Characterization of a Novel Bacterial Isolate from Louisiana Coastal Waters with the Potential for Hydrocarbon Degradation

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Little is known about the microbial community in the northern Gulf of Mexico (nGOM) coastal waters. Most studies have focused on benthic and deep-water communities, leaving coastal waters and their biogeochemical cycle contributors understudied. It is important to develop an idea of the microbial community in the nGOM so that these major contributors can be identified and characterized. In the process of the Thrash Lab efforts to isolate, identify, and characterize the major contributors of the ecosystem in the nGOM, an organism from a poorly understood clade of the Order of *Oceanospirillales* of the *Gammaproteobacteria* was isolated as LSUCC41. LSUCC41 was lost before experiments began, but a new isolate, LSUCC96, was isolated and is identical to LSUCC41 (Figure 1). LSUCC96 was used to perform the experiments proposed. When sampled, LSUCC96 was highly abundant (20th rank) and therefore is a likely contributor to the nGOM biogeochemical cycling. Other data collected by the Thrash Lab shows that LSUCC96 maintains its abundance in Louisiana coastal waters throughout the year, despite a significant salinity change. Along with this, members of this Order were found to be rapid responders in the Deepwater Horizon oil spill (Dubinsky *et al*., 2013), were among the most active and dominant taxa in the initial hydrocarbon plumes, and some were possess genes that encode for nearly complete cyclohexane degradation (Dubinsky *et al*., 2013, Gutierrez *et al*., 2013). Various physiological characterization experiments were performed on LSUCC96, including optimum temperature, salinity, and carbon utilization. The isolate also had its genome sequenced and analyzed. The initial objectives for this project were to perform complete physiological characterization and complete genomic characterization of LSUCC 41. The hypotheses for this project were that LSUCC41 would have novel physiology compared to nearest characterized neighbors and novel genomic attributes that explain its physiological variations.

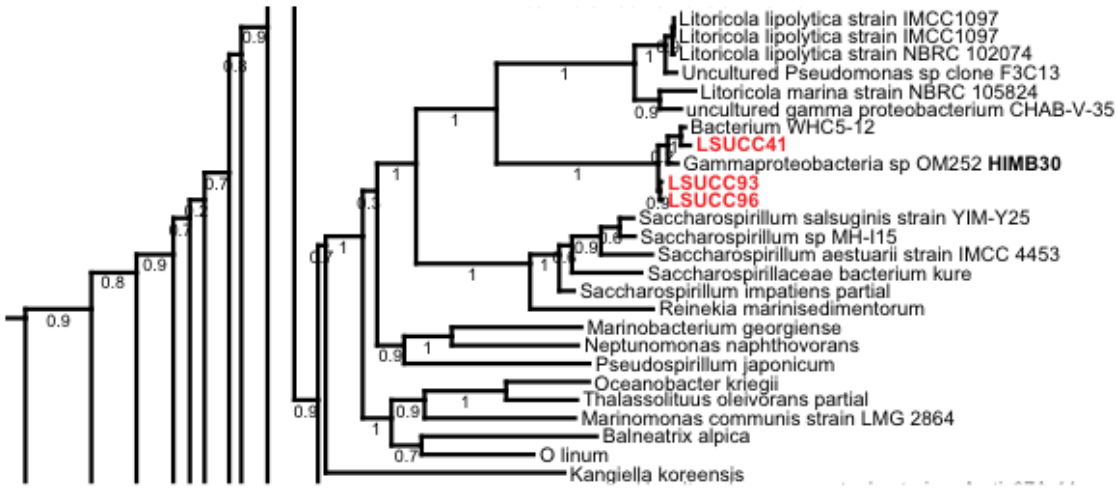

Figure 1. *Gammaproteobacteria* phylogenic tree for LSUCC41 and LSUCC96

METHODS

Counting Method. Cell counts were performed using a Guava 5HT HPL benchtop flow cytometer. Cell concentrations above 10^4 cells/mL were counted as positives.

Detailed Growth Curve. All cultures were grown in 1x JW1 media (see Table 1). A detailed growth curve was done in triplicate with a kill control. Flasks were counted twice a day, morning and evening, for 4 days.

Carbon substrate experiment. Put 1.7 mL of 1x JW1 no carbon media into wells of 96-well plate. The no carbon media was prepped by excluding carbon sources from the media recipe, such as fatty acids and amino acids. 10 uL of individual carbon sources were added to wells. Carbon sources were prepped in 8.5 mM stocks. Wells were inoculated with 6.8 uL of culture. Three of the inoculated wells had only media with no carbon source added (negative controls). Plates grew for one week then were transferred. Repeated 3 times. Counted 3rd plate after one week.

Optimum Temperature experiment. All cultures were grown in 1x JW1 media. Temperatures tested were 4°C, 12°C, 25°C, 30°C, 35°C, and 40°C. Each temperature had triplicates. Cultures were counted twice a day, morning and evening, for 5 days.

Optimum Salinity experiment. Salinity was defined as % NaCl. Percents tested were 0% NaCl, 1% NaCl, 2% NaCl, 3% NaCl, 4% NaCl, and 5% NaCl. Sets of triplicates were grown in 0% NaCl 1x JW1 media, 1% NaCl 1x JW1 media, 2% NaCl 1x JW1 media, 3% NaCl 1x JW1 media, 4% NaCl 1x JW1 media, and 5% NaCl 1x JW1 media respectively. The media was prepped by only altering the amount of NaCl added to give the respective percent values. Cultures were counted every 12 hours for 3 days.

Genome sequencing. Cells were grown in 150 mL volumes and pelleted via centrifugation. DNA was isolated using Qiagen blood and tissue kit (Qiagen Maryland, USA). Sequencing was done using PacBio SMRT technology and assembly

was performed as in Powers *et al*, 2013. Sequencing was completed at the University of Delaware. Completed scaffolds were annotated with RAST (Overbeek *et al*., 2014). Comparative analysis was completed using tools available at the SEED and pipelines such as those in Thrash *et al*, 2014.

RESULTS

LSUCC41 was lost in between semesters. The Thrash Lab isolated LSUCC96 and it was found to be identical to LSUCC41 so it was decided that it would be used in place of LSUCC41. Their phylogenic relationship can be found in Figure 1.

Physiological Characteristics. LSUCC96 has a cocci cell morphology and is 0.44 μm in diameter (Figure 3). LSUCC96 grew between 12°C and 35°C, but not at 4°C and 40°C. The optimum growth rate was at 35°C (0.45 h^{-1}). These results are shown in Figure 4. LSUCC96 could grow in 0% NaCl to 5% NaCl with an optimum growth rate at 2% NaCl (0.28 h^{-1}). These results are shown in Figure 5. Results from the carbon utilization experiment were a bit inconsistent and can be found in Table 3. For the first run of the carbon experiment there were 11 positives, 31 positives the second run, and 45 positives for the second experiment.

Genomic Anaylsis. Genomic data gathered shows that LSUCC96 possesses the RuBisCo gene (Figure 2), which may explain the inconsistent results for the carbon utilization experiment. This will tested in later experiments. LSUCC96 also shows genomic pathways for the production of various amino acids, such as L-aspartate, L-asparagine, L-cysteine, L-glutamine, L-isoleucine, L-proline, L-threonine, L-valine, and L-methionine. These amino acids may be acting as osmolytes, allowing for LSUCC96's wide range of salinity tolerance.

DISSCUSION

LSUCC96's varied salinity range allows for it to survive in various salinity conditions along the coast and throughout the year. This means that it can be a constant contributor to biogeochemical cycling throughout the year along Louisiana's coastline. The possible osmolyste productions observed from genome sequencing may explain its capability to survive such a salinity range. The presence of the RuBisCo gene suggests that LSUCC96 may be using carbon fixation, which would allow for negatives to appear as positives in the carbon substrate experiment. Some objectives were not met. There is more physiological work that can be done and will most likely be tested in future work. The hypotheses seem to be correct. LSUCC 96 has an unusually wide salinity tolerance and may be performing carbon fixation. These appear to be novel characteristics to LSUCC96 in this clade.

RESULTS/METHODS FIGURES AND TABLES

Table 1. Basic components of JW1 media

Chemicals	Concentraton
Na	479 mM
Cl	543 mM
K	10 mM
Mg	52 mM
Ca	10 mM
PO4	51 µM
SO4	30 mM
Br	800 µM
B	421 µM
Sr	90 µM
F	55 µM
Fe	101 nM
Si	71 µM
HCO3	10 mM
C and N Sources	% Fraction
Fatty Acids	29.32325175
Organic C and N	35.41199505
Amino Acids	35.2647532

Table 2. Genomic statistics

Genome Size	1935310

GC content	48.57%
Number of Scaffolds	4

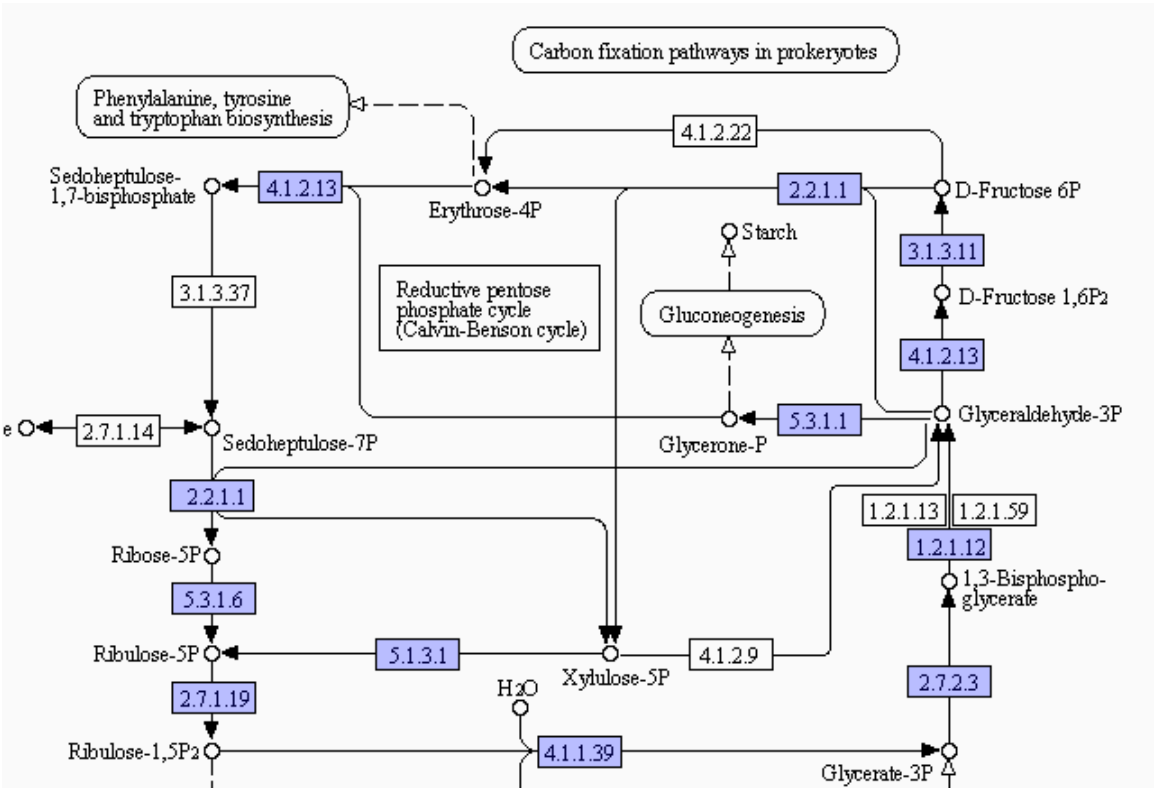

Figure 2. Kegg pathway showing the possession of the RuBisCo gene

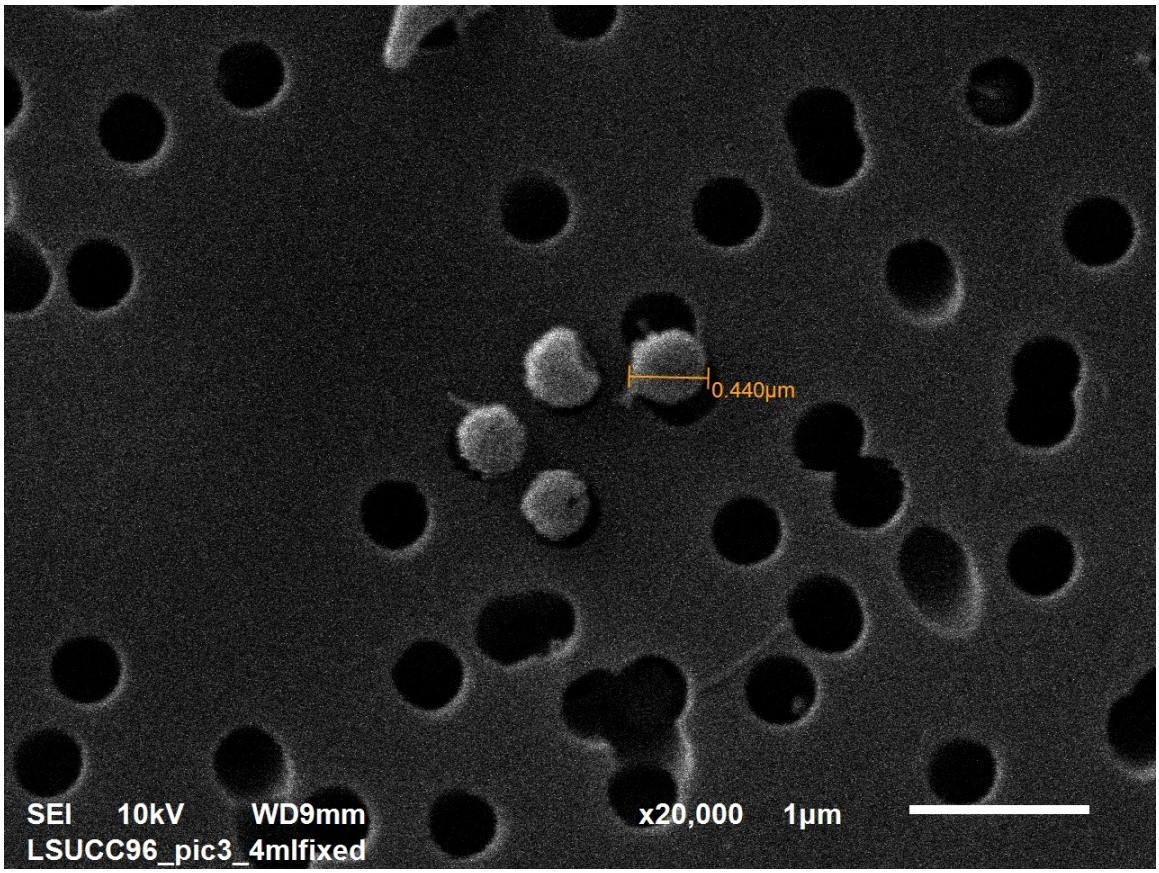

Figure 3. SEM image of LSUCC96

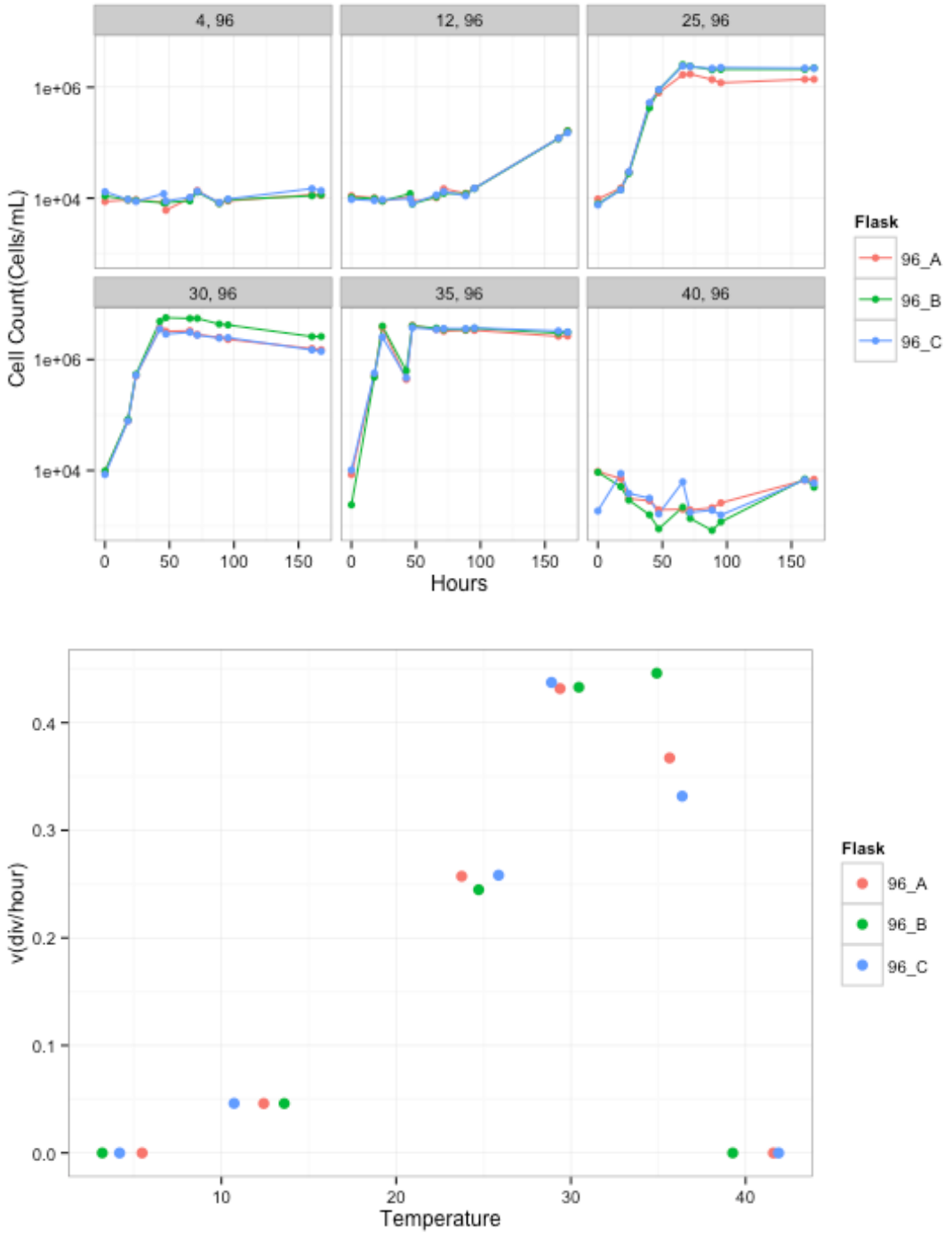

Figure 4. Growth curves and growth rates for temperature experiment

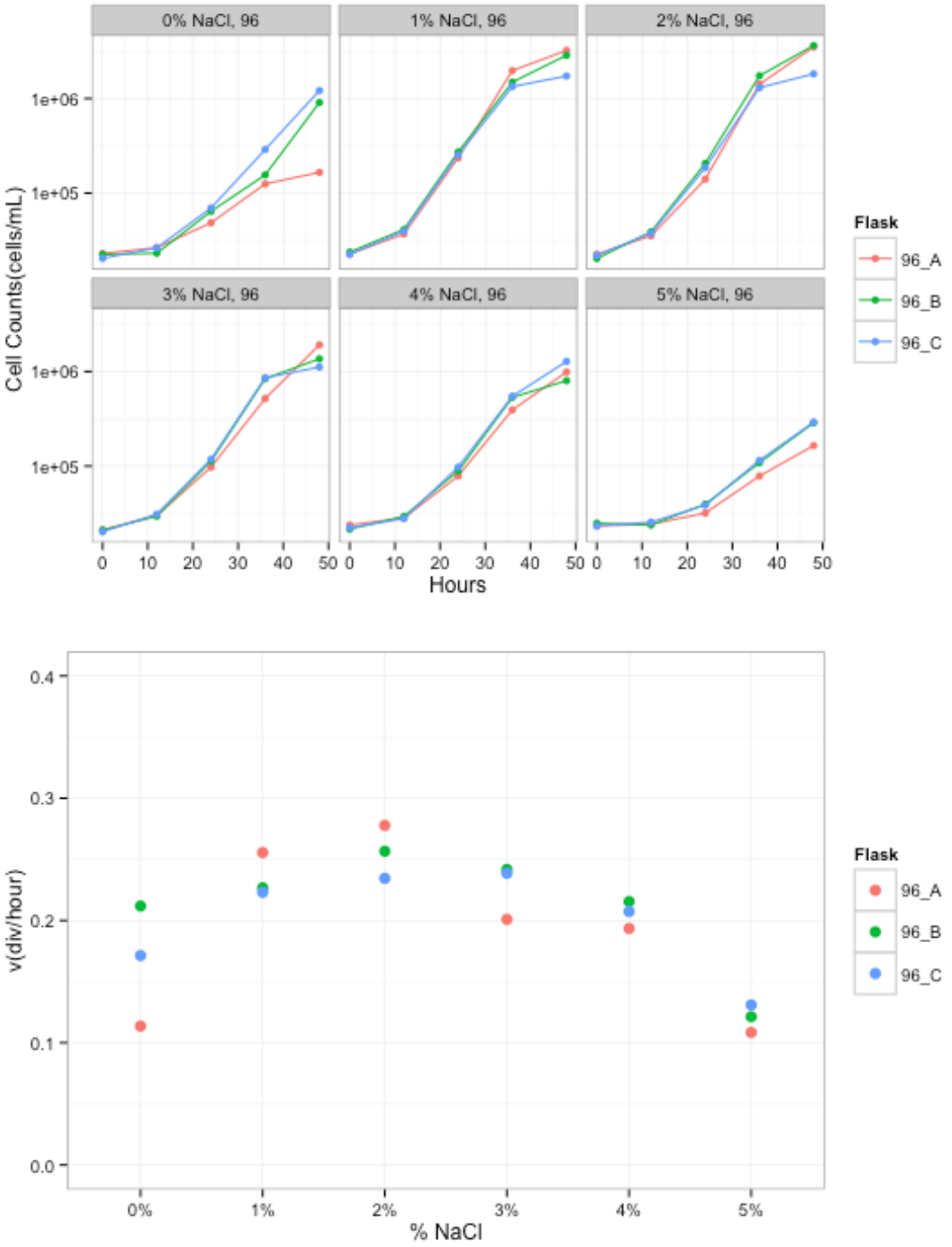

Figure 5. Growth curves and growth rates for salinity experiment

Table 3. Sole carbon substrate results

Carbon Source	Growth (1st run)	Growth (2nd run)	Growth (3rd run)
Acetate	-	+	+
Adonitol	-	+	+
Benzoate	-	+	+
Catechol	-	-	-
Citrate	-	+	+
DL-Alanine	-	+	+
DL-Arginine	+	+	+
D-Cellobiose	-	-	+
D-Gluconic acid	-	+	+
D-Mannitol	-	+	-
D-Mannose	+	+	+
D-Ribose	-	-	+
D-Trehalose	-	-	+
Ethanol	-	-	+
Formate	-	-	+
Fructose	-	+	+
Fumarate	-	+	+
Glucose	-	+	+
Glycine	+	+	+
Glycerol	-	+	+
Humic Acid	+	+	+
Inositol	-	+	+
Lacate	-	-	+
L-Aspartic acid	+	+	+
L-Glutamic acid	+	+	+
L-Leucine	+	+	+
L-Ornithine	-	-	-
L-Phenylalanine	+	+	+
L-Rhamnose	-	+	+
L-Serine	+	+	+
Maltose	+	+	+
Melibiose	-	-	+
Methanol	-	+	+
N-Acetyl-D-glucosamine	-	+	+
Propionate	-	+	+
Pyruvate	-	-	+
Succinate	-	+	+
Sucrose	+	+	+
Urea	-	+	+

Fluorene	ND	-	+
p-Hydroxybenzoate	ND	+	+
Nonane	ND	-	+
Nonadecane	ND	-	+
eicosane	ND	-	+
Octanoic Acid	ND	-	+
Decanoic Acid	ND	-	+
Isobutyric Acid	ND	-	+
Butyric Acid	ND	-	+
Valeric Acid	ND	+	-

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