Genomic and Physiological Characterization of Cave Bacteria

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Introduction and Background

Goal: Taxonomically characterize the unknown bacterial isolates collected from two caves: Mammoth Cave and Oregon Cave.

Name of Bacteria	Cave System of Origin
MACA_103	Mammoth Cave, KY
ORCA_105	Oregon Cave, OR
MACA_156	Mammoth Cave, KY
ORCA_015/_166	Oregon Cave, OR

Fig. 1: Origins of cave bacteria samples.

In order to characterize the four bacteria from the two caves, physiological tests and sequenced genomic data were used.

Materials and Methods

Below is a record of all physiological tests done on the four bacteria; falling into two categories:

- Selective(S): allows only certain organisms can grow
- Differential(D): if an organism grows during this test, it grows with a specific characteristic

Test	Kind of Test	Question
Eonise Methylene Blue	S & D	Is this organism gram positive or negative?
MacCockney Agar	S & D	Does this organism ferment lactose?
Mannitol-Salt-Agar	S & D	Can this organism survive high salt concentration?
Sulfur Indole Motility	D	Does this organism produce H_2S, Indole, or Motility?
Simmons Citrate	D	Does this organism have transport enzymes?
Carbohydrate Metabolis	D	Can this organism use complex sugars?
Triplesugar Iron	D	Does this organism produce hydrogen sulfide or CO_2?
Ornithine & Lysine	D	Does this organism eat cadavers?
Urease	D	Does this organism break down Urea?
Phenylalanine Deamina	D	Can this organism remove amino acids?
Catalase	D	Can this organism break down reactive forms of Oxygen?
Colagulase	D	Can this organism clump blood plasma?

Examples

MACA_156 R2A Agar

Urease Test

Fig. 2: Physiological Test Tube and Petri Dish Agar Test Descriptions, To the right are two examples of these tests.

BLAST stands for Basic Local Alignment Search Tool. In BLAST, 16s barcode sequences, comprised of 1600 base pairs, are input into and search and then run throughout the entire database. Results are then calculated based on the statistical probability of the two sequences being related.

EDGE stands for Empowering the Development of Genomics Expertise. With EDGE it is possible to sequence DNA strands quickly and accurately. The sample is run against many databases and possible classifications are found based on the statistical probability.

Results

Physiological Test Tube and Agar Plate Results

Test name	MACA_103	ORCA_105	MACA_156	ORCA_15/166
Indole	neg	neg	neg	neg
Motility	neg	neg	neg	neg
Citrate	pos	pos	neg	neg
All Carbohydrates	neg	neg	neg	neg
Triple Sugar Iron (TSI)	pos CO_2 neg H_2S	pos CO_2 neg H_2S	neg CO_2 neg H_2S	neg CO_2 neg H_2S
Lysine	pos	pos	pos	pos
Ornithine	pos	pos	pos	pos
Urease	pos	pos	neg	neg
Catalase	pos	pos	pos	pos
Coagulase	neg	neg	neg	neg
Phenyalanine Deaminase	neg	neg	neg	neg

Fig. 3 : Physiological Test Tube Results for each bacteria

	MACA_103	ORCA_105	MACA _156	ORCA15/166
EMB	pos	pos	pos	pos
MSA	pos	pos	pos	pos
MacConkey	neg	neg	neg	neg
R2A	pos	pos	pos	pos
LB	pos	pos	neg	neg

Fig. 4 : Physiological Agar Plate Results

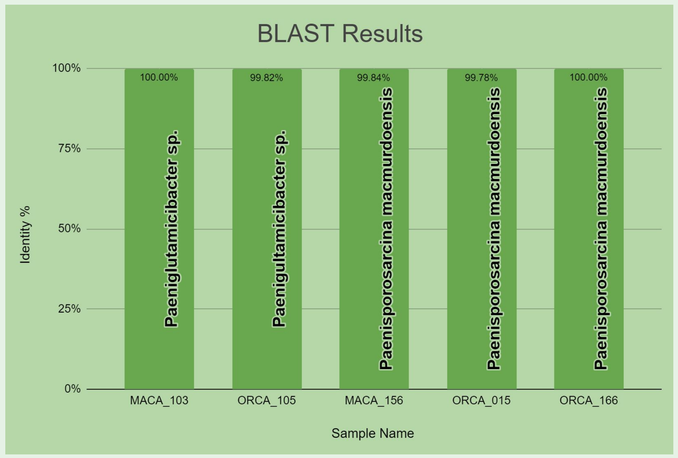

Fig. 5: Percentage of matching genome sequences according to Blast runs, Orca_015 and Orca_166 have different percentage so they are represented separately

EDGE Taxonomic ID

Sample Name	Sample Taxa
MACA_103 (KY)	Paeniglutamicibacter sp.
ORCA_105 (OR)	Paeniglutamicibacter sp.
MACA_156 (KY)	Paenisporosarcina macmurdoeinsis
ORCA_015/166 (KY)	Paenisporosarcina macmurdoeinsis

Fig. 6: Characterized and identified bacteria with their corresponding code.

Results Continued

Genomic Analysis of Physiological Tests

	MACA_103	ORCA_105	MACA_156	ORCA_015/166
Indole	No Product	No Product	No Product	No Product
Motility	Product Present	Product Present	No Product	No Product
Citrate	Product Present	Product Present	Product Present	Product Present
Dextrose	Product Present	No product	Product Present	Product Present
Lactose	No Product	No Product	No Product	No Product
Sucrose	No Product	Product Present	Product Present	Product Present
Mannitol	No Product	No Product	No Product	No Product
Lysine	No Product	No Product	No Product	No Product
Ornithine	Product Present	No Product	No Product	No Product
Urease	Product Present	Product Present	No Product	No Product
Catalase	Product Present	Product Present	Product Present	Product Present
Coagulase	No Product	No Product	No Product	No Product
Phenylalanine	No Product	No Product	No Product	No Product
EMB	Product Present	Product Present	Product Present	Product Present
MSA	Product Present	Product Present	Product Present	Product Present

Fig. 7: Genomic data was analyzed to verify physiological tests and see if bacteria genes that produced a product were present

Conclusions

- Identities of the four unknown bacteria were discovered by using the platforms BLAST and EDGE.
- BLAST gave the best estimate that the organisms were Paeniglutamicibacter sp. and Paenisporosarcina macmurdoeinsis.
- EDGE verified Paeniglutamicibacter sp.as the identities of MACA_103 and ORCA_105 and Paenisporosarcina macmurdoeinsis for MACA_156 and ORCA_015/ORCA_166.
- It was found that the isolates from the Mammoth and Oregon caves were the same species.
- Next steps: Attempt to speciate the paeniglutamicibacter bacteria
- Next steps: Attempt to understand the original mode of transport that brought the bacteria to Mammoth and Oregon Cave.

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