

读书报告

汇报人：赵卓丽 2016-7-30





ORIGINAL PAPER

Analysis of the metatranscriptome of microbial communities of an alkaline hot sulfur spring revealed different gene encoding pathway enzymes associated with energy metabolism

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Extremophiles

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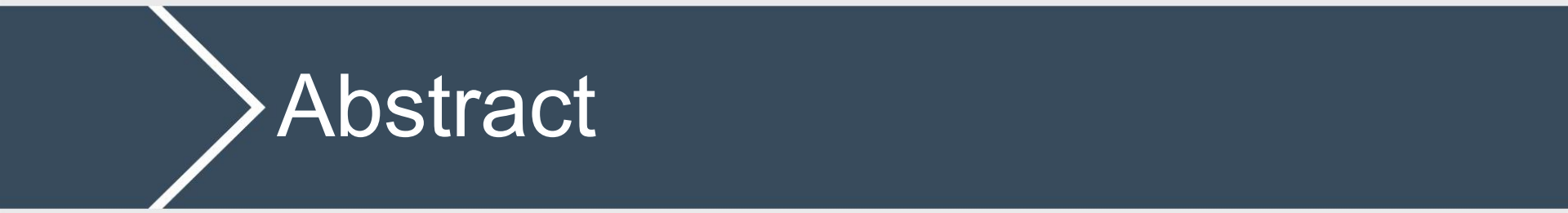
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Discussion



ONTENS

目录



Abstract

READING REPORT

> Abstract

alkaline sulfur hot spring

由于地热形成的复杂而特殊的生态系统

> 微生物的生存、适应机制与能量代谢息息相关

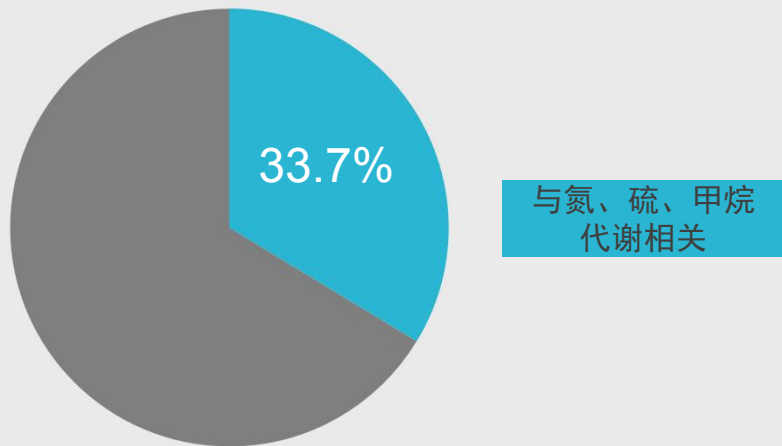
> 微生物群落的生存机制存在需氧和厌氧两条途径

> 利用宏转录组，分析能量代谢中由不同基因编码的与酶相关的厌氧途径

> Abstract

> 优势群落：变形菌门（28.1%）

与功能代谢相关基因：988reads



- > 微生物主要参与了生物体生命活动中的厌氧途径。
- > 不同基因编码的酶显示微生物参与了硝化作用、反硝化作用、异化硫酸盐还原、甲烷生成等途径。

Introduction

热泉生态系统

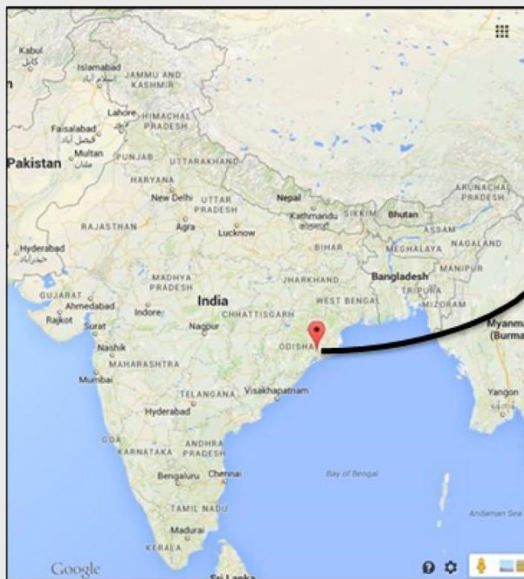


由地热而形成的重要化能合成生态系统

微生物在多元化的热泉生境中形成了一个最基本的水生食物链

微生物通过各种各样的代谢通路在热泉中生存并适应生境

Introduction



印度东南部冈瓦纳大陆东高止山脉
(20°N ; 85°E)

温度：55-58°C

PH: 8.5

富含NaCl等盐类

氮气：88-90.5%

溶解氧：1.2-6.6%

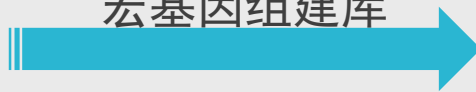
氦、氩、甲烷、CO₂

H₂S → 硫磺气味

> Introduction

新一代测序技术
(NGS)

宏基因组建库



高温环境中微生物群落
结构和功能多样性

宏转录组

分析微生物群落在极端环境中功能反应的有效部位

调查微生物mRNA的活跃部位

进一步帮助我们理解微生物群落通过不同的机制来
维持生存并适应极端环境

> Introduction



研究方法

通过宏转录组对碱性含硫热泉中微生物群落能量代谢的厌氧通路进行探究。

评估了氮代谢、硫代谢、甲烷代谢中重要的基因编码酶与微生物之间的关系。



研究成果



研究意义

通过能量代谢的不同路径对微生物群落的生存和适应机制有了一个深入的理解。

Materials and methods

01 样品采集与取样过程

02 总RNA提取与mRNA扩增

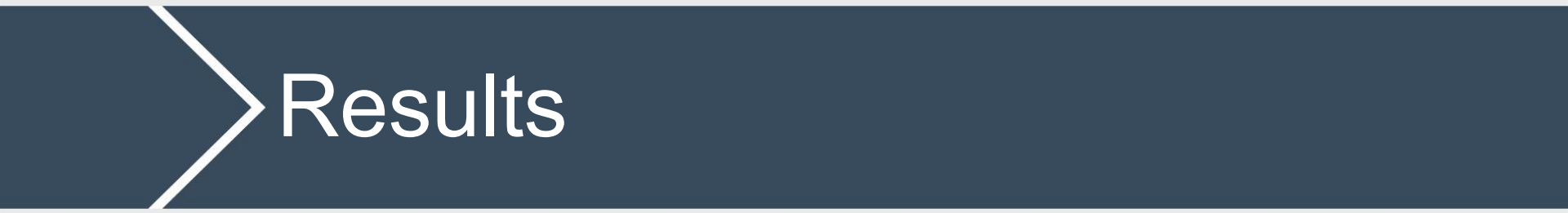
03 cDNA建库与Illumina测序

04 高质量序列的过滤与装配

05 分类依据与方法

06 功能性分类

07 数据处理



Results

READING REPORT

宏转录组数据统计

Table S1

Overview of the metatranscriptome dataset

Number of total reads	17,501,596	4.53Gb
Number of high quality reads	7,786,277	2.09Gb
Number of mRNA reads	57,480	
Total count of mRNA reads (bp)	41,695,294	
Number of reads containing predicted proteins with known function	39,958	69.5%
Number of reads containing predicted proteins with unknown function	15,245	26.5%

微生物群落组成

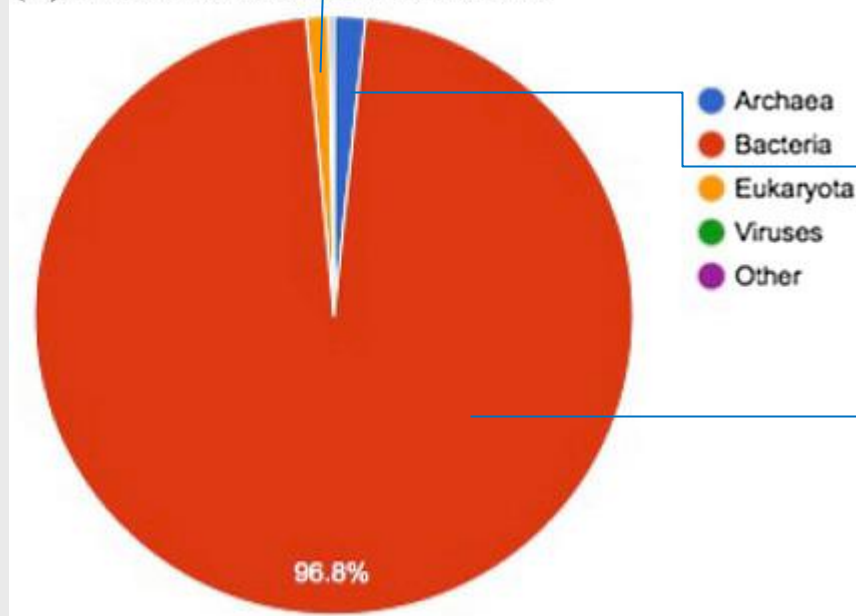
Table S2

Physico-chemical parameters of sediment samples collected from Attri hot sulfur spring

Physico-chemical parameters	Sediment samples
Temperature (°C)	55-58°C
pH	8.5
Turbidity (NTU)	1.0-1.5
Salinity (‰)	1.2
EC (mS/cm)	0.618
Nitrite-Nitrogen (µg/ml)	0.0129
Nitrate-Nitrogen (µg/ml)	6.71
Ammonia (mg/l)	0.2
Inorganic phosphate (mg/l)	0.16

微生物群落组成

(a) Distribution of Domain



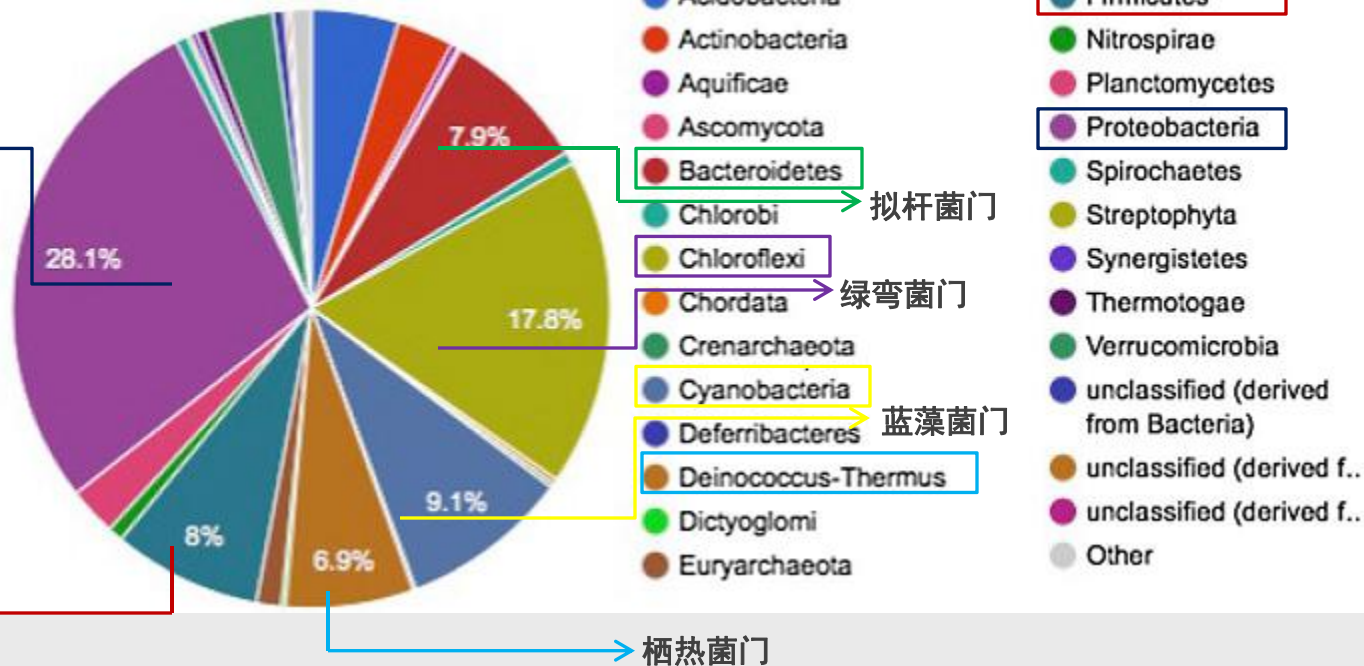
真核生物 (1.2%)

古菌 (1.7%)

细菌 (96.8%)

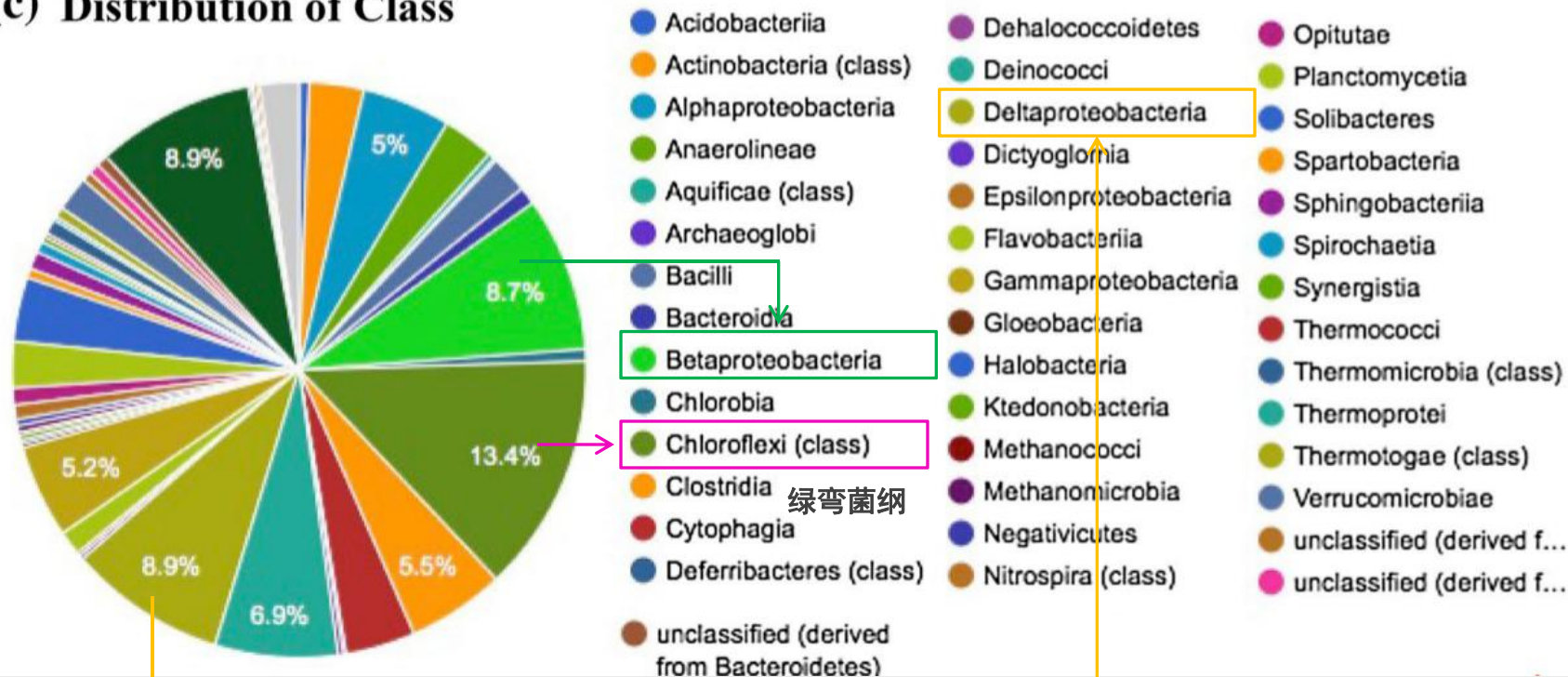
微生物群落组成

(b) Distribution of Phylum



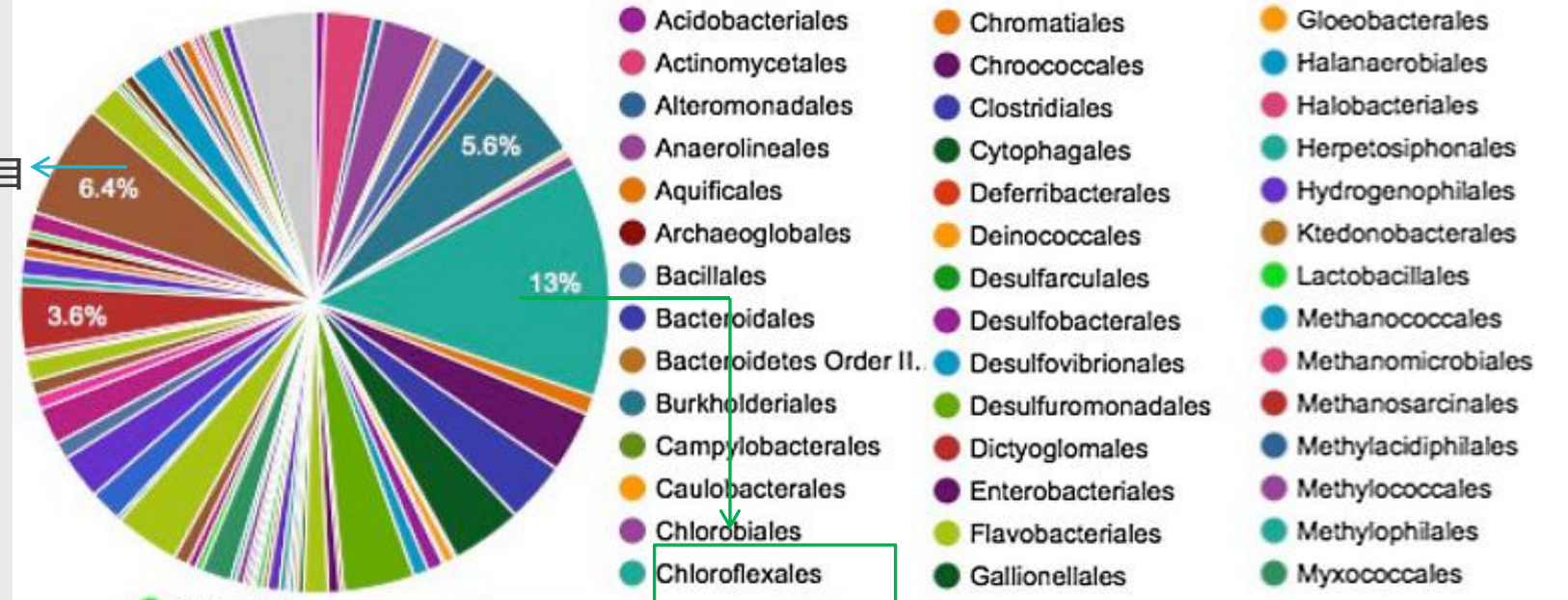
微生物群落组成

(c) Distribution of Class



微生物群落组成

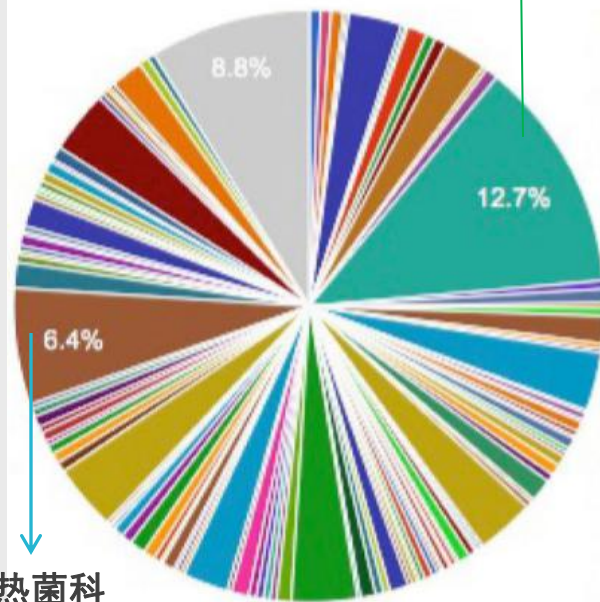
(d) Distribution of Order



绿弯菌目

微生物群落组成

(e) Distribution of Family



绿弯菌科

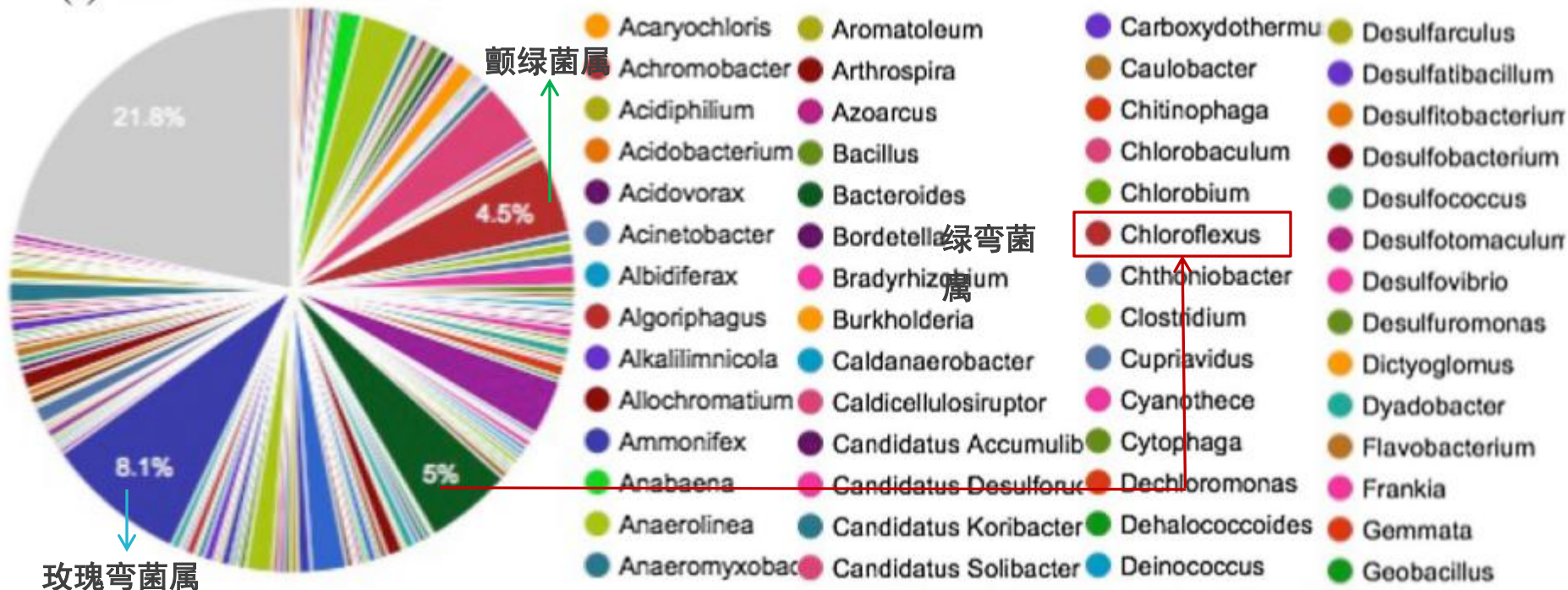


- | | | | |
|-----------------------|------------------------|--------------------------|-----------------------|
| ● Acetobacteraceae | ● Chloroflexaceae | ● Desulfohalobiaceae | ● Herpetosiphonaceae |
| ● Acidobacteriaceae | ● Chromatiaceae | ● Desulfovibrionaceae | ● Hydrogenophilaceae |
| ● Alcaligenaceae | ● Clostridiaceae | ● Desulfuromonadaceae | ● Kofleriaceae |
| ● Alicyclobacillaceae | ● Clostridiales Family | ● Dictyoglomaceae | ● Ktedonobacteraceae |
| ● Alteromonadaceae | ● Clostridiales Family | ● Ectothiorhodospiraceae | ● Lachnospiraceae |
| ● Anaerolineaceae | ● Comamonadaceae | ● Enterobacteriaceae | ● Leptospiraceae |
| ● Aquificaceae | ● Cyclobacteriaceae | ● Flammeovirgaceae | ● Methanosarcinaceae |
| ● Archaeoglobaceae | ● Cystobacteraceae | ● Flavobacteriaceae | ● Methylophilaceae |
| ● Bacillaceae | ● Cytophagaceae | ● Frankiaceae | ● Methylobacteriaceae |
| ● Bacteroidaceae | ● Deferribacteraceae | ● Gallionellaceae | ● Methylococcaceae |
| ● Bradyrhizobiaceae | ● Deinococcaceae | ● Geobacteraceae | ● Methylophilaceae |
| ● Burkholderiaceae | ● Desulfarculaceae | ● Halanaerobiaceae | ● Micromonosporaceae |
| ● Caulobacteraceae | ● Desulfobacteraceae | ● Halobacteriaceae | ● Moraxellaceae |
| ● Chlorobiaceae | ● Desulfobulbaceae | ● Heliobacteriaceae | ● Mycobacteriaceae |

栖热菌科

微生物群落组成

(f) Distribution of Genus



能量代谢相关序列分类

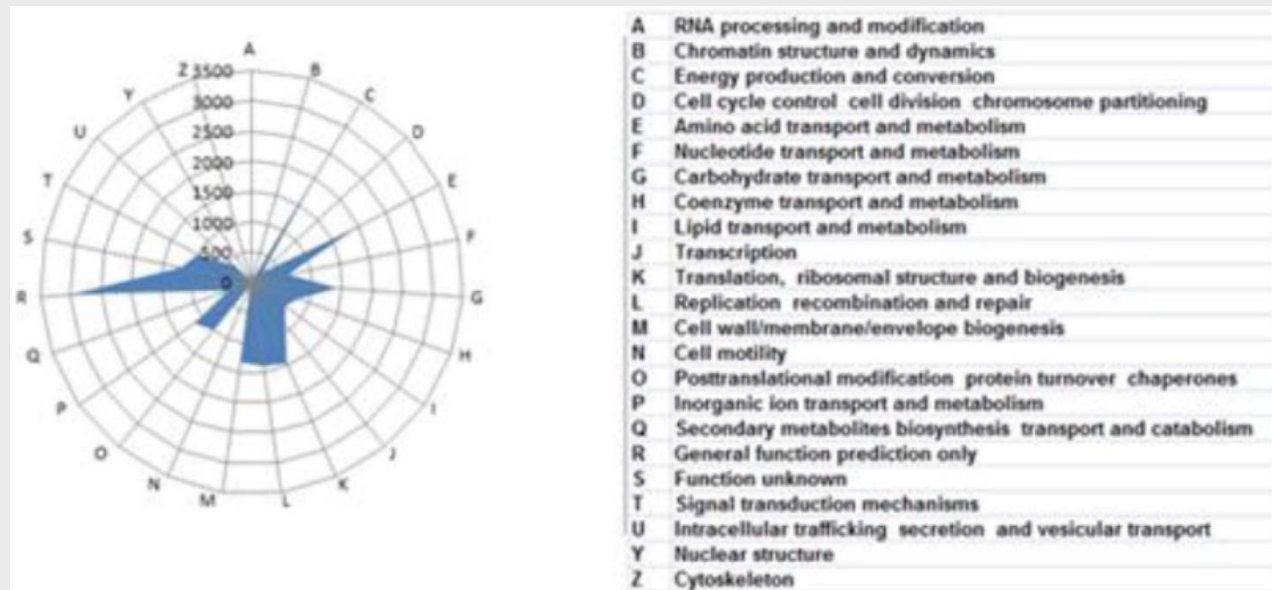


Fig. 3 Functional assignment of metatranscriptome reads using BLASTX against COG database. The number indicates the total reads affiliated to the predominant COG identifiers. Maximum reads were allotted to specific COG identifiers viz., C, E, G, K, L, M, O, P, and R

KEGG数据库:15631reads属于功能分类序列, 998reads归属于功能代谢, 其中33.7%与N、S、甲烷代谢相关。

SEED数据库:364reads与氮代谢相关, 427reads与硫代谢相关。

能量代谢相关序列分类

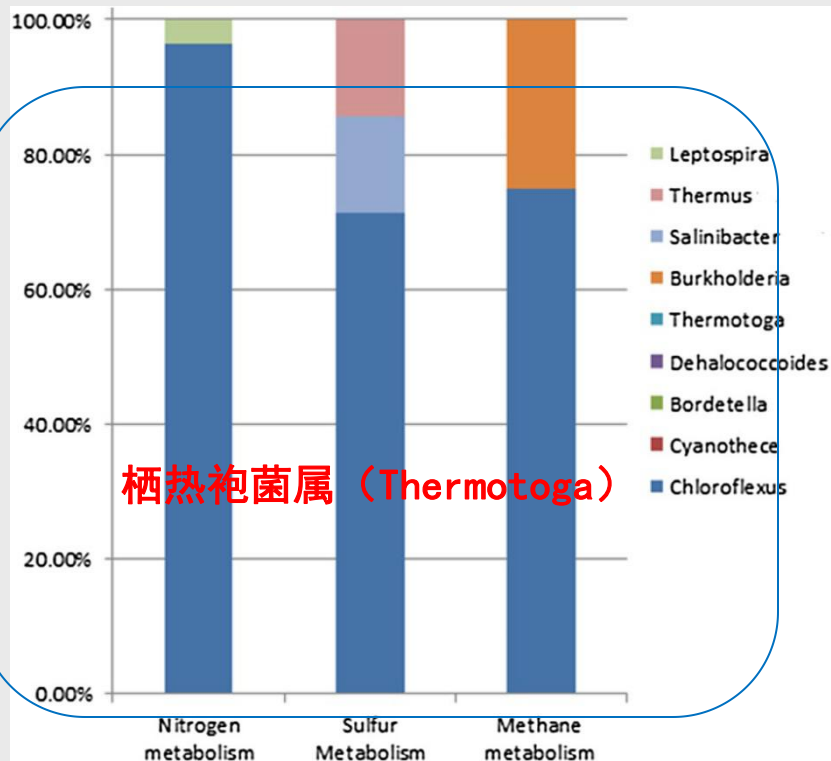


Fig. 4 Taxonomic classification of metatranscriptomic reads involved in energy metabolism (KEGG identifiers) specifically related to nitrogen, sulfur, and methane metabolism at genus level. The taxonomic assignment was performed by BLASTX against NCBI-nr database and evaluated using the MEGAN v4.0 software, which provides phylogenetic classification at various levels, based on the reads

不同属的微生物参与厌氧代谢路径表明多种微生物群落多样化的功能作用

末端电子受体：硝酸盐、硫酸盐、元素硫、二氧化碳



微生物利用不同末端电子受体来生存和适应高温环境系统

氮代谢

1

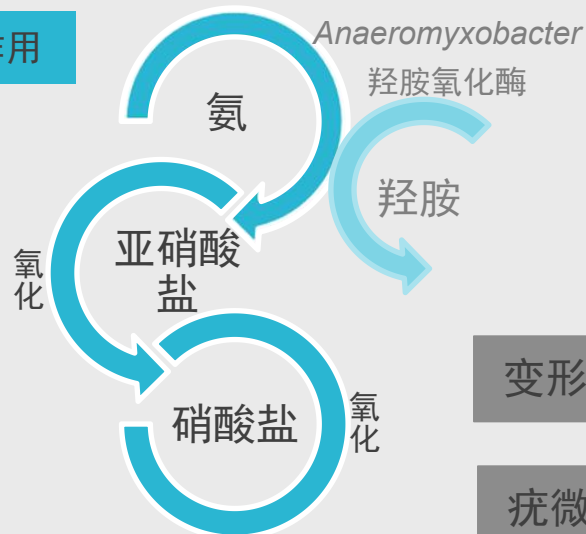
证实了大量氮代谢酶参与了热泉微生物的厌氧代谢。

2

硝化作用：微生物厌氧呼吸的末端电子受体是硝酸盐。

READING REPORT

硝化作用



Nitrogen

氮代谢

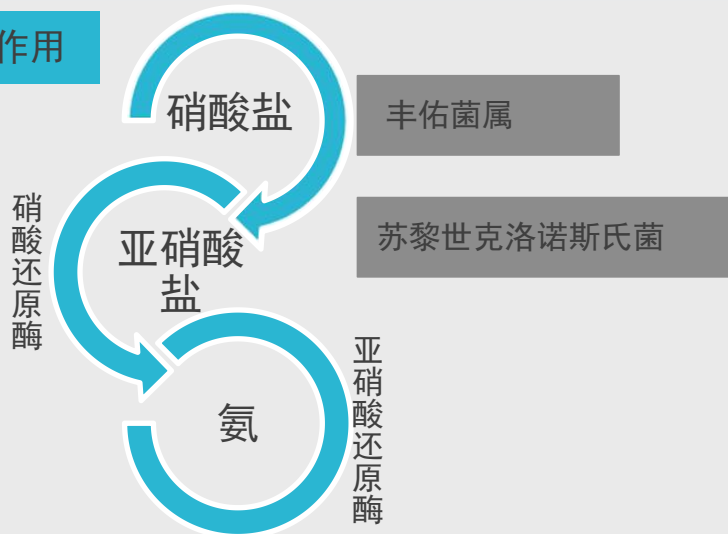
3

反硝化作用：在脱氮基因作用下进行硝酸盐还原。

不同基因编码的酶在氮代谢中与大量热泉中的微生物存在密切关系

READING REPORT

反硝化作用



Nitrogen

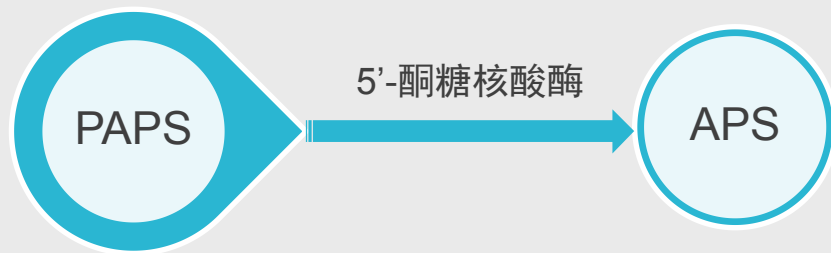
氮代谢

Table 1 Sequences associated with specific enzyme and taxa within nitrogen metabolism using KEGG pathways

Number of sequences assigned	EC number	KEGG orthology	Enzyme name	Organism	Phylum	E value
1	EC:1.7.99.4	KO0372	Nitrate reductase (<i>nar</i>)	<i>Opitutus terrae</i> <i>Cronobacter turicensis</i>	Verrucomicrobia Proteobacteria	3e-32 1e-68
1	EC:1.7.7.1	KO0366	Nitrite reductase (<i>nirA</i>)	<i>Chloroflexus</i>	Chloroflexi	0
1	EC:1.7.1.4	KO0362	Nitrite reductase (<i>nirB</i>)	<i>Burkholderia pseudomallei</i>	Proteobacteria	e-109
1	EC:1.7.3.4	K10535	Hydroxylamine oxidase (<i>hao</i>)	<i>Anaeromyxobacter</i>	Proteobacteria	8e-38
1	EC:1.7.7.2	KO0367	Ferredoxin-nitrate reductase (<i>narB</i>)	<i>Nostoc</i>	Cyanobacteria	5e-48
1	EC:1.7.1.4	KO0363	Nitrite reductase (<i>nirD</i>)	<i>Candidatus Accumulibacter phosphatis</i>	Proteobacteria	2e-31
1	EC:1.7.2.1	KO0368	Nitrite reductase (NO-forming)	<i>Cupriavidus necator</i>	Proteobacteria	4e-15
1	EC:1.7.1.1	KO0360	Nitrate reductase (NADH)	<i>Saccharopolyspora erythraea</i>	Actinobacteria	6e-07

硫代谢

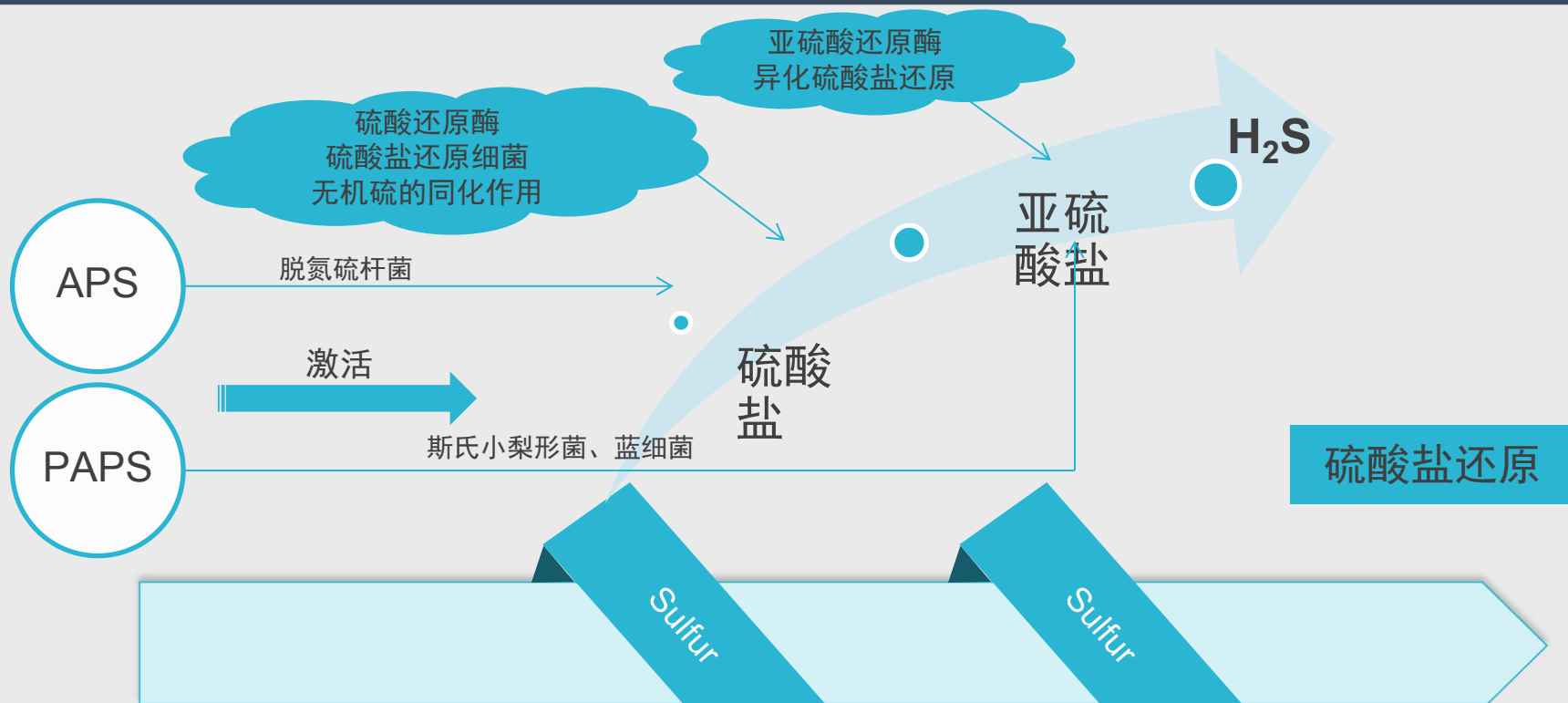
厌氧代谢末端电子受体：硫酸盐和硫。



形成硫酸
释放ATP



硫代谢



硫代谢

Table 2 Sequences associated with specific enzyme and taxa within sulfur metabolism using KEGG pathways

Number of sequences assigned	EC number	KEGG orthology	Enzyme name	Organism	Phylum	E value
1	EC:1.8.4.8	K00390	Phosphoadenosinephosphosulfate reductase (<i>cysH</i>)	<i>Thermosynechococcus elongatus</i>	Cyanobacteria	9e-93
1	EC:2.7.7.4	K00958	Sulfate adenylyltransferase (<i>met3</i>)	<i>Thiobacillus denitrificans</i>	Firmicutes	0
1	EC:1.8.1.2	K00381	Sulfite reductase (NADPH) Hemoprotein beta-component (<i>cysI</i>)	<i>Burkholderia pseudomallei</i>	Proteobacteria	1e-48
1	EC:3.1.3.7	K01082	5'-Bisphosphate nucleotidase (<i>cysQ</i>)	<i>Salinibacter ruber</i>	Chlorobi	2.80e-018
1	EC:2.7.7.4	KO0957	Sulfate adenylyltransferase (<i>cysD</i>)	<i>Opitutus terrae</i>	Verrucomicrobia	e-148
1	EC:2.7.7.4	KO0956	Sulfate adenylyltransferase subunit 1	<i>Acidothermus cellulosilyticus</i>	Actinobacteria	2e-54
1	EC:1.8.7.1	KO0392	Sulfite reductase (ferredoxin)	<i>Nostoc punctiforme</i>	Cyanobacteria	e-157

甲烷代谢

C

• 无氧呼吸末端电子受体

微生物根据不同的温度、PH利用系统中不同有机化合物中的碳进行甲烷代谢

READING REPORT

光养细菌氧化
绿弯菌属
玫瑰弯菌属
甲酸脱氢酶

乙酰-CoA
一氧化碳脱氢酶
乙酰-CoA合成酶

甲酸盐

CO₂

CO

Methane

Methane

Methane

甲烷代谢

Table 3 Sequences associated with specific enzyme and taxa within methane metabolism using KEGG pathways

Number of sequences assigned	EC number	KEGG Orthology	Enzyme name	Organism	Phylum	E value
2	EC:1.2.1.2	K00123	Formate dehydrogenase alpha subunit	<i>Chloroflexus</i> <i>Burkholderia pseudomallei</i>	Chloroflexi Proteobacteria	0 2.00e-003
1	EC:6.2.1.1	—	Acetyl-CoA synthetase (<i>acs</i>)	<i>Chloroflexus</i>	Chloroflexi	0
1	EC:2.3.1.169	K14138	Carbon-monoxide dehydrogenase/acetyl-CoA synthase subunit alpha	<i>Thermodesulfovibrio yellowstonii</i>	Nitrospirae	6e-56
1	EC:2.3.1.169	KO0190	Carbon-monoxide dehydrogenase/acetyl-CoA synthase subunit beta	<i>Carboxydotherrnus hydrogenoformans</i>	Firmicutes	e-107
1	EC:1.2.99.2C	K00198	Carbon-monoxide dehydrogenase catalytic subunit (<i>cooS</i>)	<i>Thermoanaerobacter tengcongensis</i>	Firmicutes	2e-81
1	EC:1.2.99.2F	K00196	Carbon-monoxide dehydrogenase iron sulfur subunit (<i>cooF</i>)	<i>Archaeoglobus fulgidus</i>	Euryarchaeota	4e-08
1	EC:1.2.99.2L	K03520	Carbon-monoxide dehydrogenase large subunit (<i>cutL</i> , <i>coxL</i>)	<i>Nocardioioides</i>	Actinomycetes	4e-25
1	EC:1.2.99.2 M	K03519	Carbon-monoxide dehydrogenase medium subunit (<i>cutM</i> , <i>coxM</i>)	<i>Petrotoga mobilis</i>	Thermotogae	1e-12



Discussion

READING REPORT

碱性含硫热泉



微生物群落



能量代谢

氮代谢

变形菌门
水平基因转移

硫代谢

厚壁菌门代谢和生理功能强大
对硫酸还原过程有促进作用

甲烷代谢

绿色非硫细菌与甲烷代谢相关酶甲酸
脱氢酶参与反应导致二氧化碳的形成

THANKS