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ICCC-12 Conference 2010

Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation

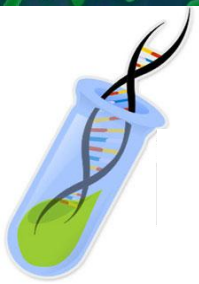
Isabel Natalia Sierra García*

Microbial Resource Division -DRM

Research Center for Chemistry, Biology and Agriculture – CPQBA / UNICAMP

State University of Campinas – UNICAMP

Campinas, SP, Brazil



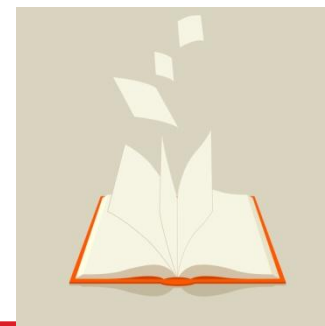
Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation



Introduction



Methodology
and results



Conclusions



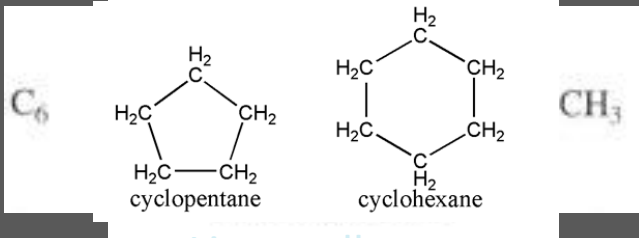
Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation

Introduction

Crude oil

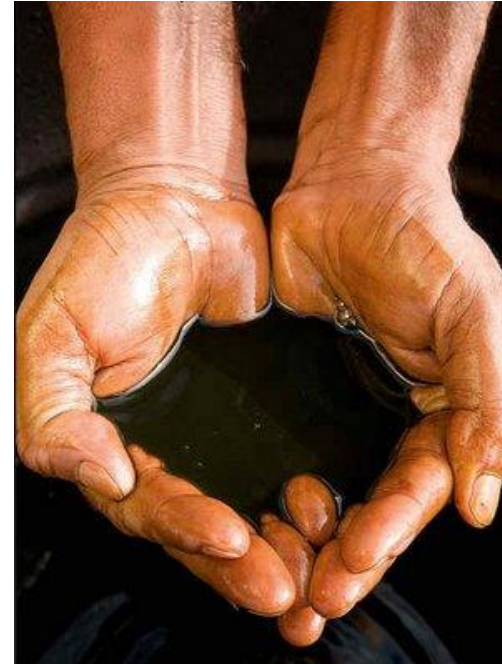
Alkanes

Saturated hydrocarbons



Linear alkanes
Branched alkanes

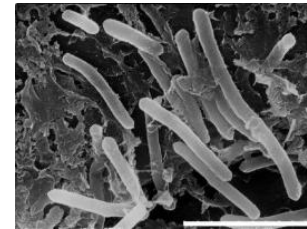
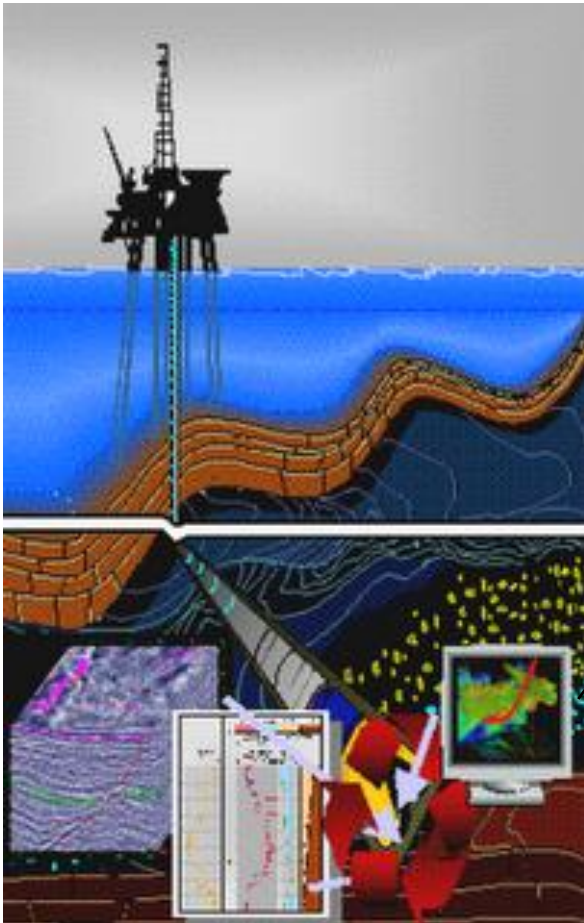
Alkanes are the major components of crude oil and the most abundant and available carbon and energy sources in reservoirs.





Introduction

□ Microbial degradation of crude oil in reservoirs



BIODEGRADATION



Crude oil → Heavy oil

Increase the oil viscosity

Amount of sulfur

Acidity



Worldwide problem for petroleum industry
recovering and refining difficult and costly



Introduction

□ Microbial degradation of crude oil in reservoirs

Effects on the composition and physical properties of crude oil are well-known



Processes of oil biodegradation in petroleum reservoirs, mechanisms, microorganisms or metabolic pathways involved are poorly understood



Limited knowledge is due to the use of

Culture – based methods

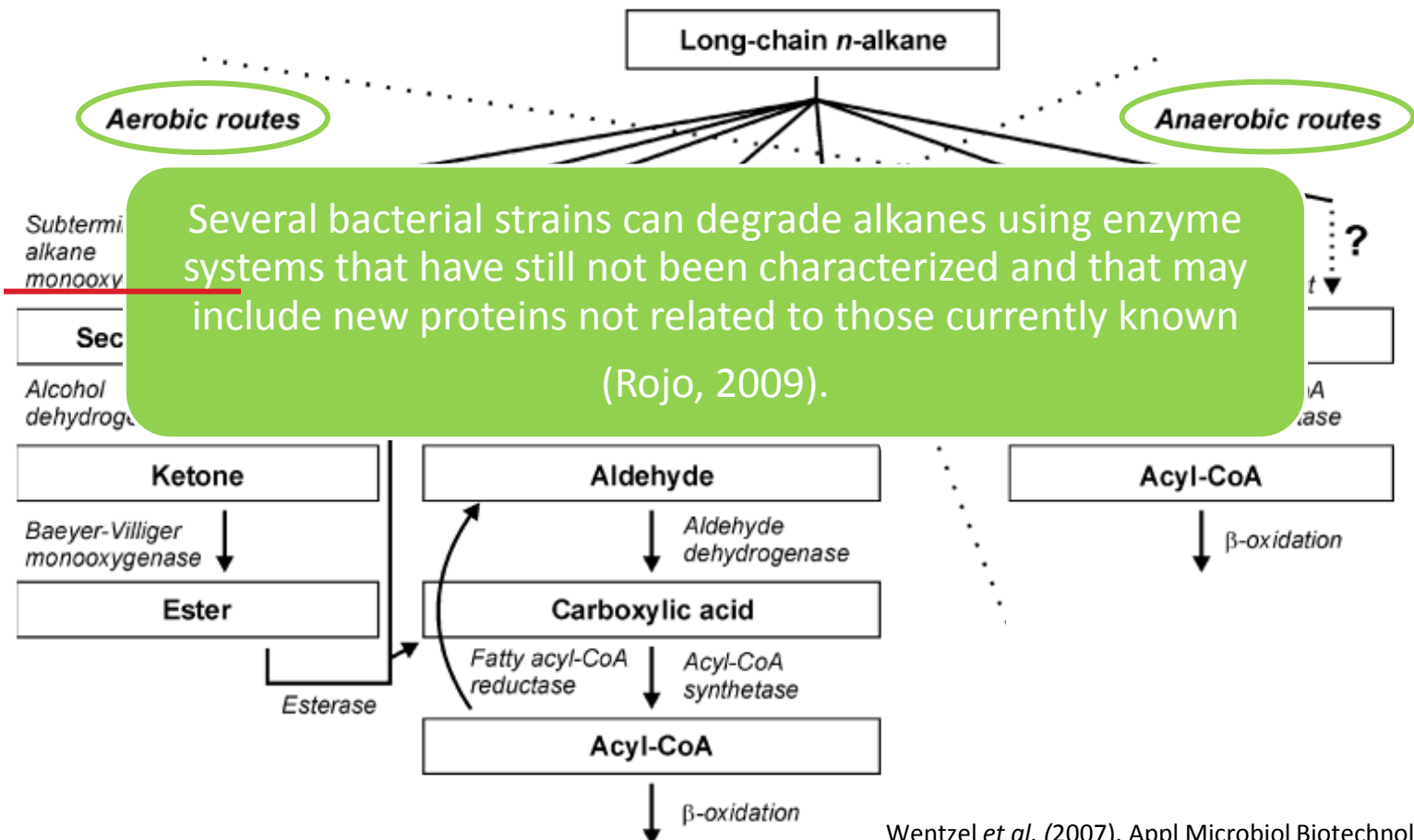
- Access only a small part of microbial communities
- Continuous isolation of the same species





Introduction

□ Pathways for aerobic and anaerobic bacterial alkane degradation





Introduction

□ Metagenomic approach



- Culture independent method which allows the access to the metabolic potential of previously uncultured microorganisms
- In petroleum environments would allow the access to new genes or complete metabolic pathways



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Introduction



Contents lists available at ScienceDirect

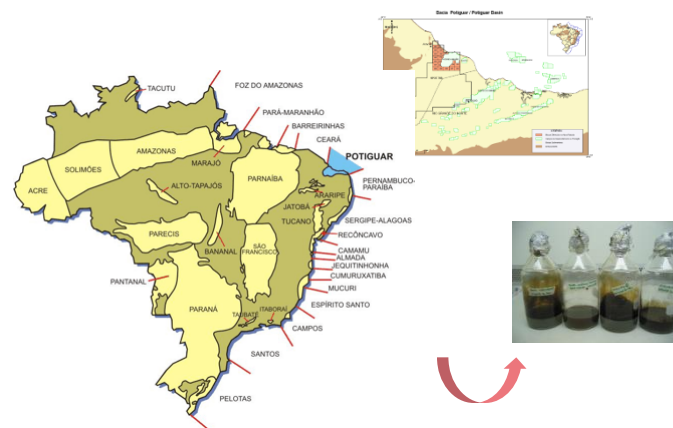
Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem



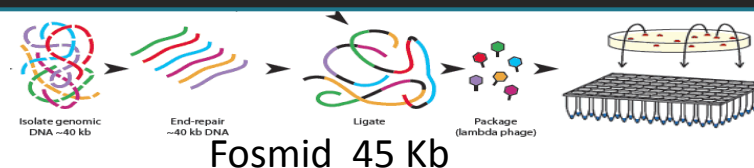
Screening for hydrocarbon biodegraders in a metagenomic clone library derived from Brazilian petroleum reservoirs

Suzan Pantaroto de Vasconcellos^a, Célio Fernando Figueiredo Angolini^b, Isabel Natalia Sierra García^a, Bruna Martins Dellagnezze^a, Cynthia Canedo da Silva^a, Anita Jocelyne Marsaioli^b, Eugenio Vaz dos Santos Neto^c, Valéria Maia de Oliveira^{a,*}

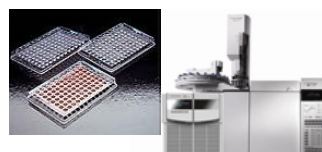


Petroleum sample collected at the Potiguar Basin in Brazil

Metagenomic fosmid library constructed
31.000 clones



Cloning-Ready Copy Control pCC2FOS (EPICENTRE)



5.000 clones screened for hexadecane degradation

FOS1A 91%
High hexadecane
degradation rate



Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation

Introduction



METAGENOMIC
LIBRARY



1 clone:FOS1A

Hexadecane degradation = 91%

Identification of metagenomic
sequences responsible for the observed
hydrocarbon degradation



Information about mechanisms of
biodegradation in a Brazilian petroleum
reservoir

New biocatalysts

Biorremediation

MEOR

Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation



Introduction



Methodology
and results

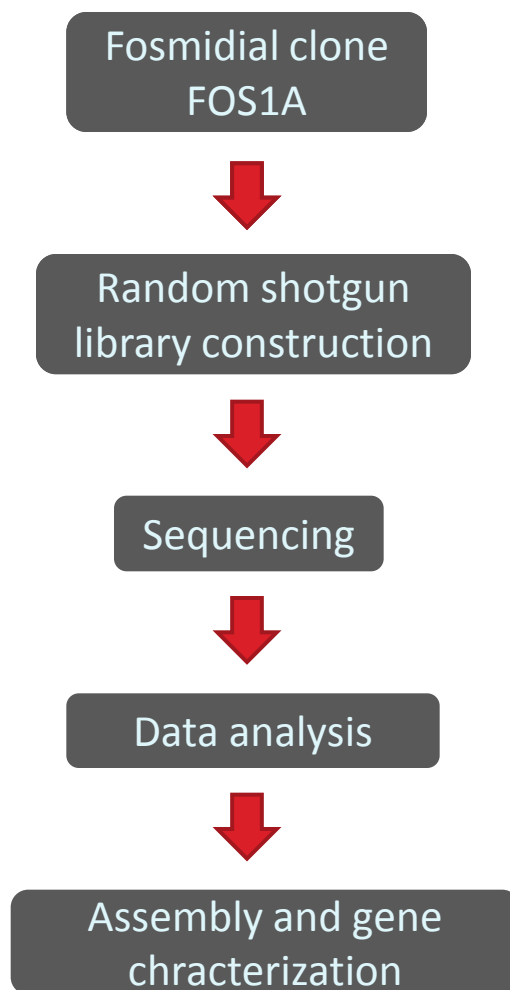


Conclusions



Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation

Methodology and Results

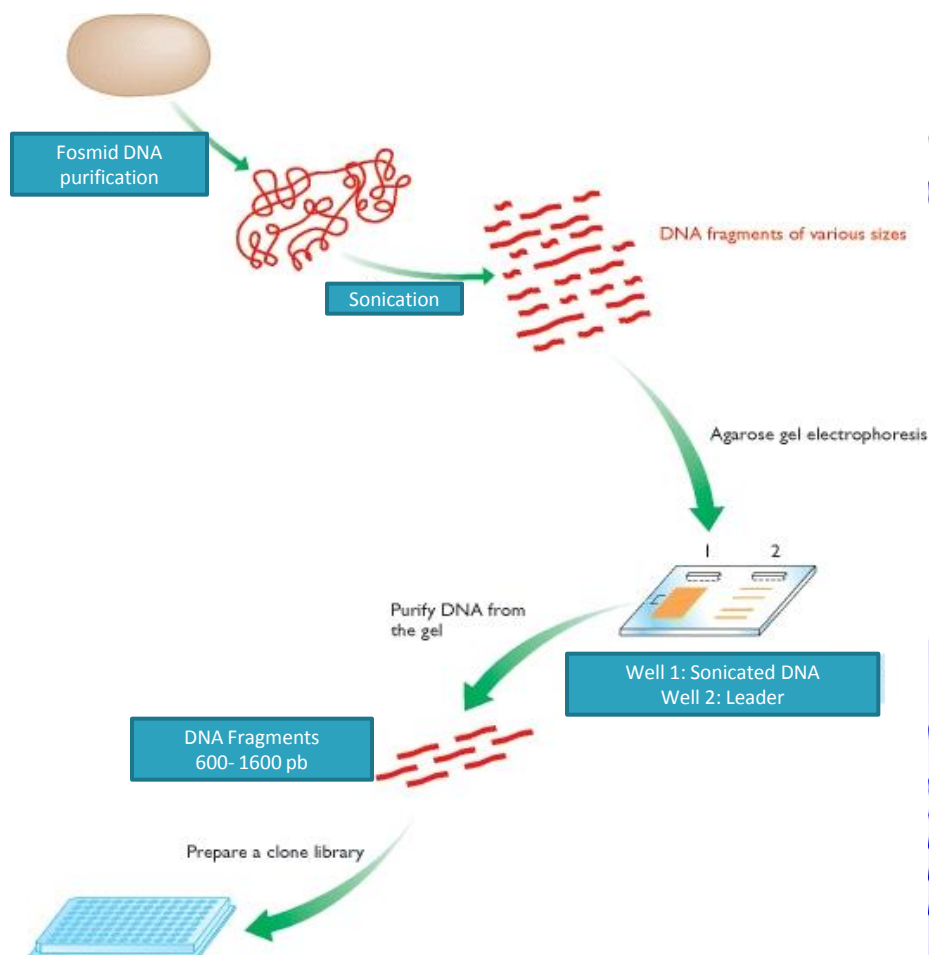




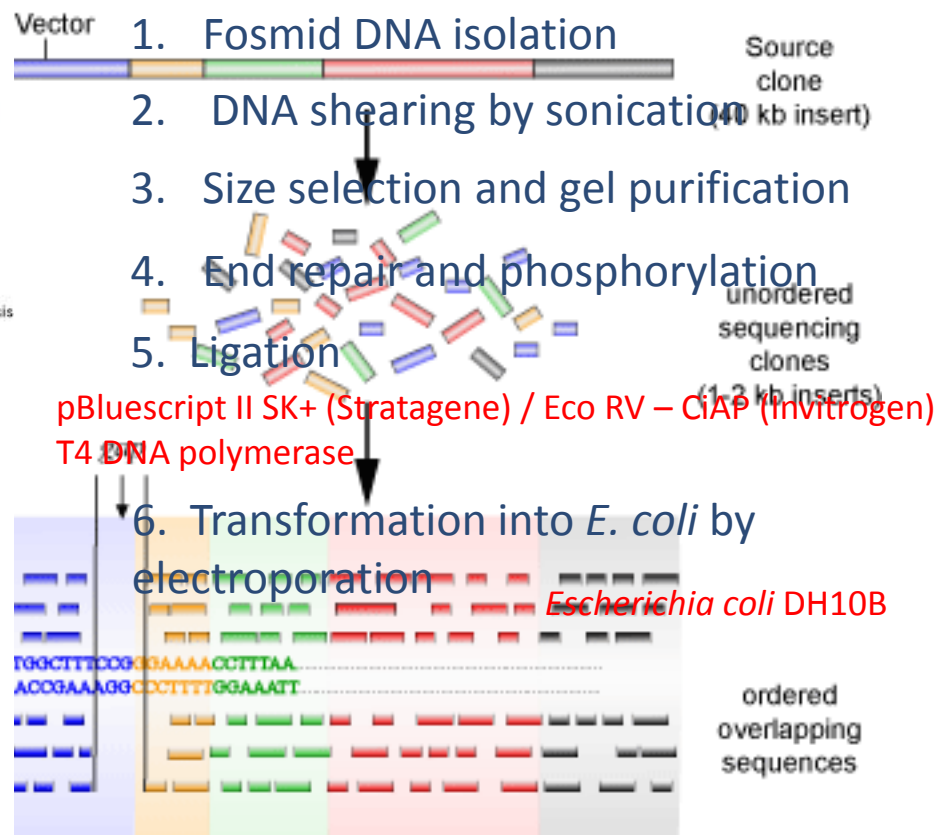
Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation

Methodology and Results

Random shotgun library construction



Shotgun Sequencing

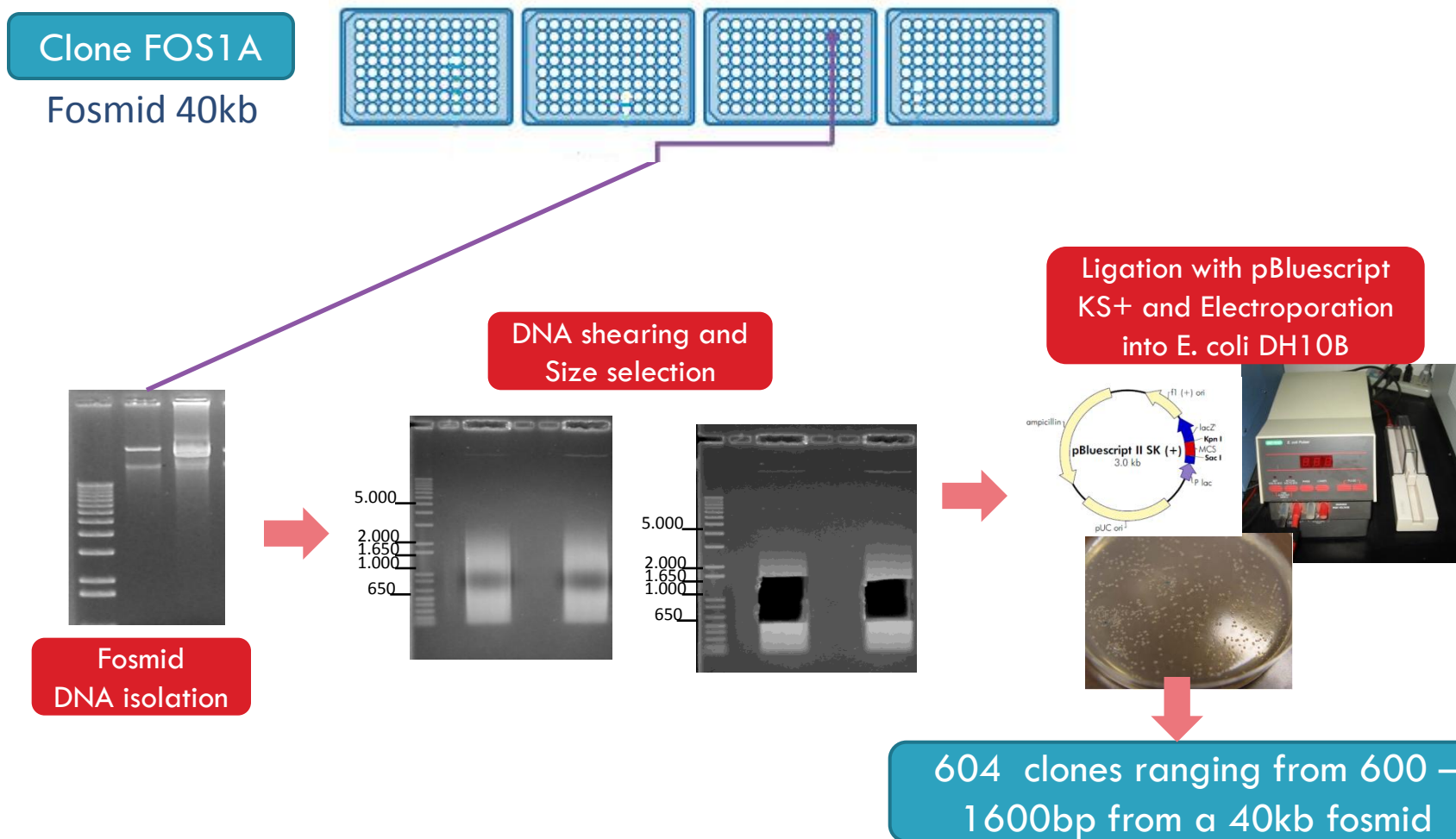




Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation

Methodology and Results

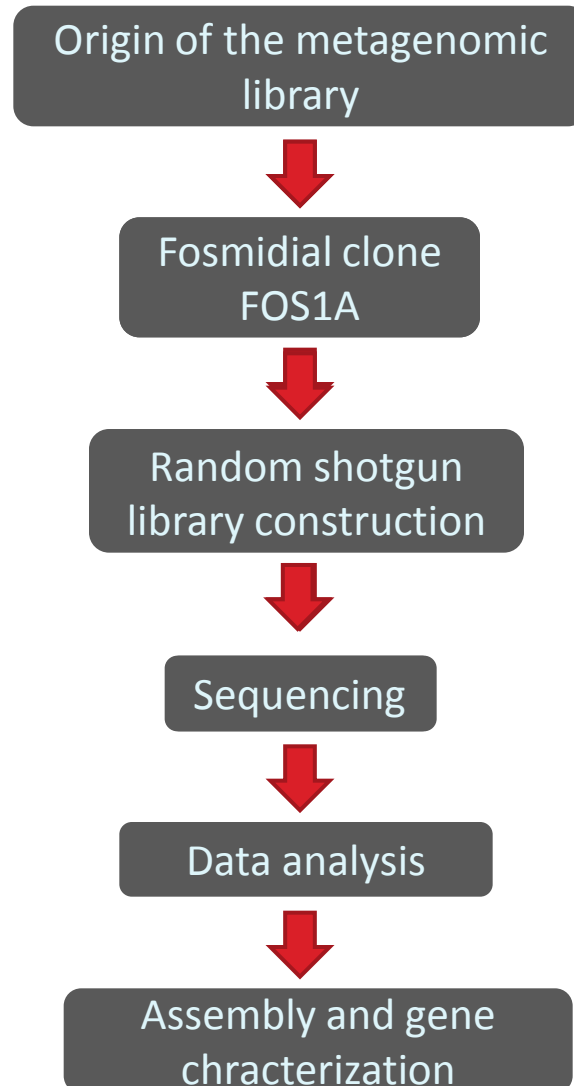
Shotgun library construction





Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation

Materials and methods





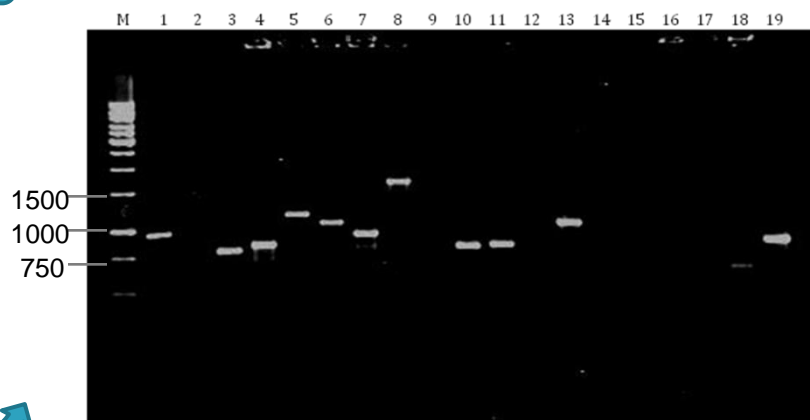
Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation

Methodology and Results

Sequencing

Sanger sequencing

604 clones



PCR tested

Primers M13F and M13R



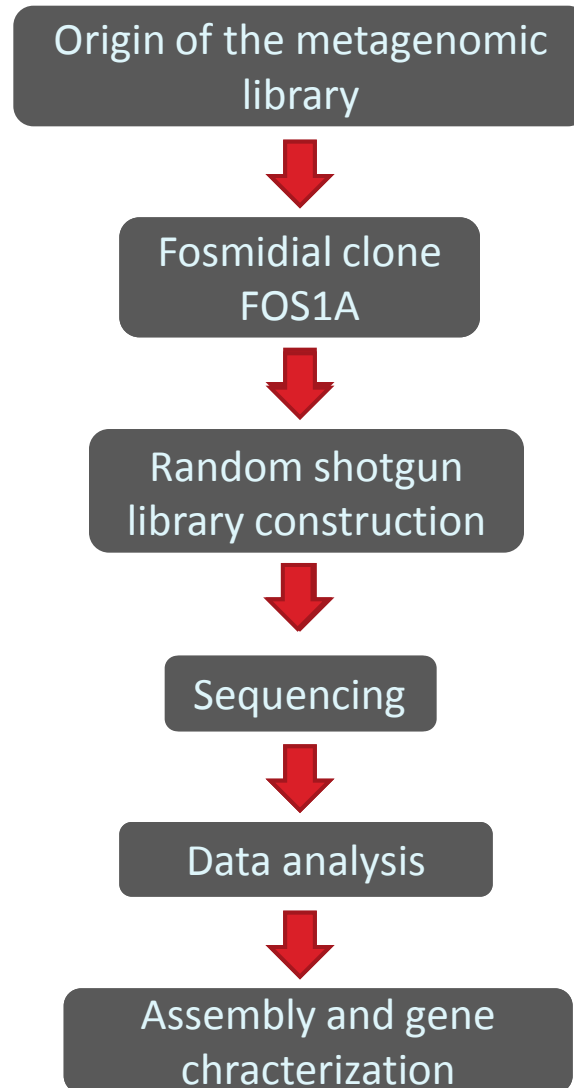
Sequencing
MegaBace 500 System

304 clones



Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation

Methodology and Results

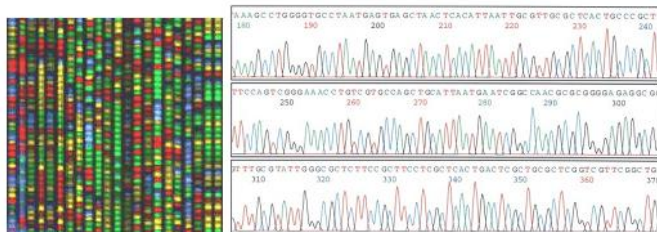




Caracterização Estrutural e Funcional de Genes de Degradação de Petróleo Originados de Metagenoma Microbiano de Reservatórios de Petróleo

Methodology and Results

Data analysis



```
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```

Phred/Phrad/Consed
Vector and contamination
screening and removal
Quality trimming
Assembly

Assembly and gene chracterization

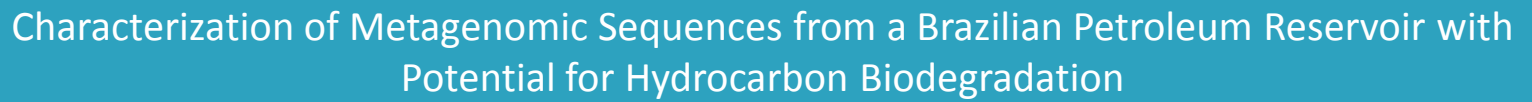
Local BLAST (blastn, blastx)
Genebank Database

E-value: $< 10^{-5}$

Identity $> 80\%$

Alignment length $> 100\text{bp}$

Open reading frame ORF
Genemark
NCBI, SwissProt, and EMBL



□ Assembly

Aligned Reads (on bioinfo01.ibi.unimab.br)

File Navigate Info Color Bin Misc

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Contig23 Some Tags Pos: 1

Search for String Compl Cont Compare Cont Find Main Min Err/10kb: 3.60

460 470 480 490 500 510

CONSENSUS

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Consed Main Window (on bioinfo01.ibi.unimab.br)

File Navigate Info Options

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Search for String Undo Edit... Print Quit Consed

Assembly View Add New Reads Digests Remove Reads Miniassembly

Single Click to Select and Click OK or Double Click to Select Contig

Contig List

Contig16 (10 reads, 1146 bps)

Contig17 (11 reads, 1411 bps)

Contig18 (12 reads, 1511 bps)

Contig19 (19 reads, 2523 bps)

Contig20 (20 reads, 2033 bps)

Contig21 (30 reads, 3152 bps)

Contig22 (63 reads, 5760 bps)

Contig23 (30 reads, 6938 bps)

You may type full contig name:

Read List

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End_sequence_F05_1R_500ng.abd Contig1 (~1712 bps from -265 to 11)

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FOS1AC13B3_M13F.abd Contig21 (~881 bps from 2193 to 3046)

FOS1AC13B4_M13F.abd Contig23 (~856 bps from 4529 to 5359)

FOS1AC13B8_M13F.abd Contig23 (~928 bps from 4811 to 5667)

FOS1AC13C1_M13F.abd Contig22 (~939 bps from 4251 to 5138)

FOS1AC13D1_M13F.abd Contig23 (~949 bps from 5430 to 6215)

Find reads containing (*s allowed): Clear

Find last read starting with:

Show Contig Close All Windows



Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation

Methodology and Results

□ Assembly

Contig	Contig size (bp)	Number of sequences in Contig	Top BLASTX hit	Identity (%)	Organism type	Target Accesion
Contig 35	990	5	Alcohol dehydrogenase zinc-binding domain protein	76.8%	<i>Thauera</i> sp. MZ1T	YP_002355505.1
Contig 39	614	2	succinate-semialdehyde dehydrogenase [NADP+]	73.7%	<i>Bradyrhizobium japonicum</i> USDA 110	NP_770638.1
Contig 11	1190	5	flavoprotein	54.4%	<u><i>Azoarcus</i> sp. BH72</u>	YP_935028.1
Contig 44	882	2	uroporphyrinogen decarboxylase	73.3%	<i>Aromatoleum aromaticum</i> EbN1	YP_158609.1
Contig 25	1774	13	putative Orn/Arg/Lys decarboxylase	78.5%	<u><i>Azoarcus</i> sp. BH72</u>	YP_934701.1
Contig 46	431	2	Orn/Arg/Lys decarboxylase	63.2%	<u><i>Azoarcus</i> sp. BH72</u>	YP_934701.1
Contig 3	1365	8	glycosyltransferase, group 2 family protein	53.9%	<i>Thiobacillus denitrificans</i> ATCC 25259	YP_315294.1

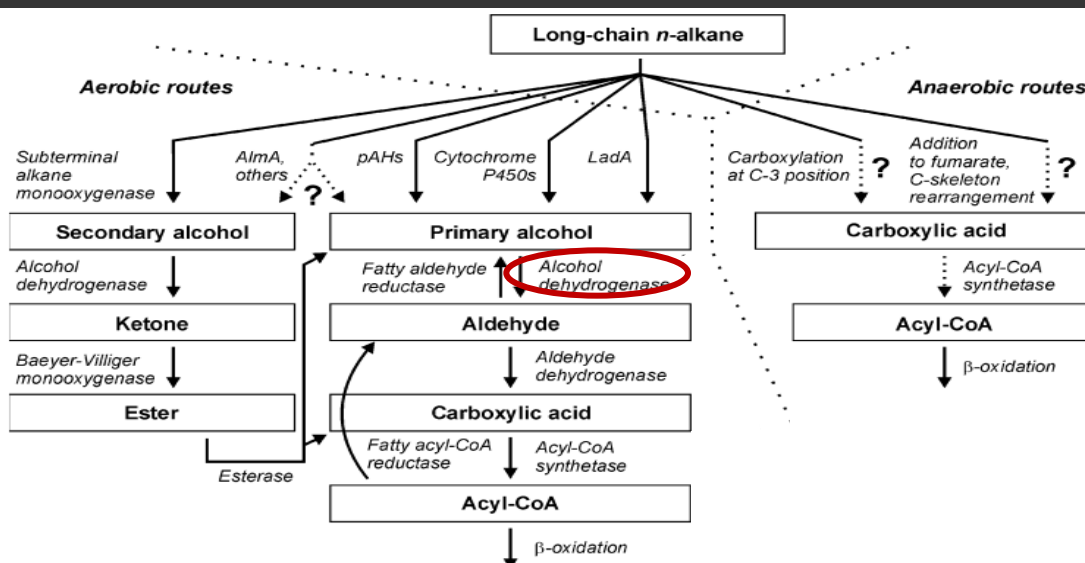


Methodology and Results

□ Alcohol dehydrogenases (ADHs)

Catalyzes the oxidation of alcohols to aldehydes,
is widely present in all organisms

In the context of alkane biodegradation, ADH catalyzes the
second reaction step of the oxidation pathway





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Methodology and Results

□ Assembly

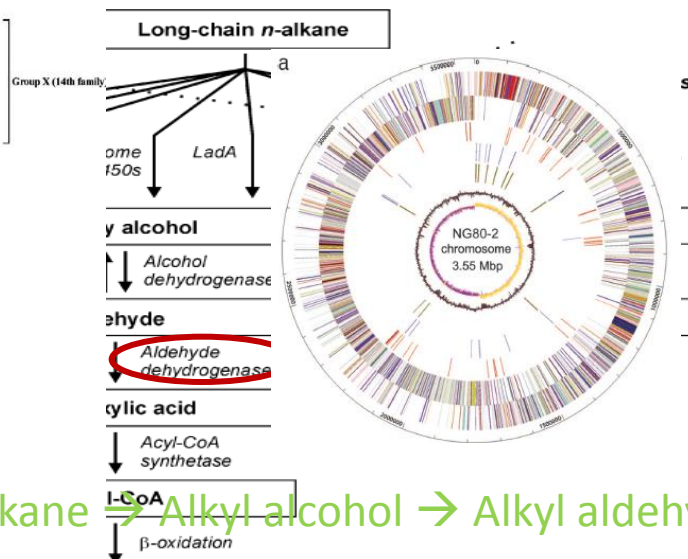
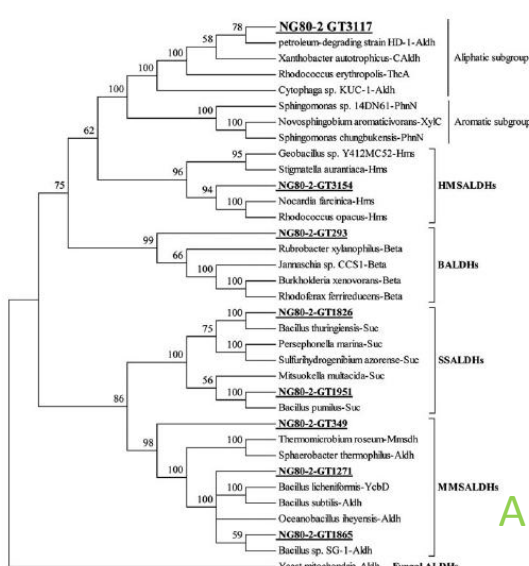
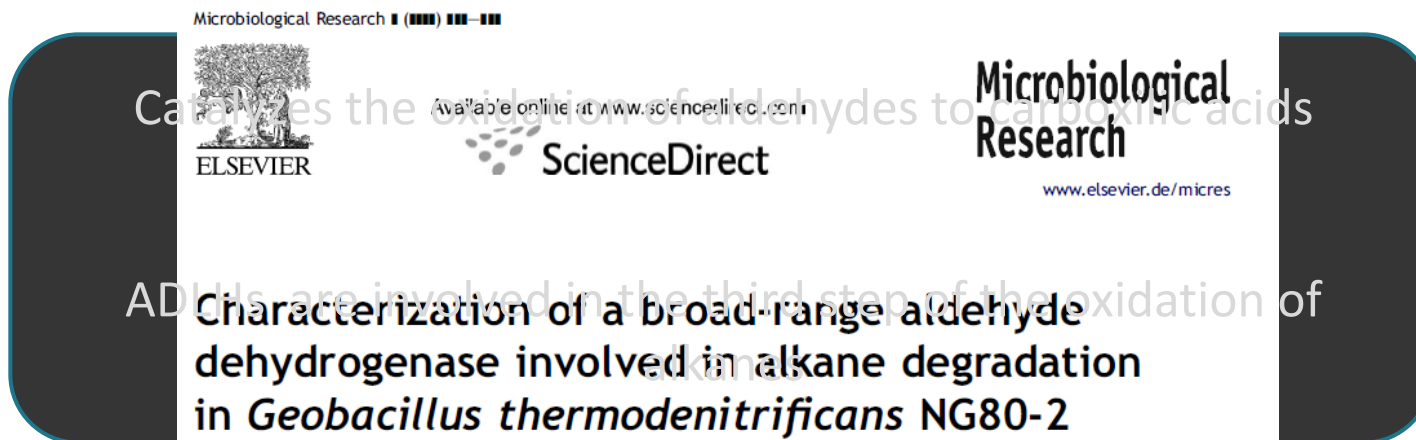
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Methodology and Results

Aldehyde dehydrogenases (ADLHs)



Alkane → Alkyl alcohol → Alkyl aldehyde → Fatty acid

Characterization of Metagenomic Sequences from a Brazilian Petroleum Reservoir with Potential for Hydrocarbon Biodegradation



Introduction



Methodology
and results



Conclusions



Conclusions

- ④ Metagenomic approach have allowed the access to potential new sequences involved with hydrocarbon biodegradation
- ④ Complete sequencing of the shotgun library may improve the assembly and may allow a complete coverage for the metagenomic fosmid responsible for hydrocarbon degradation



Conclusions

- ⌚ Accessing new catabolic properties from such a hostile environment may represent an interesting source for biotechnological use in bioremediation
- ⌚ For the first time in Brazil, gene sequences responsible for the degradation processes in oil reservoirs are being identified from microbial metagenomic data

Acknowledgements



Dr. Valéria Maia de Oliveira
Dr. Anete Pereira de Souza
Dr. Suzan Pantaroto de Vasconcellos





Questions ?