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Activities of superoxide dismutase and glutathione peroxidase in leaves of different cultivars of *Liriope spicata* L. on 10% SDS-PAGE gels

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Abstract. Activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in leaf crude extracts of different cultivars (small leaf, SL; big leaf, BL; thin leaf, TL) of *Liriope spicata* L. were detected on 10% SDS-PAGE gels. All cultivars contained different Cu/Zn SOD isozymes with molecular masses between 30 and 50 kDa, which were identified by inhibitor tests. They also contained different GPx isozymes with molecular masses between 50 and 64 kDa. It was found that SOD isozymes were stable during 40 to 65°C treatment; however, no SOD activity could be detected at either 70 or 80°C for 5 min. In contrast, all GPx isozymes were stable under 80°C for 5 min, and GPx in SL and TL cultivars could resist 80°C treatment for 30 min.

Keywords: Glutathione peroxidase; *Liriope spicata* L.; Superoxide dismutase.

Abbreviations: GPx, glutathione peroxidase; **SDS-PAGE,** sodium dodecylsulfate-polyacrylamide gel electrophoresis; **SOD,** superoxide dismutase.

Introduction

Active (or reactive) oxygen species and free radical-mediated reactions have been involved in degenerative or pathological processes such as aging (Ames et al., 1993; Harman, 1995), cancer, coronary heart disease, and Alzheimer's disease (Ames, 1983; Gey, 1990; Smith et al., 1996; Diaz et al., 1997). Meanwhile there are many epidemiological results revealing an association of a diet rich in fresh fruits and vegetables and a decreased risk of cardiovascular diseases and certain forms of cancer (Salah et al., 1995) in humans. Several reports cite natural compounds in fruits and vegetables for their antioxidant activities, such as anthocyanin (Espin et al., 2000), echinacoside in *Echinaceae* root (Hu and Kitts, 2000), phenolic compounds (Rice-Evans et al., 1997), water extracts of roasted *Cassia tora* (Yen and Chuang, 2000), and whey proteins (Allen and Wrieden, 1982). In cells, metabolic pathways are normally coupled to degrade free radicals. If the generation rates of free radicals exceed those of degradation under environmental stresses, cells suffer oxidative stresses. Two distinct pathways, nonenzymatic or enzymatic, were found in plant cells as routes of free radical scavengers. The former included chlorogenic acids (Kono et al., 1998), or ascorbate (Njus and Kelley, 1993), or vitamin E (Halliwell, 1999); the latter included different forms of SOD to metabolize superoxide free radicals to hydrogen peroxide (Bowler et al., 1992; Lin et al., 1993). The hydrogen peroxide produced was further metabolized either by catalase or different forms of peroxidase, such as glutathione peroxidase (GPx, EC 1.11.1.9).

The root of *Liriope spicata* L. is frequently used as a traditional Chinese herb, and the dried leaf powders are also used as tea drinks. In this research, we used 10% SDS-PAGE gels to identify SOD and GPx activities in leaf crude extracts of three cultivars of *Liriope spicata* L. Heat stability data were also included.

Materials and Methods

Plant Materials

Fresh leaves of three cultivars (small leaf, SL; big leaf, BL; thin leaf, TL) of *Liriope spicata* L. were provided by the Taiwan Agricultural Research Institute, Council of Agriculture, Wu-Feng, Taichung. After being cleaned with water, the leaves of different cultivars were immediately extracted with four volumes (W/V) of 50 mM Tris buffer (pH 8.3) containing 1% vitamin C (W/V) and 1% polyvinylpolypyrrolidone (W/V). After centrifugation at 12,500 *g* for 30 min, the supernatants were saved as crude extracts.

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SOD and GPx Activity Stainings on 10% SDS-PAGE Gels

Four portions of samples were mixed with one portion of sample buffer, namely 60 mM Tris-HCl buffer (pH 6.8) containing 2% SDS, 25% glycerol and 0.1% bromophenol blue without 2-mercaptoethanol at 4°C overnight. After electrophoresis at 4°C, the gel was cut into two parts. One was fixed with 12.5% trichloroacetic acid for protein staining with Coomassie Brilliant Blue G-250 (Neuhoff et al., 1985). The other was washed with 25% isopropanol in 10 mM Tris-HCl buffer (pH 7.9) to remove SDS (Hou and Lin, 1998) before activity staining. The SOD activity was identified by incubating the gel first in a 25 mL distilled water containing 50 mg nitroblue tetrazolium solution for 25 min, and then soaking it in 50 mL distilled water containing 0.53 mg riboflavin and 0.21 mL TEMED for another 25 min. The SOD activity was developed by photoreaction. To identify Cu/Zn SOD activity, 2 mM KCN was included in the riboflavin and the TEMED solution and then in the photoreaction. For hydrogen peroxide treatment, the gel was first soaked in 3 mM hydrogen peroxide in 10 mM Tris-HCl buffer (pH 7.9) containing 0.5 mM EDTA for 30 min, followed by SOD activity staining (Beauchamp and Fridovich, 1971). For GPx activity staining, the gel was submerged in a 50 mM Tris-HCl buffer (pH 7.9) containing 13 mM glutathione and 0.004% hydrogen peroxide with gentle shaking for 10 to 20 min. The GPx activity was stained by 1.2 mM 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 1.6 mM phenazine methosulfate (PMS) (Lin et al., 2002). The activity band showed a clear zone against brown backgrounds.

Temperature Stability

The crude extracts of fresh leaves of three cultivars were subjected to different temperatures (untreated, 40, 50, 60, 65, 70 and 80°C) for 5 min before being immediately cooled in an ice bath. The treated solutions were then ready for SDS-PAGE, performed at 4°C. For GPx temperature stability, the crude extracts were also subjected to 80°C for 10, 20 and 30 min and cooled immediately in an ice bath, after which they were ready for GPx activity staining.

Chemicals

All chemicals and reagents were of the highest purity available. Seeblue prestained markers for SDS-PAGE were from Novex (San Diego, CA); Other chemicals and reagents were from Sigma Chemical Co. (St. Louis, MO, USA).

Results and Discussion

Figure 1 showed the protein staining (Figure 1A), SOD activity (Figure 1B) and GPx activity (Figure 1C) stainings of leaf extracts of three cultivars of *Liriope spicata* L. It was found that all cultivars contained different SOD isozymes with molecular masses between 30 and 50 kDa (Figure 1B), and they also contained different GPx isozymes with molecular masses between 50 and 64 kDa (Figure 1C). Using inhibitor tests including KCN (Figure 2B) and hydrogen peroxide (Figure 2C) treatments, it was found that only the activity of Cu/Zn SOD isozymes could be detected in leaf extracts (Figure 2).

Figure 3 showed the temperature stability of SOD isozymes in leaf extracts of three cultivars. It was found that SOD isozymes were stable during 40 to 65°C treatments; however, no SOD activity could be detected at 70 and 80°C for 5 min. The SOD isozymes with molecular mass between 30 to 36 kDa were more heat stable than those with molecular masses between 36 and 50 kDa.

Figure 4 showed the temperature stability of GPx isozymes in leaf extracts of three cultivars. GPx isozymes were found to be stable during 50 to 80°C treatments. Figure 5 showed results of 80°C treatments for 10 min (lane 1), 20 min (lane 2) and 30 min (lane 3) in three cultivars.

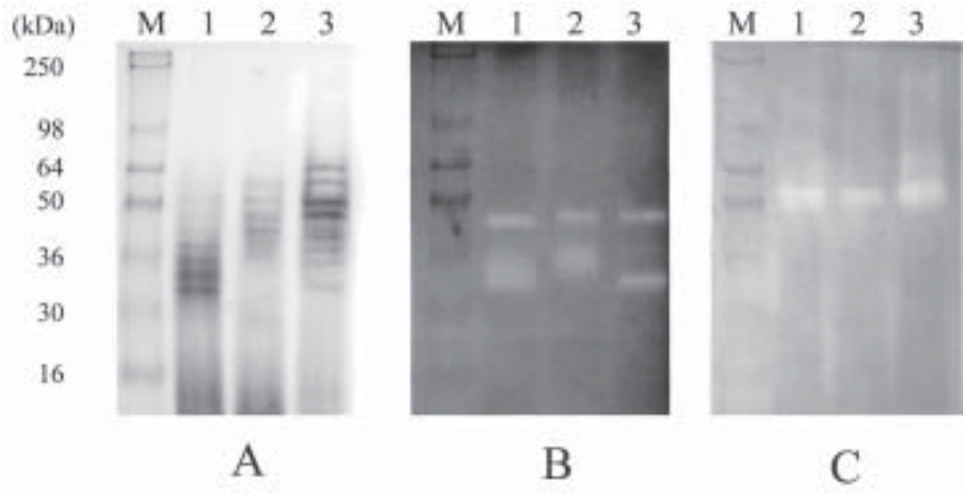

Figure 1. The protein (A), SOD activity (B) and GPx activity (C) stainings of leaf extracts of three cultivars of *Liriope spicata* L. on 10% SDS-PAGE gels. Lane 1, SL cultivar; lane 2, BL cultivar; lane 3, TL cultivar. M indicates the Seeblue prestained markers.

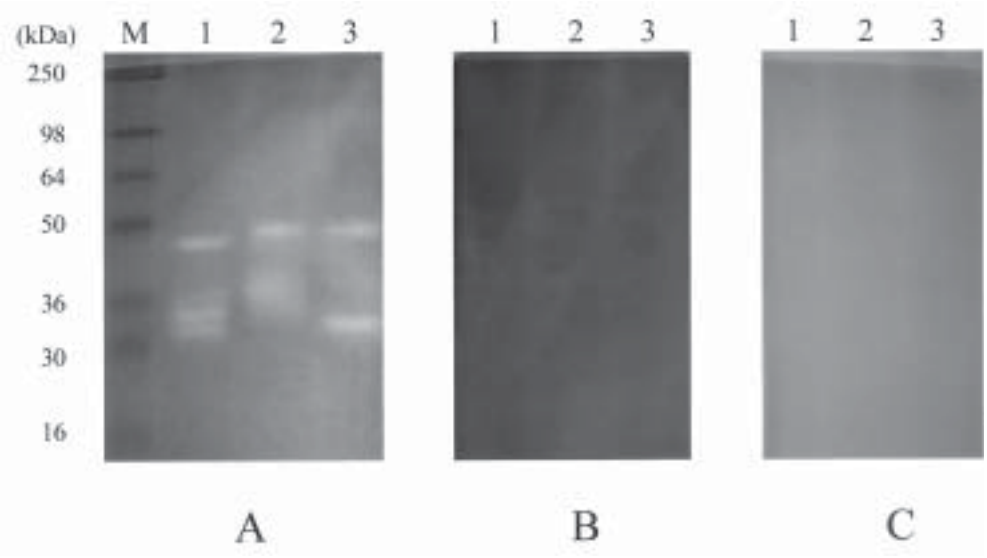

Figure 2. Inhibitor tests for SOD isozymes on 10% SDS-PAGE gels. (A) Control; (B) KCN treatment; (C) hydrogen peroxide treatment in leaf extracts. Lane 1, SL cultivar; lane 2, BL cultivar; lane 3, TL cultivar.

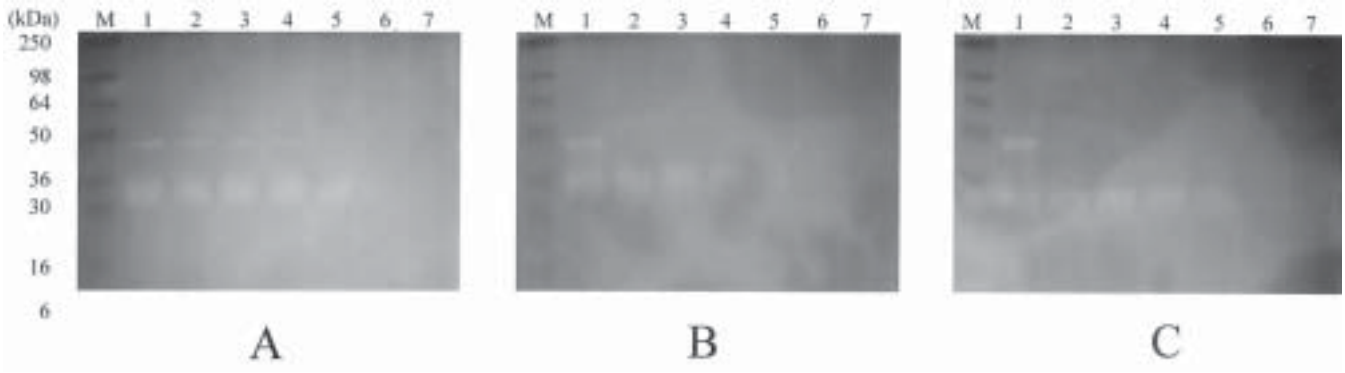

Figure 3. The temperature stability of SOD isozymes in leaf extracts of three cultivars on 10% SDS-PAGE gels. (A) SL cultivar; (B) BL cultivar; (C) TL cultivar. Lane 1, untreated; lane 2, 40°C; lane 3, 50°C; lane 4, 60°C; lane 5, 65°C; lane 6, 70°C; lane 7, 80°C for 5 min. Extracts were immediately cooled in an ice bath. The treated solutions were then ready for SDS-PAGE.

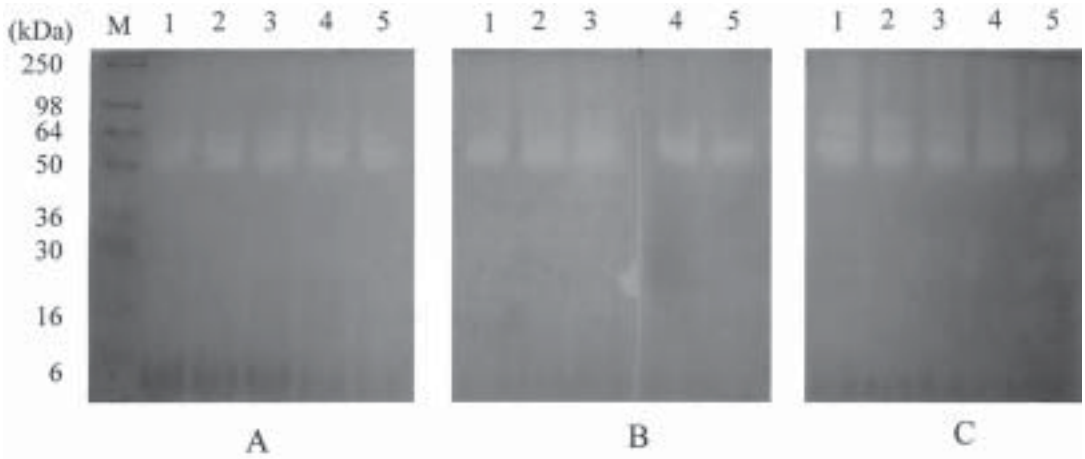

Figure 4. The temperature stability of GPx isozymes in leaf extracts of three cultivars on 10% SDS-PAGE gels. (A) SL cultivar; (B) BL cultivar; (C) TL cultivar. Lane 1, untreated; lane 2, 50°C; lane 3, 60°C; lane 4, 70°C; lane 5, 80°C for 5 min. Extracts were immediately cooled in an ice bath. The treated solutions were then ready for SDS-PAGE.

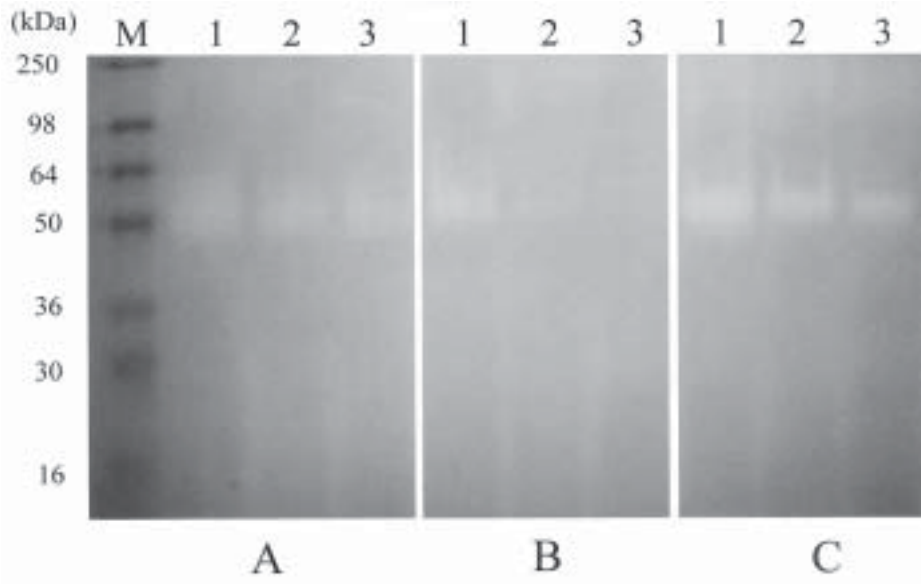

Figure 5. The GPx activity staining of SL (A), BL (B) and TL (C) cultivars' leaf samples after 80°C treatments for 10 min (lane 1), 20 min (lane 2) and 30 min (lane 3) on 10% SDS-PAGE gels.

The GPx activity in SL (Figure 5A) and TL (Figure 5C) cultivars was able to resist 80°C heating for 30 min.

In conclusion, the SOD and GPx activities in leaf extracts of different cultivars of *Liriope spicata* L. were identified. It was interesting that the dried leaf powders of *Liriope spicata* L. are also used as tea drinks. Our results were valuable for understanding the higher temperature stability of SOD and GPx activity. The purification and properties of SOD and GPx isozymes of *Liriope spicata* L. will be investigated further in the near future.

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不同栽培種麥門冬葉子的超氧歧化酶與麩胱甘肽過氧化酶活性

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利用10% SDS-PAGE方法檢測不同麥門冬栽培種葉子（小葉、大葉、細葉）粗抽液中超氧歧化酶與麩胱甘肽過氧化酶活性。利用抑制活性方法，發現所有品種都含有銅鋅超氧歧化酶，分子量介於 30到50 kDa。所有品種也都含有麩胱甘肽過氧化酶，分子量介於 50 到 64 kDa。熱安定性方面，超氧歧化酶異構酶在 40 到 65°C 都安定，但是 70 與 80°C 加熱五分鐘則失去酵素活性；但是所有品種中的麩胱甘肽過氧化酶異構酶在 80°C 加熱 5 分鐘都安定，其中小葉與細葉的麩胱甘肽過氧化酶在 80°C 加熱 30 分鐘都還有活性。

關鍵詞：超氧歧化酶；麩胱甘肽過氧化酶；麥門冬。