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COMMUNICATIONS TO THE EDITOR

Distribution of Polyketide Synthase Genes in Bacterial Populations

Sir:

Modular polyketide synthases (PKSs) are multifunctional enzyme complexes that catalyze the biosynthesis in streptomycetes and related bacteria of many medically useful drugs. Enzyme activities within the complex catalyse polyketide chain extension by repetitive decarboxylative condensations between simple organic acids. The modular nature of PKSs render the genes that encode them amenable to rational genetic engineering; combining, deleting or repositioning PKS genes has generated novel products[1]. Attempts to build libraries of genetically modified polyketides for screening against pharmaceutically relevant targets could be hampered as the availability of genes from characterized biosynthetic pathways becomes limited. Bioprospecting for novel PKS genes, particularly from microorganisms previously under-represented in natural product screening collections, might address such limitations. Bacteria producing polyketides have been isolated from various regions throughout the World, but there have been no scientific reports into which ecosystems or geographical locations might prove more favorable. Here we report the results from a study looking at the distribution of modular PKS genes in non-filamentous bacterial communities isolated from different ecosystems and geographical locations within the United Kingdom.

Samples of loam, sand and clay soils were collected from the upper aerobic layer of soil profiles in Cornwall; a further loam sample was collected in Scotland. Soils were suspended (10^{-2} dilution) in Maximum Recovery Diluent by shaking at room temperature for 30 minutes at 250 rpm. Further soil dilutions were plated out onto Cystine-Lactone-Electrolyte Deficient (CLED) medium, chosen to reduce swarming of *Proteus* spp. and to highlight the appearance of bacteria due to the inclusion of Andrade indicator for the selection of lactose and non-lactose fermenters. Soil dilutions were also plated onto Tryptone Soya Agar. Media were supplemented with additional agar to $35\,g\,l^{-1}$, again to reduce the effects of swarming, and with the antifungal agents cyclohexamide and nystatin at final concentrations of $50\,\mu g\,ml^{-1}$ each. Isolation plates were incubated for 48 hours at 30°C. Isolates that had grown as discrete colonies were sub-cultured onto nutrient agar to facilitate colony hybridization using a non-radioactive probe specific for modular PKS genes. The probe was designed based upon the nucleic acid sequence homology between β-ketoacyl-ACP (KS) condensing enzymes in modular PKSs[2]. The KS from module 1 of the erythromycin producing PKS was used as a PCR template (GenBank accession number M63676, nucleotide position 2256 to 3614); amplification incorporated digoxigenin-11-dUTP (forward primer: 5′GAACCGGTCGCGGTCGTC3′, reverse primer: 5′CAGACCTTCCGCGCTGTCT3′). Labeling of the DNA probe, colony blot hybridizations and detection protocols were performed according to the manufacturer (Boehringer Mannheim GmbH, Germany). Colonies which gave a positive reaction after hybridization with the probe where sub-cultured onto nutrient agar and then re-probed to discount any false positives.

A total of 630 non-filamentous bacteria were recovered from the four soil samples (Table 1). The two loam soils gave the highest total bacterial viable counts, which were not significantly different from each other despite the geographical location of the sampling sites. There was a significant reduction in the number of bacteria isolated from the clay ($p \leq 0.05$) and sand soils ($p \leq 0.05$) compared to the two loam samples. This observation was not unexpected since loam soil is nutrient rich, capable of supporting large and diverse microbial communities. The PKS genes were detected in all the communities studied, although of the 630 isolates only 50 (8%) carried the target gene of interest (Table 1). The largest bacterial populations (loam soils) showed the lowest gene frequency, these frequencies varying significantly between the two sample sites ($p \leq 0.05$). Variation in the distribution of the target gene was even greater between bacterial populations isolated from different habitats within the same geographical location (Cornwall). There was a significantly greater frequency of the target gene amongst bacteria isolated from the clay soil compared to bacteria isolated from loam ($p \leq 0.05$), even though the population of the clay was significantly smaller. The lowest gene frequency from this geographical location was observed in the bacterial population isolated from sand although there was no significant difference in this frequency compared to the bacterial population isolated from the loam soil collected at the Scottish sampling site.

Many polyketides exhibit antibiotic activity, it has been

Table 1. Distribution of modular polyketide synthase (PKS) genes in populations of non-filamentous bacteria.

Sampling location	Soil type	Number of bacteria (10^3 × CFU g^{-1} soil*) Recovered	Carrying PKS gene	% Bacteria carrying the PKS gene
Cornwall	Loam	26 ± 5.01	2.375 ± 3.02	9.0
	Clay	17 ± 7.14	3.125 ± 4.26	18.5
	Sand	9 ± 3.16	0.375 ± 0.74	4.0
Scotland	Loam	26 ± 8.78	0.375 ± 0.52	1.4

* Values are means from 8 isolation plates ± standard deviation

suggested that the ability to produce these compounds may offer a competitive strategy for control of nutrient supply in microbial communities[3]. Results from this study would support such a hypothesis; gene frequency was lowest amongst larger bacterial populations isolated from nutrient rich ecosystems (loam). Conversely, gene frequency was greatest amongst the smaller bacterial communities isolated from nutrient limiting ecosystems (clay and sand) where competition for nutrients presumably would be greatest[4]. It was beyond the scope of this present study to fully identify the non-filamentous bacterial isolates, however, microscopic examination of Gram stains revealed that both Gram positive and negative rods and cocci were found to carry the target gene of interest. This was an unexpected finding since PKS genes have never been described in Gram positive or negative cocci previously. Analysis of genome sequences reveals that PKSs can be encoded by low G+C content DNA (for example, the predicted type I PKS from *Bacillus subtilis*, GenBank accession number 16077068). It would be difficult to detect such sequences using the probe developed in this study since it is based on a high G+C content sequence. Future work will concentrate on testing gene probes capable of detecting low G+C content PKS genes. It is interesting to speculate that the products of such genes may be structurally quite distinct from those encoded by high G+C content DNA, despite both enzyme systems catalyzing presumably similar reactions. A more prudent bioprospecting strategy for novel PKS genes may be to access the total genetic diversity of soil microorganisms, including those of viable, yet un-culturable microbes through the isolation of total community DNA.

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