fermentation

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Nitrogen Metabolism during Anaerobic Fermentation of Actual Food Waste under Different pH Conditions

Chuyun Zhao [1,†], Luxin Yang [1,†], Huan Li [1,*] and Zhou Deng [2]

[1] Shenzhen International Graduate School, Tsinghua University, Shenzhen 518055, China; 18817295185@163.com (C.Z.); ylx_hz@163.com (L.Y.)

[2] Shenzhen Lisai Environmental Technology Co., Ltd., Shenzhen 518055, China; dengzhou2005@126.com

* Correspondence: li.huan@sz.tsinghua.edu.cn

† These authors contributed equally to this work.

Supplementary material

1. Metagenomic analyses

1.1. Sequencing data processing

The raw data processing using Trimmomatic (v.0.36): http://www.usadellab.org/cms/index.php?page=trimmomatic was conducted to acquire the clean data for subsequent analysis.

1.2. Metagenome Assembly

MEGAHIT (Version v1.0.6): https://github.com/voutcn/megahit could be used to assemble the clean data. Filter the fragment shorter than 500 bp in all of Scaftigs for statistical analysis.

1.3. Binning

Contigs obtained from the metagenomic assembly are filtered based on length. Subsequently, the filtered contigs are used for binning assembly using MetaBAT (version 2.12.1). MetaBAT employ nucleotide composition and abundance (NCA) to calculate distance matrices for the binning assembly of contigs, producing bins that are used for reconstructing draft microbial genomes from environmental samples. For quality assessment of the obtained bins, the CheckM software (version not specified) is used. The working principle of CheckM involves the identification of marker genes in the bin sequences via Hidden Markov Models (HMM). These are then aligned with universal single-copy genes using HMMER (version 3.1.2). The bins are placed into a reference phylogenetic tree using pplacer, and a specific lineage marker set is determined for quality assessment of the bins. Species annotation for the bins is carried out using the AMPHORA2 software.

1.4. Gene prediction, taxonomy, and functional annotation

Open reading frames (ORFs) from each assembled contig were predicted using Prodigal/MetaGene (http://metagene.cb.k.u-tokyo.ac.jp/). The predicted ORFs with a length ≥ 100 bp were retrieved and translated into amino acid sequences using the NCBI translation table (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=tgencodes#SG1).

A non-redundant gene catalog was constructed using CD-HIT (http://www.bioinformatics.org/cd-hit/, version 4.6.1) with 90% sequence identity and 90% coverage. High-quality reads were aligned to the non-redundant gene catalogs to calculate gene abundance with 95% identity using SOAPaligner (http://soap.genomics.org.cn/, version 2.21).

Representative sequences of non-redundant gene catalog were aligned to NR database with an e-value cutoff of 1e-5 using Diamond (http://www.diamondsearch.org/index.php, version 0.8.35) for taxonomic annotations. The KEGG annotation was conducted using Diamond (http://www.diamondsearch.org/index.php, version 0.8.35) against the Kyoto Encyclopedia of Genes and Genomes database (http://www.genome.jp/keeg/) with an e-value cutoff of 1e-5.

2. Metatranscriptomic analyses

2.1. Quality control

The raw data of fastq format was processed by Trimmomatic (v.0.36) http://www.usadellab.org/cms/index.php?page=trimmomatic to acquire the clean data. Clean data were mapping to NCBI Rfam databases, to remove the rRNA sequences by Bowtie2 (v2.33) https://github.com/BenLangmead/bowtie2.

2.2. De novo assembly

The remaining mRNA sequences were de novo assembled, which using the data by splicing software Trinity (v2.4.0) https://github.com/trinityrnaseq/trinityrnaseq.

2.3. Quantification of gene expression level

Clean data was mapped back onto the assembled transcriptome by bowtie2 (v2.33) https://github.com/BenLangmead/bowtie2. Read count of each gene in each sample was obtained from the mapping results by corset (v1.06) https://github.com/Oshlack/Corset. In order to make the expression level of genes comparable among different genes and different experiments, the RPKM of each gene was calculated.

2.4. Annotation

The Unigenes were blasted to the sequences of different database by BLASTx http://blast.ncbi.nlm.nih.gov/Blast.cgi to obtain the species annotation results (e-value＜ 1e-5). The sum of the RPKM of all genes belonging to a species was taken as the abundance of the species. At each taxonomic level, the abundance of species in each sample was counted to construct an expression abundance profile at the corresponding taxonomic level. The corresponding functional annotation and abundance statistics were obtained based on KEGG database (http://www.kegg.jp/kegg/),

3. Supplementary figures

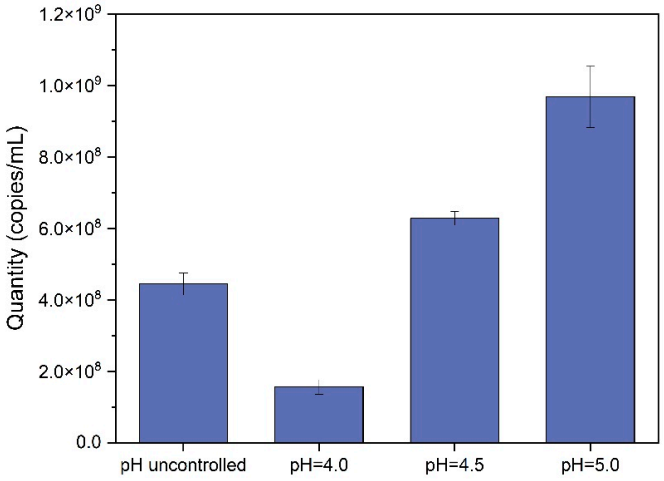


Figure S1. Quantities of bacteria in reactors using qPCR.

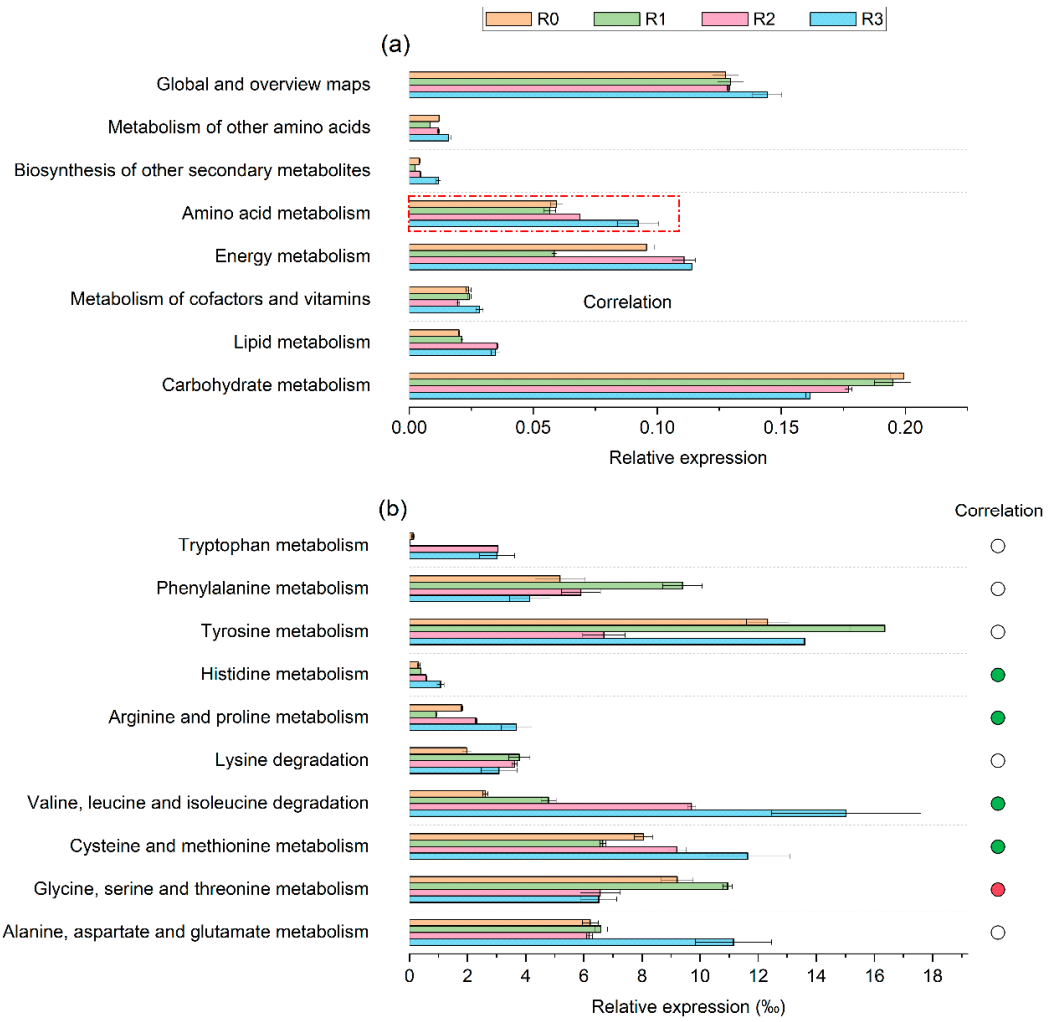
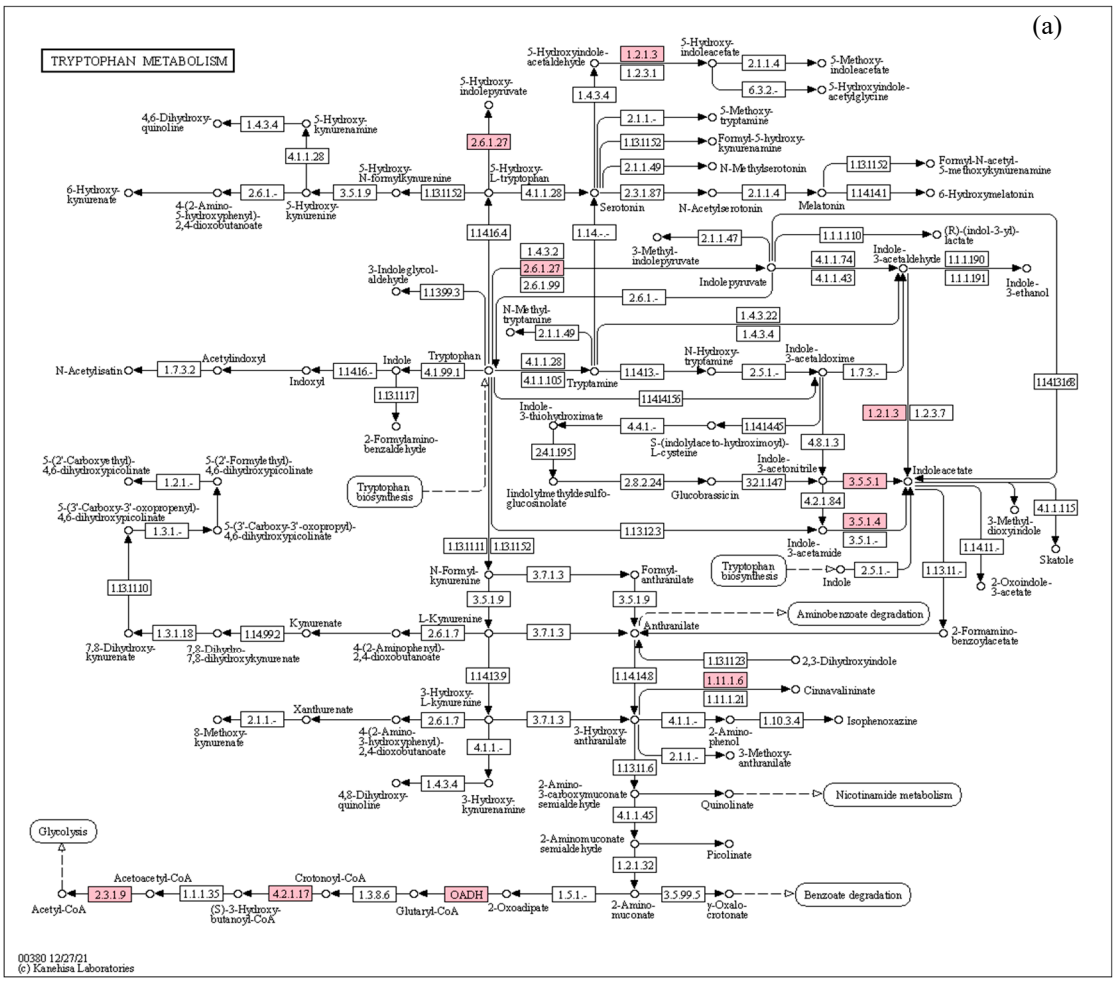


Figure S2. Relative gene expression of representative KEGG functional categories (a. metabolism at secondary level; b. key enzymes involving amino acid metabolism; Green pellets indicate positive correlation between expression level and pH, implying the Spearman's correlation coefficient R2 > 0.7 and P < 0.01, and the red pellets indicate negative correlation).


(a)
TRYPTOPHAN METABOLISM
Glycolysis
Acetyl-CoA
Acetoacetyl-CoA
(S)-3-Hydroxybutanoyl-CoA
Crotonoyl-CoA
Glutaryl-CoA
2-Oxoadipate
2-Aminomuconate
y-Oxalocrotonate
Benzoate degradation
Picolinate
Quinolinate
Nicotinamide metabolism
2-Aminomuconate semialdehyde
2-Amino-3-carboxymuconate semialdehyde
4,8-Dihydroxyquinoline
3-Hydroxykynurenamine
Xanthurenate
8-Methoxykynurenate
4-(2-Amino-3-hydroxyphenyl)-2,4-dioxobutanoate
3-Hydroxyanthranilate
2-Aminophenol
Isophenoxazine
3-Methoxyanthranilate
Cinnavalininate
2,3-Dihydroxyindole
Aminobenzoate degradation
Anthranilate
2-Formamino-benzoylacetate
Formylanthranilate
N-Formylkynurenine
L-Kynurenine
Kynurenate
7,8-Dihydroxykynurenate
4-(2-Aminophenyl)-2,4-dioxobutanoate
Tryptophan biosynthesis
Indole
Indoleacetate
Skatole
3-Methyldioxyindole
2-Oxoindole-3-acetate
Indole-3-acetamide
Indole-3-acetonitrile
Glucobrassicin
Indolylmethylsulfoglucosinolate
S-(indolylacetohydroximoyl)-L-cysteine
Indole-3-thiohydroximate
Tryptamine
Tryptophan
Indoxyl
N-Acetylisatin
Acetylindoxyl
Indole-3-acetaldoxime
N-Hydroxytryptamine
N-Methyltryptamine
Indole pyruvate
3-Methylindolepyruvate
Indole-3-acetaldehyde
Indole-3-ethanol
(R)-(indol-3-yl)-lactate
3-Indoleglycolaldehyde
5-Hydroxyindolepyruvate
5-Hydroxyindoleacetaldehyde
5-Hydroxyindoleacetate
5-Methoxyindoleacetate
5-Hydroxyindole-acetylglycine
5-Hydroxy-L-tryptophan
5-Hydroxy-N-formylkynurenine
5-Hydroxykynurenine
6-Hydroxykynurenate
4-(2-Amino-5-hydroxyphenyl)-2,4-dioxobutanoate
4,6-Dihydroxyquinoline
5-Hydroxykynurenamine
Serotonin
N-Acetylserotonin
Melatonin
6-Hydroxymelatonin
5-Methoxytryptamine
Formyl-5-hydroxykynurenamine
N-Methylserotonin
Formyl-N-acetyl-5-methoxykynurenamine
5-(2'-Carboxyethyl)-4,6-dihydroxypicolinate
5-(2'-Formylethyl)-4,6-dihydroxypicolinate
5-(3'-Carboxy-3'-oxopropenyl)-4,6-dihydroxypicolinate
5-(3'-Carboxy-3'-oxopropyl)-4,6-dihydroxypicolinate
Tryptophan biosynthesis
Phenylalanine, tyrosine and tryptophan biosynthesis
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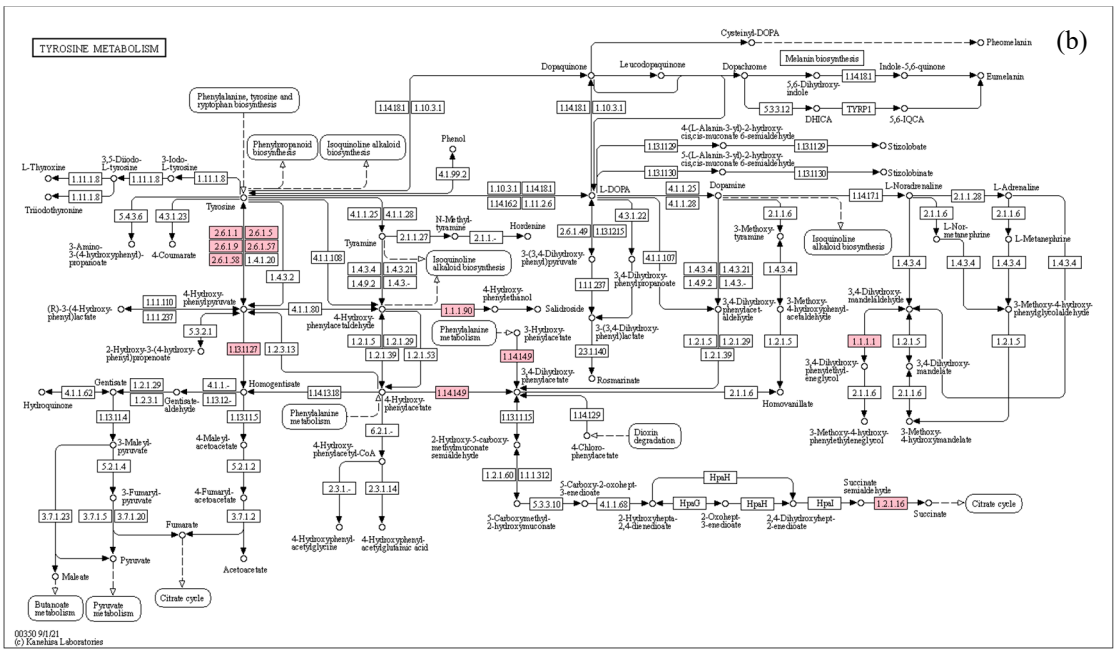
(c) Kanehisa Laboratories


(b)
TYROSINE METABOLISM
Tyrosine
L-DOPA
Dopamine
L-Noradrenaline
L-Adrenaline
Dopaquinone
Leucodopaquinone
Dopachrome
Melanin biosynthesis
Cysteinyl-DOPA
Pheomelanin
Indole-5,6-quinone
Eumelanin
5,6-Dihydroxyindole
DHICA
TYRP1
5,6-IQCA
Phenylalanine, tyrosine and tryptophan biosynthesis
Phenylpropanoid biosynthesis
Isoquinoline alkaloid biosynthesis
Phenol
Stizolobate
Stizolobinate
L-Thyroxine
Triiodothyronine
3,5-Diiodo-L-tyrosine
3-Iodo-L-tyrosine
3-Amino-3-(4-hydroxyphenyl)-propanoate
4-Coumarate
N-Methyltyramine
Hordenine
Tyramine
3-Methoxytyramine
3-(3,4-Dihydroxyphenyl)pyruvate
3,4-Dihydroxyphenylpropanoate
3,4-Dihydroxyphenylacetaldehyde
3,4-Dihydroxyphenylacetate
3,4-Dihydroxyphenyllactate
3,4-Dihydroxymandelaldehyde
3,4-Dihydroxymandelate
3,4-Dihydroxyphenylethyleneglycol
3-Methoxy-4-hydroxyphenylacetaldehyde
3-Methoxy-4-hydroxyphenylglycolaldehyde
3-Methoxy-4-hydroxymandelate
3-Methoxy-4-hydroxyphenylethyleneglycol
Salidroside
Rosmarinate
Homovanillate
Homogentisate
Hydroquinone
Gentisate
Gentisate-aldehyde
4-Hydroxyphenylacetaldehyde
4-Hydroxyphenylacetate
4-Hydroxyphenylpyruvate
4-Hydroxyphenylethanol
4-Hydroxyphenylacetyl-CoA
4-Hydroxyphenylacetylglycine
4-Hydroxyphenylacetylglutamic acid
4-Maleylacetoacetate
4-Fumarylacetoacetate
Fumarate
Maleate
Pyruvate
Acetoacetate
3-Maleylpyruvate
3-Fumarylpyruvate
Phenylalanine metabolism
Tyrosine metabolism
Butanoate metabolism
Pyruvate metabolism
Citrate cycle
Succinate
Succinate semialdehyde
Dioxin degradation
4-Chlorophenylacetate
2-Hydroxy-5-carboxymethylmuconate semialdehyde
5-Carboxymethyl-2-hydroxymuconate
5-Carboxy-2-oxohept-3-enedioate
2-Hydroxyhepta-2,4-dienedioate
2-Oxohept-3-enedioate
2,4-Dihydroxyhept-2-enedioate
HpaH
HpaG
HpaI
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(c) Kanehisa Laboratories

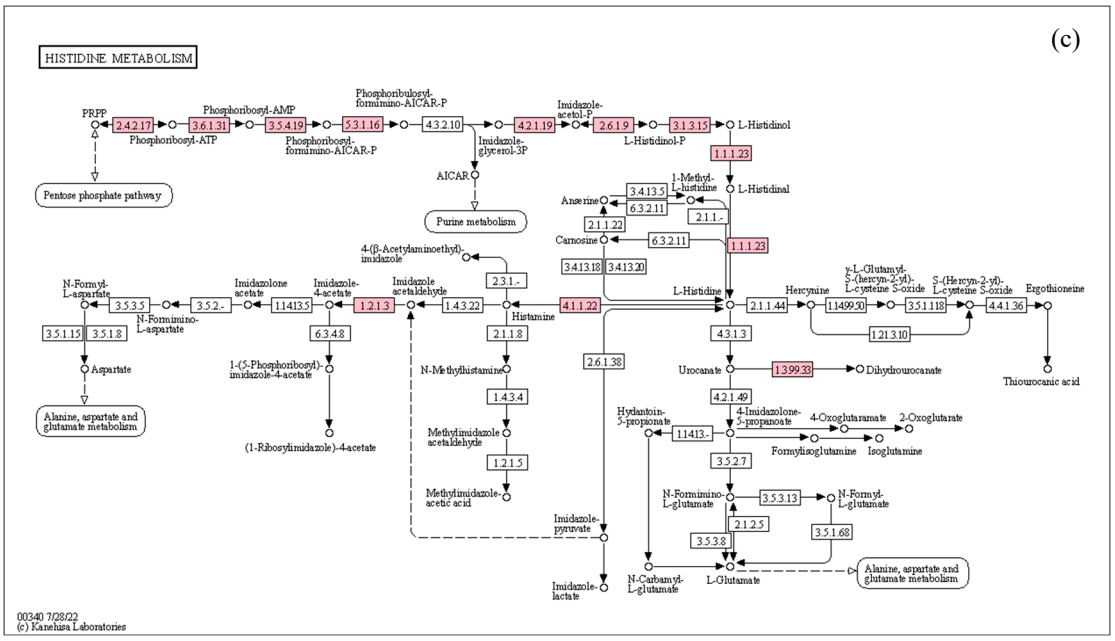

Figure S3. Tryptophane metabolism pathway (ko00380) (a), tyrosine metabolism pathway (ko00350) (b), and histidine metabolism pathway (ko00340) (c) in FW fermentation reactors. The detected and expressed enzymes are colored in pink.