial topology, metabolite access, tolerance, or transport capacity needed for productive flux.

The mismatch can begin at the enzyme level. Plant proteins evolved within cellular environments that differ from yeast or bacterial hosts in codon usage, protein-folding context, membrane architecture, post-translational environment, organelle targeting, redox partners, and metabolite availability. Work on "yeastizing" plant enzymes therefore frames heterologous performance as partly an adaptation problem between protein properties and host physiology rather than simply an expression problem [4]. Poor product formation cannot, however, be attributed to enzyme incompatibility by default. Low protein abundance, insufficient precursor supply, competing native reactions, incorrect localization, metabolite loss, or culture conditions may produce a similar phenotype.

The key analytical problem is therefore attribution. A low-titer strain reports an outcome, not its cause. The same phenotype may arise because a catalyst is inactive, a cofactor is depleted, a substrate cannot reach the next enzyme, native metabolism diverts precursor, an intermediate inhibits growth, or the final product is retained intracellularly. These possibilities require different measurements and different interventions. A useful compatibility framework must preserve that diagnostic ambiguity while still organizing the evidence strongly enough to guide experimental choice.

This article proposes that these failures are more usefully interpreted through host–pathway compatibility. Compatibility is defined here as the degree to which a host can accommodate the biochemical, spatial, physiological, and transport demands of a specified heterologous pathway under specified conditions. It is deliberately relational: the same host may be well suited to one pathway and poorly suited to another, while the same pathway may expose different bottlenecks in different hosts. The model does not assign numerical weights or assume that its dimensions are independent. Instead, it organizes questions that should be answered before optimization is interpreted as a generic problem of "low flux."

The argument proceeds from failure phenotypes to incompatibility classes, then to interactions among constraints. Those distinctions are subsequently used to construct a proposed compatibility model, guide chassis choice, and connect diagnosed failure modes with engineering responses. Throughout, empirical pathway reconstructions are treated as evidence for particular mechanisms or boundaries, not as proof that one chassis, intervention, or pathway architecture is universally superior.

Why transplanted pathways fail in new hosts

A transferred pathway can be genetically intact yet catalytically incomplete. Plant cytochrome P450 enzymes illustrate this problem because productive heterologous activity depends not only on enzyme expression but also on membrane context, electron-transfer partners, and supporting redox machinery [5]. Cofactor availability is a distinct constraint: engineering supply and recycling in yeast can alter phenolic-acid biosynthesis, demonstrating that reaction stoichiometry may exceed what the unmodified host can sustain [6]. These mechanisms should remain analytically separate because increasing enzyme abundance will not necessarily correct inadequate redox supply, and raising cofactor availability will not rescue an inactive or mislocalized catalyst.

Spatial reconstruction creates a second route to failure. Plant pathways can rely on organelles, membrane surfaces, and selective metabolite movement that disappear when enzymes are simply coexpressed in one microbial compartment [7]. In yeast tropane-alkaloid engineering, transporter identification and manipulation were required to support movement of pathway intermediates among cellular locations, providing direct evidence that trafficking itself can become limiting [8]. Thus, an apparently weak enzymatic step may instead reflect poor substrate delivery.

A third failure route is physiological. Heterologous biosynthesis can divert energy, precursors, transcriptional and translational capacity, or membrane resources in ways that reduce robustness and production [9]. Product or intermediate accumulation can also create a removal problem; efflux-transporter engineering provides evidence that export can be an active determinant of microbial production rather than a passive endpoint [10]. These states are not interchangeable. Slow growth does not by itself identify toxicity, and intracellular accumulation does not establish that export is the dominant limitation.

Flux failure adds a further ambiguity. Increasing precursor formation can be ineffective when downstream capacity is saturated, and it can worsen intermediate accumulation when transport or catalytic steps remain limiting. Conversely, relieving an export or trafficking restriction can change the apparent demand on upstream reactions. The proposed model therefore treats "flux" not as a single adjustable quantity but as an emergent pathway state shaped by reaction capacity, precursor competition, spatial access, and product handling. This interpretation is a synthesis of the observed engineering problems rather than a claim that every interaction has been causally decomposed.

Complex reconstructions show why these explanations often coexist. In engineered yeast producing steviol glycosides, improvements involved coordinated interventions affecting pathway flux, stress, channeling, and product handling rather than correction of a single universal bottleneck [11]. The appropriate inference is therefore conditional: low output is a phenotype requiring diagnosis, and the dominant incompatibility may change as other constraints are relieved.

Classes of host–pathway incompatibility

Recent reconstructions of QS-21, protoberberine and benzophenanthridine alkaloids, and monoterpene indole alkaloids show that long plant pathways can function in yeast, but only after extensive adaptation of enzyme expression, pathway organization, and host metabolism [12–14]. These studies support classifying incompatibility by the kind of host capacity that a pathway demands rather than by pathway length alone. A long pathway can be tractable when its requirements are accommodated, whereas a shorter pathway may remain poorly productive if one indispensable reaction or transport step is mismatched.

Catalytic and cofactor incompatibility should remain distinct even when they occur in the same reaction module. P450-centered pathways may fail because the heterologous enzyme is poorly expressed or improperly supported by membrane and redox partners [5], whereas direct cofactor engineering shows that the host's regeneration capacity can independently restrict biosynthetic output [6]. Toxicity and burden form another useful distinction: both can reduce host performance, but burden may arise from resource allocation even when the product itself is not toxic [9]. The classification therefore describes diagnostic hypotheses, not mutually exclusive biological compartments.

Compartmentation deserves its own class because the host must provide not only the correct location but sufficient capacity within that location. Machine-learning-guided expansion of yeast peroxisome capacity demonstrates that an organelle can become a limiting resource for a multienzyme pathway [15]. At the same time, peroxisome engineering literature shows that compartmentation can create benefits and liabilities involving precursor access, transport, enzyme environment, and organelle maintenance [16]. Engineering peroxisome assembly to increase acetyl-CoA-derived 5-deoxyflavonoid production further shows that host organelle architecture can itself be modified as part of pathway design [17].

Transport constitutes another analytically distinct incompatibility. Exporter mining for terpenoid secretion and engineered secretory production of tocotrienols show that product removal can be designed rather than assumed [18, 19]. Export nevertheless should not be conflated with intracellular trafficking: one governs movement between cellular compartments or toward enzymes, whereas the other concerns removal from the producing cell or accumulation in an external phase. Both can influence toxicity and flux, but they imply different measurements and interventions.

The resulting distinctions are summarized in **Table 1** as proposed diagnostic classes rather than as fixed causal assignments.

Table 1. Proposed analytical classes of host–pathway incompatibility and their diagnostic boundaries

Proposed incompatibility class	What may be mismatched	Diagnostic question	Interpretation boundary
Catalytic context	Enzyme folding, membrane environment, partner proteins	Is active catalyst available where needed?	Expression alone does not establish activity
Cofactor/redox context	Cofactor supply, recycling, electron transfer	Can reaction demand be sustained?	Low flux is not specific to cofactor shortage
Spatial/trafficking context	Organelle location, membrane access, intermediate movement	Can substrate reach the next reaction?	Compartmentation may help or constrain
Precursor/flux context	Precursor supply, competing sinks, pathway balance	Is carbon and reducing power directed appropriately?	Flux limitation can shift after intervention
Toxicity/burden context	Product stress, intermediate toxicity, resource demand	Does pathway operation impair host physiology?	Poor growth has multiple possible causes
Export context	Product removal, secretion, membrane transport	Can accumulated product leave the producing cell?	Export is distinct from intracellular trafficking

Note: The classification is an original proposed synthesis. Categories are diagnostic lenses, not mutually exclusive causal labels or validated quantitative scores.

Interactions among cellular and metabolic constraints

The usefulness of separate incompatibility classes depends on not mistaking separability for independence. Modern pathway-design strategies increasingly use multiplex experimentation and machine learning because pathway performance can depend on combinations of expression levels, enzymes, host modifications, and operating choices that are poorly explored one factor at a time [20]. For host–pathway compatibility, this means that a cofactor deficit, spatial mismatch, precursor limitation, burden state, or export restriction may alter the importance of another constraint. A model that assigns each dimension a fixed independent penalty would therefore claim more than the current evidence supports.

Several interaction routes are mechanistically plausible and, in particular systems, experimentally observable. Relocating enzymes can change substrate access and local cofactor availability; altered precursor supply can increase intermediate accumulation; export can reduce intracellular exposure to a toxic product; and burden can lower the expression or maintenance of the very machinery introduced to increase flux. These relationships also create feedback. An engineering intervention that relieves one bottleneck may expose a previously hidden one, so the relevant compatibility state can change during strain development rather than remain fixed.

This interaction logic has an important consequence for interpretation: an intervention can change the diagnostic landscape. If export is improved, intracellular toxicity may fall while upstream precursor demand rises; if compartmentation concentrates enzymes and substrates, local reaction rates may improve while transport across the new boundary becomes more important. Such shifts mean that compatibility is better viewed as a state that can be reassessed after engineering rather than a permanent label attached to a pathway. The model therefore allows recursive diagnosis and explicitly avoids fixed weights among dimensions.

Host context further modifies these interactions. Comparative evaluation of microbial cell factories indicates that production capacities differ across chassis, arguing against a universal host ranking independent of product and pathway demands [21]. Nonconventional hosts illustrate the same point from the opposite direction: *Yarrowia lipolytica* offers metabolic properties attractive for particular plant-natural-product classes, but those native advantages do not imply superiority for unrelated pathways [22]. Compatibility should therefore be evaluated as a pairing between a defined pathway architecture and a defined host, with process conditions treated as part of that pairing.

The interaction architecture and its downstream implications are summarized in **Figure 1.**

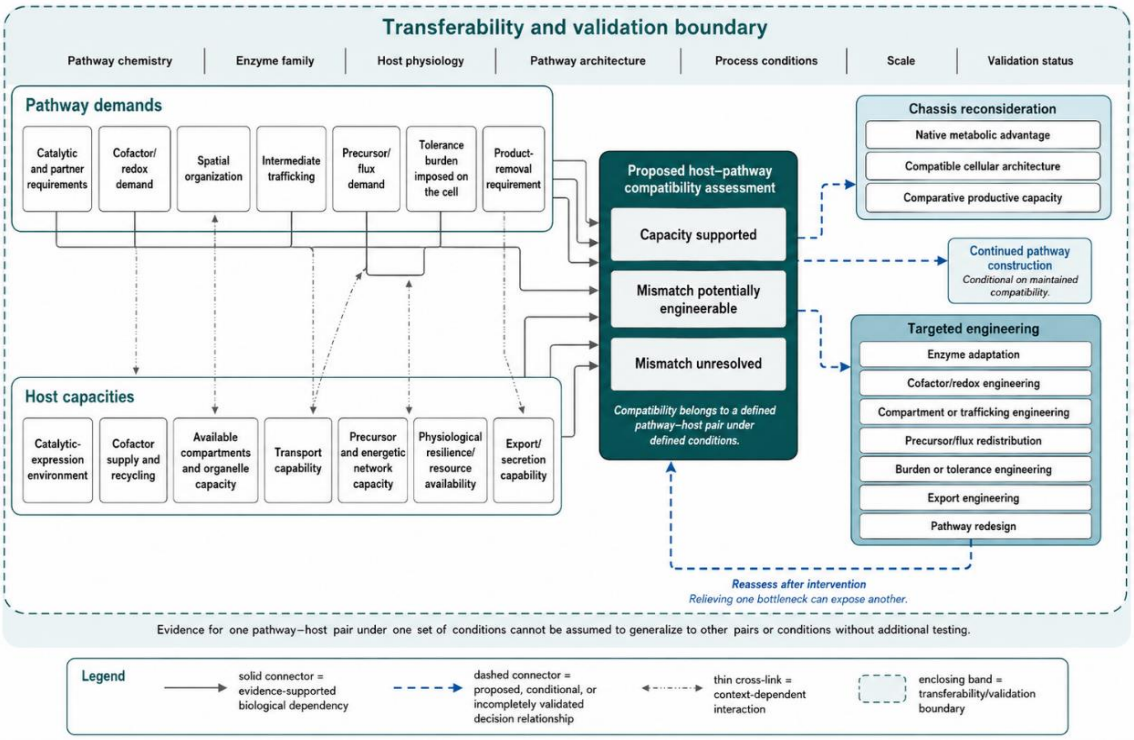

Figure 1. Host–pathway compatibility as an interacting constraint system linking failure diagnosis to chassis choice and engineering response

A compatibility model for heterologous biosynthesis

The preceding evidence suggests that compatibility should be assessed before low production is reduced to a generic optimization problem. Integrated yeast-manufacturing strategies already require coordination among pathway construction, regulation, screening, strain engineering, and process development [23]. The model proposed here adds a diagnostic layer to that workflow. It defines a host–pathway compatibility state as the qualitative correspondence between what a biosynthetic pathway demands and what a microbial host can provide under specified conditions. Compatibility is therefore neither a permanent property of a pathway nor a fixed property of a chassis.

The first model operation is to describe pathway demands without assuming their severity. These include requirements for catalytically competent enzymes and partners, cofactors and redox recycling, specific intracellular locations, metabolite movement, precursor and energetic supply, physiological tolerance, and product removal. Large reconstructions illustrate why this demand profile matters. Microbial production of vinblastine required extensive coordination across a highly complex biosynthetic architecture, demonstrating that genetic reconstruction can expose dependencies that are invisible when enzymes are considered individually [24]. Pathway complexity nevertheless should not be converted into a compatibility score: many steps do not necessarily imply poor compatibility, and one indispensable mismatched step may dominate a shorter route.

The second operation is to characterize host capacities against those demands. The tropane-alkaloid reconstruction in yeast demonstrates that successful biosynthesis may depend on recreating spatial and transport relationships rather than merely expressing the correct catalytic sequence [25]. The proposed model therefore distinguishes capacity present, capacity engineerable, and capacity currently unresolved. These are interpretive states, not quantitative categories. A host with insufficient native capacity may remain an appropriate chassis if the missing function can be introduced without creating a larger burden elsewhere.

The third operation is diagnosis through mismatch rather than outcome. Low product formation, intermediate accumulation, reduced growth, or unstable performance should trigger competing compatibility hypotheses. Evidence that a pathway needs more cofactor supply should not be inferred from low flux alone; evidence that intracellular product accumulates should not automatically identify export as the dominant problem. Where several hypotheses remain plausible, the model favors discriminating measurements or rescue experiments before extensive redesign.

Finally, compatibility is treated as dynamic. Once an intervention changes enzyme activity, compartmentation, precursor supply, or transport, another limitation can become dominant. The model therefore uses recursive reassessment rather than a one-time pass/fail decision. **Table 2** translates this proposed framework into an analytical comparison that preserves the distinction between pathway demand, host capacity, diagnostic evidence, and permissible interpretation.

Table 2. Proposed host–pathway compatibility matrix for diagnosing heterologous biosynthetic constraints

Compatibility dimension	Pathway demand to establish	Host capacity to examine	Evidence needed before assigning mismatch	Permissible interpretation
Catalytic function	Active enzyme and required partners	Expression, folding, membrane and partner support	Protein/activity evidence	Catalytic mismatch remains one candidate cause
Cofactor/redox	Sustainable cofactor or electron supply	Native supply and recycling capacity	Cofactor, redox or rescue measurements	Demand may exceed host support
Spatial organization	Correct reaction location and access	Suitable compartment and carrying capacity	Localization and compartment-function evidence	Spatial mismatch may restrict productive flux
Trafficking	Intermediate movement between required locations	Relevant transport routes	Transport perturbation or rescue evidence	Substrate delivery may be limiting
Precursor/flux	Adequate precursor and pathway balance	Native supply, competing sinks, energetic support	Flux or targeted metabolic evidence	Network allocation may constrain pathway operation

Toxicity/burden	Tolerable pathway operation	Physiological and resource resilience	Growth, stress and pathway-specific perturbation evidence	Host impairment may contribute to failure
Export	Removal compatible with product chemistry	Membrane/export capacity	Intracellular–extracellular distribution or transporter evidence	Product handling may limit performance

Note: This matrix is an original proposed analytical framework. A mismatch assignment requires pathway-specific evidence and does not constitute a validated numerical score.

Figure 2 extends the same logic from diagnosis to decision-making while retaining chassis choice, engineering response, and generalization limits as separate analytical layers.

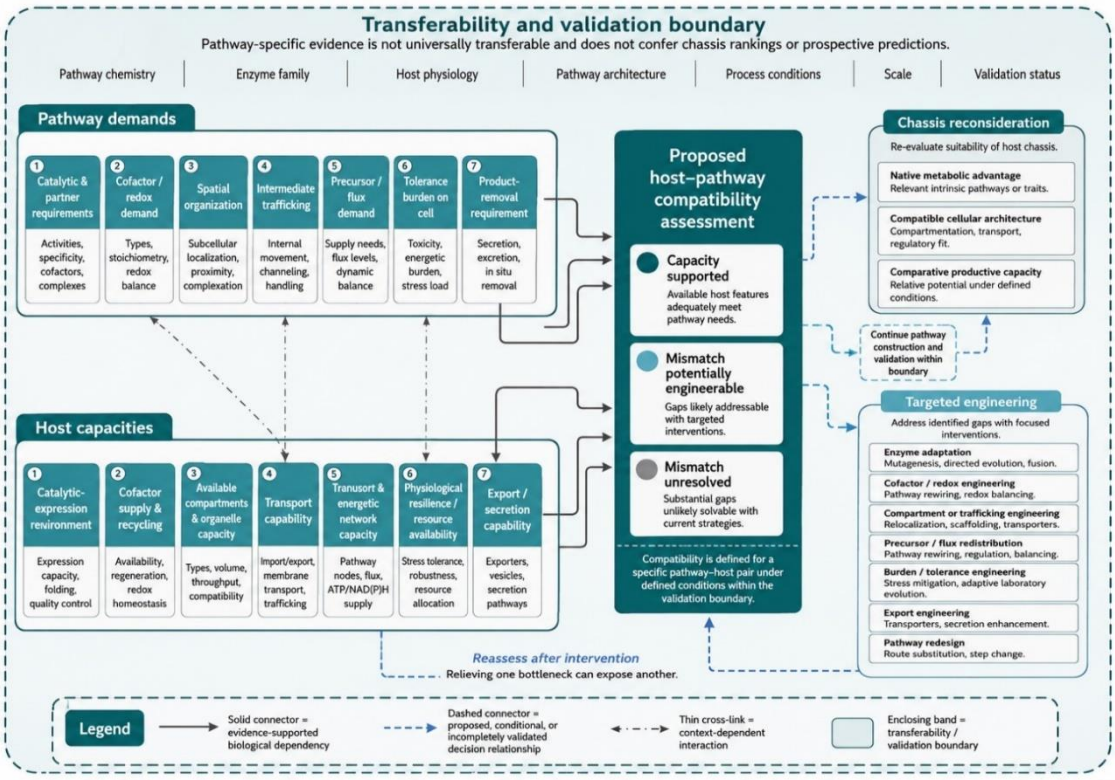

Figure 2. A demand–capacity compatibility architecture for pathway diagnosis, chassis comparison, and iterative engineering

Using compatibility to guide chassis choice

Chassis selection is often framed as a choice among familiar production organisms, but the compatibility perspective changes the question from "Which host is best?" to "Which host supplies the most relevant capacities for this pathway, and which missing capacities are realistically engineerable?" Complete cannabinoid biosynthesis in yeast illustrates that a chassis can support chemically complex plant metabolism after substantial host and pathway refactoring [26]. That success establishes feasibility for a particular engineered system; it does not establish yeast as an intrinsically compatible host for all plant pathways.

The same limitation applies within natural-product classes. Engineered production of strictosidine and related analogues demonstrates that yeast can accommodate a tailored alkaloid pathway after appropriate enzyme and pathway design [27]. It would nevertheless be unsafe to infer that all benzylisoquinoline, monoterpene indole, tropane, or other alkaloid pathways impose equivalent demands. Compatibility assessment should therefore begin with pathway-specific requirements rather than taxonomic labels such as "plant pathway" or even broad chemical labels such as "alkaloid pathway."

Comparative evidence strengthens this conditional approach. Microbial cell factories differ in their capacities for different biosynthetic tasks [21]. Likewise, *Yarrowia lipolytica* possesses metabolic characteristics that can be advantageous for selected plant-natural-product chemistries [22]. Such differences make native precursor supply, organelle architecture, redox environment, membrane composition, tolerance, genetic tractability, and process

knowledge legitimate chassis-selection variables. None should be converted into an absolute ranking because advantages in one dimension can coexist with liabilities in another.

The proposed decision rule is therefore demand–capacity matching followed by an engineering-cost judgment. A chassis with a strong native match may be preferable when the pathway depends heavily on difficult-to-recreate physiology. A less naturally matched but more tractable host may remain preferable when the mismatches are well characterized and readily engineerable. **Table 3** converts this reasoning into a workflow while preserving unresolved states rather than forcing every candidate host into a winner–loser classification.

Table 3. Proposed workflow for compatibility-informed microbial chassis selection

Decision state	Question	Appropriate action	What must not be inferred
Strong apparent match	Are critical pathway demands already supported?	Prioritize experimental reconstruction	Production success is guaranteed
Localized engineerable mismatch	Is the missing capacity identifiable and tractable?	Engineer the specific deficit, then reassess	Other constraints are absent
Multiple coupled mismatches	Will one intervention plausibly create another constraint?	Use staged or multiplex testing	A single bottleneck explains failure
Unresolved mismatch	Is the failure mechanism distinguishable experimentally?	Measure before major redesign	Low output identifies the cause
Alternative chassis advantage	Does another host better match difficult pathway demands?	Compare hosts under equivalent evidence standards	The alternative host is universally superior
Persistent incompatibility	Do required adaptations exceed plausible host accommodation?	Consider pathway redesign or a different chassis	The pathway is intrinsically impossible

Note: This decision workflow is proposed as qualitative guidance. It does not assign universal weights, engineering-cost thresholds, or chassis rankings.

Engineering responses to different failure modes

A compatibility model is useful only if diagnosis changes engineering behavior. In benzylisoquinoline-alkaloid production, targeted optimization of pathway bottlenecks and process variables demonstrates how specific constraints can be addressed once they are sufficiently localized [28]. The broader principle proposed here is response proportionality: intervene at the level supported by diagnostic evidence rather than applying every available metabolic-engineering tool to every low-producing strain.

Catalytic failure may justify enzyme replacement, sequence adaptation, expression tuning, partner optimization, or altered localization. Cofactor limitation instead calls for supply, recycling, or redox-balancing strategies; direct cofactor engineering shows why this should remain a separate intervention class [6]. If intermediates fail to reach their required locations, transporter or compartment engineering is more relevant than indiscriminate precursor amplification, as demonstrated by transport-focused tropane-pathway engineering [8]. Conversely, evidence of physiological burden should motivate resource, expression, or tolerance interventions rather than being treated automatically as a transport defect [9].

Export problems likewise require evidence that product handling is limiting. Exporter mining in yeast demonstrates that secretion can be deliberately improved when appropriate transporter candidates are identified [18]. However, forcing export of an intermediate that must remain intracellular could reduce rather than improve pathway performance. The direction of intervention therefore depends on pathway topology and product chemistry.

A more fundamental response is to change the pathway itself. A concise enzyme cascade for protoberberine production shows that alternative pathway architecture can improve manufacturing tractability [29]. Pathway redesign should consequently be considered when recreating native topology requires disproportionate cellular engineering. This does not imply that the shortest route is always preferable: native routes may provide selectivity, regulation, accessible cofactors, or desirable intermediates that an abbreviated cascade does not reproduce.

These response categories are deliberately nonexclusive. Improving an enzyme can expose cofactor demand; raising precursor flux can create toxicity; compartmentation can solve one localization problem while introducing a transport boundary. Engineering should therefore be coupled to recursive compatibility reassessment. In this

model, an intervention is not interpreted simply by whether titer rises. It is also informative because the pattern of rescue, nonresponse, or newly exposed limitation can refine the diagnosis of the host–pathway relationship.

Limits of generalization across pathways

The strongest limitation of any compatibility framework is the temptation to convert recurring engineering problems into universal rules. Reviews of plant-natural-product metabolic engineering document major achievements while also emphasizing persistent limitations in enzyme performance, pathway reconstruction, host engineering, and translation across systems [30]. The proposed model should therefore be read as an organizing framework for questions and experiments, not as evidence that all plant pathways share one architecture of incompatibility.

Chemical class is an obvious moderator. Triterpenoid production in yeast depends on pathway-specific relationships among precursor generation, membrane-associated reactions, enzyme activity, and downstream modification, and the corresponding engineering strategies are shaped by that chemistry [31]. Comparable specificity exists for alkaloids, glycosides, phenolics, and other natural products. A principle such as "cofactor compatibility matters" may travel across classes, while the relevant cofactor, enzyme partner, compartment, and acceptable intervention may not.

Host identity adds another boundary. Findings obtained in *S. cerevisiae* cannot automatically be transferred to *Y. lipolytica*, bacteria, or other platforms because native metabolism, membrane organization, organelles, redox balance, regulatory capacity, and process behavior differ. Even within one species, strain background and engineering history may alter the available capacity. Culture conditions can also change which incompatibility dominates by altering oxygenation, growth rate, redox state, substrate uptake, membrane composition, and stress.

Measurement uncertainty is equally important. Low pathway output can be observed more easily than its intracellular cause. Transcript abundance is not catalytic activity; protein localization does not prove metabolite access; intracellular accumulation does not by itself prove toxicity; increased product after an intervention does not isolate the mechanism through which improvement occurred. Where several modifications are introduced together, attribution becomes especially difficult.

The proposed model therefore requires prospective validation before it can support prediction. Multiplex experimentation provides a methodological route for testing interacting engineering variables rather than assuming independence [20]. Comparative host evaluation likewise provides a basis for testing whether compatibility judgments transfer across chassis [21]. Strong validation would require pathways and hosts not used to construct the framework, prespecified dominant-failure hypotheses, orthogonal measurements of enzyme activity, cofactors, localization, transport, flux and host physiology, and intervention-rescue tests capable of contradicting the initial diagnosis.

Failure to predict correctly should refine or reject parts of the framework rather than be absorbed post hoc as another "compatibility factor." The model will be scientifically useful only if its categories remain falsifiable, if unresolved states are preserved, and if pathway-specific exceptions are allowed to challenge rather than merely decorate the proposed synthesis.

Conclusion

Transferring the genes of a plant biosynthetic pathway into a microorganism transfers catalytic potential, not the cellular system that originally supported that potential. Functional heterologous biosynthesis depends on whether the new host can accommodate the pathway's demands for active enzymes, cofactors and redox support, intracellular organization, metabolite trafficking, precursor and energetic flux, physiological tolerance, and product removal. These demands can coexist and interact, making low production an outcome that requires diagnosis rather than a mechanism that can be assumed.

The pathway-compatibility model proposed here reframes this problem around the relationship between pathway demands and host capacities under defined conditions. It separates candidate incompatibilities before reconnecting them through interaction analysis, uses that comparison to inform chassis choice, and links diagnosed failure modes to proportionate engineering responses. Compatibility is treated as dynamic because interventions can reveal previously masked constraints, requiring reassessment rather than a one-time judgment.

The framework deliberately stops short of numerical compatibility scores, fixed weights, universal chassis rankings, or claims of prospective prediction. Individual pathway successes establish what can be engineered in

particular systems; they do not prove that the same architecture, host, or intervention will transfer to unrelated chemistries. The most useful next step is therefore not broader retrospective classification but prospective testing across pathway–host pairs with measurements designed to discriminate among competing failure mechanisms. If supported through such testing, compatibility analysis could become a disciplined front end to heterologous-pathway engineering. Until then, its value is more modest but still practical: it provides a structured way to ask what cellular capacities a transferred pathway actually needs before treating every unsuccessful reconstruction as simply another problem of insufficient gene expression or flux.

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