

where co-localized resistance genes, if present, can reveal the biochemical function of their products²². The therapeutic activities of many pharmaceutically relevant metabolites, however, differ from their native functions²³, and most biosynthetic pathways can, when appropriately reconfigured, yield entirely new—and, perhaps, more effective—therapeutic molecules^{12,24}.

Microbial systems have emerged as powerful platforms for the biosynthesis of natural products from unculturable or low-yielding organisms.^{25,26} Recent work showed that such systems can also permit the discovery and evolution of metabolic pathways with specific, therapeutically relevant activities (PCT/US2019/40896).

SUMMARY

Disclosed herein are systems, methods, reagents, apparatuses, vectors, and host cells for the discovery and evolution of metabolic pathways that produce small molecules that modulate enzyme function. For example, a microorganism is provided in which a first genetically encoded system links cell growth to the activity of a target enzyme and in which a second genetically encoded system—to be discovered or evolved—produces a metabolite that modulates the activity of the target enzyme. This disclosure applies this approach to a subset of target enzymes that post-translationally modify proteins, to metabolic pathways that produce phenylpropanoids or nonribosomal peptides, and to the discovery of cryptic metabolic pathways. Some aspects of this disclosure provide specific reconfigured or evolved pathways that produce specific modulators of enzyme activity, that yield improved titers of such modulators (relative to a starting pathway), and/or that exhibit reduced host toxicity (relative to a starting pathway). Metabolic products with specific inhibitory effects are also disclosed.

In one aspect, provided herein is a method for the discovery and evolution of metabolic pathways that produce molecules that modulate protein function, comprising: contacting a population of host cells that each comprise a protein of interest with a population of expression vectors that each comprise a metabolic pathway from a library of different pathways, wherein the host cells are amenable to transfer of the population of expression vectors; expressing each metabolic pathway of the population of expression vectors in the population of host cells, wherein a cell or subset of the population of host cells produce a detectable output when a metabolic pathway comprised in an expression vector of the population of expression vectors produces a product that modulates the protein of interest;

5 screening the cell or the subset of the population of host cells under conditions that enable
measurement of the detectable output in the cell or the subset of the population of host cells;
isolating the cell or the subset of the population of host cells that produce the detectable
output; isolating the expression vector that produces the detectable output that is higher than a
reference output of a reference vector that harbors a reference pathway, wherein the reference
pathway does not produce molecules with concentrations and/or potencies sufficient to
modulate the protein of interest in the cell or the subset of the population of host cells; and
characterizing one or more products of the metabolic pathway comprised in the expression
vector that produced the detectable output that is higher than the reference output of the
10 reference vector in the cell or the subset of the population of host cells.

15 According to one aspect, methods for the discovery and evolution of metabolic pathways that produce molecules that modulate protein function are provided. The methods include contacting a population of host cells that comprise a protein of interest, such as an enzyme of interest, with a population of expression vectors comprising different metabolic pathways, wherein the host cells are amenable to transfer of the population of expression vectors; expressing the metabolic pathways in the population of host cells, wherein a cell or subset of the population of host cells produce a detectable output when the metabolic pathway within said cell or population of host cells produces a product that modulates the protein of interest, such as the enzyme of interest; screening the population of host cells under