Research progress in Microbial Ecology Research on Chinese Liquor

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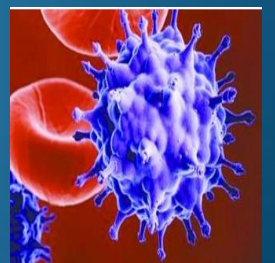


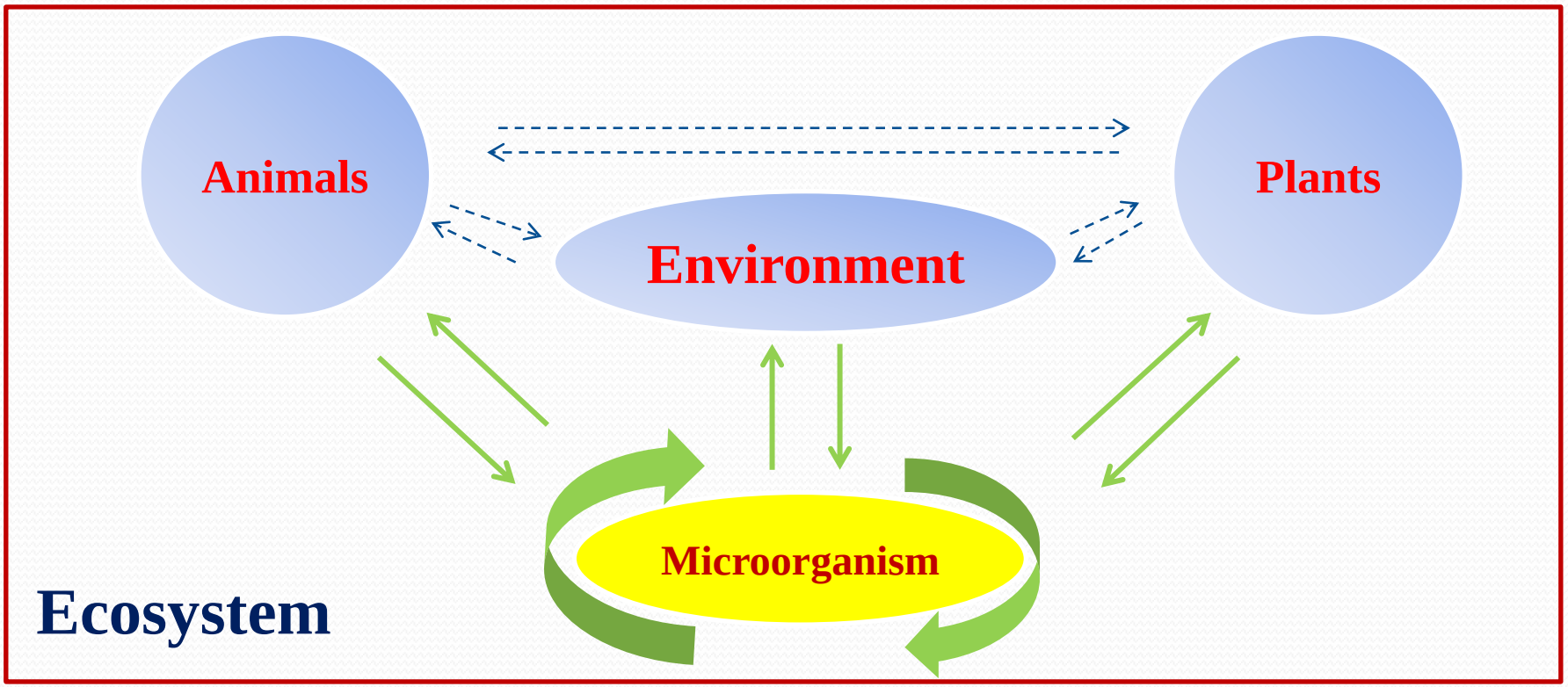
Zhengzhou, Oct. 2014

Work flow

- Definition of Microbial Ecology
- Research fields of Microbial Ecology
- Methods used in the research
- Background of Chinese Liquor
- Research of Microbial Ecology on Chinese Liquor
- Two research samples

Definition

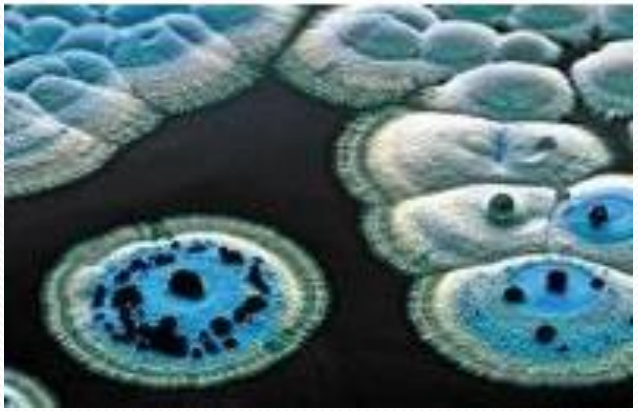
Microbial Ecology is a kind of investigations of how microorganisms interact with the environment, with each other and with their hosts.



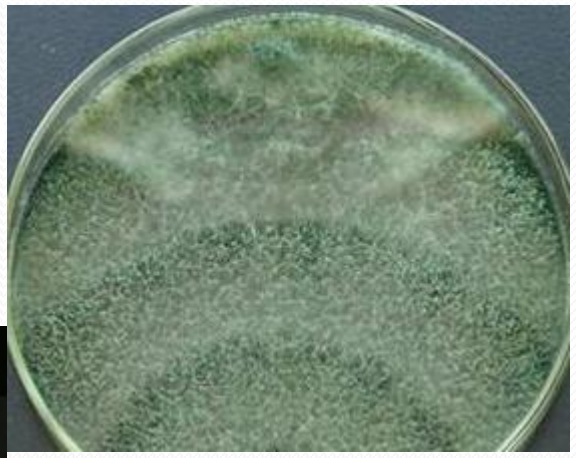
Microorganism

Bacteria

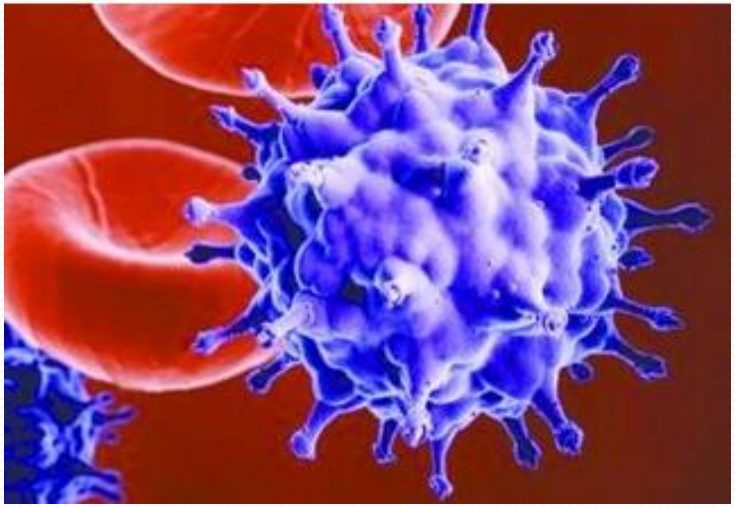
Actinomyces



Fungus

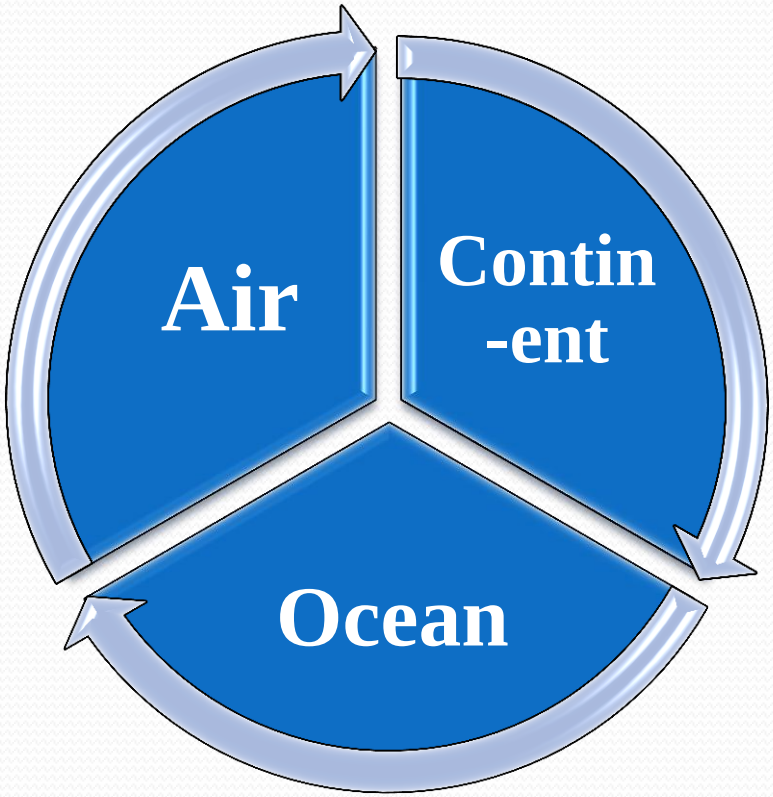


Virus



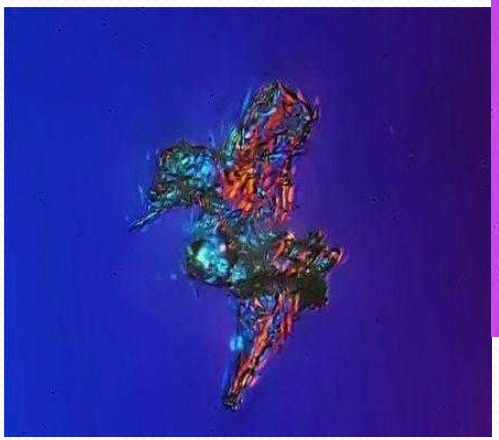
Fungus

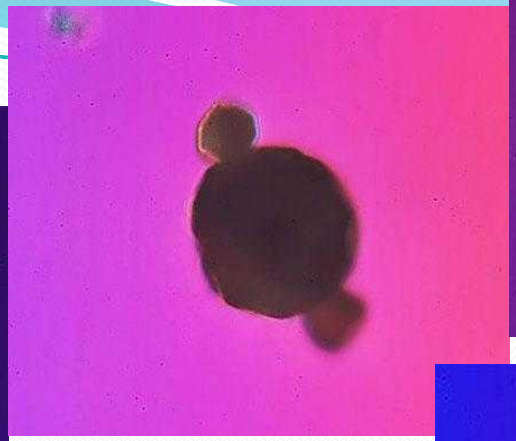
Research fields of Microbial Ecology

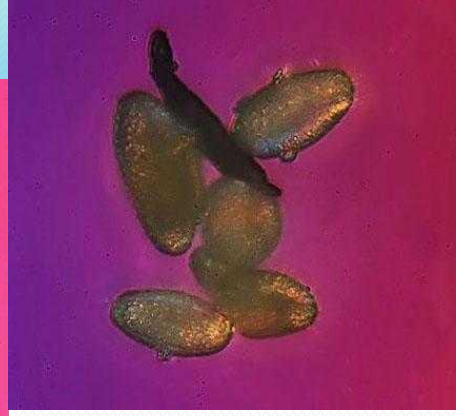


Everywhere!

Air



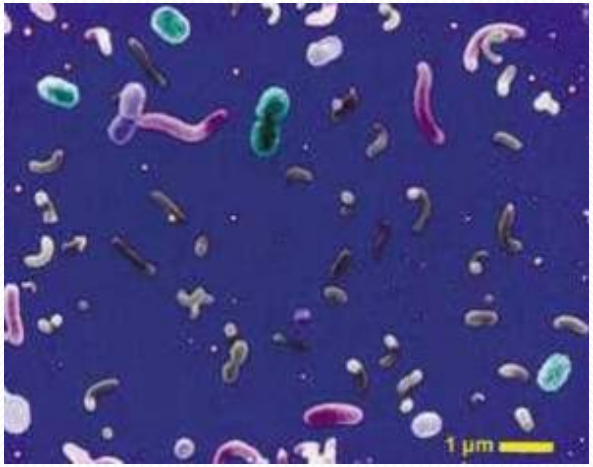




Air pollution

Haze

Organisms



Microbial diversity

Pollution

Ocean

Cyanophyte

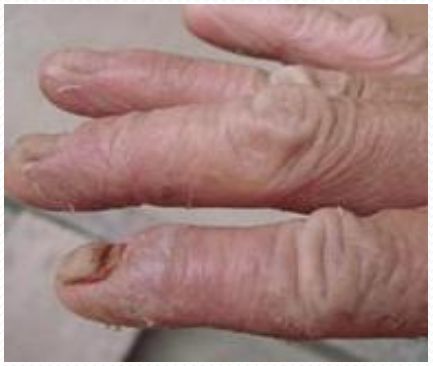
Red tide

Food

Agriculture

Contin
-ent

Medicare

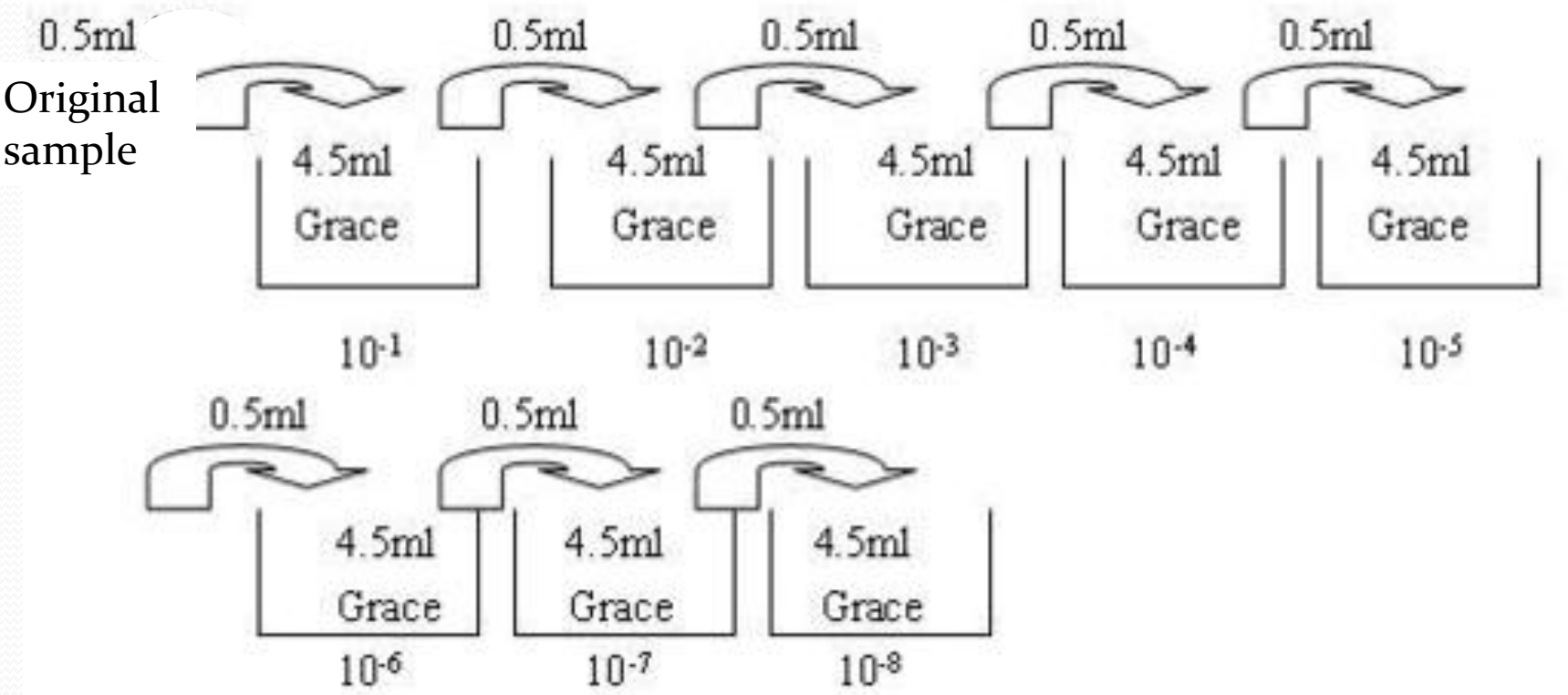


Environment

Methods used in the research

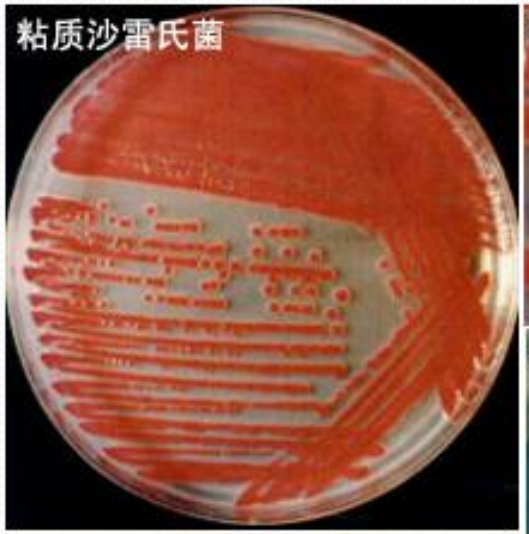
- **Traditional culturable methods** were used to test the strains through phenotypic characteristics such as the colonial morphology, microscopic morphology, physicochemical characteristics and so on.
- **Modern biochemical and molecular biological methods** were taken to identify the strains by using fatty acids assay, polar lipids profiles, whole cell protein electrophoresis, RFLP (Restriction Fragment Length Polymorphism), DGGE (Denaturing Gel Gradient Electrophoresis), cloning libraries, metagenomics and so on.

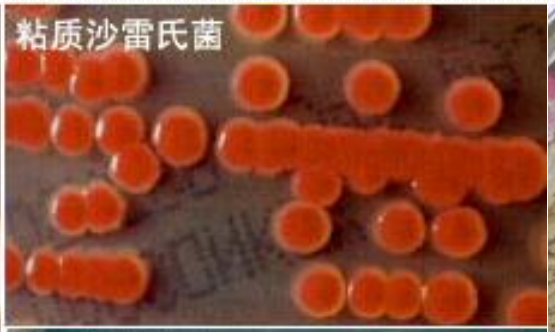
Methods used in the research

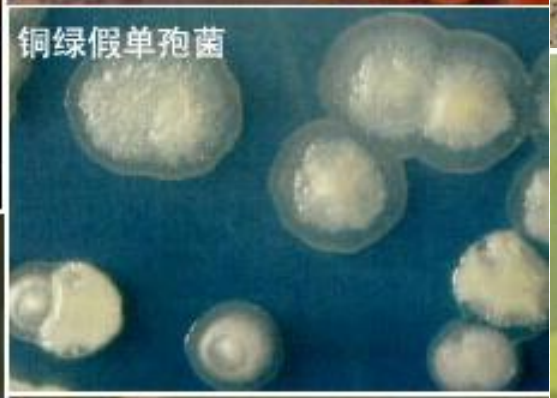


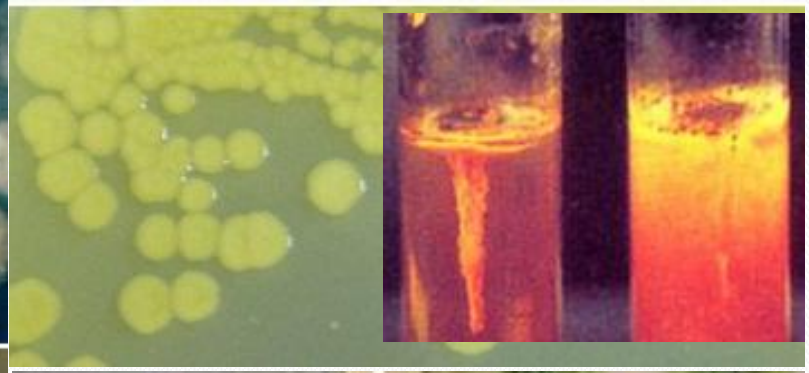
Traditional culturable methods

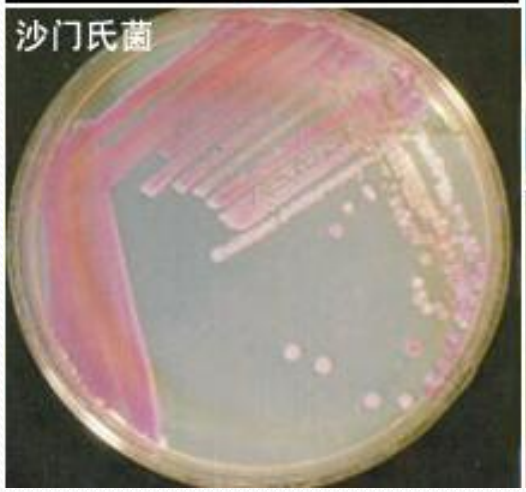
Traditional culturable methods



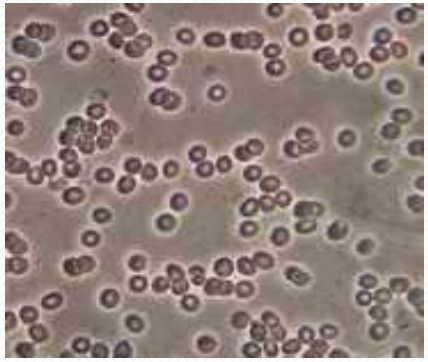
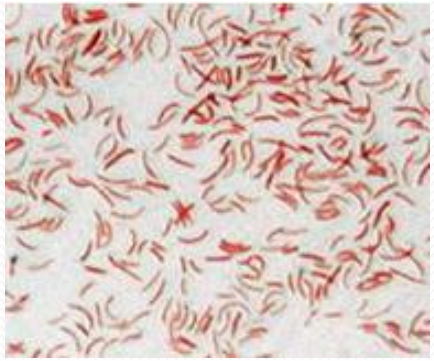
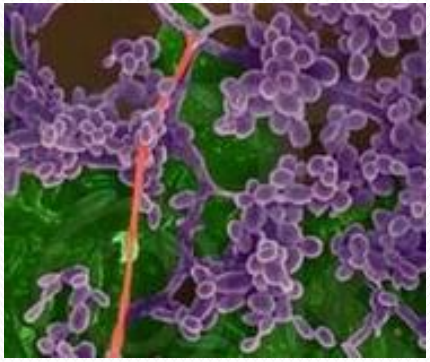
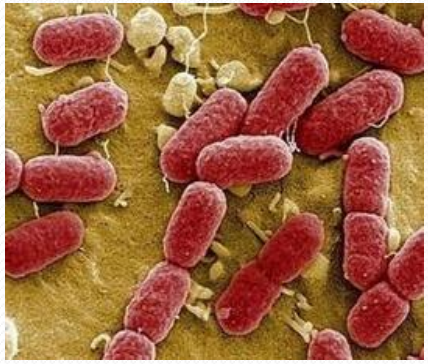
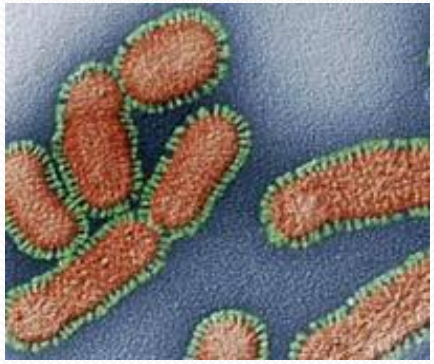


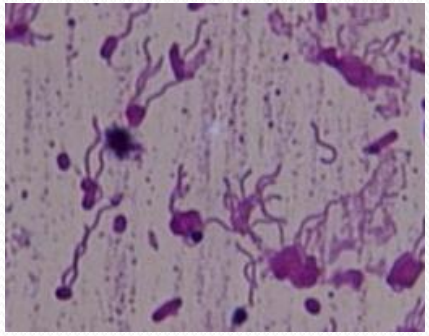
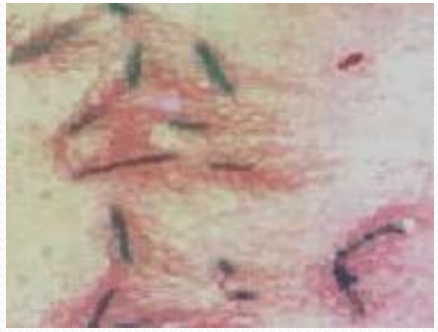


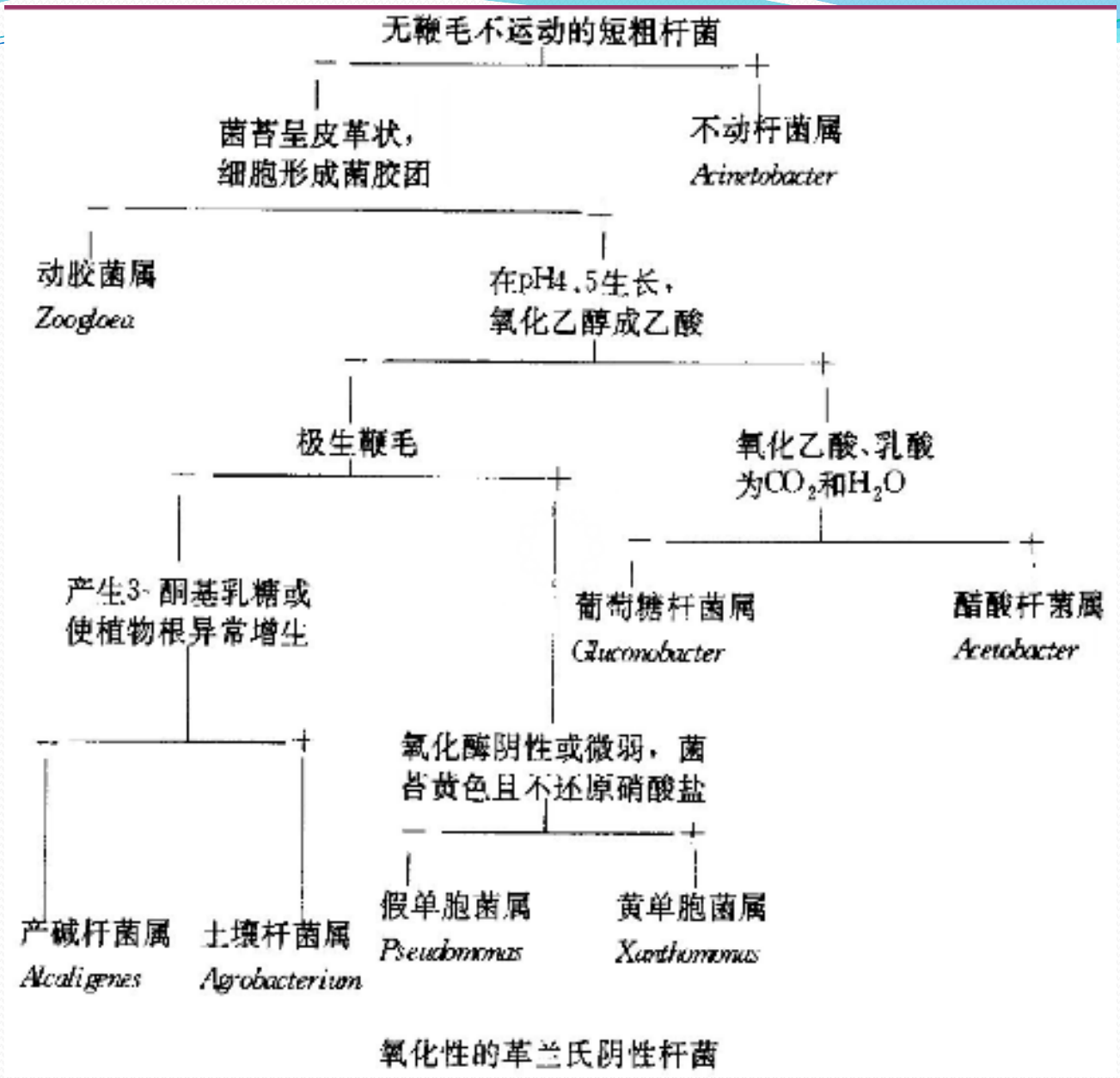




Traditional culturable methods

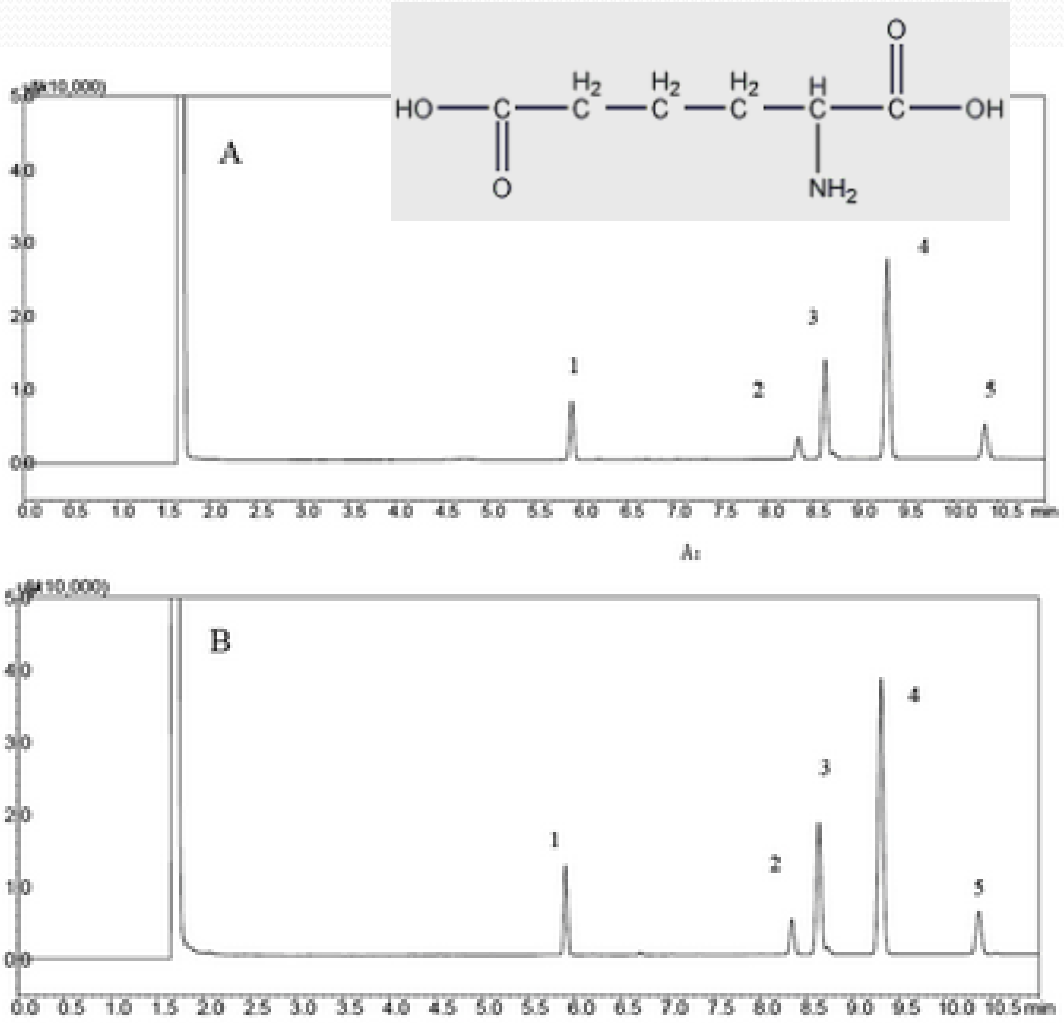






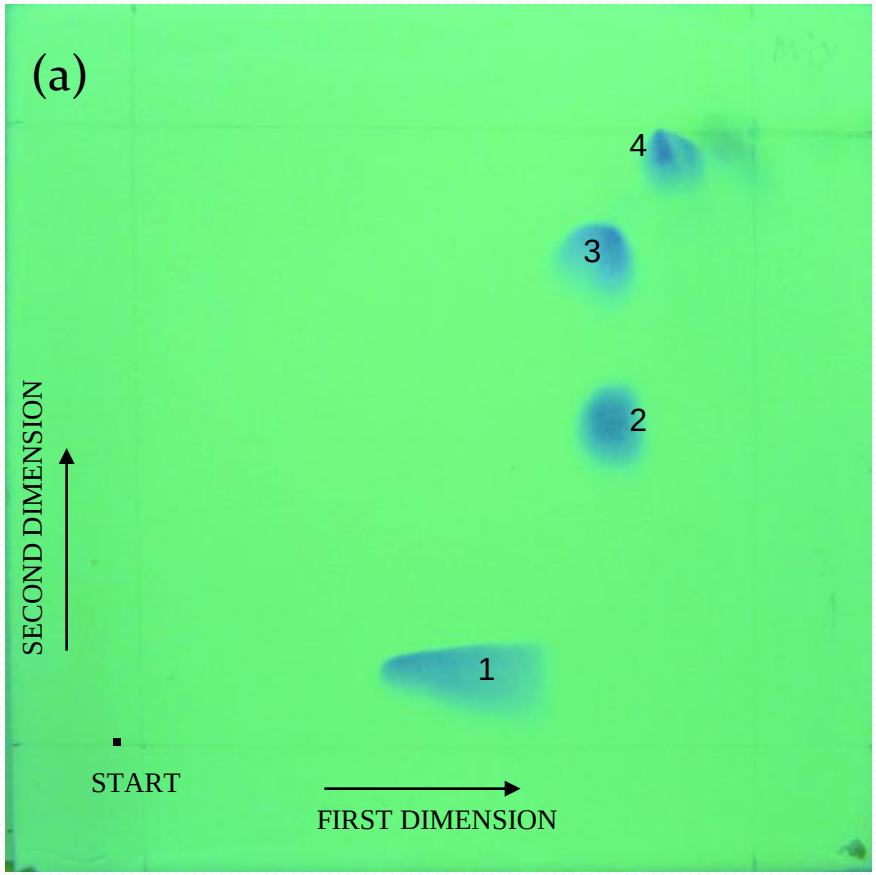
Traditional culturable methods

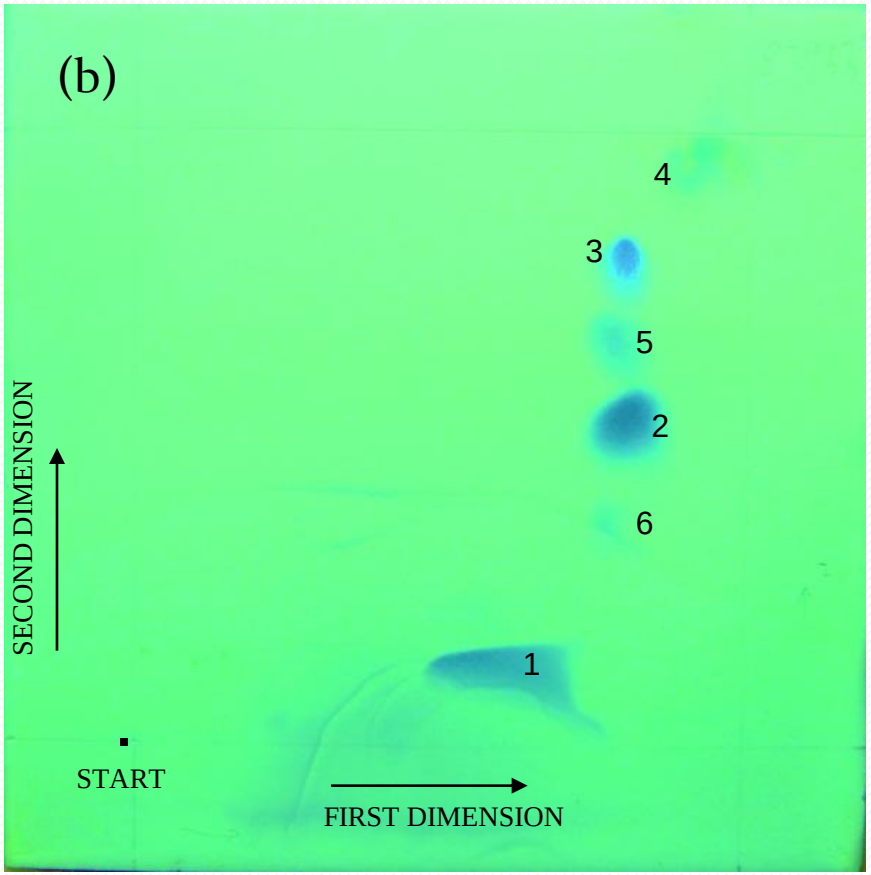
Modern biochemical methods



fatty acids assay

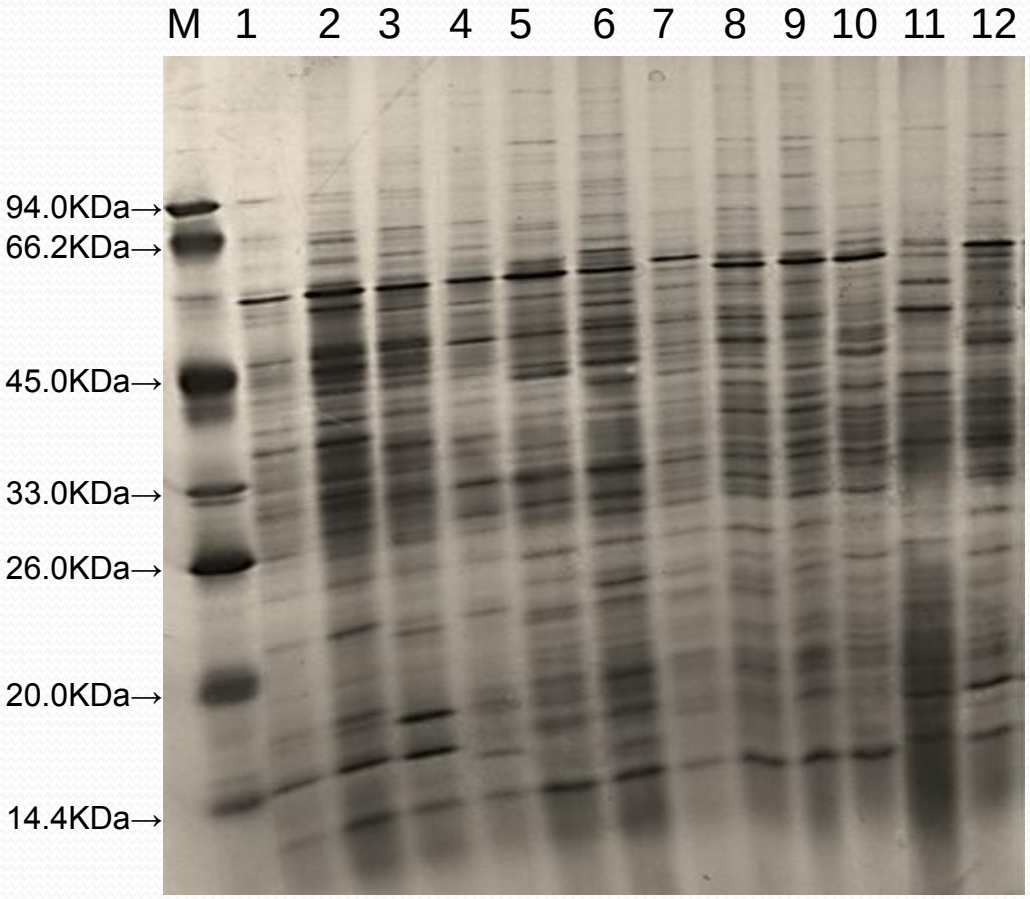
Modern biochemical methods

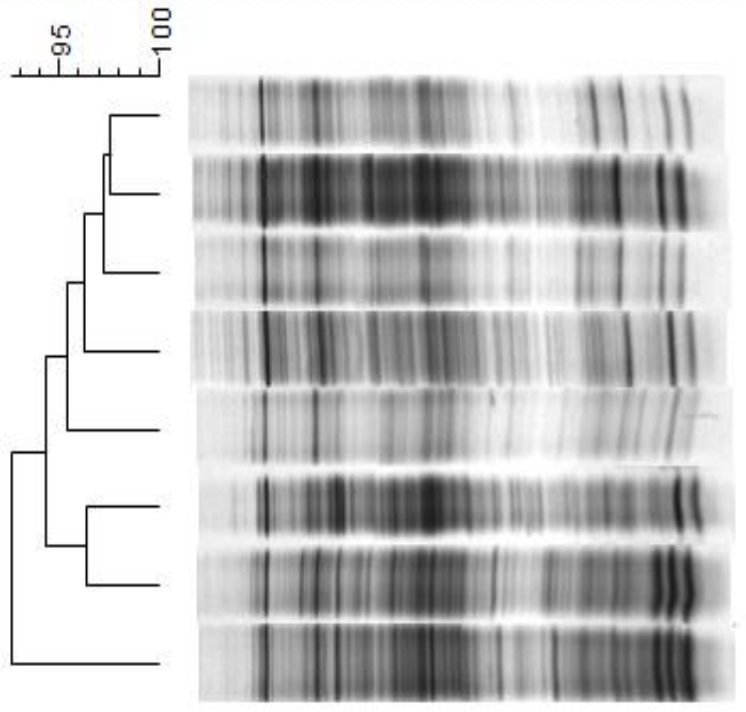




Polar lipids profiles

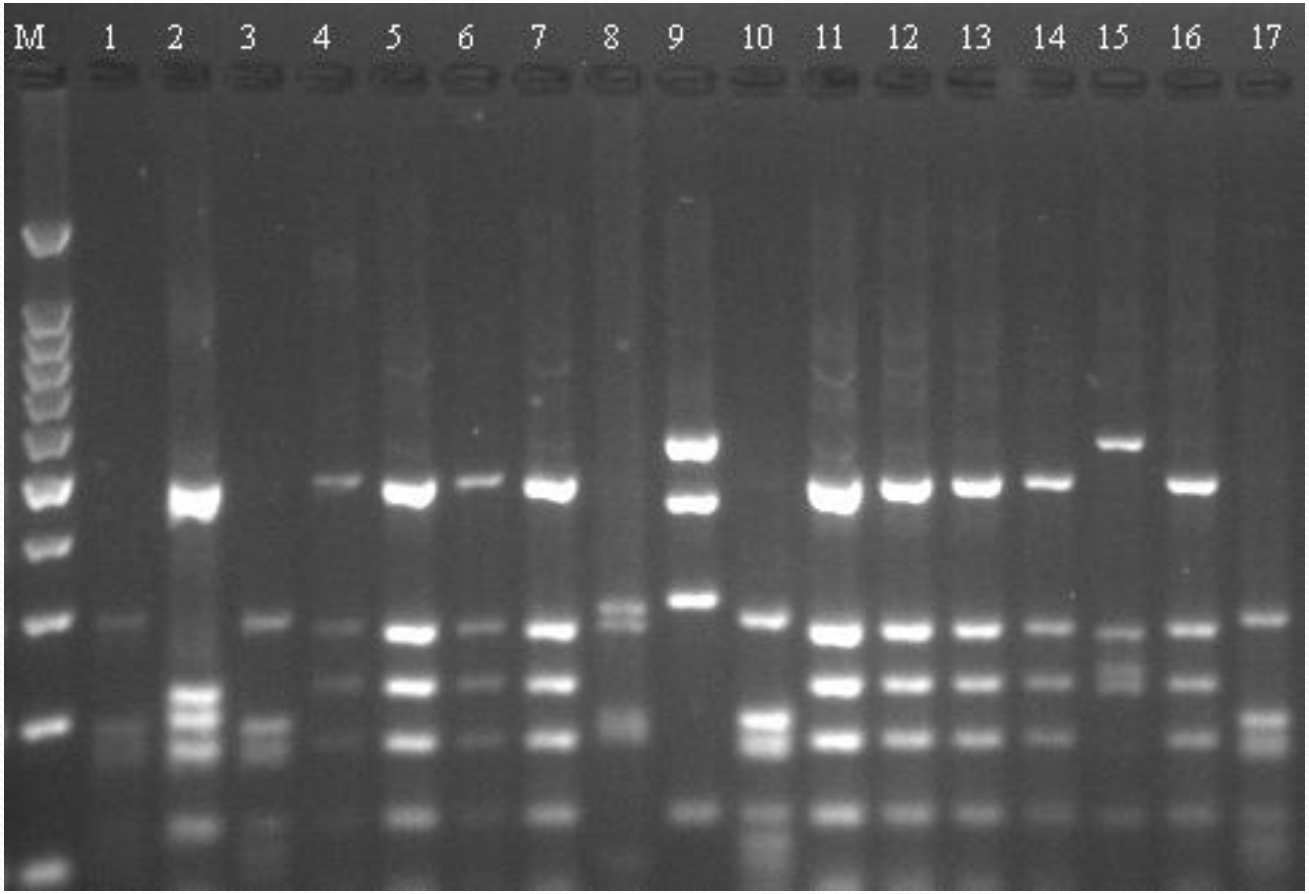
Modern biochemical methods

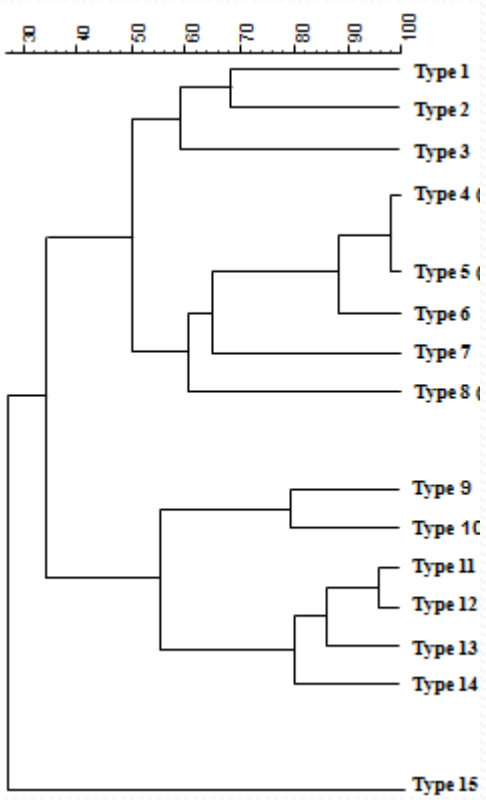




whole cell protein electrophoresis

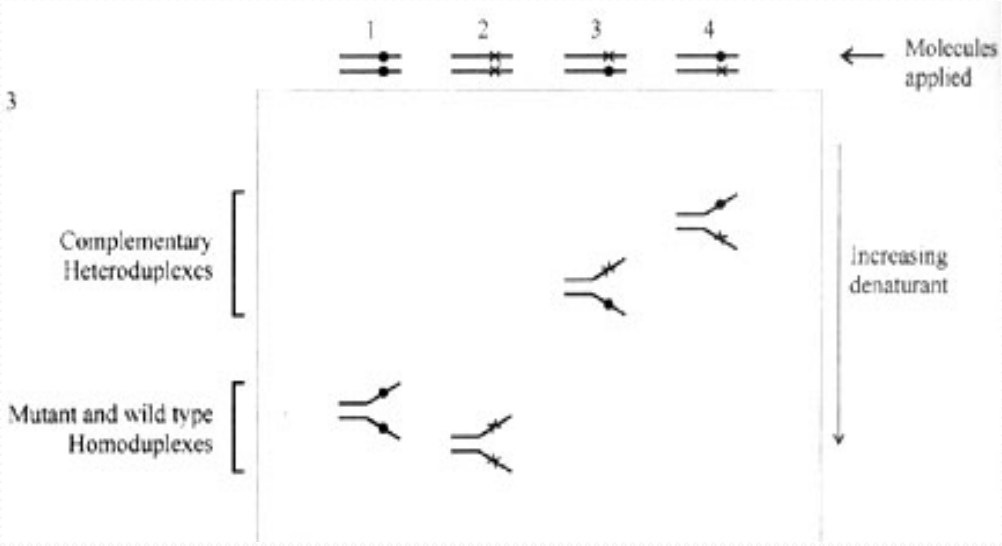
Modern molecular biological methods

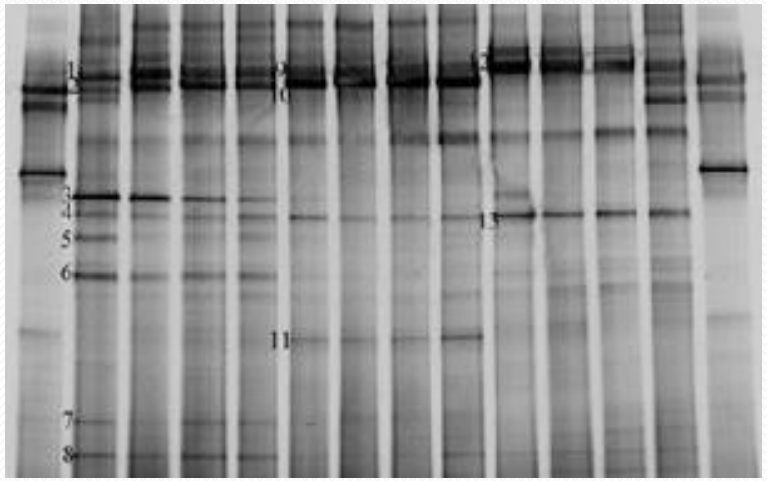




RFLP (Restriction Fragment Length Polymorphism)

Modern molecular biological methods





DGGE (Denaturing Gel Gradient Electrophoresis)

Modern molecular biological methods

Prepare the genomic DNA of samples

PCR to get the genes want

Electrophoresis and gel restoring the aiming band

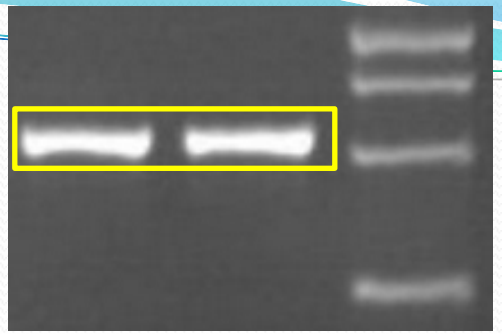
Cloning into T vector

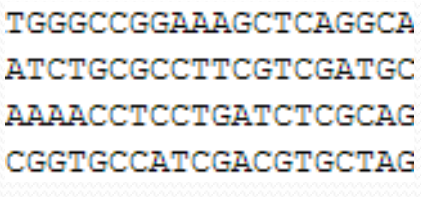
Picking positive colonies & Do double restriction cutting

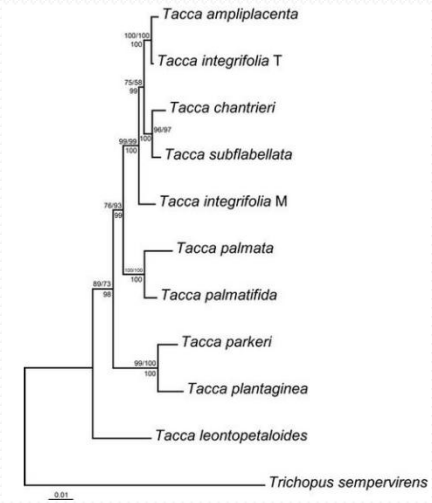
Taken representatives for sequencing and analysis

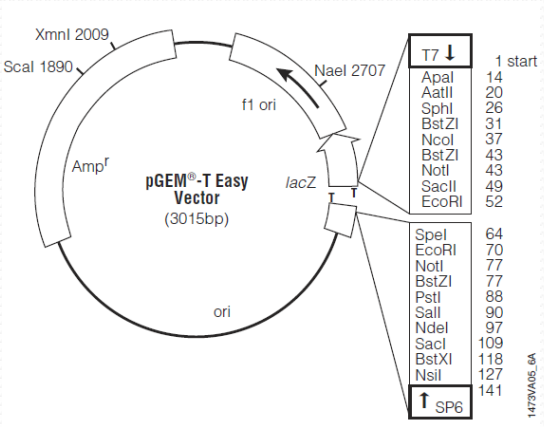
Construction of Cloning Libraries

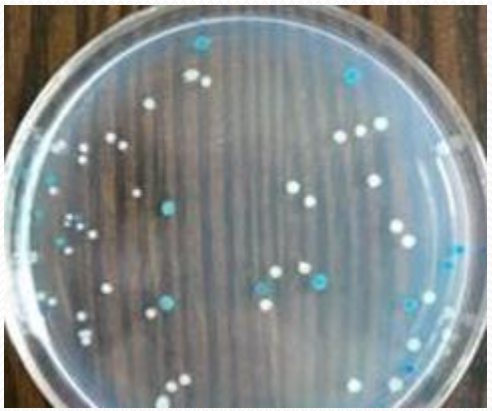
Construction of Cloning Libraries



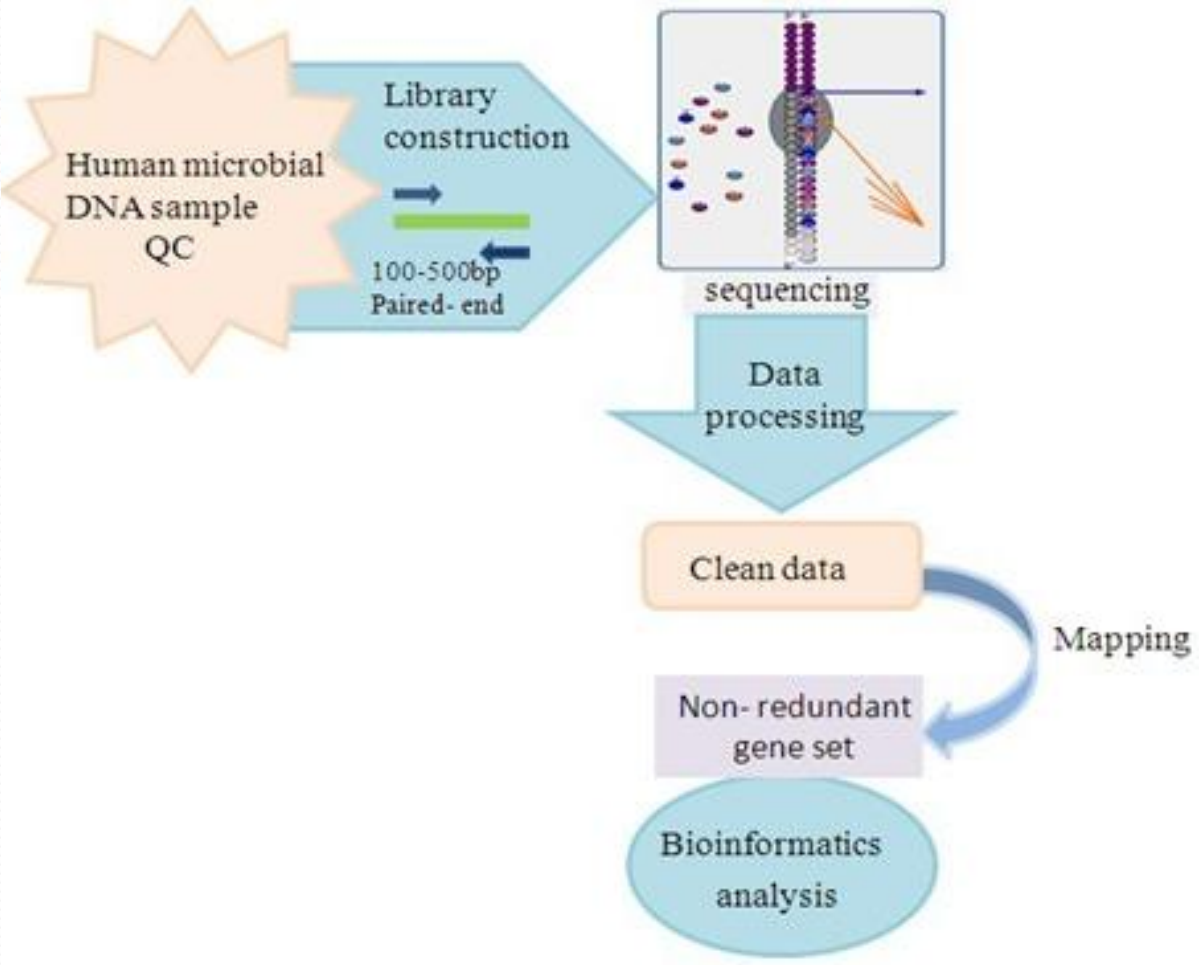




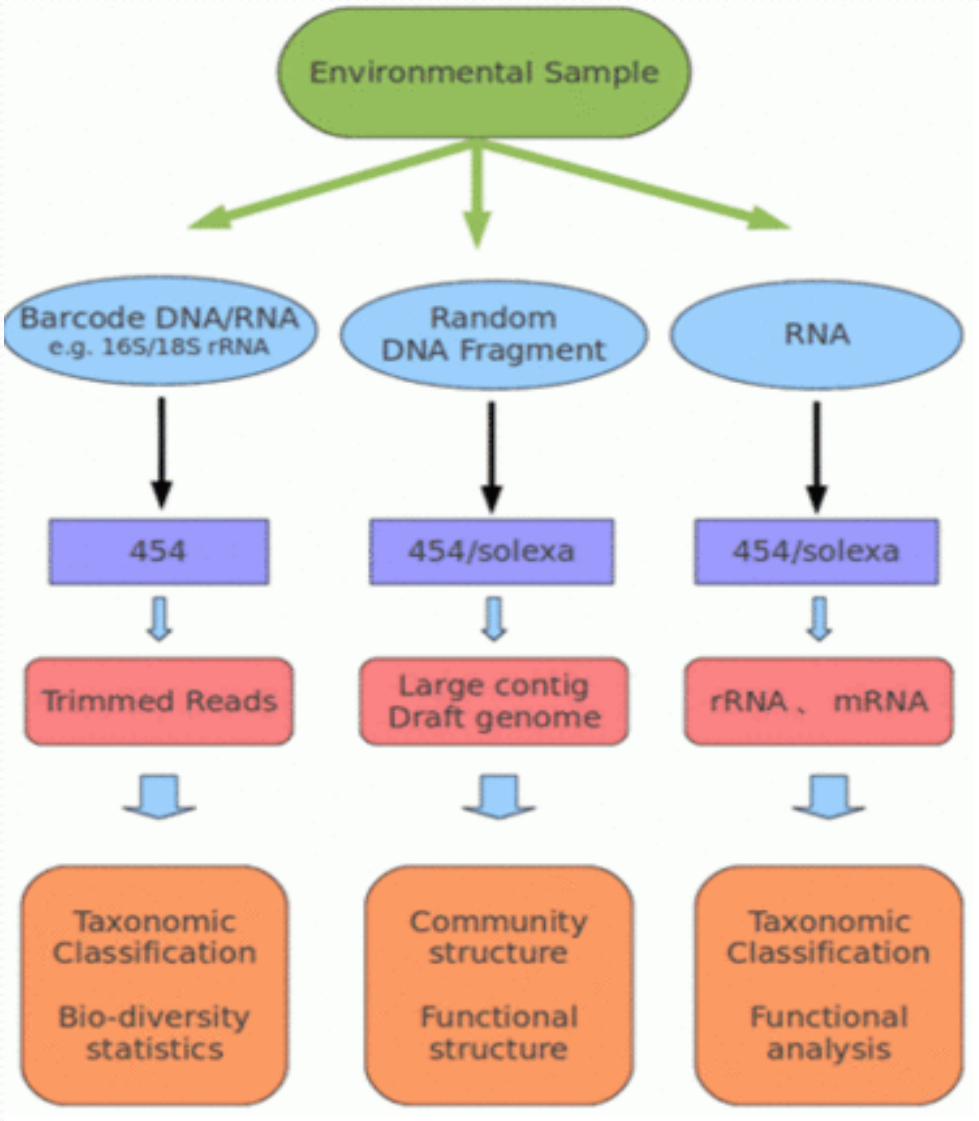




Modern molecular biological methods



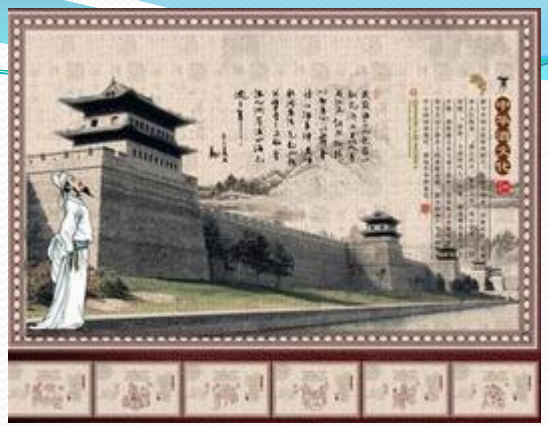
Metagenomics



Metagenomics

Background of Chinese Liquor

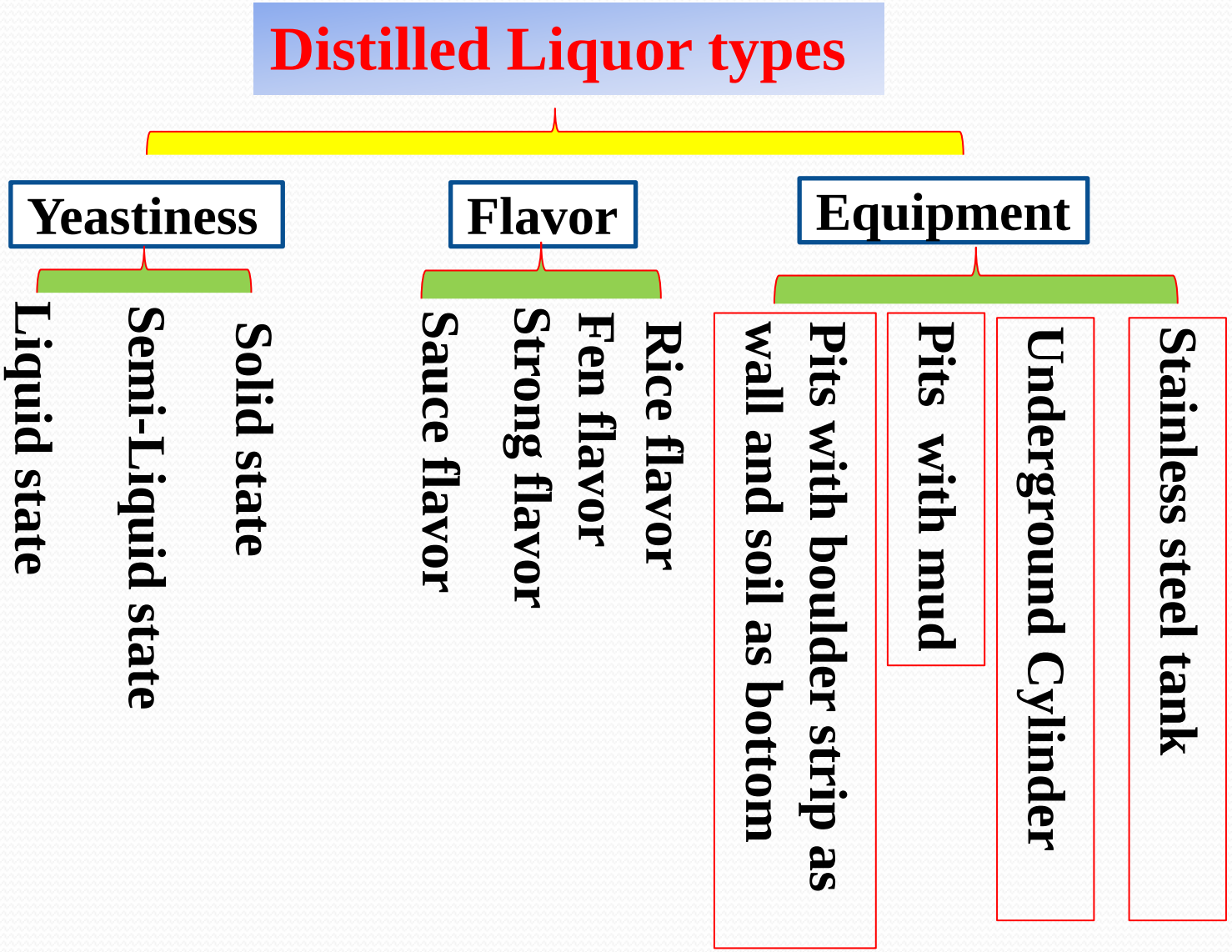
Liquor Culture



Background of Chinese Liquor

The Producing Procedure → **distilled liquor**

Background of Chinese Liquor



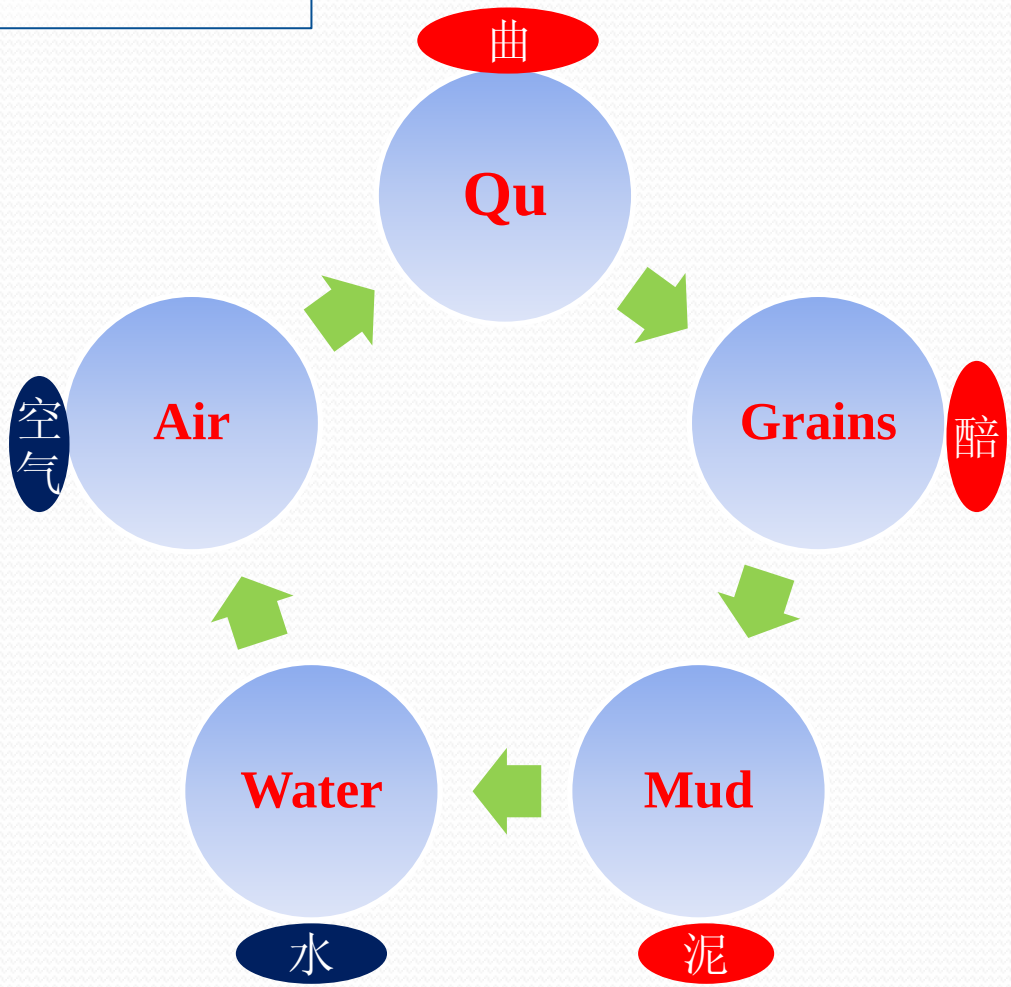
Microbial Ecology Research on Chinese Liquor

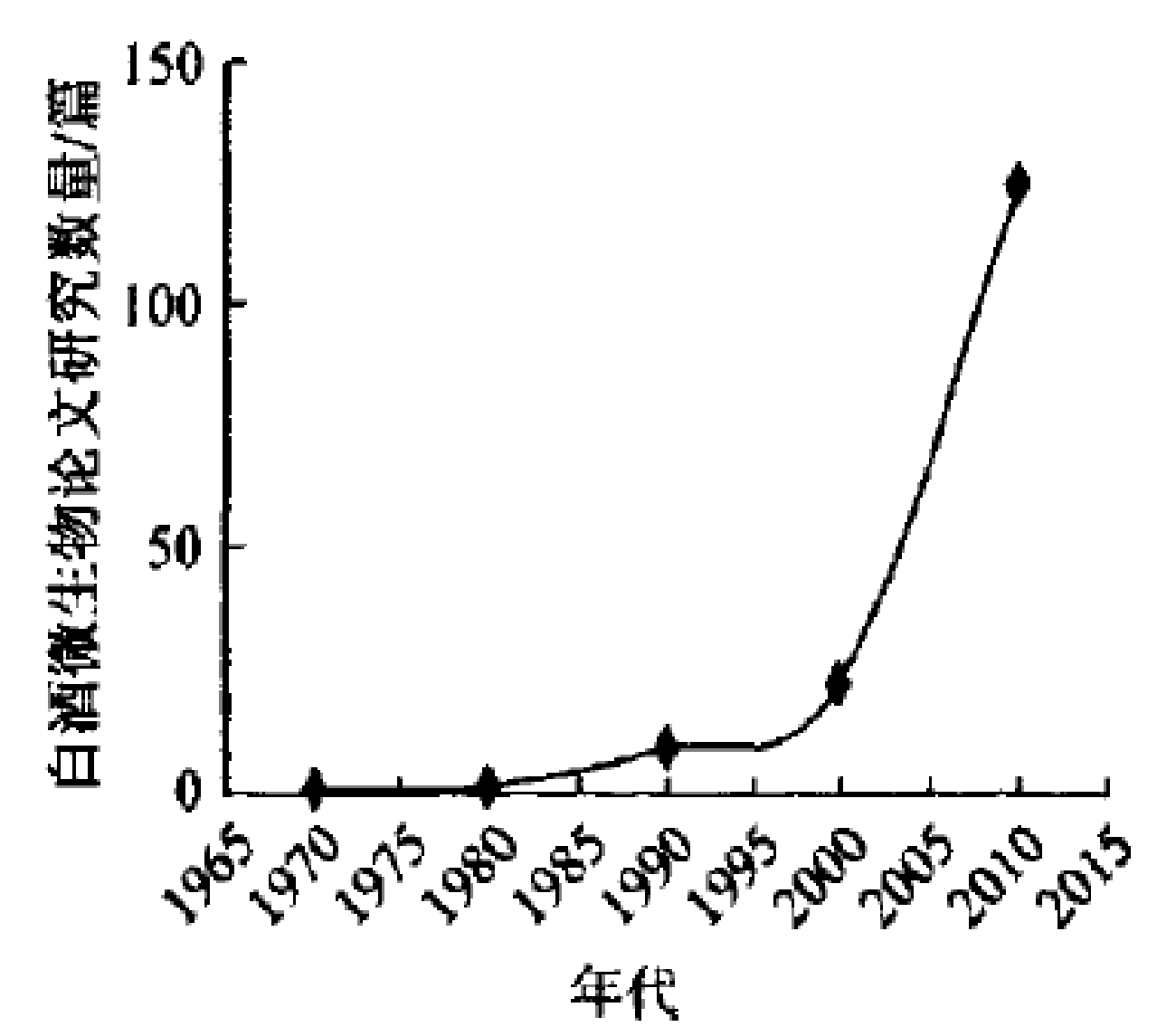
Advances

Sample-1

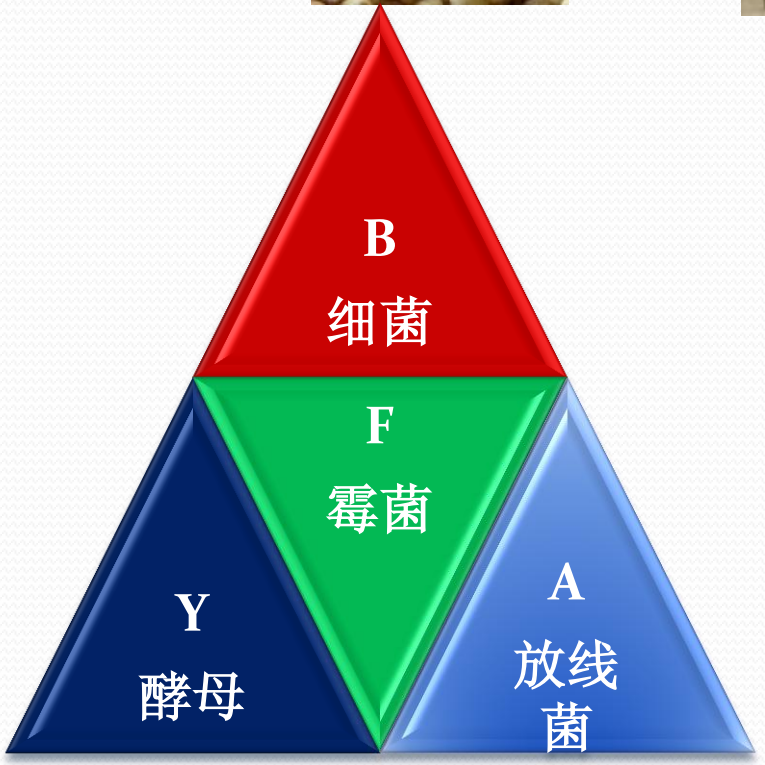
Sample-2

Advances




白酒微生物论文研究数量/篇
150
100
50
0
1965
1970
1975
1980
1985
1990
1995
2000
2005
2010
2015
年代

Qu



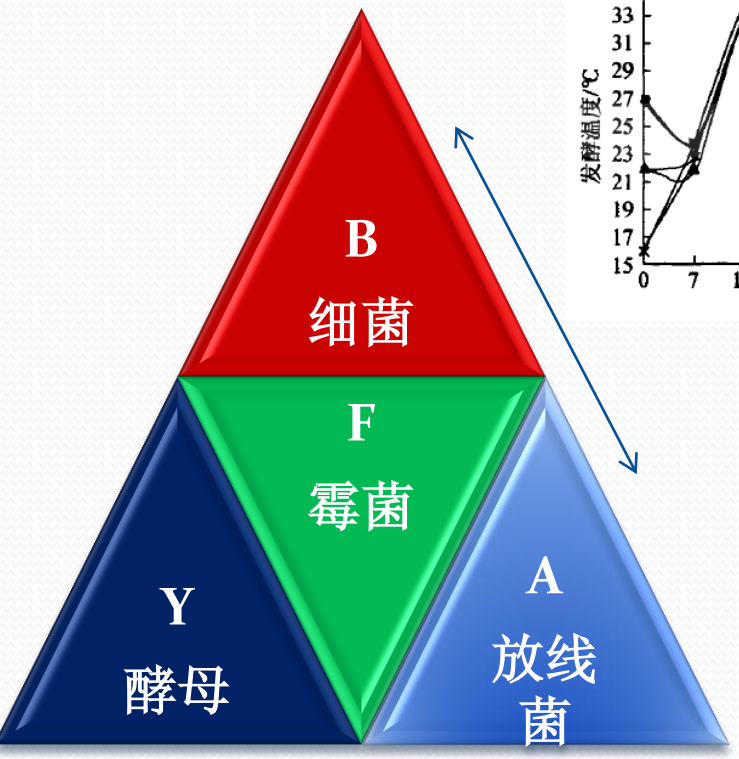
Bacillus, Lactobacillus and so on.

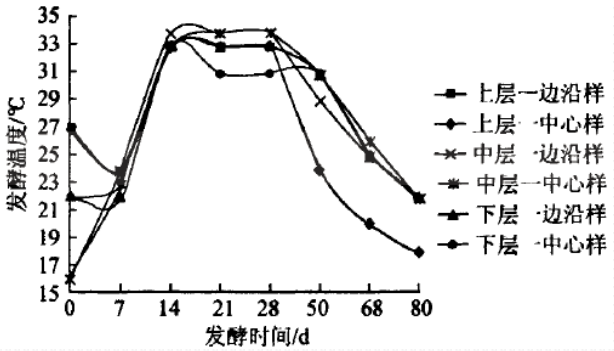
***Rhizopus sp., Mucor sp., Aspergillus* and so on.**

***Saccharomyces cerevisiae, Hansenula sp., Candida sp.* and so on.**

Actinomycetes

Grains





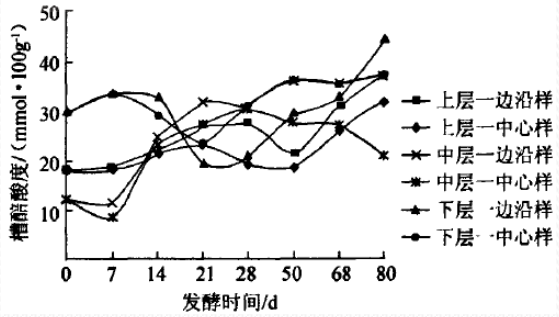
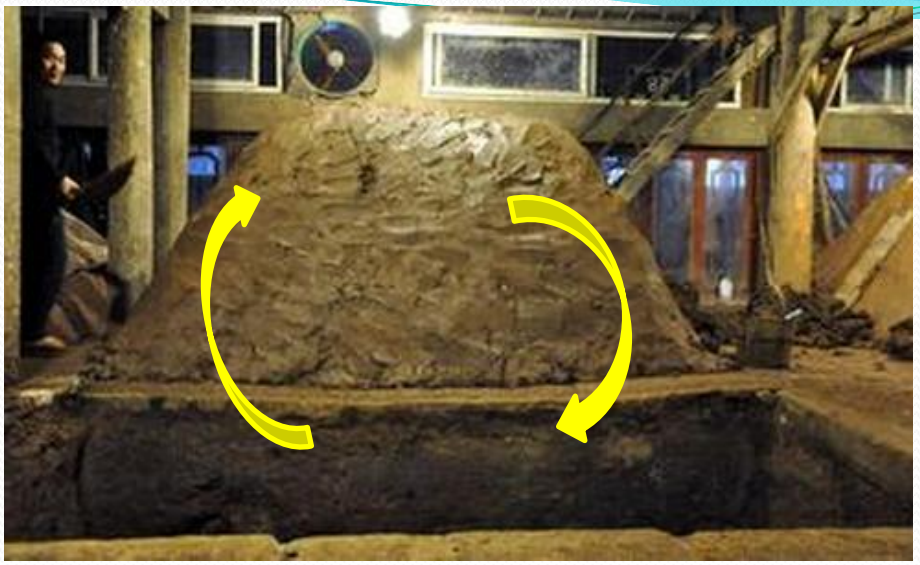
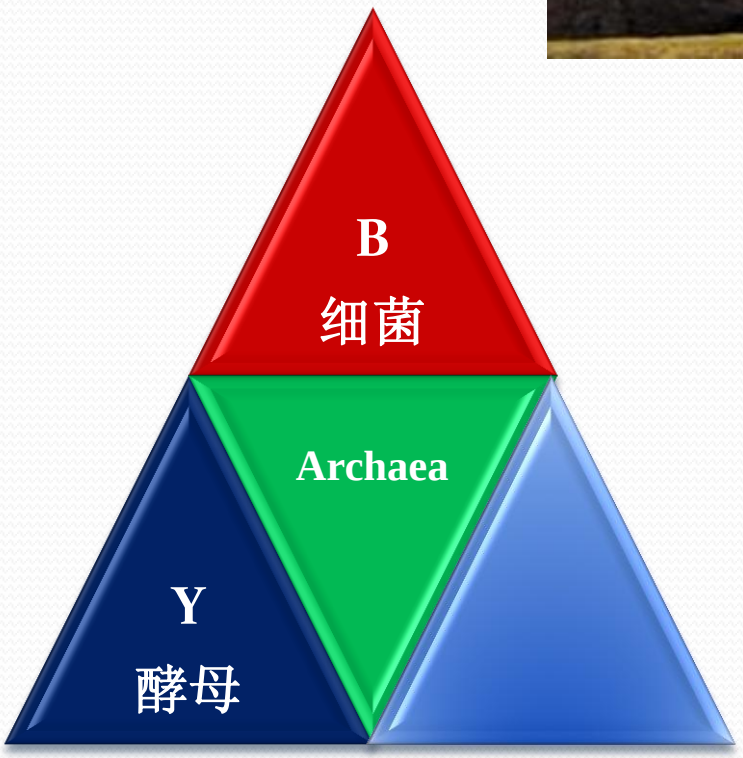


表1 酒醅发酵中好氧细菌及芽孢菌的数量

发酵时间(d)	上层		中层		下层	
	好氧细菌(10^6个/g)	芽孢菌(10^4个/g)	好氧细菌(10^6个/g)	芽孢菌(10^4个/g)	好氧细菌(10^6个/g)	芽孢菌(10^4个/g)
4	11	5.5	12	2	6	3
7	25	3	7	4	8	2
18	8	7	3	3	1	2

Mud





Bacillus, Lactobacillus, Clostridium and so on.

***Methanoculleus, Methanosarcina* and so on.**

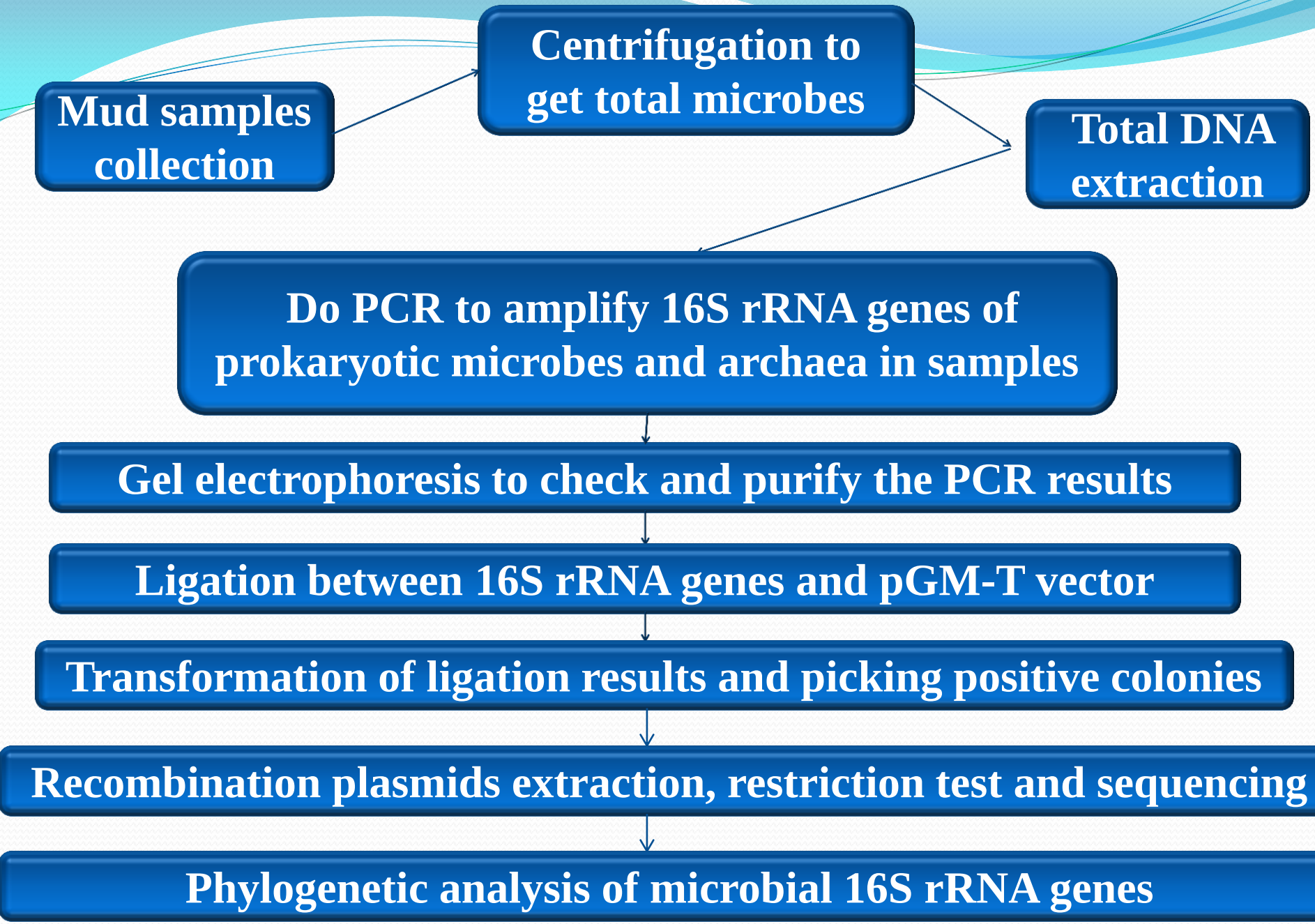
Saccharomyces cerevisiae

Sample-1

中国浓香型白酒窖池窖泥中原核微生物群落空间异质性研究 西華大學

Pit mud is very important for the fermentation of Chinese Liquor as it contains very complex microbial resources whose metabolism is related with the fermentation procedure.

Mud samples from the top, middle and bottom levels of a 20 years old pit in Sichuan province were collected to test the microbial composition and differentiation between different levels.


Mud samples collection
Centrifugation to get total microbes
Total DNA extraction
Do PCR to amplify 16S rRNA genes of prokaryotic microbes and archaea in samples
Gel electrophoresis to check and purify the PCR results
Ligation between 16S rRNA genes and pGM-T vector
Transformation of ligation results and picking positive colonies
Recombination plasmids extraction, restriction test and sequencing
Phylogenetic analysis of microbial 16S rRNA genes

Mud samples collection

-80℃ fridge

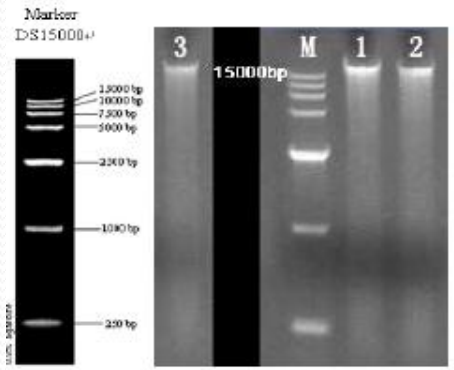
Centrifugation to get total microbes → **Total DNA extraction**

Vortex in PBS

Centrifuge to get microbial samples

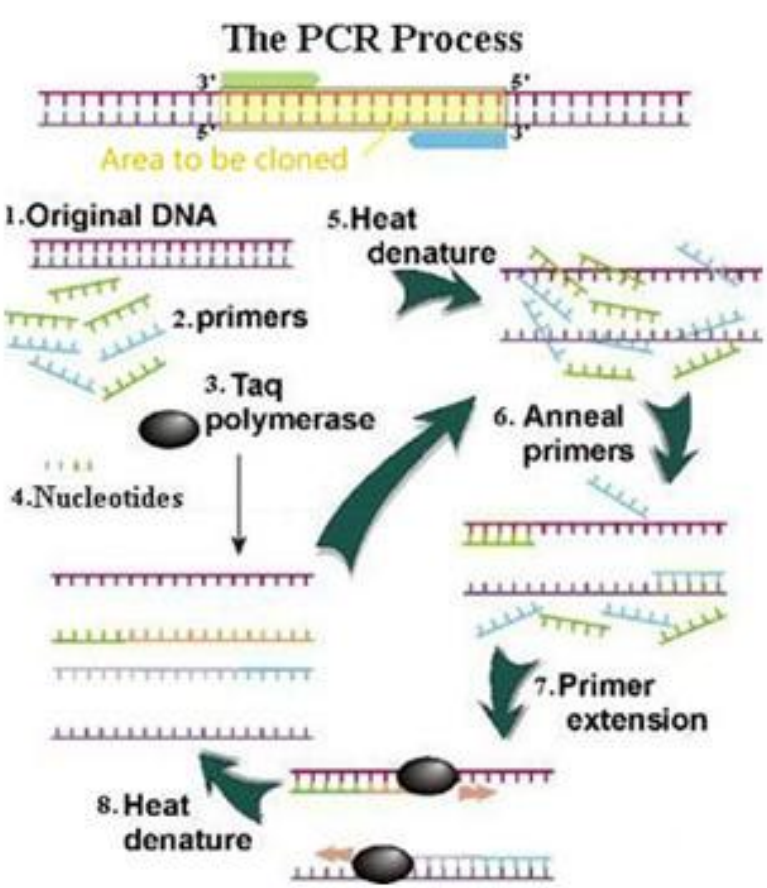
Extract DNA using KIT

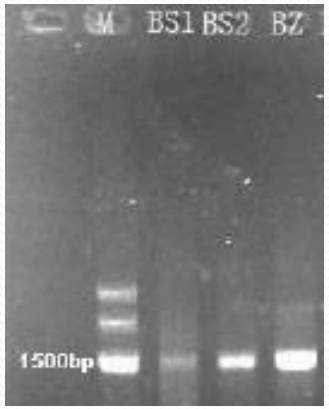
Sample total genomic DNA



Do PCR to amplify 16S rRNA genes of prokaryotic microbes and archaea in samples

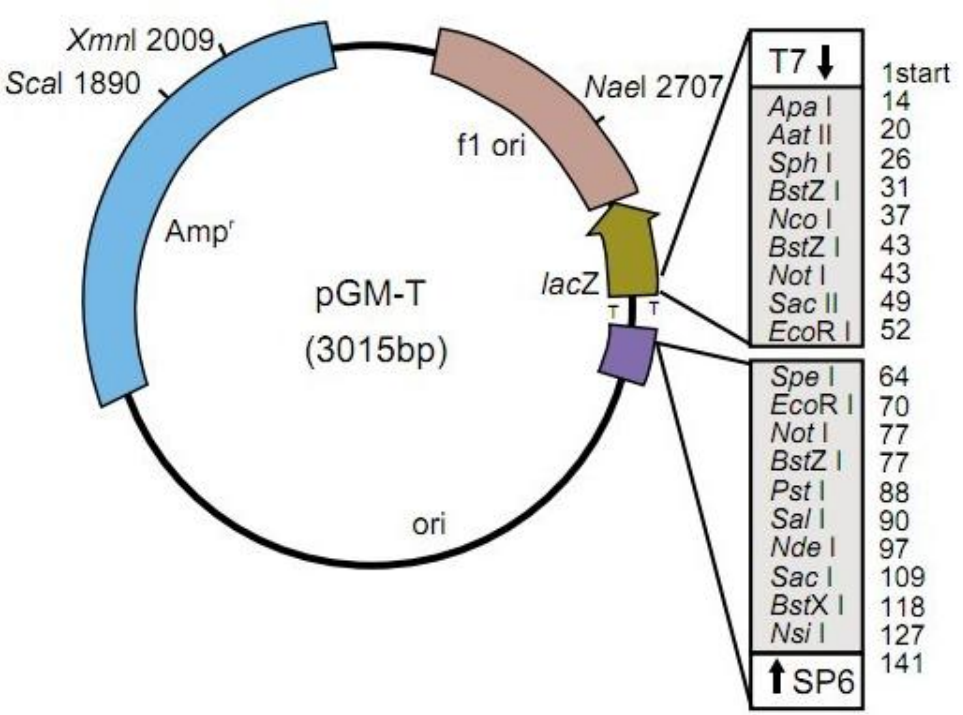
Gel electrophoresis to check and purify the PCR results





Purify **16S rRNA using kit**

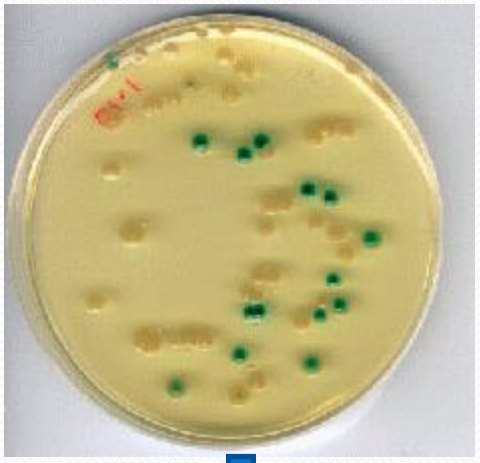
Ligation between 16S rRNA genes and pGM_T vector

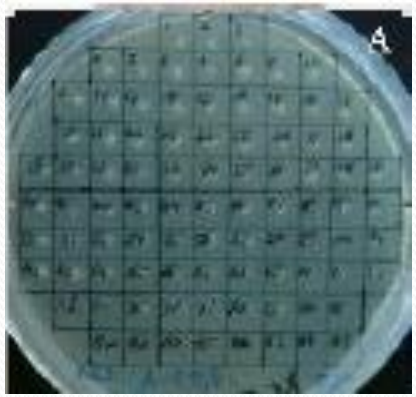


+

Ligase

Transformation of ligation results and picking positive colonies

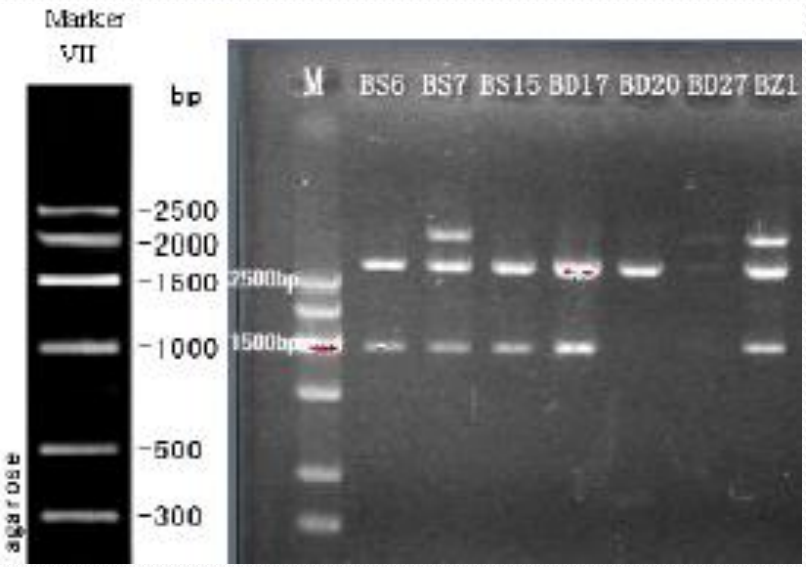


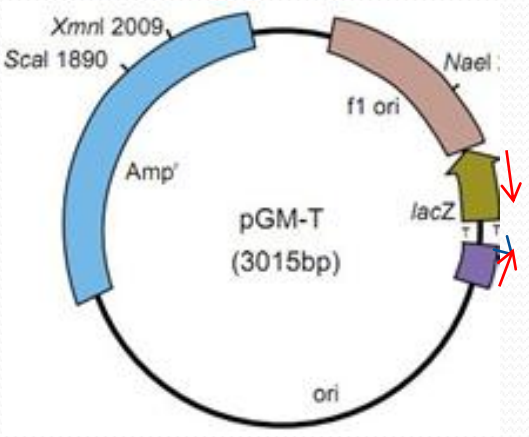


IPTG(异丙基硫代-β-D-半乳糖苷)

X-gal(5-溴-4-氯-3-吲哚-β-D-半乳糖苷)

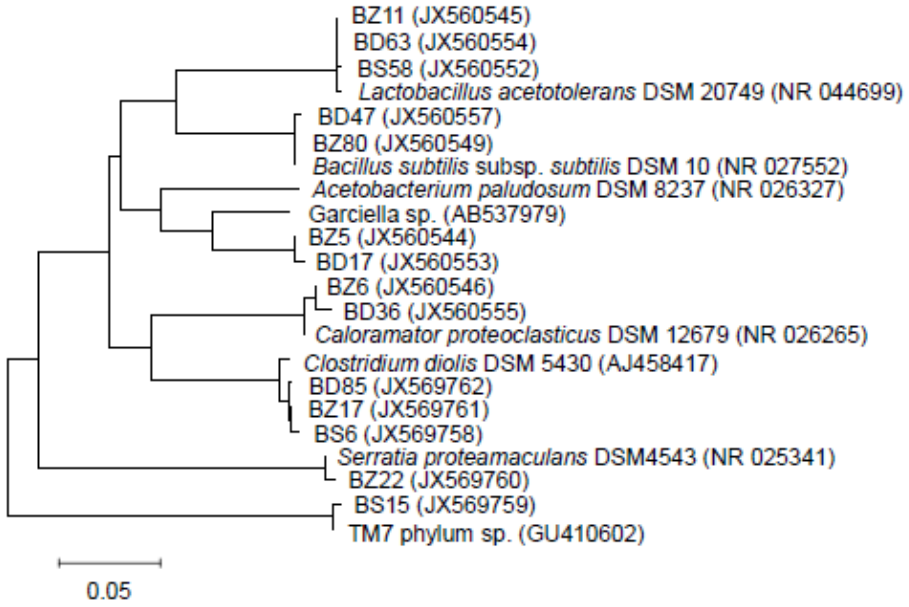
Recombination plasmids extraction, restriction test and sequencing





NCBI (The National Center for Biotechnology Information)-Vecscreen

Phylogenetic analysis of microbial 16S rRNA genes



层面	代表克隆子
上层	BS6
	BS15
	BS58
中层	BZ11
	BZ6
	BZ5
	BZ22
	BZ17
	BZ80
底层	BD63
	BD17
	BD36
	BD85
	BD47

图 10　基于 16S rRNA 构建的各层窖泥细菌系统发育树图

Fig.10 Phylogenetic tree of bacteria in the pit mud based on 16S rRNA sequence comparison

层面	代表克隆子
上层	AS64
	AS81
中层	AZ104
	AZ107
	AZ44
底层	AD37
	AD68

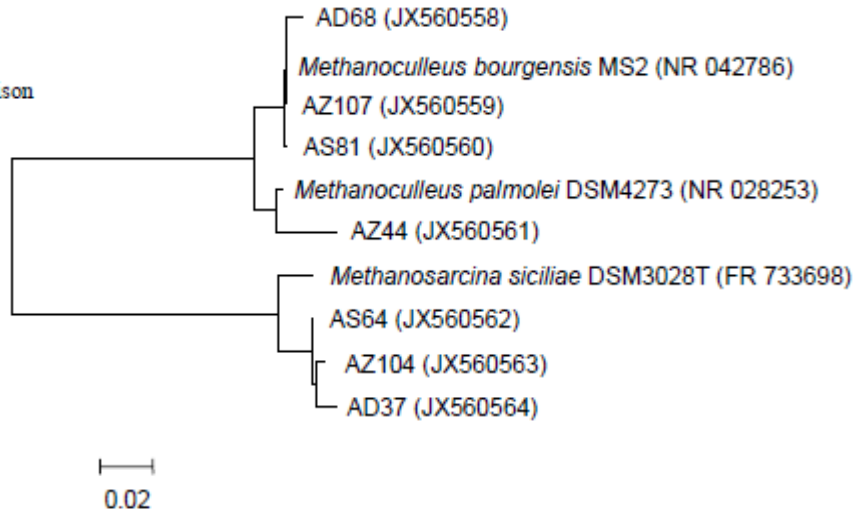


图 11　基于 16S rRNA 构建的各层窖泥古细菌系统发育树图

Fig.11 Phylogenetic tree of Archaea in the pit mud based on 16S rRNA sequence comparison

Sample-2

Short Communication

SCI

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(wileyonlinelibrary.com) DOI 10.1002/jsfa.6058

Microbial community structure in fermentation process of Shaoxing rice wine by Illumina-based metagenomic sequencing

Sample collection and storage of wheat Qu

↓

DNA extraction, DNA library construction and sequencing

↓

Illumina Hiseq2000 short-read *de novo* assembly

↓

Gene prediction and taxonomic assignment

↓

eggNOG, KEGG and pathway annotation

Sample collection and storage of wheat Qu

Samples of wheat Qu incubated at room temperature for 5 and 30 days collected.

Aliquots of 10 g were snap-frozen in liquid N_2 and transported to the lab on dry ice

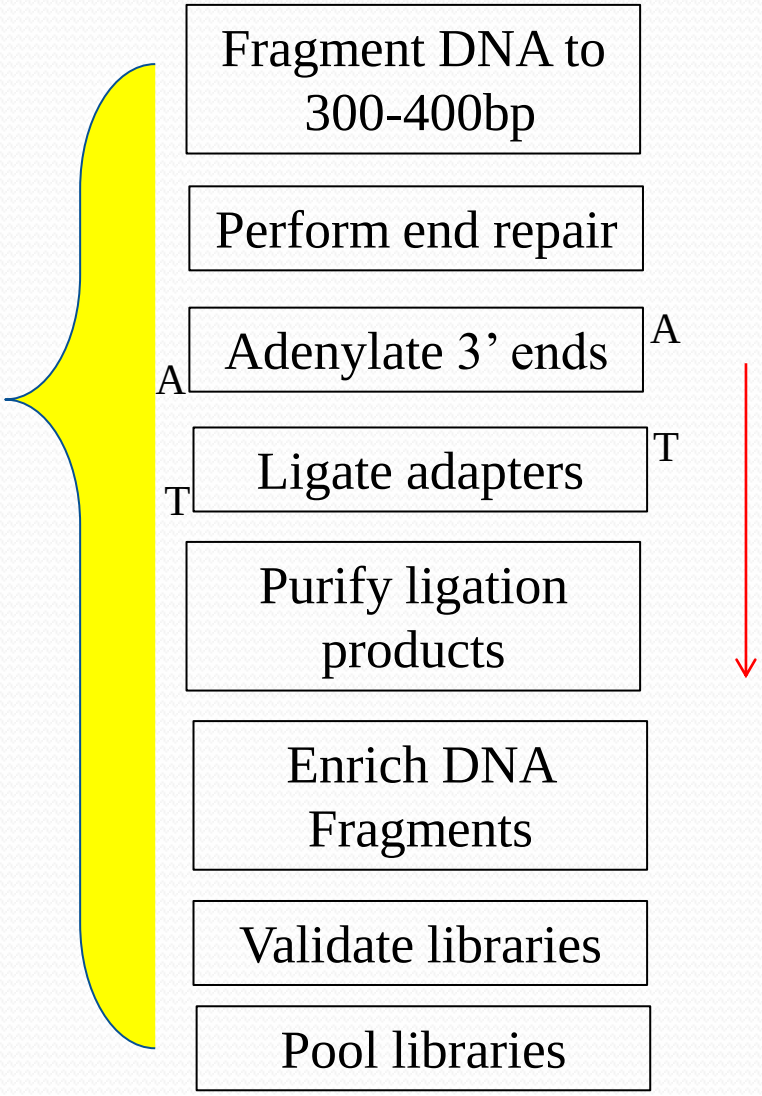
DNA extraction, DNA library construction and sequencing

Genomic DNA of was extracted using a QIAamp DNA mini kit.

DNA libraries were constructed with 2 µg starting genome DNA according to the Illumina TruSeq DNA SamplePrep v2 Guide.

The quality was evaluated using Agilent bioanalyser with a DNA LabChip 1000 kit.

Sequencing was performed by Illumina Hiseq2000



Illumina Hiseq2000 short-read *de novo* assembly

↓

Gene prediction and taxonomic assignment

↓

eggNOG, KEGG and pathway annotation

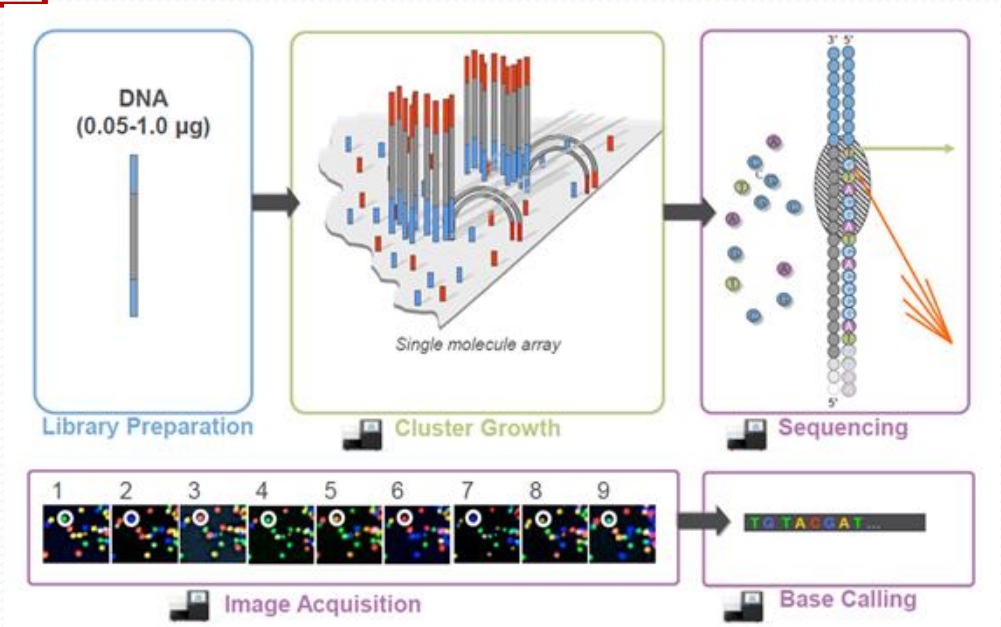
Short reads were removed and contig assembly by SOAPdenovo.

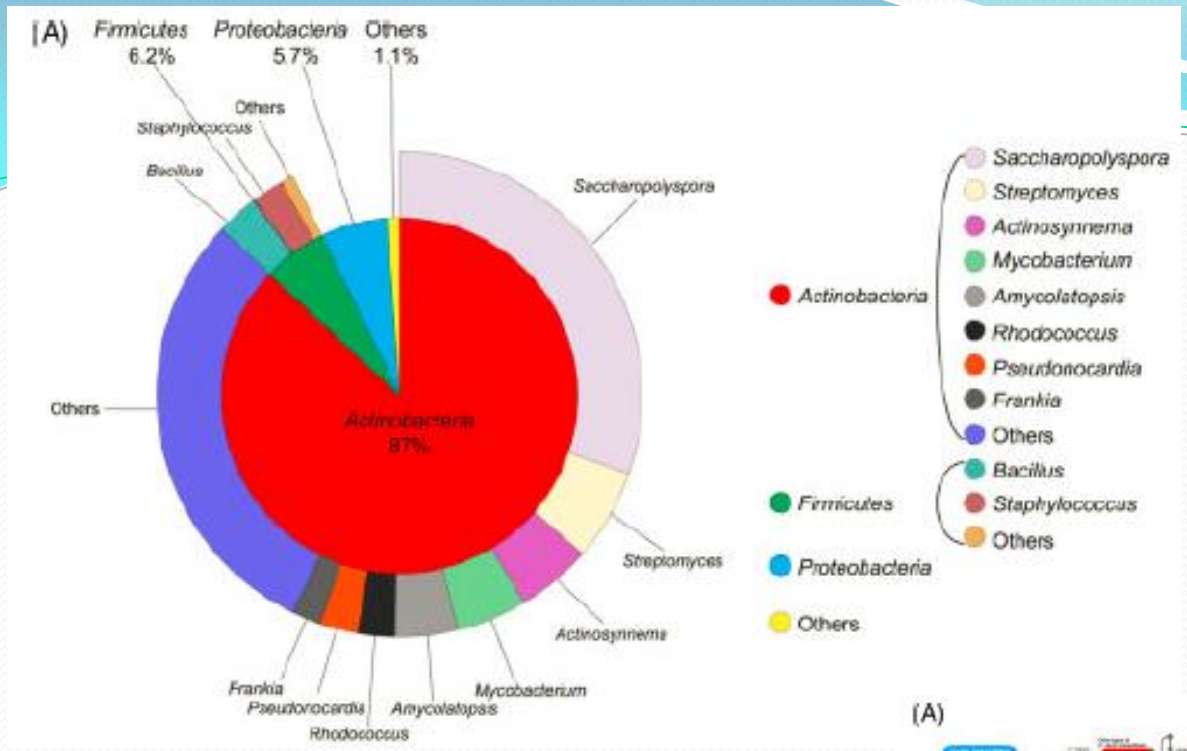
↓

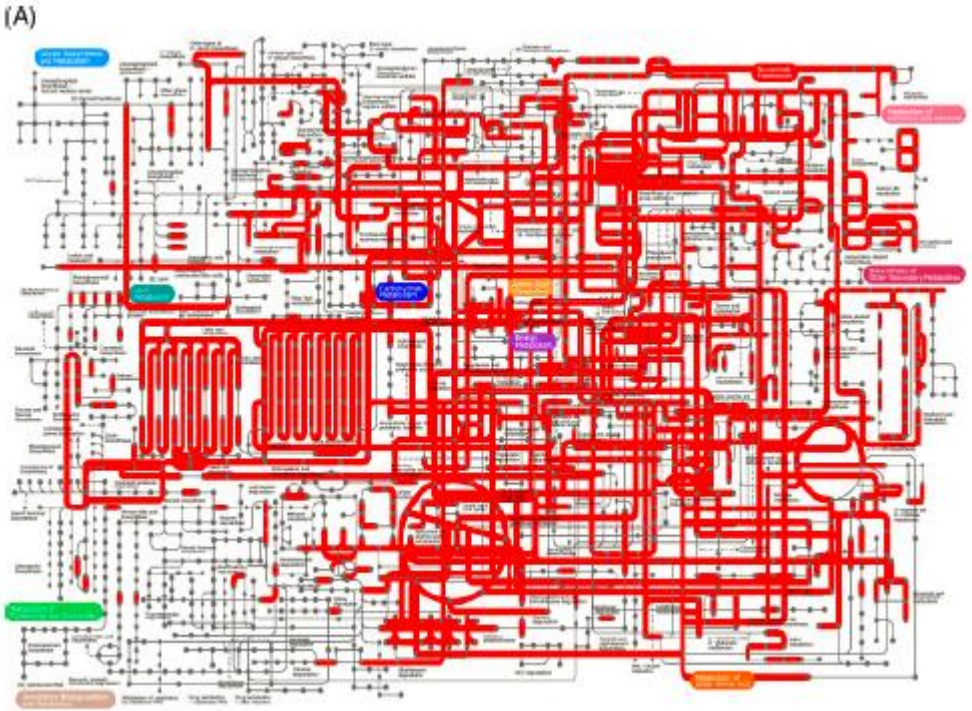
Predict genes using MetaGeneMark

↓

eggNOG protein database and KEGG annotation




(A)
Firmicutes
6.2%
Proteobacteria
5.7%
Others
1.1%
Others
Staphylococcus
Bacillus
Saccharopolyspora
Others
Actinobacteria
87%
Streptomyces
Actinosynnema
Frankia
Pseudonocardia
Amycolatopsis
Rhodococcus
Mycobacterium
Actinobacteria
Saccharopolyspora
Streptomyces
Actinosynnema
Mycobacterium
Amycolatopsis
Rhodococcus
Pseudonocardia
Frankia
Others
Firmicutes
Bacillus
Staphylococcus
Others
Proteobacteria
Others


(A)