# Review of genetics-free high-throughput approach for activating production of cryptic microbial secondary metabolites

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## Abstract

Natural products account for a significant fraction of approved antibiotics, anti-inflammatory and anti-cancer drugs. Produced by complex biosynthetic pathways encoded by biosynthetic gene clusters (BGC), natural products remain a difficult class of compounds for study. Specifically, genome sequencing efforts have revealed many putative BGC with unknown secondary metabolites. Laboratory culture experiments, however, have failed to induce the expression of many BGC, where the expression level was either zero or very low levels under standard laboratory growth conditions. Different methods have been utilized in eliciting the expression of such silent BGC; however, most of the approaches either rely too heavily on time consuming genetic manipulations or require significant efforts in systematic study of culture conditions. Given the diversity of natural products and their chemical complexity that present particular challenges to instrumental analysis, difficulty also exists in profiling secondary metabolites. Thus, there is a need to develop high throughput facile methods for inducing the expression of silent BGC and visualizing the secondary metabolome generated. Writing in *Nature Chemical Biology*, Xu and coworkers described a genetics-free approach for high throughput activation and visualization of the cryptic metabolome of different natural products producing bacteria. Specifically, high throughput elicitors screening was coupled to imaging mass spectrometry where the latter help visualize the set of secondary metabolites activated by different inducers. By profiling for secondary metabolites from three bacterial species: *Pseudomonas protegens* Pf-5, *Streptomyces*, and actinomycetes, the work demonstrated the utility of the approach for different bacterial species and useful natural products were isolated from individual strains. For example, a novel lasso peptide was isolated from *Streptomyces*, while an antimicrobial and antiviral compound was obtained from actinomycetes. However, the paper would benefit from more detailed explanation of the methodology of high throughput elicitors screening as well as the mechanisms of action of different elicitors (inducers). More importantly, the high throughput screen of useful elicitors could be done at different growth conditions to help expand the secondary metabolome of cells that could be profiled. Collectively, a useful approach for high throughput activation and profiling of secondary metabolites in different bacterial species without the use of genetics methods was proposed and validated. This fills an important gap in the need for fast and high throughput methods for activating silent BGC. Use of imaging mass spectrometry for high throughput profiling of the secondary metabolites produced also expands the analytical toolkit of the field. Overall, the proposed approach would find application in drug discovery efforts in profiling natural products from microbial sources.

***Keywords:*** natural products, secondary metabolites, *Streptomyces*, actinomycetes, silent biosynthetic gene cluster, heterologous expression, *Pseudomonas protegens*, lasso peptide, antimicrobial compounds,

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Writing in *Nature Chemical Biology*, Xu et al., articulated "A genetics-free method for high throughput discovery of cryptic microbial metabolites". The article sought to close an important experimental gap in developing rapid approaches for inducing the production of secondary metabolites from silent biosynthetic gene cluster (BGC). Such secondary metabolites, also known as natural products, have important medicinal uses.[1,2] Using imaging mass spectrometry (IMS) for readout of a high throughput screen of inducers used in activating production of cryptic metabolites, the authors successfully illustrated a faster approach for activating and profiling the secondary metabolome of bacterial species compared to conventional genetic reporter assay methodologies.[3-6] However, the paper would benefit from more detailed elaboration of the types of elicitors (inducers) used, their mechanisms of action, and the proportion of elicitors that successfully induced the secondary metabolome of the bacterial species. In addition, different growth conditions should be used for screening the elicitors library given that not all elicitors would be able to function at the same growth condition.

High throughput elicitor screening approach was used to assess a library of inducers for possible endogenous activation of secondary metabolites synthesis in bacterial species. This is followed by using highly sensitive IMS for profiling the secondary metabolites induced by each elicitor. The approach was validated through experiments conducted using carefully chosen model organisms spanning the bacterial domain, each with silent BGC with unknown secondary metabolites. In the first example, production of orfamides was sought from *Pseudomonas protegens* bacterium. Secondly, a novel lasso peptide was profiled from the secondary metabolome of a *Streptomyces* bacterial strain. Thirdly, novel metabolites with antimicrobial activities were profiled and biosynthesized from a rare actinomycetes bacteria. Putative elicitors were confirmed with large scale cultivation experiments. Detailed structural characterizations were carried out for the isolated secondary metabolites, yielding structural information useful for compound classification and functional studies. Finally, utility of the isolated compounds in inhibiting various Gram-positive bacteria and virus pathogens was demonstrated; thereby, helping identify a potential new class of antimicrobial compounds.

Previous work investigated the use of different genetics, growth and nutrient conditions for inducing the biosynthesis of natural products, but such approaches are inherently time-consuming.[3,7] What Xu and coworkers brought to the field is an alternative approach of using inducers to activate secondary metabolite biosynthesis, and searching through the library of inducers in a high throughput manner where multiple BGC could be activated by a single inducer. In addition, secondary metabolites provide direct readout of the experiment, in contrast to conventional indirect transcriptional and translational assay.

Given that visualizing the secondary metabolome typically require the use of time-consuming high performance liquid chromatography-mass spectrometry (HPLC-MS), demonstration of the use of IMS for profiling induced secondary metabolite production expands the analytical toolkit of the field to enable faster screening of inducers. More importantly, coupled to IMS, the 96 well format afforded the simultaneous screening of multiple inducers for secondary metabolites production. Organisms chosen for study were also representative of the microbial species with silent BGCs, and thus, yielded a dataset rich in new understanding of silent BGC and their encoded secondary metabolites. Finally, another plus point of the paper is that the authors extended the investigation to include the structural elucidation of the newly profiled cryptic metabolites and analysing their potential antimicrobial activity.

However, lack of clarity in explaining the elicitor screening approach such as the types of compounds in the library and their likely mechanisms of action obfuscate understanding of the approach. Additionally, use of 96 well plates for profiling the elicitors render uniform induction conditions for all elicitors, which might not be the case as different elicitors might work at different growth conditions. In addition, the study could be extended to include a larger set of growth conditions for maximising the potential of the different elicitors in inducing the production of secondary metabolites. Ion suppression in IMS might also preclude the detection and identification of some secondary metabolites; thereby, reducing the compound space attributed to a bacterial species. Finally, details on the proportion and types of elicitors effective in inducing the production of secondary metabolites were lacking in the paper. Such information would provide a clearer view

of the types of inducers able to elicit secondary metabolites biosynthesis, and their putative mechanisms of action.

Collectively, the authors proposed and validated a new genetics-free high-throughput experimental strategy for rapidly inducing and visualizing the production of secondary metabolites in bacterial species. The approach was validated with the isolation of novel secondary metabolites with antimicrobial activity. One weakness of the approach is in the use of uniform growth conditions for different elicitors (inducers) of secondary metabolome of cells where it was likely that different elicitors might work at different growth conditions. Overall, the new approach increases the speed and extent in which the secondary metabolome of microbial species could be activated and probed, which will likely find applications in drug discovery from microbial sources.

## Conflicts of interest

The author declares no conflicts of interest.


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