

Research Article

Research of Sichuan Paocai and Lactic Acid Bacteria

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Abstract: Sichuan Paocai with long history is a typical representative of Chinese pickles, which has been inherited for thousands years. Its strong vitality was largely due to its unique microbial fermentation style. In this study, molecular microbial technology and traditional microbial technology were employed to study the distributions and changes of the microorganisms during the fermentation. The results showed that the lactic acid bacteria such as *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus brevis* etc., were dominant microorganisms during the Sichuan Paocai fermentation. In addition, the current situation of the Sichuan Paocai industries, classification of the Sichuan Paocai and the lactic acid bacteria was also reviewed.

Keywords: Dominant microorganisms, lactic acid bacteria, sichuan paocai

INTRODUCTION

An overview of Sichuan Paocai: Sichuan paocai, which boast a long history and a rich culture, are representative of Chinese pickles. They live up to the title of “national essence” and are called “the backbone of Sichuan dishes”. Characterized by being crisp, fragrant and healthy, Sichuan paocai are quite popular with people. Industrial clusters of pickles are formed and they are located mainly in Chengdu and Meishan, whose products can take up 50% of the national market share. In 2011, the output of Sichuan paocai reached 1.8 million tons and its value 15 billion Yuan (with 16 enterprises whose sales revenue exceed hundreds of millions Yuan). The quantity of newly processed pickles was almost 5 million tons and therefore the income of farmers in the raw material bases which cover an area of 1.5 million mu increased nearly 800 million Yuan and 600 Yuan/capita. Sichuan paocai industry has become a pillar for the development of Sichuan agriculture and economy and even a specialty industry in our country.

Sichuan is located in the upper reaches of the Yangtze River. It is blessed by a beautiful scenery and good ecology and has a subtropical humid climate. It is a typical area of biological diversity. It is rich in vegetables and other agricultural products. For thousands of years the unique water quality, the soil, the climate and environment all contribute to the formation of the micro-ecological system in Sichuan

basin, which is the precondition for the processing and production of Sichuan paocai.

The main ingredients of Sichuan paocai are fresh vegetables or salted vegetables either with dressing or not. Salt or salty water is used to ferment vegetables. Then salted vegetables are processed following three steps: mixing (dressing), packing (or not), sterilizing (or not). Pickles are traditional biological food and are produced by anaerobic fermentation dominated by microorganisms (probiotics). They are rich in dominant microfloras which are full of lactic acid bacteria (Chen, 2010a). They are easy to eat and for storage. So pickles are the precious heritage of our Chinese culture. In 2010 Sichuan paocai have been conferred “Geographical Indications of Agricultural Products” (Code 1347). (The scope of protection is geographically longitude 92 21'-108 12' east, latitude 26 03'-34.19' north and 220-2000 m above sea level. It covers an area of 20 million ha and 144 counties). And all these create the conditions for the protection of this precious national heritage.)

By applying different skills, Sichuan paocai industry falls into three categories: fermented pickles, instant pickles and other pickles (Chen, 2009).

Soaked pickles, also called fermented pickles, are made by fresh vegetables or salty soaked vegetables as the raw materials with or without accessories. They are firstly soaked in salty water with low concentration to ferment and then added by bitter or solution and so on. Soaked pickles are also called fermented pickles or soup pickles since the vegetables will not separate from

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the liquid and the solids contained are not less than 50%.

Instant pickles, also called dressed pickles, are made by fresh vegetables or salty soaked vegetables as the raw materials with or without accessories. They are firstly soaked in salty water and then reshaped, desalted, dressed, packaged and sterilized. The water contained is usually no more than 90% (Chen and Xia, 2010c).

Other pickles are made by other fresh vegetables such as edible mushrooms, legumes, seaweed, mountain vegetables, etc and are supplemented by accessories like livestock and poultry meat, aquatic products and so on. They are firstly soaked by using edible salt or in edible salty water to ferment and then reshaped, desalted, dressed, packaged and sterilized.

However, there is little information regarding the succession of LAB flora during the fermentation of Sichuan paocai. Thus the aim of our present study was to demonstrate the LAB species occurring throughout the natural fermentation.

RESEARCH ON PICKLES AND LACTIC ACID BACTERIA

General information about lactic acid bacteria: Lactobacillus is lactic acid bacteria, shorted as LAB, is a kind of bacteria which can produce a lot of lactic acid

using carbohydrates. Its diameter is usually 0.5~1.5 μm (cocci) or its length is (0.5~1) μm \times (2~10) μm (bacilli). Gram-positive bacteria (G+) usually do not move and do not produce spores (Zhang, 2007). Their main metabolite is lactic acid in the metabolism of fermentation. They are facultative anaerobe bacteria and are not sensitive to oxygen. They can grow with or without bacteria and the culture fluid is not clear. The bacterial colony morphology of lactic acid is usually humid, smooth, transparent and sticky. It usually have ridgy colony and round edges and are milky white and gray in color. Lactic acid bacteria are widely spread in nature and are quite complex. Most of them belong to the probiotic group essential to human bodies. They usually colonize the human intestine and are thus called probiotics.

According to «Bergey's Manual of Systematic Bacteriology» (Garrity *et al.*, 2004), there are 23 genera and more than 200 kinds of lactic acid bacteria, among which 5 genera such as *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Bifidobacterium* and *Pediococcus* are common. The identification and classification of lactic bacteria are more precise, scientific and convenient due to the development of modern molecular biology techniques.

Morphologically, there are cocci and bacilli of lactic acid. The former is round or oval in shape and is arranged in short or long chains while the latter is usually long and thin and is arranged in chains.

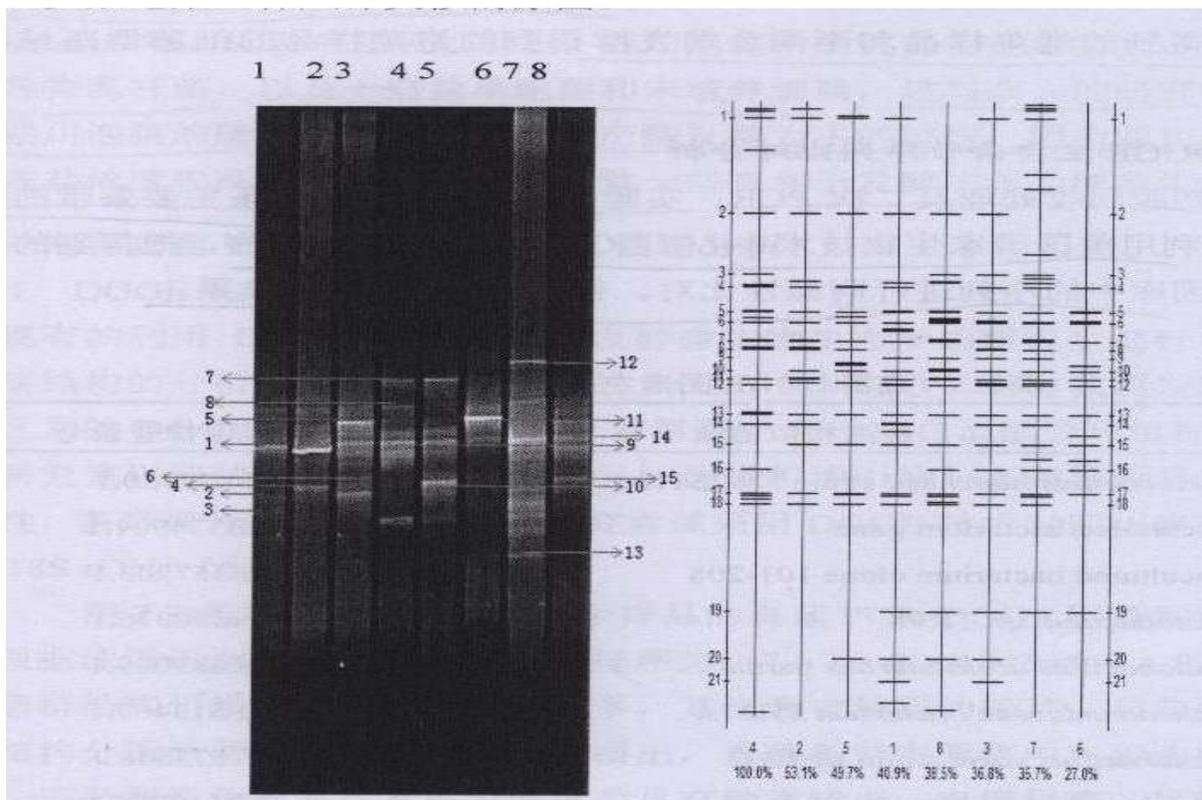


Fig. 1: Image of PCR-DGGE amplified electrophoresis structure

From origin, lactic acid related to food can be classified into two types: one is of animal origin and the other of plant origin. Lactic acid of animal origin is taken from animals. This kind is often in a relatively unstable state and the excessive intake of it easily makes people suffer protein allergies, which are also called rejection. Lactic acid of plant origin, on the contrary, is easily absorbed by people and no rejection will be resulted despite its intake quantity. Besides, Lactic acid of plant origin is of more vitality compared with lactic acid of animal origin.

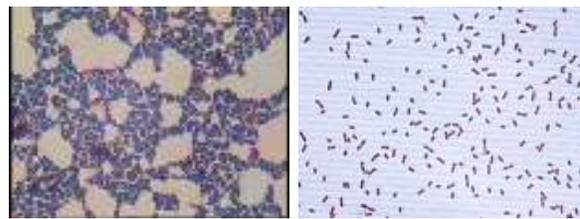
Confirmation of the dominant microorganisms: Traditional high qualified Sichuan paocai are used as objects. Use the direct decomposition method to extract total DNA and use PCR amplification to conduct variable region sequencing of V7-V8 in the 16SrDNA and do DGGE image analysis of the amplified products. That is, to study the colonial structure of dominant microorganisms to confirm the composition and abundance of elements by using the modern molecular technology of PCR-DGGE.

PCR-DGGE amplified electrophoresis structure of pickles can be seen on Fig. 1, each band represents a certain DNA fragment and bands of the same position are generally believed to have the same DNA sequence, so bands of different positions stand for bacteria of varied genera or kind. And the brighter the signal of the band is, the bigger the relative number of such bacteria. From the graph 15 main sequences of bands can be identified. Blast program of NCBI is employed and a contrast of the sequences obtained from scientific research and those of the lactic acid bacteria in the gene bank show that all sequences have a similarity of 97-99% except 2, whose similarity is lower. Therefore, it could be initially determined that the dominant bacteria of Sichuan paocai are the lactic acid genera, which are responsible for the fermentation of dominant microorganisms. They include: *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermenti*, *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Lactobacillus coryniformis*, *Lactobacillus buchneri*, *Lactobacillus casei*, *Pediococcus ethanolidurans*, *Lactobacillus alimentarius* and so on (Table 1). Uncultivated bacteria are also found. So the soaked fermentation of pickles is the typical lactic acid fermentation.

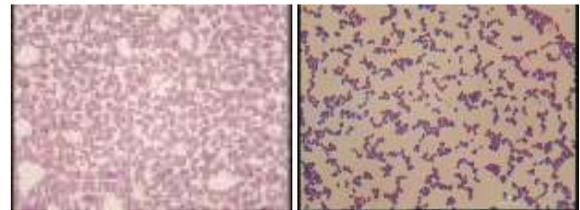
Micro-ecological distribution of dominant microorganisms of pickles: In order to further understand the lactic acid bacteria of dominant microorganisms of Sichuan paocai and its distribution, we collect pickles from different areas in Sichuan of varied sea levels with different ecological conditions. The focus is in Chengdu and the area extends to the

Table 1: Main lactic acid bacteria of Sichuan Paocai

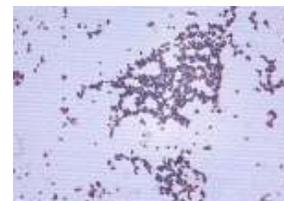
Bacteria	
	<i>Leuconostoc mesenteroides</i>
	<i>Lactobacillus plantarum</i>
	<i>Lactobacillus brevis</i>
	<i>Lactobacillus fermenti</i>
	<i>Lactobacillus sakei</i>
	<i>Pediococcus pentosaceus</i>
	<i>Lactobacillus coryniformis</i>
	<i>Lactobacillus buchneri</i>
	<i>Lactobacillus casei</i>
	<i>Pediococcus ethanolidurans</i>
	<i>Lactobacillus alimentarius</i>
	<i>Lactobacillus parafarraginis</i>
	<i>Lactobacillus versmoldensis</i>



(a) *Lactobacillus plantarum* (b) *Lactobacillus brevis*



(c) *Leuconostoc mesenteroides* (d) *Pediococcus pentosaceus*



(e) *Pediococcus ethanolidurans*

Fig. 2: Microscopic cell maps of lactic acid bacteria

eastern, western and southern parts of Sichuan. To be specific, the area covers nearly 20 places: Chengdu (Xinfan, wenjiang, pengzhou, dayi), Meishan, Leshan, Neijiang, Zizhong, Jiangyang, Xichang, ya'an, Ganzi and A'ba. Over 50 kinds of pickles have been collected: more than 500 in the first selection and more than 300 in the second selection. Some lactic acid cell images can be seen on Fig. 2. Some bacteria strains which are typical in shape are chosen from those selected from different regions and the result is the lactic acid bacteria in Sichuan paocai are no less than 3 genera and 13 kinds, which is basically consistent with the above conclusion.

Table 2: Change of lactic acid bacteria of Sichuan Paocai

Days for fermentation	Total acid (%)	Total number of lactic acid colonies (cfu/mL)	Other information
3-7	0.2-0.5	$1 \times 10^6 - 1 \times 10^8$	Fermentation under room temperature; total acid measured by lactic acid
7-10	0.5-1.0	$1 \times 10^6 - 1 \times 10^7$	

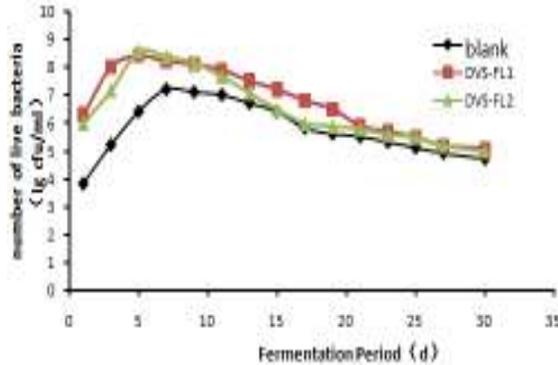


Fig. 3: Live lactic acid bacteria in fermented pickles with DVS bacteria (fermentation under constant temperature)

By a primary research on the distribution of biological zones of pickles microorganisms, it could be determined that the lactic acid bacteria of dominant microorganisms in Chengdu mainly include *Lactobacillus plantarum*, *Lactobacillus brevis*, *Leuconostoc mesenteroides*, *Lactobacillus fermenti*, *Pediococcus pentosaceus*, *Lactobacillus sakei*, *Lactobacillus coryniformis*, *Lactobacillus buchneri*, etc.; in Wenjiang *Lactobacillus plantarum*, *Lactobacillus brevis*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, *Lactobacillus ethanolidurans*, *Lactobacillus alimentarius*, etc.; in Neijiang *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Lactobacillus casei*, *Lactobacillus brevis*, *Leuconostoc mesenteroides*, *Pediococcus ethanolidurans*, etc.; in Zizhong *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus sakei*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, etc.; in Xi'chang *Lactobacillus plantarum*, *Lactobacillus brevis*, *Leuconostoc mesenteroides*, *Lactobacillus casei*, *Lactobacillus coryniformis*, etc.; in Ya'an *Lactobacillus plantarum*, *Lactobacillus coryniformis*, *Lactobacillus brevis*, *Lactobacillus fermenti*, *Lactobacillus casei*, etc.; in Jiangyang *Lactobacillus plantarum*, *Lactobacillus alimentarius*, *Lactobacillus brevis*, *Leuconostoc mesenteroides*, *Pediococcus ethanolidurans* 002C *Pediococcus pentosaceus*, etc.; in A'ba *Leuconostoc mesenteroides*, *Lactobacillus fermenti*, *Lactobacillus plantarum*, *Lactobacillus brevis*, etc.; and in Ganzi *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermenti*, *Pediococcus ethanolidurans*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, etc., due to the diversity and complexity of lactic acid bacteria system and pickles samples in different places, this initial biological distribution of zones awaits further research. But it could be found that

Lactobacillus plantarum, *Leuconostoc mesenteroides*, *Pediococcus ethanolidurans*, *Lactobacillus brevis*, *Pediococcus pentosaceus* and so on can be found almost everywhere in Sichuan.

Changes of dominant microorganisms of pickles fermentation: Fresh vegetables such as Chinese cabbages, carrots and lettuce are chosen as the experimental objects to ferment and DVS lactic acid bacteria are used to contrast the dominant microorganisms in the process of fermentation of Sichuan paocai. The initial findings are: the number of live lactic acid bacteria of pickles which are added by DVS bacteria in fermentation is larger than that of those not. The live lactic acid bacteria of those not (Sichuan paocai) decrease as times extends (Table 2 and Fig. 3).

CONCLUSION

Sichuan paocai are typical fermented vegetable products without high temperature. Fermentation of beneficial microorganisms last throughout the whole process of pickles soaking and the dominant microorganisms is lactic acid bacteria.

Abundant in fibers, various vitamins, minerals and small amount of carbohydrate and so on, Sichuan paocai can achieve the balance of dietary nutrition which is essential for human bodies. And they are good food with low calorie. Meanwhile, Sichuan paocai are rich in lactic acid bacteria and thus intestinal pathogenic bacteria reproduction can be inhibited and micro ecological balance in intestines can be achieved. Besides, eating pickles can stimulate appetites and help digestion, which can promote human health. In recent years, our research show that lactic acid bacteria separated from pickles can be made into DVS drugs, which can significantly reduce the fat accumulation in the livers of obese big rats and thus can resist fat livers.

The above research can indicate that lactic acid bacteria in Sichuan paocai mainly include *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus fermenti*, *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Lactobacillus coryniformis*, *Lactobacillus buchneri*, *Lactobacillus casei*, *Pediococcus ethanolidurans*, *Lactobacillus alimentarius*, etc., they fall into categories of no less than 3 genera and 13 kinds.

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