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Predicting Useful Microbial Biotransformations of Plant Natural Products by Matching Functional-Group Liability, Enzyme Class, Stereochemical Outcome, and Downstream Value

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ABSTRACT

Microbial biotransformation offers access to selective oxidation, glycosylation, halogenation, methylation, amination, and other modifications of structurally complex plant natural products, yet catalytic possibility alone provides a poor basis for choosing useful transformations. A candidate reaction may be chemically plausible but fail because the relevant enzyme does not accept the scaffold, generates an unsuitable regioisomer or stereoisomer, performs differently in a microbial host, produces competing products, or creates an intermediate with little downstream value. This article develops an original, evidence-bounded biotransformation prioritization model that treats route selection as a conditional matching problem rather than a reaction-enumeration exercise. Four analytical dimensions are integrated: functional-group liability as a context-dependent transformation opportunity; enzyme-class capability tempered by substrate recognition and catalytic architecture; regio- and stereochemical outcome as independent determinants of product utility; and downstream value defined by diversification potential, pathway compatibility, recoverability, or production relevance. The analysis further separates isolated-enzyme feasibility from whole-cell feasibility and distinguishes catalytic success from route-level usefulness. Evidence from late-stage enzymatic modification, cytochrome P450 oxidation, glycosyltransferases, halogenases, methyltransferases, heterologous enzyme expression, microbial pathway reconstruction, transport engineering, and stereoselective biocatalysis is used to establish the constraints that a useful prioritization framework must respect. The resulting model is proposed as a qualitative decision architecture, not a validated predictive score. Its principal limitation is the scarcity of systematically reported negative transformations and prospective cross-enzyme benchmarks needed to estimate generalizable success probabilities.

Keywords: Microbial biotransformation, Plant natural products, Enzyme selectivity, Late-stage functionalization, Metabolic engineering, Route prioritization

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Introduction

Plant natural products occupy chemical spaces in which high stereochemical density, multiple similarly reactive positions, dense oxygenation patterns, and limited synthetic accessibility can make selective modification difficult. Microbial and enzymatic engineering increasingly provide complementary ways to access such chemistry, particularly when enzyme engineering is coordinated with pathway and host optimization [1]. The practical significance of a biocatalytic reaction, however, depends on more than whether turnover can be detected. Modern biocatalysis has advanced through improvements in enzyme discovery, engineering, reaction design, and process integration, emphasizing that activity, selectivity, operational compatibility, and process performance

jointly determine whether a catalytic transformation becomes useful [2]. For plant-derived scaffolds, this distinction is especially important because the chemically most obvious position for modification need not be the position an enzyme recognizes, and the product easiest to form need not be the product that advances a synthetic or biosynthetic objective.

Microbial production of plant natural products similarly demonstrates that pathway reconstruction cannot be reduced to installing a sequence of nominally appropriate reactions. Precursor supply, heterologous enzyme performance, intracellular toxicity, transport, cofactor availability, competing metabolism, and scale-dependent physiology can each constrain a nominally complete route [3]. Synthetic-biology strategies for plant natural products therefore operate simultaneously at host, pathway, and individual-enzyme levels rather than at the level of reaction identity alone [4]. These observations create an important conceptual problem for biotransformation planning: candidate reactions are commonly generated from functional groups or known enzyme classes, while practical route selection requires decisions about an interacting substrate–enzyme–host–product system.

This article proposes that useful microbial biotransformations should be predicted through conditional matching, not through transformation enumeration. “Functional-group liability” is used here in a deliberately restricted sense: a structural feature creates a *candidate opportunity* for enzymatic conversion, but it is not treated as an intrinsic numerical probability of reaction. That opportunity must subsequently survive enzyme-class matching, substrate-recognition constraints, regio- and stereochemical assessment, microbial-context assessment, and evaluation of the product’s downstream purpose. The resulting framework is an original qualitative prioritization model. It does not claim prospective predictive accuracy, universal enzyme rules, or an implementation-ready scoring system. Instead, it organizes experimentally supported determinants into a route-selection logic intended to make explicit why a transformation that is chemically possible may nevertheless deserve low priority, whereas a less conspicuous reaction can become valuable because it creates controlled stereochemistry, unlocks a later pathway step, enables analogue diversification, or improves production compatibility.

Natural-product biotransformation as a selection problem

Enzymatic late-stage modification establishes the central opportunity behind natural-product biotransformation: structurally complex molecules can sometimes be modified selectively after much of their molecular architecture is already present [5]. This is attractive because the enzyme can exploit three-dimensional recognition rather than relying only on intrinsic functional-group reactivity. Specialized metabolism further expands the accessible catalyst space by supplying enzymes evolved to perform transformations that are uncommon, difficult, or poorly selective in conventional synthesis [6]. Together, these observations justify broad exploration of biotransformation opportunities, but they do not establish which opportunity should be pursued first.

The selection problem appears when a natural-product scaffold contains several plausible sites, several potentially relevant enzyme classes, and several acceptable but nonequivalent products. A chemically feasible oxidation may generate an unproductive regioisomer; a glycosylation may improve solubility but block a later enzyme; a highly selective reaction may depend on an expensive cofactor system; or an excellent isolated-enzyme conversion may fail when embedded in a whole-cell route. Compatibility among catalytic operations is already recognized as a central constraint when chemical and enzymatic steps are combined, because solvent, reagent, intermediate, and catalyst requirements can conflict even when each step functions independently [7]. The same route-level reasoning should apply when selecting among microbial biotransformations.

Accordingly, the proposed selection logic separates four questions that are often conflated. First, what structural feature creates a credible transformation opportunity? Second, what enzyme family or catalytic architecture could act on that opportunity with useful selectivity? Third, what product identity, regioisomer, or stereoisomer is likely to emerge? Fourth, does that product improve the intended route, diversification strategy, or production system? The first two questions concern opportunity and feasibility; the third concerns outcome control; the fourth concerns value. A candidate can therefore progress through some dimensions while failing another.

This framing also prevents enzyme promiscuity from being interpreted automatically as route advantage. Promiscuity enlarges searchable substrate space, but increased substrate acceptance can coexist with uncertain product distributions. Conversely, a narrow enzyme may be preferable if it produces the required stereochemical outcome reproducibly. The prioritization task is thus not to maximize the number of reactions considered. It is to reduce a broad reaction space into a smaller set whose chemical opportunity, biological feasibility, product control, and downstream consequence are mutually compatible.

Chemical features that create transformation opportunities

Structural inspection remains an appropriate starting point, but substrate structure does not itself determine usable enzyme reactivity. Biocatalyst discovery and engineering can expand catalytic scope beyond a native substrate relationship, meaning that apparently difficult chemistry may become accessible when an appropriate enzyme architecture is identified or constructed [8]. Conversely, an obvious functional group may remain unproductive if the scaffold cannot bind in an orientation compatible with catalysis. Whole-cell P450 experiments on flavonoids illustrate this distinction by showing that selective hydroxylation can be achieved in a microbial system while remaining dependent on the particular substrate–P450 context [9].

For oxidative transformations, the local environment around a candidate C–H bond or other reactive site therefore matters alongside nominal reaction class. Mechanistic and structural work with Rieske oxygenases demonstrates that substrate positioning and active-site organization can govern which site is hydroxylated [10]. Functional-group liability should consequently be interpreted as *conditional accessibility*: a candidate site becomes more credible when its steric environment, orientation, neighbouring groups, and enzyme-binding geometry are compatible with a known catalytic mode. It should not be assigned a universal reactivity rank independent of the enzyme.

Glycosylation provides a different form of opportunity. Some plant glycosyltransferases display sufficient acceptor promiscuity to create useful glycodiversity across natural-product substrates [11]. Yet whole-cell glycosylation also depends on intracellular nucleotide-sugar availability, so acceptor recognition by the transferase does not by itself establish production feasibility [12]. Similar distinctions arise in less common late-stage reactions. Engineering of the halogenase WelO5* demonstrated that asymmetric functionalization of complex soraphen scaffolds can be altered through enzyme engineering [13]. Engineered methyltransferases have likewise enabled selective C-methylation of otherwise unactivated alkenes in terpenoid substrates, extending the transformation space beyond reactions that would be predicted from conventional polar functional groups alone [14].

These examples support a classification based not simply on functional-group names but on the interaction between chemical opportunity and catalytic context. **Table 1** summarizes the manuscript-derived distinctions that should be preserved when a structural feature is converted into a biotransformation candidate.

Table 1. From structural opportunity to a biotransformation candidate: concept-and-evidence classification

Opportunity class	What creates the candidate	What must still be established	Principal failure mode
Oxidative site	Accessible C–H or oxidizable functionality within a recognizable scaffold	Enzyme-dependent site control and acceptable product distribution	Wrong position, over-oxidation, multiple products
Glycosylation site	Suitable nucleophile plus compatible acceptor geometry	Transferase recognition and intracellular donor supply	No conversion or pathway-incompatible conjugate
Halogenation site	Enzyme-accessible scaffold position	Regio- and stereochemical control within the target scaffold	Alternative site or stereochemical outcome
C-methylation opportunity	Catalytically accessible unsaturation or related acceptor	Enzyme architecture capable of non-native transfer chemistry	Narrow substrate scope
Proposed cross-class criterion	Structural opportunity supported by a plausible catalytic mode	Product identity, host feasibility, and downstream value	Chemical possibility mistaken for route priority

The classification deliberately avoids treating these categories as exhaustive or quantitatively ranked. Its purpose is to prevent the earliest stage of prioritization from collapsing distinct evidence questions into a single judgment of “reactivity.” A structural feature can justify searching a relevant enzyme class; it cannot, by itself, establish either the product or the usefulness of producing it.

Enzymatic and microbial determinants of reaction outcome

Once a plausible transformation opportunity is identified, enzyme class provides a useful reaction prior but remains an incomplete predictor of outcome. Microbial cytochrome P450 repertoires encompass oxidation chemistry relevant to pharmaceuticals and natural products, yet individual P450s differ markedly in substrate

acceptance, regioselectivity, stereoselectivity, redox-partner requirements, and operational behavior [15]. The distinction between **class** capability and enzyme-specific outcome is therefore fundamental. "P450-compatible oxidation" is a reasonable hypothesis; a particular hydroxylated product is a stronger claim requiring enzyme- and substrate-level evidence.

The microbial host adds another layer. Plant enzymes transferred into yeast can encounter differences in expression, subcellular localization, membrane environment, precursor context, cofactors, redox partners, and protein processing. Strategies described as "yeastizing" plant enzymes address precisely this mismatch between native biochemical capability and heterologous performance [16]. Consequently, enzyme feasibility should be represented twice in a prioritization model: first as intrinsic catalytic plausibility and then as host-conditioned feasibility. These are related but not interchangeable judgments.

Pathway reconstruction makes the importance of this distinction visible. Reconstitution of early paclitaxel biosynthesis revealed a complex oxidative network in which pathway enzymes can generate multiple products and context-dependent chemical outcomes rather than behaving as idealized one-enzyme/one-product modules [17]. Microbial architecture can also change what is feasible. A tripartite co-culture has been used to distribute plant phenylpropanoid biosynthesis across microbial partners, showing that a difficult pathway may sometimes be enabled by allocating functions among hosts rather than forcing every transformation into one cell [18]. Product handling imposes another independent constraint: engineered metabolite trafficking can permit secretion of terpenes that are otherwise poorly exported from yeast [19]. A transformation whose product cannot be transported, tolerated, or recovered may therefore have less process value than its catalytic performance implies.

Stereochemistry requires explicit treatment rather than being hidden within a general notion of "selectivity." Structure- and data-driven transaminase engineering has shown that catalytic activity and stereoselectivity can be improved as related but separable properties [20]. This distinction generalizes as a decision principle even though the specific transaminase models cannot be assumed to transfer to plant-natural-product enzymes. Conversion, regioselectivity, and stereoselectivity answer different questions: whether a reaction occurs, where it occurs, and which three-dimensional product is formed.

The combined evidence therefore supports a multilevel outcome model. Chemical structure constrains what might be attacked; enzyme architecture determines how a substrate can be recognized and transformed; the host alters whether that catalytic potential is expressed; pathway context introduces competing reactions and resource dependencies; and transport or recovery determines whether the product remains operationally useful. The proposed prioritization framework consequently treats microbial reaction outcome as a conditional system property rather than a lookup from functional group to enzyme class.

Defining useful rather than merely possible transformations

A transformation becomes useful only when its product advances an intended chemical or biotechnological objective. This criterion is stricter than catalytic success. Engineered yeast has been used to produce cannabinoids together with unnatural analogues, demonstrating that pathway engineering can create value through controlled access to chemical variants rather than simply reproducing a native metabolite [21]. Reconstruction of medicinal tropane-alkaloid biosynthesis in yeast illustrates a different form of usefulness: individual transformations acquire significance because they connect otherwise inaccessible stages of a complex biosynthetic route [22]. In both cases, value is relational. It depends on what the transformation makes possible afterward.

Production provides another route-dependent definition of utility. Engineering yeast cell factories for bioactive isoflavonoids shows that a transformation may contribute value because it supports a broader product family within a functioning production architecture [23]. Conversely, chemically modest reactions can have disproportionate pathway importance. In limonoid biosynthesis, identification of a carboxylesterase-dependent protection/deprotection strategy improved pathway progression and provided mechanistic insight into later structural formation [24]. Such cases caution against ranking candidates only by apparent reaction novelty. A deprotection, conjugation, or apparently simple redox adjustment may deserve higher priority than a more unusual transformation if it enables downstream enzymes, stabilizes an intermediate, redirects flux, or exposes the functionality required for later chemistry.

The proposed model therefore defines usefulness across four nonexclusive outcomes. A transformation may be valuable because it expands diversification space, creates the required regio- or stereochemical product, improves compatibility with subsequent pathway operations, or contributes to recoverable production. These outcomes should not be collapsed into one universal objective. A medicinal-chemistry program seeking analogues may

rationally prioritize diversity, whereas a cell-factory program may favor pathway continuity, host tolerance, secretion, or reduced product heterogeneity.

This distinction creates a practical rejection rule. Detectable conversion should not automatically advance a candidate. Low regioselectivity, an unwanted stereoisomer, difficult product recovery, host incompatibility, formation of unstable intermediates, or obstruction of the next pathway step can all reduce priority even when catalytic activity is substantial. Conversely, modest conversion may justify optimization when the product has unusually high route value. The model therefore treats catalytic feasibility as necessary evidence but not as sufficient evidence of usefulness.

The resulting logic is summarized in **Figure 1**, which separates transformation opportunity, catalytic outcome, microbial feasibility, route value, and the boundary between evidence-supported feasibility and the proposed prioritization interpretation.

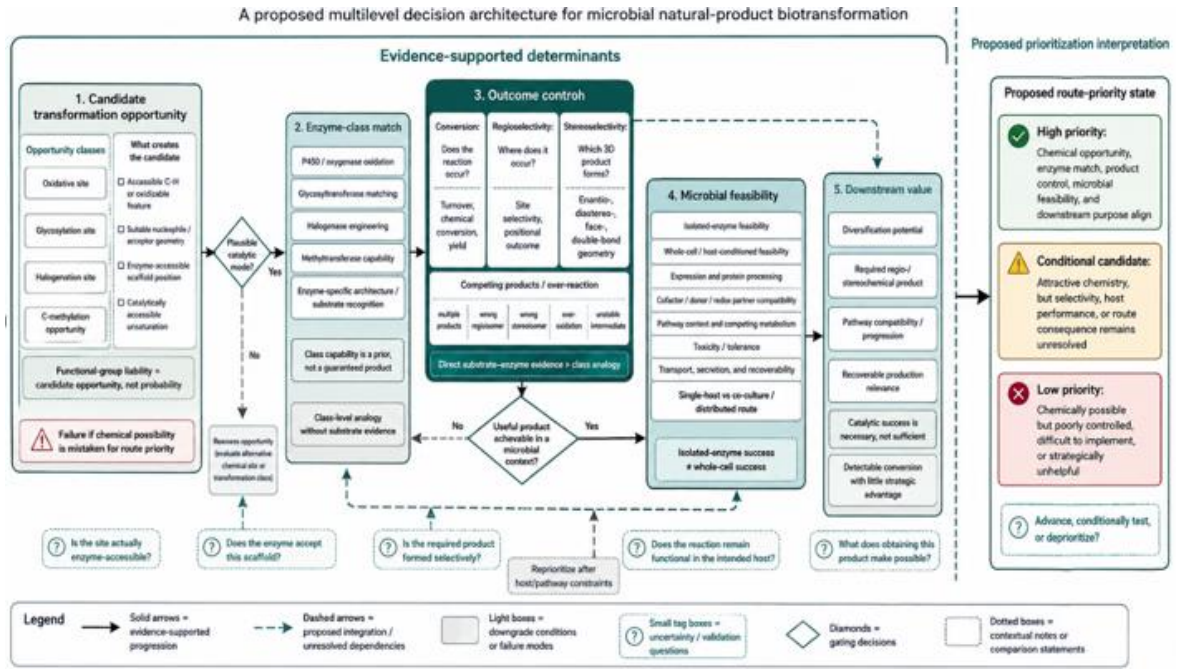

Figure 1. From chemical opportunity to route-level utility: a proposed multilevel decision architecture for microbial natural-product biotransformation

A prioritization model for route selection

The proposed model begins with candidate generation, but candidate generation and candidate prioritization are deliberately separated. Structural and reaction-class knowledge can nominate plausible transformations; it cannot establish their rank. Computational approaches already show that biological search spaces can be reduced by combining sequence, pathway, or other contextual information and then testing the nominated candidates experimentally. Machine-learning identification of missing links in plant-alkaloid biosynthesis provides such a precedent: prediction functioned as a way to prioritize plausible biological relationships, while experimental confirmation remained necessary [25]. The same epistemic discipline should govern microbial biotransformation prediction.

Candidate assessment is proposed to proceed through five linked dimensions. Chemical opportunity asks whether the scaffold presents a credible site or functionality for an enzymatic transformation. Enzyme match asks whether a catalyst class and, preferably, a specific enzyme architecture can recognize that opportunity. Outcome control evaluates conversion, regioselectivity, stereoselectivity, and competing products. Microbial feasibility asks whether the catalytic system can operate in the intended host or community context. Downstream value asks whether the resulting product advances diversification, pathway progression, recoverability, or production. These dimensions are not additive scores; a severe failure in one can outweigh favorable evidence in the others.

The need for this nonadditive logic is illustrated by enzyme multifunctionality. A cytochrome P450 involved in Taxol biosynthesis has been shown to catalyze functionally distinct chemistry associated with oxetane-ring formation, demonstrating that an enzyme-family label can conceal a more complex catalytic repertoire than a one-

reaction lookup would suggest [26]. Conversely, detailed functional and structural analysis of glycosyltransferases underlying lycibarbarspermidine glycodiversity shows that enzyme–substrate matching can be strongly organized by structural determinants [27]. Together, these cases argue for treating enzyme class as a prior that must be refined by enzyme-specific structural or empirical evidence.

Priority should therefore be expressed qualitatively. A high-priority candidate has a credible chemical opportunity, an enzyme or enzyme family with directly relevant catalytic precedent, acceptable expected regio-/stereochemical control, a plausible microbial implementation, and a clearly specified downstream purpose. A conditional candidate has attractive chemistry but unresolved selectivity, host performance, or route consequence. A low-priority candidate may be chemically possible yet poorly controlled, difficult to implement, or strategically unhelpful. These are proposed decision states, not calibrated probabilities.

The model further requires that an uncertainty tag travel with each decision. Direct substrate–enzyme evidence should carry greater interpretive weight than enzyme-class analogy; whole-cell evidence should carry greater implementation relevance than isolated-enzyme feasibility; and confirmed downstream compatibility should be distinguished from assumed usefulness. **Table 2** converts these principles into a comparative analytical matrix intended for route triage rather than numerical scoring.

Table 2. Proposed qualitative decision states for prioritizing microbial transformations of plant natural products

Decision dimension	Evidence pattern favoring priority	Downgrade condition	Validation question
Chemical opportunity	Accessible feature plus relevant catalytic precedent	Reactivity inferred only from functional-group presence	Is the target site actually enzyme-accessible?
Enzyme match	Direct or structurally persuasive substrate–enzyme evidence	Class-level analogy without substrate evidence	Does the enzyme accept this scaffold?
Product control	Desired regio- and stereochemical outcome is plausible	Multiple, incorrect, or unstable products likely	Is the required product formed selectively?
Microbial feasibility	Host, donor/cofactor, transport, and pathway context are compatible	Expression, resource, toxicity, or export constraints	Does the reaction remain functional in the intended host?
Downstream value	Product enables diversification, pathway progression, recovery, or production	Conversion yields little strategic advantage	What does obtaining this product make possible?
Proposed decision state	Multiple dimensions align without a critical unresolved gate	One severe incompatibility dominates otherwise favorable evidence	Advance, conditionally test, or deprioritize?

Note: Decision states and their integration are proposed conceptual synthesis; they are not experimentally calibrated scores or thresholds.

Applications in diversification and production

The framework is intended to support two overlapping but distinct applications. In diversification, the objective is not necessarily maximum biosynthetic flux but controlled access to derivatives whose production by conventional chemistry may be cumbersome. Microbial or enzymatic transformations can be particularly attractive when they introduce late-stage oxidation, glycosylation, halogenation, methylation, or stereochemically controlled functionality while preserving a complex scaffold. The prioritization question is then which transformation offers the highest likelihood of generating analytically identifiable, structurally useful products rather than the largest number of detectable derivatives.

In production, the optimization target changes. A reaction has value only insofar as it supports pathway completion, acceptable flux, host performance, and product recovery. Recent reconstruction of protoberberine and benzophenanthridine alkaloid production in engineered yeast demonstrates how coordinated metabolic engineering can extend microbial synthesis to complex plant-alkaloid families [28]. Such results do not mean that every installed transformation contributes equally. Within a long pathway, a modest but selective step that prevents intermediate loss or connects two productive modules can be more valuable than an unusually versatile catalyst whose products cannot be efficiently consumed downstream.

The relevant production unit may also exceed a single cell. Microbial production of vinblastine has been demonstrated through a distributed supply architecture, illustrating that system-level usefulness can arise from coordination among biosynthetic modules rather than identification of one globally optimal host or enzyme [29]. This expands the meaning of microbial feasibility within the proposed model. A transformation that performs

poorly in a monolithic pathway might remain attractive if pathway partitioning, co-culture, intermediate exchange, or modular production changes its operational context.

Diversification and production should therefore not share an automatic ranking. A promiscuous catalyst may be desirable for analogue generation yet undesirable in a manufacturing route requiring a single defined intermediate. Conversely, a highly specific transformation may contribute little chemical diversity but be essential to efficient pathway progression. The downstream-value dimension must be defined before candidates are ranked, because the same substrate–enzyme combination can occupy different priority states under different objectives.

In practical use, the model is best interpreted as a triage device. It can direct experimental effort toward transformations in which the chemistry, catalyst, product identity, microbial context, and intended use are mutually coherent. It may also expose where the decisive uncertainty lies—for example, whether a promising candidate requires enzyme screening, stereochemical confirmation, host engineering, transport optimization, or downstream-pathway testing before further route investment is justified.

Uncertainty and predictive limits

The principal limitation of any generalized biotransformation-prioritization scheme is heterogeneity. Plant-natural-product pathways differ in substrate class, enzyme evolution, compartmentation, cofactor requirements, host compatibility, toxicity, transport, and process behavior. Critical assessment of metabolic engineering for plant natural products continues to identify unresolved enzyme and pathway knowledge, heterologous-host limitations, and translation challenges despite substantial synthetic-biology progress [30]. A framework that ignores this heterogeneity would convert useful qualitative priors into false precision.

Functional-group similarity is one major source of over-extrapolation. Two molecules may contain apparently equivalent oxidizable positions while presenting different steric environments, binding orientations, electronics, or conformational constraints. Likewise, two enzymes placed in the same family may differ enough in active-site architecture to generate distinct products. The framework therefore cannot assign transformation probability from substructure alone. Chemical features should initiate a search for enzyme evidence, not substitute for it.

Negative evidence is another problem. Published biotransformation literature disproportionately communicates successful or optimizable reactions. Failed substrate screens, weak conversion, unresolved products, unstable intermediates, poor heterologous expression, and unsuccessful host transfers are less systematically available. A model developed mainly from positive examples can consequently overestimate the density of useful transformations. Prospective benchmarking should include failed candidates and competing products rather than asking only whether known successful transformations can be retrospectively ranked highly.

Analytical certainty is equally important. Conversion signals do not establish complete structural identity. When regioisomers, stereoisomers, sequential oxidation products, rearrangements, or conjugates are possible, route prioritization requires product assignments strong enough for the intended decision. An apparent transformation should not be promoted to a mechanistic or production claim merely because a mass change is compatible with the expected reaction.

Future validation should consequently occur at several levels. Candidate sets should contain both plausible positives and credible negatives; enzyme-class predictions should be tested across chemically diverse scaffolds; product identity and stereochemistry should be resolved explicitly; isolated-enzyme performance should be compared with whole-cell implementation; and downstream pathway consequences should be tested rather than assumed. For production-directed use, additional evaluation must address host burden, transport, pathway competition, reproducibility, and process transfer.

These requirements define the present framework's status. It is a proposed prioritization architecture supported by experimentally established determinants of biotransformation outcome, but its integrated decision logic has not been prospectively calibrated. Its intended value is therefore organizational: to make assumptions visible, separate levels of evidence, identify the weakest gate in a candidate route, and define what must be tested next. Demonstrating that it improves prospective transformation discovery or production decisions requires dedicated benchmarking beyond the evidence synthesized here.

Conclusion

Useful microbial biotransformation of plant natural products cannot be predicted reliably by identifying a reactive functional group and assigning a familiar enzyme class. Transformation choice is a multilevel decision in which

structural opportunity, enzyme–substrate recognition, regioselectivity, stereoselectivity, heterologous-host behavior, pathway context, transport, and downstream purpose can each change the value of the same nominal reaction.

The model proposed here reorganizes these determinants into a conditional route-prioritization architecture. Functional-group liability generates candidate opportunities rather than probabilities. Enzyme class provides a reaction prior rather than a guaranteed product. Catalytic activity is separated from regio- and stereochemical control, and isolated-enzyme feasibility is separated from whole-cell feasibility. Most importantly, a transformation is not defined as useful merely because it occurs: its product must contribute to a specified diversification, pathway, recovery, or production objective.

This architecture is deliberately qualitative. It does not assign universal weights, numerical thresholds, predicted success rates, or implementation-readiness classes. Its principal contribution is to expose the sequence of judgments that is otherwise compressed into a vague expectation that a natural product "should be biotransformable." That transparency also makes failure informative. Poor substrate recognition, competing products, host incompatibility, transport limitations, and downstream obstruction identify different experimental problems and therefore require different responses.

Prospective value will depend on datasets that include negative transformations, rigorous structural and stereochemical product assignment, cross-enzyme and cross-scaffold benchmarking, whole-cell confirmation, and pathway-level validation. Until such evidence accumulates, the proposed model should be used to organize candidate selection and experimental priorities rather than as a validated predictor. Its central principle is therefore conservative but actionable: prioritize the transformation whose chemical opportunity, catalytic control, microbial feasibility, and downstream consequence align most convincingly, while keeping uncertainty visible at every decision gate.

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