

Identification of Novel Open Reading Frames from Metagenomic Libraries Generated from Extremophilic Organisms: Application of Metagenomics and High Throughput Screening for Novel Enzyme Isolation

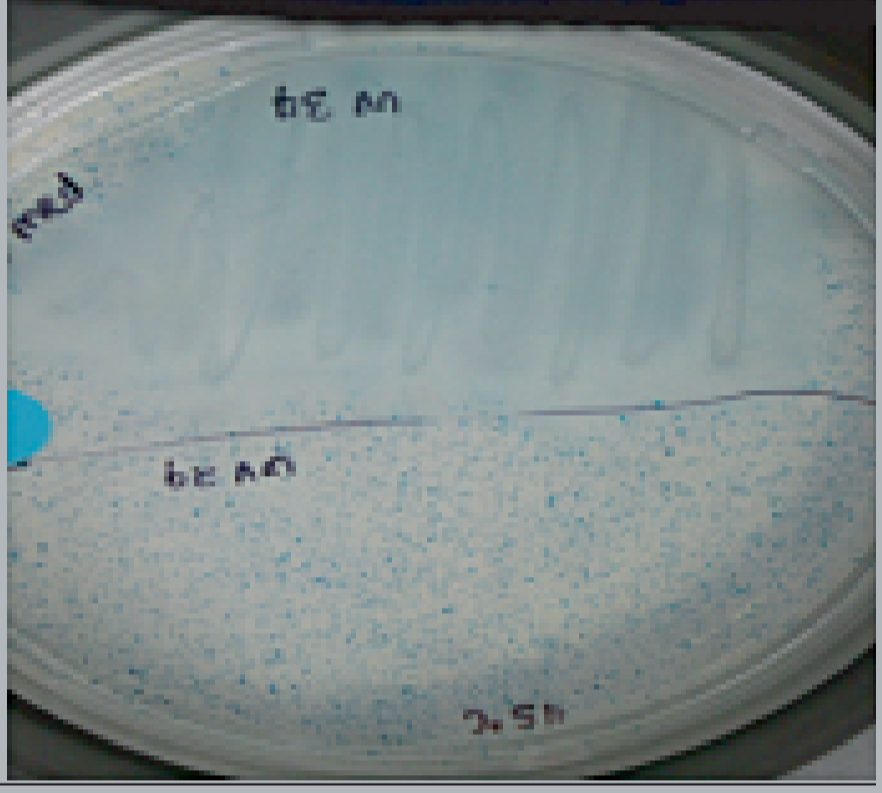
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INTRODUCTION

Isolating novel enzymes to enhance a commercial process is an area of active research. We have established screening methods to isolate genes for novel enzymes from extreme environments. Samples were obtained from four South African mines. Genomic DNA was isolated from these biofilms, and various metagenomic libraries generated. These libraries were in turn screened for industrially important enzymes, in particular proteases and lipases. Resultant hits had plasmid DNA isolated; this DNA was sequenced and analysed using BLAST.

Amylase

Positive



Negative

Agar Based Selection
Dye-linked amylose
Metabolism of dye

Transposase
Spo IVB
Duf 520

Nitrilase

Agar Based Enrichment:
Aliphatic & Aromatic Nitriles / amides
as sole nitrogen source (5mM)

Growth vs no growth

N-acetylmuramoyl-L-alanine amidase



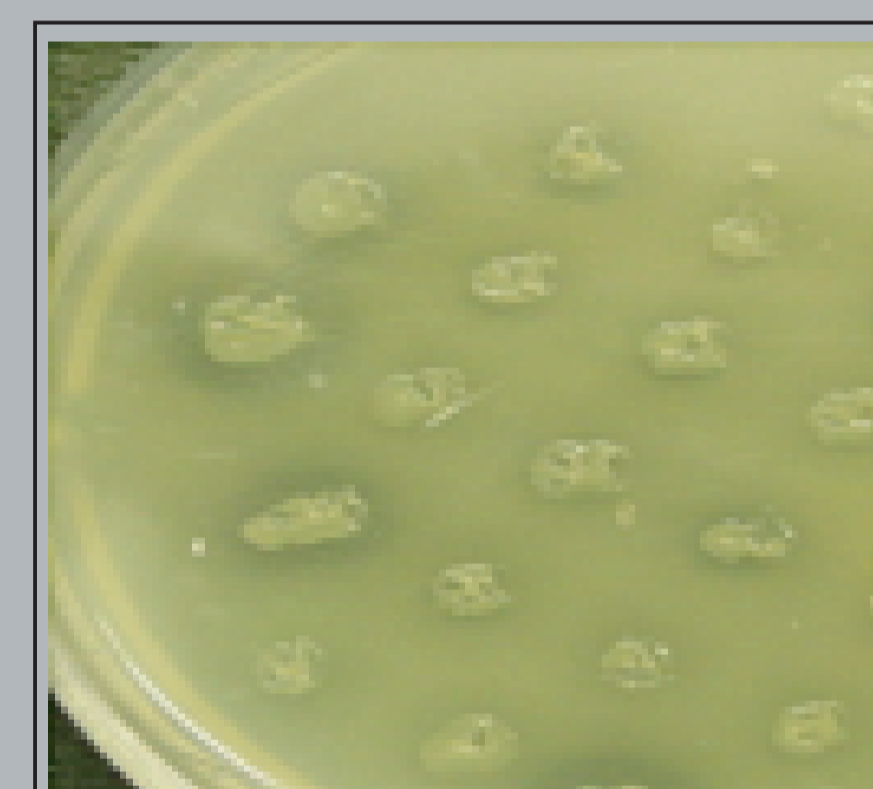
Positive

Protease

Primary Screening –
Agar Based Selection on 1%
Skim Milk (Casein)

Zones of Clearing

Secondary Screening –
Colorimetric – Universal
Protease Substrate,
Temperature and pH profiling



Positive

Actin / Hsp70
ATPase
ring hydroxylating
dioxygenase
Acyl co-A
dehydrogenase

Lipase

Primary Screening
Agar Based Selection:
Olive Oil / Rhodamine B
Tributyrin

Fluorescence under UV

Secondary Screening Colorimet-
ric - PNP-ester substrates (C2, C4,
C18);
Substrate, Temperature and pH
Profiling



Negative

Positive

16S ribosomal RNA gene (DNA level)
Amidase
ATP-dependant protease
Beta-galactosidase alpha peptide
DNA mismatch repair protein
Electron transfer flavoprotein
Excinuclease ABC subunit A
Fe-S oxidoreductase
Flavin-containing monooxygenase
ELI2453 (unknown protein)
Metallo-beta-lactamase
Methyl-accepting chemotaxis protein
Monooxygenase, flavin-binding

N-acetyl-gamma-glutamyl-phosphate
Phosphate regulon sensor protein
Polyribonucleotide nucleotidyltransferase
Pyridoxine-5-phosphate synthase
rRNA methylase / hemolysin A
Subtilisin-like serine protease
Succinate dehydrogenase
Transcriptional regulator sensor histidine
kinase
tRNA pseudouridine synthase B
Tyrosine-protein kinase

CONCLUSIONS

- High variation in hits
- Duplicate results
- Smaller inserts still gave activity despite small ORF's
- Weak correlation between activity and sequence results
- Unusual activity may require functional screens

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