Based on the Analysis of 16S rDNA Sequence, the Change Rule of Microorganism in the Process of Processing Jianchang Banya was proposed

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Abstract: In the whole fermentation process of Jianchang salted duck, the change of microbial quantity, species and dominant population in different periods were studied. Seven different culture media were used to isolate and purify all kinds of microorganisms, and 16S rDNA sequencing method was used to identify the microorganisms. The results showed that the dominant populations were lactobacillus, yeast and glucose bacteria. In this study, the DNA of the bacterial strain was analyzed by the OUT analysis method, and the corresponding classification information of each species was obtained. The results showed that among the three dominant populations, the main bacterial colonies were lactobacillus acidophilus, pichia Pasteur yeast and staphylococcus rimidis, which had a relatively large impact on the quality and flavor of the products.

1. Introduction

Jianchang salted duck is one of Sichuan's specialty products, mainly produced in Xichang and Dechang of Liangshan Prefecture. Because of its plump appearance, bright dry skin, fat but not greasy, chewy, rich fragrance, rose red cut, high nutritional value, it is well known at home and abroad [1]. Its production mode is traditional manual, so if we want to adapt to the needs of social production, we must optimize its processing technology. It is the objective need to improve the processing technology to modern transformation to carry out the analysis of the change rule of Jianchang salted duck microorganism and the dominant population.

During the production process of Jianchang salted duck, the main factor affecting the surface color, taste and flavor of the finished salted duck is microbial fermentation. If the fermentation of the product is not mature, it is easy to cause changes in meat quality during storage and transportation, and poses a threat to the food safety of consumers. In this experiment, 16S rDNA technology was used to separate and identify microorganisms of Jianchang salted duck at different stages in the fermentation process, and to analyze the variation law of microorganisms in the fermentation process of salted duck, which provided basic data for optimizing the traditional processing method of Jianchang salted duck.

2. Materials and methods

2.1 Material, culture medium

Jianchang duck: Sold in Xichang, Sichuan Province

Malt juice culture medium, MRS agar culture medium, mannitol sodium chloride agar culture medium, M17 agar culture medium, tiger red agar culture medium, intensified clostridium agar culture medium, PCA culture medium

2.1.1 Laboratory instruments, equipment and reagents

High-speed centrifuge, gel imaging system, electrophoresis instrument, vortex spot shaker, Bio-RAD CFX96 fluorescence quantitative PCR instrument, Agilent 1260 high performance liquid chromatograph, Illumina HiSeq sequencing platform, FLASH v1.2.7 software, Trimmomatic v0.33 software QIIME, software genome extraction kit, genome purification kit, AceQTMqPCR SYBR Green Master Mix, DNA Marker, dNTP

2.2 Experimental method

2.2.1 Production method of Jianchang salted duck

Fasting (time ≥24 hours) → postmortem treatment → evisceration → pickling (at room temperature, evenly mix with edible salt and natural crushed spices, smear on duck inside and outside, and put into a jar for pickling for 12 hours. Turn over the jar to replace the upper and lower pickled ducks, and take them out after pickling for 24 hours) → air dry naturally (hang and air dry the raw duck after preliminary shaping. Air-dry naturally indoors for 5-7 days) → ferment naturally (expose 5-7 cervical vertebrae and whiten ribs after air-drying. When the muscle turns black and red, stacking fermentation is carried out under the condition of natural temperature ≤15°C, stacking height ≤ 0.6m, stacking fermentation is carried out for 24 hours and then molding is carried out) → natural air drying (natural drying until water content ≤30%) [2]

2.2.2 Sampling method

Samples were taken from the fleshy part of the breast of the salted duck before salting, and several small pieces were randomly taken from the fleshy part of the breast of the salted duck every 1d after salting and mixed.

2.2.3 Isolation of strains

Take samples from each time period, mix with 90 mL of sterile saline, and oscillate ultrasonically for half an hour. After that, 100 μL of the mixed solution of different time periods was applied to the solid medium of C. fortified, M17, MRS, and PCA, tiger red, mannitol sodium chloride and wort. Mark the coated media in sequence as S1, S2, S3, S4, S5, S6, and S7, and incubate each medium for 1 to 2 days in a suitable temperature incubator. Count the colonies according to GB 4789.15-2010, and record the colony morphology. [3]

2.2.4 Extraction of total DNA from samples

Using DNA extraction kit to extract the total DNA of microorganisms requires that the DNA concentration of all samples is above 10ng/μL and A260/A280 is between 1. 8-2.0 [4]

2.2.5 Identification of strains

The corresponding genome extraction kit was selected to extract the total genome in the culture medium of each strain, and the results were tested using the double-end sequencing method. The bacterial samples used various genomes as templates, and Eu27F and 1492R were used as primers to perform PCR amplification on the bacteria. The 16S rDNA and 18S rDNA products amplified by the PCR reaction were used. The PCR products were purified using PCR and enzyme reaction product purification kits, and the purified results were sent to Bioengineering Technology Co., Ltd. for inspection.

2.2.6 Phylogenetic analysis of 16S rDNA

The sequence obtained from the experimental test is compared with the sequence in the GenBank database. The QIIME software was used to select the sequence of the most abundant OTU at the taxonomic level as the representative sequence to construct the phylogenetic tree.

3. Results and analysis

3.1 Changes in the total number of microorganisms during the processing of Jianchang salted duck

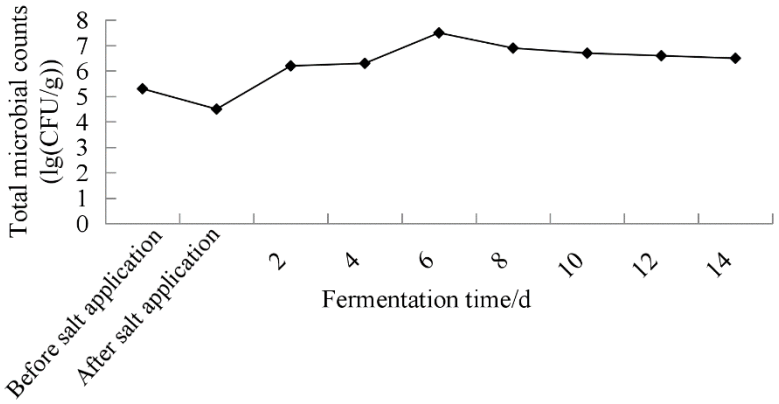

Figure 1. Change of the total number of micoorganisms during fermentation

As can be seen from Figure 1, the total number of microorganisms in Jianchang salted duck rapidly decreased to the lowest value after salt application, because a large number of microorganisms died in high osmotic pressure environment. In the time after salt application, the viable microorganisms in the duck and the microorganisms in the air began to move, and the total number of microorganisms began to increase, and became stable after a period of time.

3.2 Changes of microorganisms on different media during the processing of Jianchang salted duck

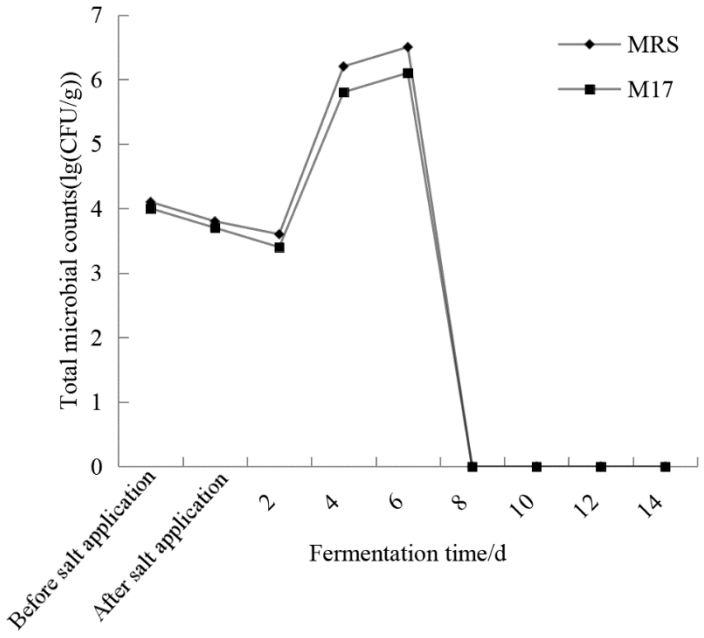

Figure 2. Changes in the total number of microorganisms on MRS and M17 medium

From Figure 2, it can be seen that the total number of lactic acid bacteria in M17 and MRS media varies similarly, and the number of lactic acid bacteria decreases from the start of fermentation to the second day of fermentation. The reason is that the metabolic products of lactic acid bacteria reduce the acidity in the culture medium. However, lactic acid bacteria are more suitable for growth and reproduction in weakly acidic environment, so the number of lactic acid bacteria increases rapidly within a few days after fermentation. However, after a period of fermentation, the PH in the culture medium was too low, which inhibited the reproduction and growth of lactic acid bacteria. Therefore, the total number of lactic acid bacteria began to decline

rapidly when it was fermented to the 6th day, and the number of lactic acid bacteria became 0 when it was fermented to the 8th day, and the result remained unchanged until the fermentation was finished.

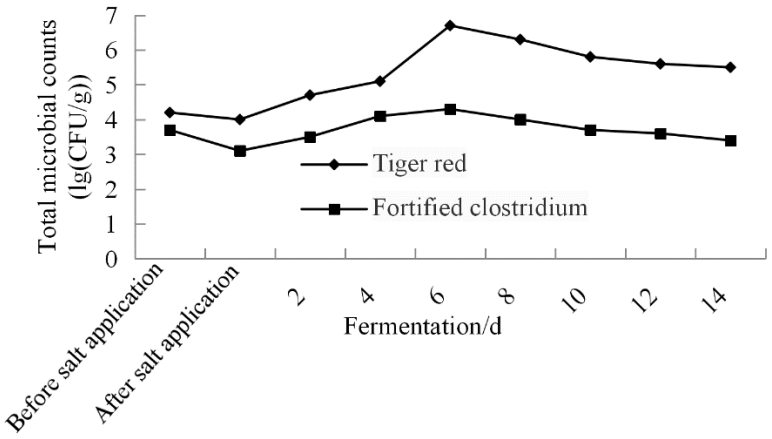

Figure 3. Changes in the total number of microorganisms on the culture medium of Tiger Red and Fortified Clostridium

As can be seen from Figure 3, the microbial changes in the enhanced Clostridium culture medium and tiger red culture medium are also similar, i.e., the changes of bacteria are similar. At the beginning of fermentation, due to the high salt content in the salted duck, the microbial cells in the culture medium dehydrate and die in high osmotic pressure environment, so the change trend of microorganisms decreases first and then increases gradually. When the fermentation reached the 6th day, the number of bacteria in the culture medium reached the highest value, thus it can be inferred that all kinds of microorganisms in the fermentation of salted duck are in the most active state during this period of time, which is also the key time for the fermentation and flavor generation of salted duck. Since then, the number of bacteria has been slowly decreasing, which is due to the low acidity of the surrounding environment caused by lactic acid bacteria metabolism.

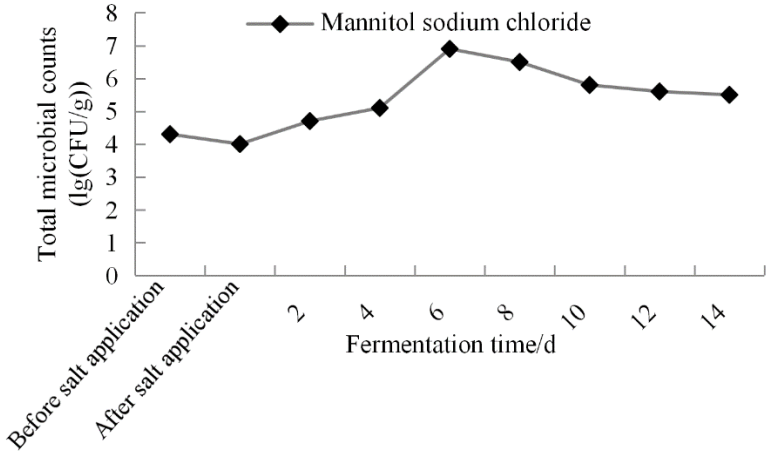

Figure 4. Change of total number of microorganisms on mannitol sodium chloride medium

As can be seen from Figure 4, the microorganism in mannitol and sodium chloride medium, namely glucose bacteria, has a similar change trend to that of bacteria, i.e., the total number decreases first, then increases and then decreases after salt application. At last, it tends to a stable value before the fermentation is completed, and the change of the total number is also caused by the high salt content in the early stage and the acidity of the environment in the later stage.

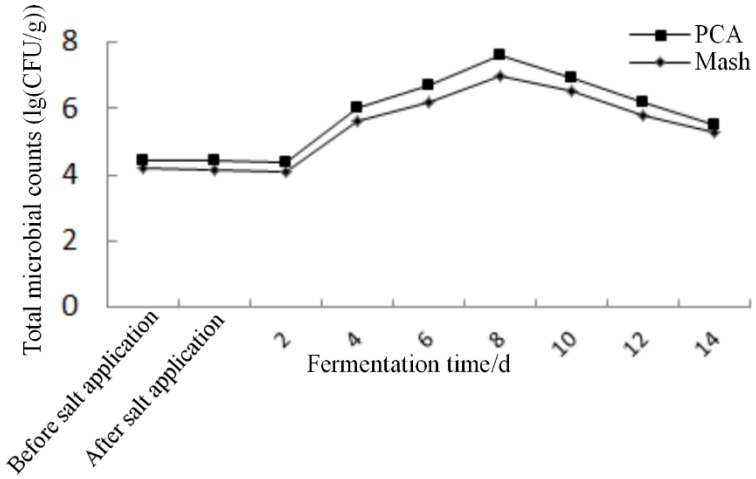

Figure 5. Changes of the total number of microorganisms in wort and PCA medium

As can be seen from Figure 5, the changes in the total number of yeasts, which are microorganisms in wort and PCA culture media, are similar. After salt application, the total number of yeasts decreases due to the increase in salt concentration caused by the intrusion of some salts into the duck meat. After that, the PH of the growth environment of the yeast is 3.8-6.0, so that the metabolites of lactic acid bacteria do not inhibit the growth and reproduction of the yeast, and the number of the yeast is increased to a certain extent. When the number of yeast reached the highest, the number began to decrease due to the accumulation of microbial metabolites.

3.3 OUT analysis

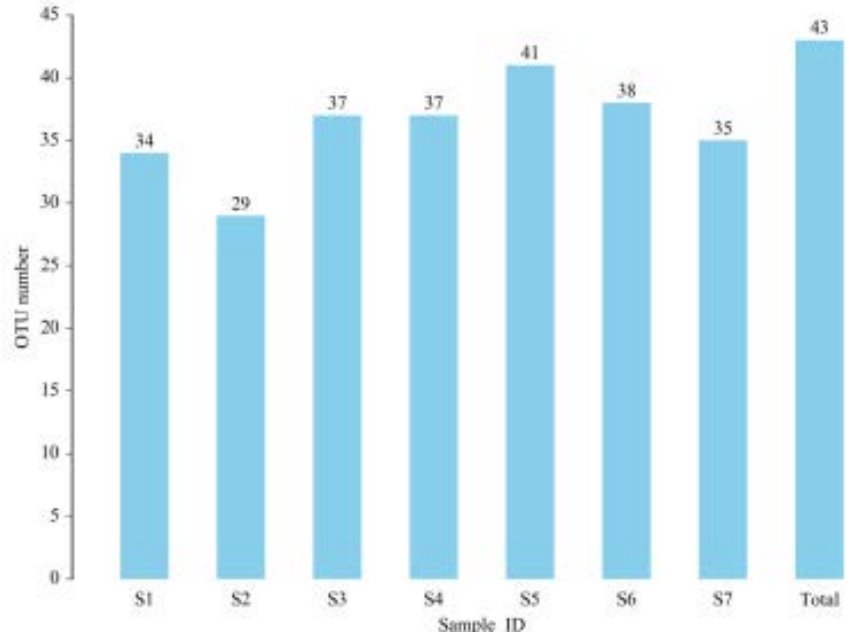

Figure 6. Distribution of OUT number of each sample

The figure above shows the number of OTUs of each sample by clustering. The number on the column is the number of OTUs of the corresponding sample. It can be seen from Figure 6 that the number of out in S5 medium is the most, and that in S2 medium is the least. It can be seen that the growth and reproduction of colonies on tiger red medium is better, while that on M7 medium is relatively weak.

3.4 Display and description of clustering results

Remove the low content of OTU. The original OTU clustering results may contain very low abundance OTU (species abundance is less than 0.005%), and the final OTU list is obtained and the tags number annotated to species of each grade in each sample is counted.

Table 1. Tags statistics of samples at different levels

Sample	Kindom	Phylum	Class	Order	Family	Genus	Species
S1	16,050	16,050	16,050	16,046	16,046	14,678	2,673
S2	7,327	7,327	7,327	7,327	7,327	7,011	4,375
S3	62,144	62,144	62,144	62,140	62,140	55,071	27,274
S4	63,457	63,457	63,457	63,457	63,457	57,990	34,721
S5	65,070	65,070	65,070	65,070	65,070	54,051	17,407
S6	65,472	65,472	65,472	65,470	65,470	60,040	14,455
S7	98,245	98,245	98,245	98,089	98,089	86,708	6,440

Table 1. Is a statistical table of the tags of each grade of the sample at the classification level, where the value represents the total number of tags covered by the sample at that grade. It can be seen from the figure that the level of species in S7 medium is more than that in other mediums, and the level of species in S2 medium is relatively less.

Table 2. Sample species statistics

Sample	Kindom	Phylum	Class	Order	Family	Genus	Species
S1	1	4	5	9	13	18	8
S2	1	3	4	6	12	16	6
S3	1	4	5	8	13	18	9
S4	1	3	4	8	14	19	8
S5	1	3	4	10	16	22	10
S6	1	4	5	8	14	19	9
S7	1	4	5	7	13	17	7

Table 2 is a statistical table of species in each grade of the sample, showing the number of species types in each grade of the sample. The number of species types of order, family, genus and species in S5 medium is large. It can be seen that the microbial content of salted duck in this environment is large, which has greater influence on the flavor of salted duck products.

3.5 Phylogenetic tree

The QIIME software was used for selection, the sequences that belong to the most abundant OTU at the taxonomic level were used as representative sequences, multiple sequence comparisons were made, a phylogenetic tree was constructed, and then graphics were drawn by Python language tools. The following figure shows the phylogenetic tree of OTU at the taxonomic level: the ring chart shows the phylogenetic tree, the branch represents the species, the length represents the degree of difference between species, and the same color genus name represents the same door.

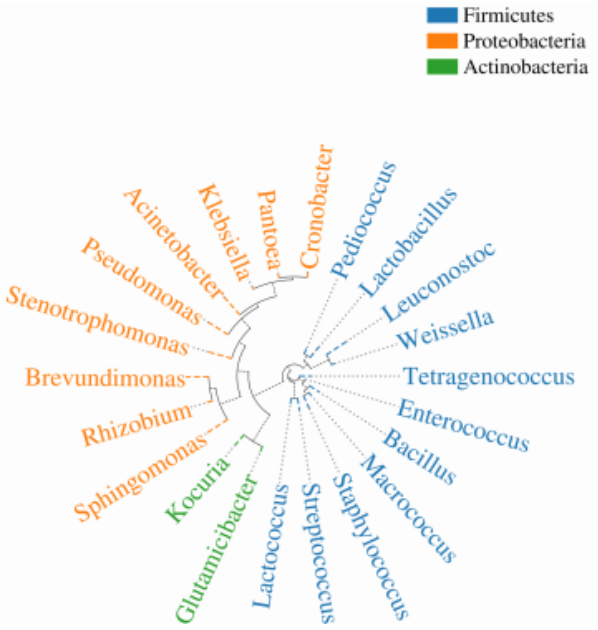

Figure 7. Phylogenetic tree of species

It can be seen from Figure 7 that the bacteria of the microorganisms are the most and the bacteria of the actinobacteria are the least. The bacteria species of Proteobacteria are in the middle, so the bacteria of phylum sclerecta and Proteobacteria have great influence on the processing of duck.

3.6 Alpha diversity analysis

Table 3. Alpha diversity index statistics

Sample ID	OTU	ACE	Chao1	Simpson	Shannon	Coverage
S1	34	35.1074	34.2	0.1191	2.381	0.9999
S2	29	32.2503	34.0	0.1957	1.9672	0.9993
S3	37	39.6726	40.0	0.2203	1.9071	0.9999
S4	37	40.6171	40.3333	0.3357	1.5416	0.9999
S5	41	44.0276	44.0	0.1561	2.1948	1.0
S6	38	39.5109	38.25	0.1552	2.2047	1.0
S7	35	35.5204	35.0	0.3396	1.7149	1.0

In order to test the Alpha diversity index of bacteria in 7 media, Mothur (version v.1.30) software was used for analysis, and the index at 97% similarity level was counted as above table. The data in the above table reflect the richness and diversity of species in different media. The table shows that Simpson index in S1 medium is the lowest and Shannon index is the highest. It shows that the species diversity in this medium is more than that in the other 6 media, and further the diversity changes of microorganisms participating in the processing of salted duck can be more reflected in this medium.

3.7 Analysis of dominant strains

Based on OUT analysis data and Alpha diversity analysis index, dominant strains on different media were selected for quantitative analysis. Pichia pastoris is selected from tiger red culture medium, staphylococcus lenamine is selected from mannitol and sodium chloride culture medium, lactobacillus acidophilus is selected from MRS culture medium, and the quantity changes of the selected microorganisms in the fermentation process are as follows:

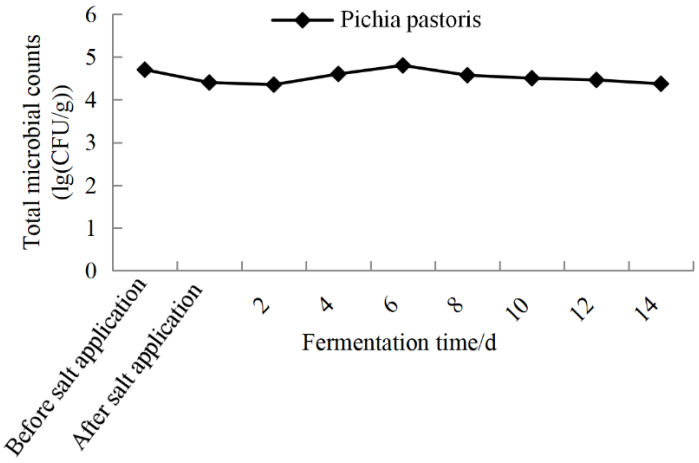

Figure 8. Changes in the total number of Pichia pastoris

As can be seen from Figure 8, the number of Pichia pastoris decreased slightly but changed little after salt application, which may be due to its strong tolerance to high permeability environment. The quantity increased slightly in the later period, but it tended to be stable in general, which indicated that this yeast may exist in the salted duck and the environmental changes had little influence on it.

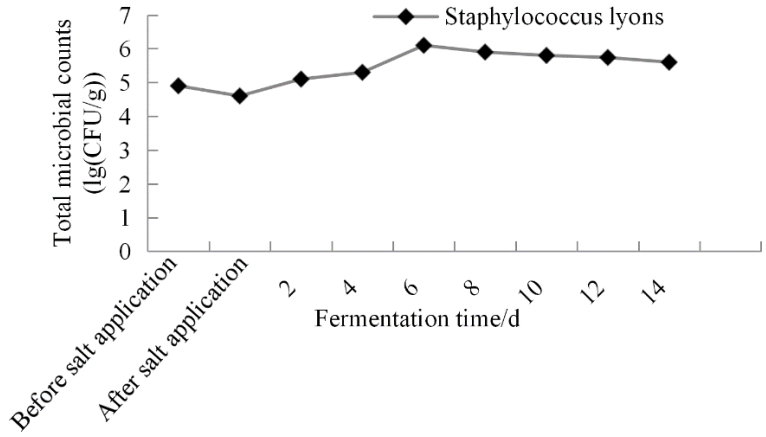

Figure 9. Changes in the total number of staphylococcus aureus

It can be seen from Figure 9 that S. rimici has always been the dominant strain during the processing of Jianchang salted duck. In the early stage of fermentation, due to the effect of high concentration of salt on the growth and reproduction of S. In a few days after salt application, due to the environment suitable for its growth and reproduction conditions, the number of S. rimici showed an upward trend, which was stable in the late stage of fermentation, but still slightly reduced.

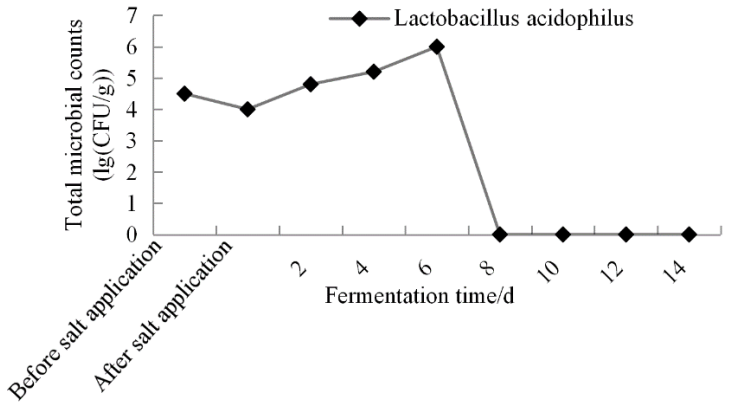

Figure 10. Changes in the total number of Lactobacillus acidophilus

As the dominant group in the natural fermentation process of many foods, lactobacillus is considered to be safe [5], which also plays a very important role in the processing of duck. It can be seen from Figure 10 that Lactobacillus acidophilus has always been in an advantageous position in the early stage of fermentation. Before applying salt, Lactobacillus acidophilus first decreased and then increased. After the 6th day of fermentation, the number of Lactobacillus acidophilus rapidly decreased to 0, indicating that it was not suitable for the environment of over acid.

4. Summary

In the course of this inquiry, the change law of microorganism in the fermentation process of Jianchang salted duck was compared and analyzed. Its dominant population is lactic acid bacteria, and the metabolites of lactic acid bacteria reduce the PH of the surrounding environment, which inhibits the growth of some spoilage microorganisms, thus improving the quality of salted duck products. At the same time, the product can be better stored and the flavor, texture, color and nutritional components of the raw materials can be changed in a better direction, thus giving the salted duck a better taste. [6] On the other hand, the content of nitrite in the product is reduced to a certain extent due to the metabolism of acidic substances by lactic acid bacteria, thus avoiding the harm of carcinogenic substances to human body and ensuring the safety of salted duck products. [3] In addition, yeast in microorganisms can also reduce the content of acetic acid and lactic acid in salted duck products, and increase the content of amino acids therein, so that the nutritional value of the products is higher. [7]

The results of this experiment show that after salt application, except lactic acid bacteria, yeast and glucose bacteria have strong adaptability to high osmotic pressure environment, other miscellaneous bacteria are inhibited to a certain extent in this environment. Quantitative analysis and identification of the three dominant populations showed that staphylococcus serotonin, Pichia pastoris and Lactobacillus acidophilus played a crucial role in the fermentation process of salted duck. However, in the fermentation process, some bacteria have strong viability and still exist in large quantities in the later stage of fermentation. Therefore, further research on how to reduce the bacterial content is needed in the future to ensure the health of consumers.

Acknowledgments

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