COMMENTARY

Co-evolve strategy for the discovery of genetic "dark matter"

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Natural products derived from plants, animals, fungi and bacteria serve as a rich source of medicinal compounds and have extensively been used in traditional and modern systems of medicine. From 1981 to 2019, 61% of the over 1200 small molecule drugs that entered the market were derived from natural products, underscoring their critical role in driving pharmaceutical innovation. As many natural products are relatively unexplored, they continue to provide a valuable resource for novel, therapeutic compounds in the fields of pharmacology and biotechnology, which is necessary to cope with various infectious diseases caused by important pathogens.[1] A key player in this field is the bacterium *Streptomyces*, known for its extraordinary capability in producing various natural products. Compounds derived from *Streptomyces* account for 30% of the top 20 best-selling small molecule drugs, such as tetracycline, avermectin, doxorubicin, and rapamycin, which are used as antibiotics, anti-tumor agents, or immunosuppressant. Further exploration into the *Streptomyces* genome has revealed an abundance of transcriptionally silent biosynthetic gene clusters, which are called genetic "dark matter". The potential of engineering *Streptomyces* for enhancing the production of current products as well as activating the dormant genes offers a beacon of hope in decreasing the price of current small molecule drugs and developing new drugs.

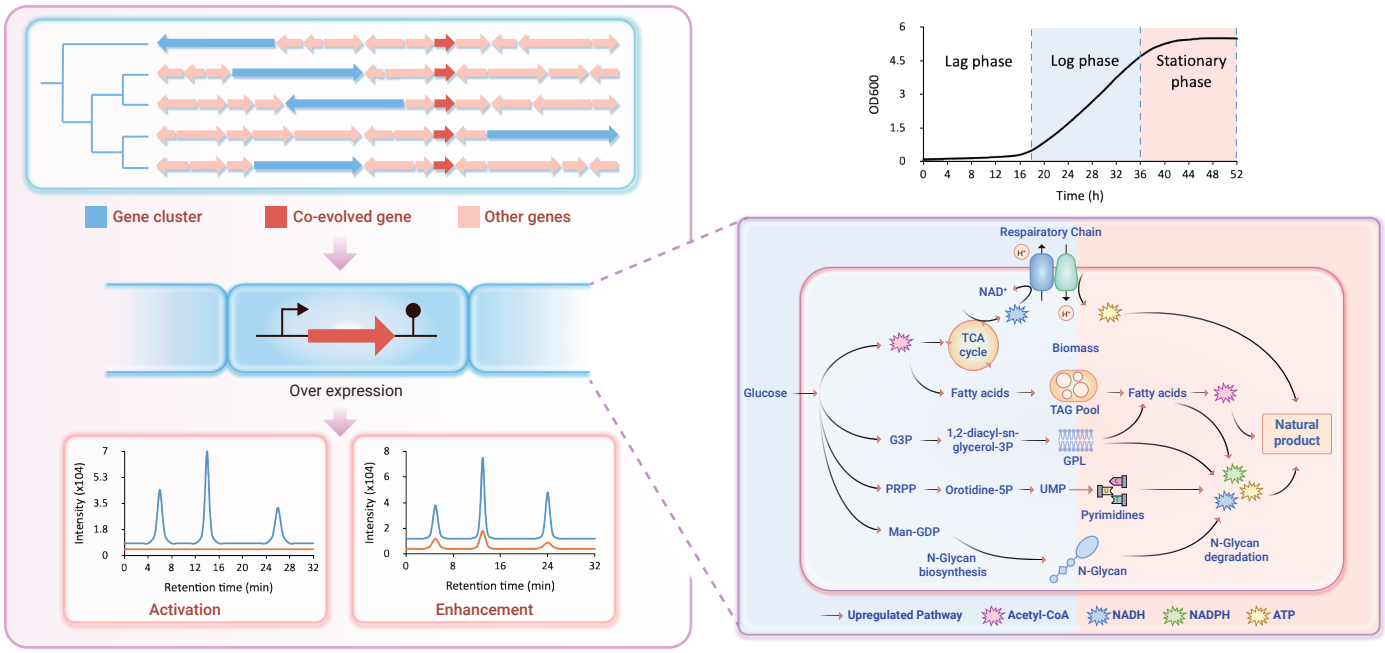

Figure 1. Illustration of the co-evolve strategy workflow for natural products discovery and overproduction (left) and the underlying mechanism for natural product overproduction resulted from introduction of the PQQ gene cluster in Streptomyces coelicolor (right). TAG: triacylglycerol; GPL: glycerol-phospholipid.

The unique metabolic and physiological characteristics of different strains complicate the development of a universal engineering strategy in *Streptomyces*. Biosynthesis of natural products usually occurs in stationary phase and is regulated under complex network. Genetic differences among strains further complicate efforts to understand universal metabolic and regulatory patterns. For instance, in *Streptomyces coelicolor*, the global regulator AbsA2 controls the biosynthesis of Ca^{2+} dependent antibiotic, actinorhodin and undeylglobin;[2] however, the absence of this regulator gene in *Streptomyces albus* indicates a different regulatory pattern in this strain. Consequently, a comprehensive understanding of universally applicable regulatory genes and their impact on secondary product biosynthesis remains a challenging and ongoing field of research.

Current strategies for engineering natural product biosynthesis are grounded in detailed knowledge of metabolic and regulatory mechanisms. One interesting discovery revealed that triacylglycerol (TAG) accumulates during the growth phase, which subsequently degrades into acetyl-CoA to fuel polyketide biosynthesis in the stationary phase. This finding led to the development of a method to enhance TAG degradation, which significantly increased the production of four different antibiotics.[3] However, creating new methods based on in-depth understanding of these mechanisms often requires considerable time and effort. This is because it involves long cultivation periods and complex handling procedures, which can slow down the development of innovative engineering strategies for *Streptomyces*. These challenges highlight the need for more efficient approaches to overcome the barriers posed by the complex nature of natural product biosynthesis.

The concept of 'co-evolution' originated from the investigation of secondary metabolic gene clusters in the gram-positive bacterium, *Streptomyces*, which is ubiquitous in diverse environments like soil, water, and plant surfaces. Due to nutritional scarcity and immobility, *Streptomyces* has evolved to generate antibiotics for self-defense against predators and competitors. Studies hypotheses a nuanced relationship between different biosynthetic gene clusters, which did not evolve independently but instead seemed to co-evolve. Extensive genomic studies and atlas analyses of biosynthetic gene clusters have revealed that certain bacterial and fungal taxa are more adept than others at producing a diverse array of natural products, hinting at the existence of productivity-related key genes.[4] Identifying

these essential genes could drive the development of metabolic engineering and synthetic biology strategies to boost production and expedite the discovery of invaluable molecules.

Research teams from the Shenzhen Institute of Advanced Technologies, University of California, Berkeley, and Shenzhen Bay Laboratory collaborated to investigate the genes co-evolved with natural product proficiency.[5] After analyzing 202 *Streptomyces* genomes from NCBI GenBank using pangenomic analysis, 597 genes co-evolved with polyketide gene cluster proficiency were identified. They used the gene cluster of pyrroloquinoline quinone (PQQ) as a proof of concept to assess its impact on natural product biosynthesis. Introducing the PQQ gene cluster into 11 *Streptomyces* wild type strains and 2 industrial *Actinomycetes* strains was found to significantly boost the natural product yield, enhancing at least 16,385 compounds including 36 known natural products with notable applications such as anti-bacterial, anti-fungal or anti-tumor properties.

The research also identified several silent gene clusters upregulated following the PQQ introduction, suggesting that PQQ not only can enhance the biosynthetic rate of known natural products but also stimulate the production of potentially new compounds. Further investigation exhibited the emergence of new activity against crucial clinical infective strains, highlighting the potential for discovering antibiotics with promising bio-activities. A multi-omic analysis post PQQ gene cluster introduction showed elevated ATP and key cofactor levels, as well as a surge in TAG degradation into acetyl-CoA, which is the key precursor to many natural products.

The alterations induced by PQQ in this study align with the changes in mitochondrial respiration in mammalian cells caused by PQQ, as reported in previous studies. However, unravelling the mechanistic details underlying these effects on natural product biosynthesis necessitates further research. It was noted that the *pqq* gene cluster in *Streptomyces* often correlates with carbohydrate-active enzymes such as chitinases, along with some dehydrogenases and catalases. This suggests that PQQ may also function in these catabolic pathways. It is possible that PQQ functions through binding to its target proteins, techniques such as photo-affinity chromatography or drug affinity responsive target stability can be employed to pinpoint PQQ's intracellular protein target and fully elucidate the internal mechanism of the PQQ gene cluster in *Streptomyces*.

This study provided an assessment of the 'co-enzyme' clade genes, whilst the impact of the other 586 co-evolved genes remains untested. Testing all co-evolved genes is essential to gain a comprehensive understanding of co-evolved genes' interplay with natural product biosynthesis. Despite the PQQ gene cluster's multiple effects on metabolism, a precise correlation between co-evolved characteristics and specific BGCs' productivity has not been elucidated, consequently impeding the prediction of optimal co-evolved candidates for a particular cluster. Leveraging bioinformatics tools, including machine learning algorithms, combined with high throughput evaluation could dramatically improve the accuracy of predictions concerning these genes' influence on the biosynthesis of targeted compounds, therefore facilitating more precise genetic interventions.

Work with *Streptomyces* is often delayed due to the slow growth rates, intricate handling procedures, and genetic heterogeneity. In the future, standard and automated methods for handling *Streptomyces* need to be developed to address these issues, alongside super-hosts that can offer higher growth rates, shorter fermentation times, and increased homogeneity.

In conclusion, this study utilized pan-genomic analysis on *Streptomyces* species to search for genes that co-evolved with natural product biosynthetic gene clusters. The association of PQQ with biosynthetic proficiency was confirmed in 11 *Streptomyces* strains and two other industrial *Actinomycetes* strains. This research provides innovative engineering strategies using big-omic data in natural product and metabolic engineering field and highlights targets for developing platforms for polyketide biosynthesis production and drug discovery.

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X.W. and X.L. were responsible for conceptualization of the original draft and figure. X.W., N.C. and X.L. wrote and edited the manuscript. Z.L. was responsible for the figure. All authors contributed to the article and approved the submitted version.

DECLARATION OF INTERESTS

X.L. is an Editorial Board member of The Innovation Life. He was blinded from reviewing or making final decisions on the manuscript. The article was subject to the journal's standard procedures, with peer review handled independently of this Editorial Board member and their research groups. The other authors declare that they have no conflicts of interest.