EVALUATION OF THE SPOILAGE POTENTIAL OF BACTERIA ISOLATED FROM CHILLED CHICKEN

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Abstract – Microorganisms play an important role in the spoilage of chilled chicken. A total of 53 isolates, belonging to 7 species of 3 genera, were isolated using a selective medium based on the capacity to spoil chicken juice. Four isolates of *Aeromonas salmonicida* 35, *Pseudomonas fluorescens* H5, *Pseudomonas fragi* H8 and *Serratia liquefaciens* 17 were further characterized to assess their spoilage potential on raw chicken breast fillets. The study showed that the major spoilage isolate was strain H8, which exhibited a fast growth rate, slime formation and increased pH and total volatile basic nitrogen (TVBN). This study demonstrated the characteristics of 4 potential spoilage bacteria isolated of chilled yellow-feather chicken and provides a simple and convenient method to assess spoilage bacteria during quality management.

Key Words – Raw-meat juice agar, Spoilage bacteria, Sensory analysis.

I. INTRODUCTION

Chicken is one of the most traded and consumed meats worldwide. The yellow-feather chicken, as a special species in Asia, has a more distinctive flavor than many other commercial broilers. Chicken meat is prone to deterioration in a short time, even under chilled conditions [1]. Microbiological contamination is one of the most important factors contributing to quality loss, resulting in slime, colony formation, compromised food texture, off-flavors and off-odors [2]. Many studies have reported that the specific spoilage organisms (SSOs) in refrigerated poultry are *Pseudomonas* spp., *Enterobacteriaceae*, lactic acid bacteria, and *Brochotrix thermosphacta* [2]. However, few studies have examined microbial organisms with strong catabolic capacities on chicken meat at refrigeration temperatures. These bacteria may destroy cell structure, promoting the outflow of nutrients that can be utilized by other spoilage bacteria. Therefore, it is necessary to assess the spoilage potential of these bacteria. Such information may eventually be used to better inform processing and preservation strategies to enhance chilled chicken quality and shelf-life.

II. MATERIALS AND METHODS

The selective medium was prepared by mixing raw chicken juice and 1% agar. This raw-meat juice agar (RJA) was sterilized by irradiation at a dose of 6 KGy via the ^{60}Co source. Yellow-feather chickens were collected from local slaughterhouses. Samples were stored until spoilage at 8 °C for isolation.

Surface samples were homogenized in sterilized 0.85% NaCl solution and appropriate dilutions was plated on RJA. Fifty-three isolates were selected from selective medium with a large decomposition zone, they were identified by 16S rRNA gene sequence and then confirmed using a VITEK2 automated system (BioMerieux, France). All strains were cultured in tryptone soy broth (TSB), cell density was adjusted to an optical density of 600 nm (OD_{600}) of 0.4. Four replicate of isolates were spotted onto RJA. Decomposition was measured after 3 days of incubation at 25 °C. The strains *Pseudomonas fragi* H8, *Aeromonas salmonicida* 35 and *Pseudomonas fluorescens* H5 were selected because they provided the three largest decomposition zone diameters (DZDs) among the 53 isolates. The spoilage potentials of these strains were compared with that of a well-known spoilage bacterium, *Serratia liquefaciens* 17, which was isolated from chicken previously.

Raw chicken breast fillets were sterilized via irradiation and the four isolates was spread onto the surface of the samples at a concentration of 3 log CFU/g. All samples were stored at 8 °C for 7 days. Total viable counts (TVC), Total volatile basic nitrogen (TVBN), pH and sensory analysis were performed after 2, 3, 4, 5, 6 and 7 days. All experiments were performed using 4 replicates. The results are presented as the mean ± standard deviation (SD). Statistical analyses were performed using the SPSS statistics program (Version 22, America), and ANOVA was used to valuate differences between means.

III. RESULTS AND DISCUSSION

In this work, 53 isolates, belonging to 7 species of 3 genera, were selectively isolated from RJA, and their spoilage potential was preliminarily evaluated.

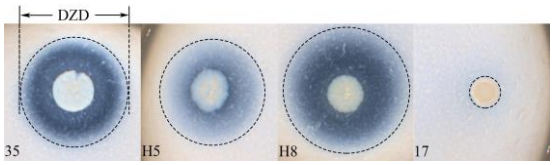
Table 1 Raw-meat juice agar (RJA) assay of the isolates.

Genera	Species	Number of the isolates	The largest decomposition zone diameter (mm)
Aeromonas	*Aeromonas salmonicida*	35	19.3±0.89^{ab}
	Aeromonas hydrophila	2	17.1±0.38^{bc}
	Aeromonas media	1	17.1±0.23^{bc}
Chryseobacterium	*Chryseobacterium shigense*	1	14.7±2.52^{c}
Pseudomonas	*Pseudomonas fragi*	4	21.8±2.63^{a}
	Pseudomonas fluorescens	8	17.3±1.12^{bc}
	Pseudomonas putida	2	15.1±0.31^{c}

Values are expressed as the mean ± standard deviation (n =4).
a-c: Values with different lower case letters in superscript are significantly different (P<0.05).

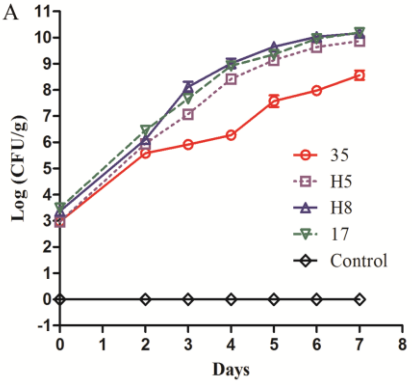
According to the RJA assay (Table 1 and Figure 1), different strains exhibited different DZD profiles. The DZDs of H8 and 35 were significantly larger (P<0.05) than the others, which indicated strong catabolic capacity on chicken meat. However, the decomposition zone of 17 was rarely observed. The bacterial species, selected in this experiment, were consistent with our metagenomic results for chilled yellow-feather chicken (data not published).

Figure 1. The decomposition zone diameter (DZD) profiles of *A. salmonicida* 35, *P. fluorescens* H5, *P. fragi* H8 and *S. liquefaciens* 17.



The results of the TVC are shown in Figure 2. After two days of storage, the growth of the 4 strains reached between 5 and 6 log CFU/g; however, strain 35 tended to exhibit slower growth over the next three days. At the end of storage, strains H8 and 17 reached titers of 10.2 log CFU/g, whereas strain 35 attained a titer of approximately 8.6 log CFU/g.

Figure 2. Microbiological changes during storage of chicken samples inoculated with *A. salmonicida* 35, *P. fluorescens* H5, *P. fragi* H8 and *S. liquefaciens* 17 stored at 8 °C.

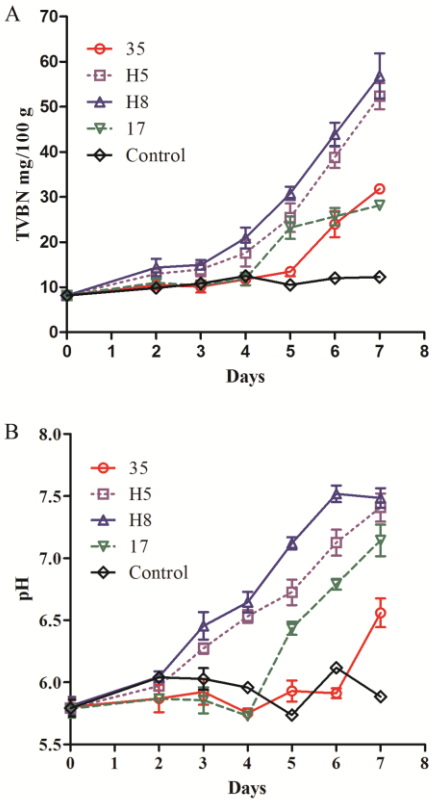


P. fragi is present in almost all the samples during storage under various conditions [3]. By contrast, *P. fluorescens* is more abundant on fresh meat than *P. fragi*, but the latter becomes dominant over time [4]. This finding is also in general agreement with a report demonstrating that *S. liquefaciens* represented the dominant isolate of *Enterobacteriaceae* during storage of minced beef and pork at a chilled temperature [4]. However, *Aeromonas* spp. displayed a lower trend in fresh samples than the other 3 isolates, consistent with

Pennacchia et al. [5], who detected *Aeromonas* spp. in chilled beef at the beginning of storage but found that it disappeared after 7 days. Therefore, due to their strong occurrence of H8 and 17 strains in samples, chicken meat may be considered their ecological niche.

The evolution of TVBN and pH values is depicted in Figure 3. The initial concentration of TVBN was 8.2 mg/100 g. Subsequently, TVBN increased rapidly in strains H8 and H5 compared with strains 17 and 35. At the end of storage, the concentrations of TVBN in strains H8 and H5 were higher than 50 mg/100 g.

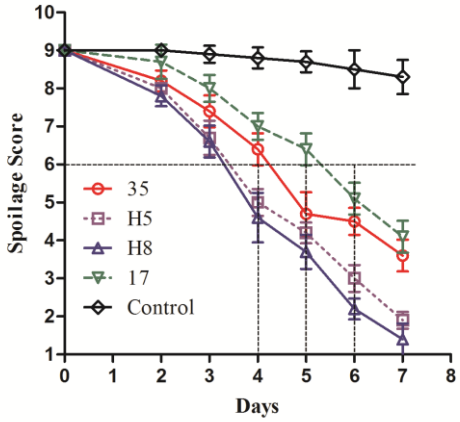
Figure 3. TVBN (A) and pH (B) changes of chicken samples inoculated with *A. salmonicida* 35, *P. fluorescens* H5, *P. fragi* H8 and *S. liquefaciens* 17 stored at 8 °C.



The limiting concentration of TVBN in meats for human consumption in China (GB 16869-2005) is 15 mg/100 g meat. On the 4th day of storage, strains H8 and H5 had exceeded this limit and became the fastest group. The pH value was initially 5.8. During storage, the pH values in strains H8 and H5 increased substantially, reaching values of approximately 7.4. Similar to TVBN, the final pH values were significantly higher than the initial values in strains H8 and H5. The pH value of normal chicken breast is 5.7 to 6.1. Many factors have been attributed to changes in the pH value of meat. It is well established that LAB metabolism produces compounds such as lactic acid, resulting in a slight decrease in pH, whereas in non-lactic cultures, the pH increases slightly [6]. Furthermore, the majority of *Pseudomonas* spp. produce only one type of proteinase with pH optima ranging from 6.5-8 [7]. In this study, the growth of strains H8 and H5 resulted in significant increases in pH, ranging from 5.8 to 7.4, thus achieving the most suitable pH value for proteinases. This finding may also explain why strains H8 and H5 showed the largest growth rate and TVBN values on chicken.

As shown in Fig. 4, chicken fillet freshness was excellent over the first two days, and the freshness characteristics diminished gradually over time. The differences in appearance and odor among the four isolates became apparent from the 4th day of storage. For example, samples inoculated with strains H8 and H5 exhibited slight surface slime, and strain 35 displayed special off-odors, whereas strain 17 did not produce any typical characteristic slime or intense odors. After 6 days of storage, all groups were considered spoiled.

Figure 4. Spoilage scores of chicken samples inoculated with *A. salmonicida* 35, *P. fluorescens* H5, *P. fragi* H8 and *S. liquefaciens* 17 stored at 8 °C for 7 days, as determined in the sensory panel. The dashed lines show the time of organoleptic rejection.



Slime production gives bacteria an advantage because it constitutes a protective layer that keeps bacteria moist and allows microorganisms to grow at a lower temperature [8]. This protection allows these bacteria to survive and to compete with other bacteria in meat. Consequently, the use of a low temperature for meat storage does not prevent the formation of slime, although refrigeration results in longer product shelf-life. In this study, there were some correlations between sensory rejection time and the quantity of TVBN, suggesting that TVBN was likely to be a good indicator of the sensory detection of chicken spoilage.

IV. CONCLUSION

Strain H8 led to a strong occurrence, rapid increases in TVBN and pH, off-odors and serious slime formation on chicken breast fillets. Therefore, it is likely that *P. fragi* H8 plays a significant role in the spoilage of chilled chicken. Furthermore, the availability of RJA may be useful for effective analyses in studies aimed at identifying the potential spoilage bacteria during the natural spoilage of meat and meat products.

ACKNOWLEDGEMENTS

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