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Antifungal genome mining and genetics in filamentous actinomycete bacteria isolated from local soils

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Abstract

Actinomycetes are gram positive, filamentous bacteria that produce useful antibiotics, antitumor agents, and agricultural products. A series of enrichments were undertaken to isolate actinomycetes from local soils, varying enrichment media, antibacterials, and soil treatments (including heat and CaCO₃). Isolates were characterized by 16S rDNA sequencing, phenotypic and morphological observations, and antibiotic production. The genetic tractability of select isolates was analyzed using a panel of integrating vectors derived from ΦC31, ΦBT1, and Oryzah. Phage using intergeneric conjugation. Further, a semi-degenerate multiplex PCR assay to detect QDTI genomic integrants was designed and tested for the first time. Finally, PCR screens were used to test if the isolates genetically encode for the production of Polycyclic Tetramate Macrolactams (PTM), a common class of antifungal natural products. We designed and tested PCR screens in silico that predicted specific PTM biosynthetic genes in order to predict PTM chemical variability arising from gene cluster diversity. PTM production from positive isolates was assessed using coupled liquid chromatography-mass spectrometry (LC-MS). Our results indicate that we have isolated a variety of Actinomycetes, many of whom produce antifungal and antibacterial compounds, which are genetically tractable with a subset predicted to produce PTM compounds.

Environmental Isolation

METHODS

- Dried local soils
- Heat shock (95°C) + CaCO₃ soil treatment
- Bioassays, intergeneric conjugation

RESULTS

Actinomycete Recovery Varies with Media and CaCO₃ Treatment

Intergeneric Conjugation

METHODS

- MS donor Z. cell with Streptomyces
- Select with antibiotics after 16h @30 C
- Partly conjugants by microscopy
- In vitro ΦC31 and ΦBT1 antibiotic recombination in Streptomyces strains

RESULTS

- 12/17 strains had bioactivity against E. coli (green negative)
- 27/27 strains had bioactivity against B. subtilis (green positive)
- 14/17 strains had bioactivity against S. epidermidis (fungi)
- 7/27 strains had bioactivity against C. albicans (fungi)

Conclusion: Environmental actinomycetes inhibited Gram + bacteria most frequently, followed by Gram − E. coli, with fewer strains inhibiting either of the yeast strains. Are antifungal producers rarer in nature?

Genome Mining

METHODS

- Prior Chiles PCR screen for juxtaposed flaA and flaB
- Chiles detected into PTM producers in our environmental strains
- On the other hand, our novel DH1/DH2 PCR assay detected PTM clusters from the same environmental strains

RESULTS

- Discovery of K33 as a new dehydro-3-hydroxylindole PTM producer
- 5, 5, 6 ring system PTMs require both DH1 and DH2 genes, while 5, 5, 6, 6 ring system PTMs require only DH2
- Two lines of evidence suggests K33 lacks flaA

Figure 9: PTMs and gene clusters encoding 5,5,6 and 5,6,5 ring variants

Figure 10: PTM primer annealing locations for Chiles, flaA, DHI, DH2 screens

Conclusion

- We optimized conditions for the enrichment culture of Actinomyces, and 16S rDNA sequencing provided genus level identification of our environmental isolates. Most were Streptomyces, but Kribbella, Rhodococcus and Nocardia were also isolated
- We were successful in integrating phage vectors (ΦC31, ΦBT1, and novel phage OzzyJ) into known Streptomyces strains and environmental Streptomyces isolates using intergeneric conjugation
- Through our analysis of PTM gene clusters, we found most PTM producing strains among our isolates lack a flaA gene. In addition, we developed a more effective PCR screen for detecting and characterizing PTM clusters in soil isolates using the DH1 and DH2 genes compared to previous screens that detect juxtaposed flaA and flaB PTM genes

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