Reviving natural product and drug discovery pipelines with fungal artificial chromosome (FAC) technology

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ABSTRACT

We have developed the fungal artificial chromosome [FAC, or autonomously replicating *E. coli*- fungal shuttle bacterial artificial chromosome (BAC)] technology. The FAC system or technology enables unbiased capturing of 263 full-length large secondary metabolite (SM) gene clusters (each 100kb or larger) as individual FAC clones, from 6 sequenced fungal genomes for one-step transformation and heterologous expression. The FAC technology also enables precise SM gene and gene cluster editing and activating silent and cryptic SM gene clusters. We have demonstrated that the FAC technology is extremely effective for natural product and drug discovery by using a liquid chromatography-fourier transform mass spectrometer (LC-FTMS) with novel data analysis pipeline. This novel FAC platform provides a major advance in the study of fungal biosynthesis, enabling rapid detection of novel SMs, identification of their biosynthetic machinery, and dissection of their biosynthesis. As an example, three SM compounds: benzomalvin A, ophiobolin G, and a novel lipopeptide has been discovered from a FAC clone: AtFAC9J20 containing a super-SM gene cluster. This disruptive technology could revive natural product and drug discovery platform from sequenced fungi, fungal metagenomes and environmental microbial metagenomes.

Keywords: fungal artificial chromosome (FAC), secondary metabolite (SM) gene clusters, natural product, drug discovery.

INTRDUCTION

Fungal genomes each encode up to 70 secondary metabolite (SM) gene clusters ranging from 30 to 80 kb in size for pharmacologically important molecules such as lovastatin and penicillin. However, due to the difficulties of culturing and genetically manipulating, many fungi including the overwhelming majority of fungal-encoded chemical space remains untouched. With thousands of fungal genomes being sequenced, breakthrough techniques are needed to characterize these SM wealth.

MATERIALS AND METHODS

High molecular weight (HMW) genomic DNAs from the protoplasts of 6 sequenced fungi: *Aspergillus aculeatus*, *A. terreus*, *A. wentii*, *Fusarium solani*, *Penicillium expansum*, and *P. marneffei*, were prepared respectively, followed by cloning into fungal artificial chromosome [FAC, or autonomously replicating *E. coli*- fungal shuttle bacterial artificial chromosome (BAC)] vectors. Full-length SM gene cluster-containing FACs (SM-FACs) were identified by BAC end sequencing and PCR-screening and then the SM-FACs were transformed into *A. nidulans* strains for heterologous expression. SM gene and gene cluster editing were done by using the Red/ET based homologous recombination system. SM extracts derived from FAC-strains were then analyzed using LC-FTMS coupled novel data analysis pipeline. The AtFAC9J20 was selected for intensive study by gene editing and chemical analysis to confirm gene-product associations and dissect biosynthetic steps.

RESULTS AND DISCUSSION

We describe a revolutionary FAC technology which enables unbiased capturing of 263 full-length SM gene clusters as individual FAC clones from 6 sequenced fungal genomes for one-step transformation and heterologous expression. We demonstrate precise SM gene and gene cluster editing with FACs. FAC-strains analyzed by LC-FTMS were rapidly evaluated through XCMS spectral feature detection (≈5,000 features/FAC) and assignment of a FAC-score to each feature, leading to identification of 17 out of 56 FACs with high scoring features. Accurate mass comparison of top scoring features to databases of fungal SMs showed that 8 of these 10 FAC-strains likely produced novel SMs and one upregulated product of the host fungus. As an example, AtFAC9J20 produces three high scoring features. Two of these were known SMs, benzomalvin A and ophiobolin, but until now lacked identified gene clusters. The third was a lipopeptide which does not match any previously reported SM, to our knowledge.

To confirm the relationship between AtFAC9J20 and its three distinct products, deletants were prepared utilizing

facile *E. coli* gene/cluster editing tools as above. These deletants allowed identification of the NRPS and methyl transferase enzymes involved in benzomalvin biosynthesis, the terpene synthase involved in ophiobolin biosynthesis, and the hybrid NRPS-PKS enzymes involved in the production of the novel lipopeptide. Using data from deletions of the NRPS enzymes BenY and BenZ, as well as the methyl transferase BenX, we also propose for the first time the biosynthetic model of benzomalvins.

The FAC platform can be used to capture the entire set of intact SM gene clusters and/or pathways from any fungal species sequenced or even not sequenced yet, fungal metagenomes for heterologous expression in *A. nidulans*, as well as environmental microbial metagenomes for bacterial heterologous expression.

Wide application of the FAC and untargeted metabolomics pipeline should have a major impact on fungal natural products research and drug discovery in the near future. For example, the application of the FAC technology to a collection of over 200 Wisconsin *Aspergillus* strains collected by Dr. Martha Christensen, with an average of 50 SM gene clusters per strain and an estimated hit rate of >20%, would lead to validate the assignments of at least 2,000 compounds.

REFERENCES

[1] Bok JW, Ye R, Clevenger KD, Mead D, Wagner M, Krerowicz A, Albright JC, Goering AW, Thomas PM, Kelleher NL, Keller NP, Wu CC. Fungal artificial chromosomes for mining of the fungal secondary metabolome. BMC Genomics. 2015 Apr 29;16(1):343

[2] Clevenger KD, Bok JW, Ye R, Miley GP, Velk T, Chen C, Yang K, Gao P, Lamprecht M, Thomas PM, Islam MN, Wu CC, Keller NP, Kelleher NL. Fungal Artificial Chromosomes with Untargeted Metabolomics (FAC-MS) Drives Natural Product Discovery and Facile Dissection of Their Biosynthesis. In Preparation. 2016

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