ANTIOXIDANT POTENTIAL OF A UNIQUE LAB CULTURE ISOLATED FROM HARBIN DRY SAUSAGE *IN VITRO*

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Abstract –The lactic acid bacteria *Pediococcus pentosaceus, Lactobacillus curvatus, Lactobacillus brevis,* and *Lactobacillus fermentum* isolated from Harbin dry sausage were evaluated for their potential antioxidant activity. The *in vitro* results showed that *P. pentosaceus* had the strongest hydrogen peroxide resistance, radical scavenging activity, reducing power, and inhibition of lipid peroxidation ($P<0.05$). Additionally, superoxide dismutase and glutathione peroxidase activities in *P. pentosaceus* were higher than those observed in three other strains ($P<0.05$). These results demonstrate that *P. pentosaceus* has the potential to be employed as an antioxidant starter culture in fermented meat products.

Key Words –Lactic acid bacteria; Antioxidant potential; *In vitro*;

I. INTRODUCTION

Excessive lipid oxidation is the main cause of quality deterioration in meat products. Aldehyde formation during oxidation is been considered to be directly related to the deterioration of meat quality, protein stability and functionality. Additionally, protein oxidation is another issue in meat quality evaluation. One effective method for reducing or preventing oxidative processes in muscle food is to use an antioxidant to block the radical reaction. The role of LAB in fermented meats has been studied widely [1], and certain LAB strains have been determined to contain antioxidant properties *in vitro*.

In our previous studies, the LAB *Pediococcus pentosaceus*, *Lactobacillus brevis*, *Lactobacillus curvatus*, and *L. fermentum* were isolated from Harbin dry sausage. The objective of the current study was to evaluate the antioxidant potential of these four strains of LAB and determine their antioxidant potential to be used as meat starter cultures. The antioxidant activity of these four strains was examined for their resistance to hydrogen peroxide, radical scavenging activity, reducing power, inhibition of lipid peroxidation and antioxidant enzyme activities.

II. MATERIALS AND METHODS

1. Bacterial cultures and growth media

Four species of LAB, designated *P. pentosaceus* R1, *L. brevis* R4, *L. curvatus* R5 and *L. fermentum* R6, were isolated from Harbin dry sausage (a traditional Chinese fermentation meat product) and identified by sequencing of 16S rDNA [2]. All strains were stored at the College of Food Science, Northeast Agricultural University, China and kept on Man Rogosa Sharp (MRS) agar plates at 4 °C.

2. Preparation of cell and intracellular cell-free extracts

After 12 h of cultivation in MRS at 30 °C, LAB cell pellets were harvested by centrifugation (10,000 × *g*) for 10 min at 4 °C, washed twice with 0.02 M sodium phosphate buffer (pH 6.5), re-suspended in deionised water. The bacterial counts in the cell suspension (intact cells) were adjusted to 10^9 CFU/mL. Thereafter, the cell suspensions were subjected to ultrasonic disruption (five 1-min intervals in an ice bath), the cell debris was removed by centrifugation (10,000 × *g*) for 10 min at 4 °C, and the resulting supernatant, which was the intracellular cell-free extract, was collected.

3. Resistance of intact cells to hydrogen peroxide

Overnight cultures of *P. pentosaceus*, *L. brevis*, *L. curvatus* and *L. fermentum* were inoculated at level of 10^7 CFU/mL in isotonic saline containing 0, 0.4, 0.8 and 1.2 mM hydrogen peroxide at 30 °C for 8 h. Bacterial counts were enumerated on MRS agar after incubation at 30 °C for 48 h.

4. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging assay was conducted by a modification of the Fenton reaction method [3]. Hydroxyl radical scavenging

activity (%) was calculated as $(A_S - A_0) \times 100 / (A - A_0)$, where A_S is the absorbance of the samples; A_0 is the absorbance of the control in the absence of the sample, and A is the absorbance of the blank without the hydrogen peroxide.

5. DPPH radical scavenging activity

The DPPH radical scavenging activity was determined according to Wu [4]. The resulting DPPH radical scavenging activity (%) was expressed as $[1 - (A_S - A_B) / A_C] \times 100$, where A_S is the absorbance of the sample; A_B is the absorbance of the blank (ethanol and sample); A_C is the absorbance of the control (deionised water and DPPH solution).

6. Reducing power

The method described by Oyaizu [5] was used to determine the reducing power. In this assay, an increase in the observed absorbance indicates an increase in the reducing power of the sample.

7. Lipid peroxidation inhibition activity

The antioxidant activity of LAB strains was assessed by the thiobarituric acid (TBA) method, which is based on monitoring the inhibition of linolenic acid (chosen as the source for unsaturated fatty acid) peroxidation by intact cells and intracellular cell-free extracts [6]. The percentage of inhibition of linoleic acid peroxidation was expressed as $(1 - A_S / A_B) \times 100$, where A_S is the absorbance of sample; A_B is the absorbance of deionised water instead of sample.

8. Antioxidant enzymes activity

The activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the intracellular cell-free extracts were evaluated spectrophotometrically using commercial assay kits (Beyotime Institute of Biotechnology, China) following the manufacture`s protocols. The enzyme activities of CAT, SOD and GSH-Px were expressed as U/mg sample.

9. Statistical analysis

All the data were analyzed statistically using the General Linear Models procedure of the Statistix 8.1 software package (Analytical Software, St Paul, MN, USA), and presented as mean ± standard deviations (SD). One-way analysis of variance (ANOVA) with the Tukey's multiple comparison was used to measure the significance of the main effects ($P < 0.05$).

III. RESULTS AND DISCUSSION

1. Resistance of intact cells to hydrogen peroxide *in vitro*

Table 1 Bacterial counts (lg CFU/mL) of different LAB strains after incubation in different hydrogen peroxide concentrations for eight hours

	0 mM	0.4 mM	0.8 mM	1.2 mM
P. pentosaceus	7.44 ± 0.01^{aA}	7.40 ± 0.02^{bA}	7.32 ± 0.01^{cA}	7.25 ± 0.02^{dA}
L. brevis	7.48 ± 0.04^{aA}	7.32 ± 0.02^{bB}	7.23 ± 0.02^{cA}	7.08 ± 0.01^{dB}
L. curvatus	7.47 ± 0.07^{aA}	7.31 ± 0.04^{bB}	7.26 ± 0.08^{bA}	5.55 ± 0.04^{cD}
L. fermentum	7.45 ± 0.02^{aA}	7.31 ± 0.02^{bB}	7.04 ± 0.02^{cB}	6.87 ± 0.02^{dC}

a–d Means within the same row with different lowercase letters differ significantly ($P < 0.05$).
A-D Means within the same column with different uppercase letters differ significantly ($P < 0.05$).

The influence of hydrogen peroxide on the survival of these four LAB strains is shown in Table 1. These four strains exhibited a strong resistance to 0.4 mM hydrogen peroxide for eight hours. When *P. pentosaceus*, *L. brevis*, *L. curvatus* and *L. fermentum* were subjected to 1.2 mM hydrogen peroxide, the bacterial counts decreased approximately 0.19, 0.40, 1.92 and 0.58 logarithmic cycle, respectively, indicating that *P. pentosaceus* was the most resistant strain against hydrogen peroxide, followed by *L. brevis* and *L. fermentum*.

Hydrogen peroxide could be catalytically converted to hydroxyl radical in presence of metal ions, which a much stronger oxidant. The reactive oxygen species (ROS), hydrogen peroxide and hydroxyl radical have deleterious effects on many molecules, including protein, lipid, RNA and DNA due to their highly reactive nature. Therefore, the resistance of cells to oxidative stress such as H_2O_2-treatement could reflect their antioxidant properties.

2. Radical scavenging activity

Both intact cells and intracellular cell-free extracts of these four LAB strains exhibited the inhibition against hydroxyl and DPPH radicals, as shown in Fig. 1A and B. The hydroxyl radical scavenging activity of intact cells was stronger than that of the intracellular cell-free extracts, however and opposite trend was found in DPPH radical scavenging activity ($P < 0.05$). The intact cells of *P. pentosaceus* had the highest hydroxyl radical scavenging activity with an inhibition rate of 54.01%, followed by the intracellular cell-free extracts of *P. pentosaceus* ($P < 0.05$). For the DPPH radical, the intracellular cell-free extracts of *P. pentosaceus* showed 64.49% scavenging activity, which was higher than other strains ($P < 0.05$). The *L. brevis* and *L. fermentum* also had higher radical scavenging activity than *L. curvatus*.

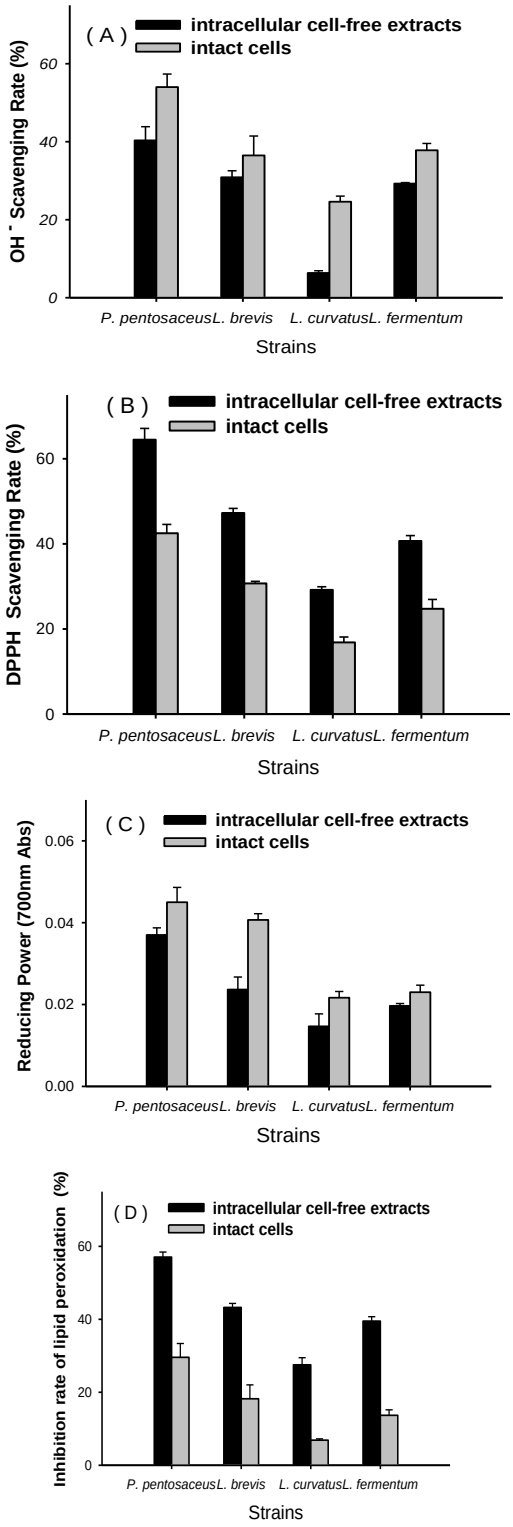
3. Reducing power

Similar to hydroxyl radical scavenging activity, the reducing power (absorbance at 700 nm) of these four intact strain cells were higher than the intracellular cell-free extracts, and *P. pentosaceus* showed the highest reducing activity ($P < 0.05$) (Fig. 1C). Reducing power of the cells is non-specific, but refers to all enzymatic or non-enzymatic compounds that contain the ability to reduce oxygen radicals or iron and therefore make it unavailable for oxidative reactions. Therefore, the reducing power of compounds could serve as a significant indicator of its potential antioxidant activity.

4. Inhibition of lipid peroxidation

As shown in Fig. 1D, in the intracellular cell-free extract, the *P. pentosaceus* had the highest inhibitory effect on linoleic acid peroxidation with the rate of 57.1% followed by *L. brevis* and *L. fermentum* ($P < 0.05$). The inhibitory effect of intact cells of the four LAB strains showed the same trend as the effect in intracellular cell-free extracts, with rates ranging from 29.6% to 6.9%. In general, the inhibitory rate of intracellular cell-free extracts was found to be greater than that of intact cells ($P < 0.05$).

Figure 1. Hydroxyl radical scavenge (A), DPPH free radical scavenge (B), reducing power (C), and inhibition of lipid peroxidation (D) by intracellular cell-free extracts and intact cells of different LAB strains. Error bars refer to the standard deviations obtained from triplicate sample analysis



5. Antioxidant enzyme activities

Table 2 CAT, SOD and GSH-Px activities (U/mg protein) of different LAB strains

	P. pentosaceus	*L. brevis*	*L. curvatus*	*L. fermentum*
CAT	n. d.	n. d.	n. d.	n. d.
SOD	0.48 ± 0.02^{a}	0.35 ± 0.01^{b}	0.14 ± 0.01^{d}	0.30 ± 0.02^{c}
GSH-Px	8.32 ± 0.60^{a}	1.48 ± 0.50^{c}	n. d.	4.22 ± 0.42^{b}

a-c Means within the same column with different lowercase letters differ significantly ($P < 0.05$).
n. d.: not detected.

SOD and GSH-Px activities of these four LAB strains were shown in Table 2. CAT activity was not detectable in all the strains indicating that they did not possess CAT due to the lack of heme. Although these selected strains were CAT negative, it is possible that they may decompose hydrogen peroxide by the reduced form of nicotinamide-adenine dinucleotid peroxidase. Eliminating superoxide in nearly all the cells and cellular organisms is attributed to SOD. In our study, SOD activity of these four LAB strains ranged from 0.14 to 0.48 U/mg proteins, which was the highest in *P. pentosaceus* ($P < 0.05$). These results are consistent with previously reported findings on SOD activity in intracellular cell-free extracts of LAB [7]. GSH-Px is known as an important cellular scavenger of hydroxyl radicals. Aside from the strain *L. curvatus*, GSH-Px was found in all the strains, which was significantly higher in *P. pentosaceus* than other strains ($P < 0.05$).

Hence, it can be concluded that *P. pentosaceus* contained strong antioxidant properties, which was also consistent with the metabolic activity of LAB. Antioxidant enzymes including SOD and GSH-Px in the strain cells play an important role in scavenging free radicals, and the reducing power of LAB strains contribute to reducing the content of oxygen radicals, which exhibited resistance to hydrogen peroxide and high inhibition activity of lipid peroxidation.

IV. CONCLUSION

The antioxidant activity of four LAB strains, isolated from Harbin dry sausage, was evaluated using *in vitro*. The results *in vitro* showed that *P. pentosaceus* had the strongest hydrogen peroxide resistant ability, hydroxyl radical and DPPH radical scavenging activity, and highest reducing power and inhibition of lipid peroxidation, as well as high SOD and GSH-Px activities. Therefore, *P. pentosaceus* isolated from Harbin dry sausage could be considered to be a potential antioxidant starter culture for applications in fermented meat products.

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