PURIFICATION, BIOCHEMICAL CHARACTERISTICS OF TRANSGLUTAMINASE FROM STREPTOVERTICILLIUM KENTUCKENSE AND ITS APPLICATION IN MEAT PROCESSING

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Background

Transglutaminase Tgase can catalyze the formation of covalent bond between inter-or intra-molecules of proteins to improve the function of protein in food. Traditionally, most sources of Tgase derived from animal organs, but by the economics it is necessary to look for more convenient and economical method for production.

Objectives

The purpose of this study is to select the most suitable strains from ten strains of Streptoverticillium to produce transglutaminase, and applied to catalyze polymerization of chicken muscle protein and to study the levels and optimal action conditions.

Methods

Ten strains of *Streptoverticillium* sp purchased from CCRC of FIRD in Taiwan were used to produce Tgase and compare their activity of Tgase and production. The organisms were inoculated in the media which described by Ando et al. (1989) and incubated at 28°C. After 96 hr incubation, the culture fluid was taken every 6 hr and filtered by centrifugation at 3000rpm for 15 min.
The culture filtrate was determined dry matter, protein concentration, pH, enzyme activity (using the method described by Folk, 1970).
The crude enzyme was precipitated with 55-57% ammonium sulfate and purified with CM-cellulose cation exchange chromatography. The SDS-PAGE electrophoresis was used to analyze the purity of Tgase (Laemmli et al., 1970). Biochemical properties of Tgase were also tested. Consequently, Tgase was employed to catalyze the polymerization of myofibrillar proteins from chicken breast muscle, which were extracted by the method of Zerifi et al.(1992) and reacted with crude and purified Tgases at different concentrations, times and temperatures. The polymerization between muscle proteins catalyze by Tgase was detected by SDS-PAGE electrophoresis. Rheological properties of chicken paste gel were also analyzed.

Results and Discussion

The results showed that a suitable strain for producing Tgase was *S. kentuckense* CCRC 12429 because it had highest Tgase activity-1.924 unit/mL obtained after 60 hr. culture, and took less time than other strains. In addition, the correlation between Tgase activity and protein concentration, pH of medium, dry matter weight, and total bacterial counts increased as time increasing in the former incubation period, but decreased in the later period.
The purity multiples of $(NH_4)_2SO_4$ prcipitated Tgase from *S. kentuckense* increased 5.19 times, and yield was 32%. Furthermore the samples was purified with CM-cellulose cation-exchange chromatography elute multiples increased 4.81 times and 24% yield was obtained (Table 1).
The crude Tgase from *S. Kentuckense* had the highest stability at pH7.0. The optimal reactive temperature was at 45°C. The metal ions, Zn^{2+} and Hg^{2+} inhibited the activity of Tgase. Although Ba^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Mg^{2+} inhibited partial activity of Tgase, and the enzyme activity still remained 80%.The addition of Mg^{2+} did not affect the activity of Tgase. Thiol reagent-NEM (N-ethylamaleimide) inhibited the activity of Tgase, but reductant increased the activity of Tgase.
The results of action of Tgase on the myofibrillar proteins of chicken breas muscle showed that the polymers of large molecule were found in the top of SDS-PAGE electrophoretogram when 0.1 unit crude Tgase incubated at 37°C for over 30 min or 0.3 unit crude Tgase incubated at 10°C for over 1 hr (Fig.1,2).
In rheological properties of gel, significantly effective improvement for the gel strength, breaking strength and gel hardness of chicken breast meat paste when added with 0.5% and 1.0% crude Tgase powder or 0.05%, 0.1%, 0.3% purified Tgase powder and incubated at 37°C, but no significantly effective improvement when incubated at 10°C.

Conclusion

1. *Streptoverticillium kentuckense* CCRC 12429 is the most suitable strain for producing Tgase of ten strains used in this study. It has the highest Tgase activity and yield %. The optimal activity conditions for the Tgase from *S. kentuckense* were also studied.
2. The catalysis of *S. kentuckenese* Tgase on the myoifibrillar proteins of chicken breast muscle was analyzed by observing protein electrophoresis and the polymers of large molecule were found in the top of SDS-PAGE electrophoretogram.
3. In rheological properties of gel, the gel strength, breaking strength and gel hardness of chicken breast meat paste were significantly improved by treated with Tgase from *S. kentuckenese*.

Pertinent Literature

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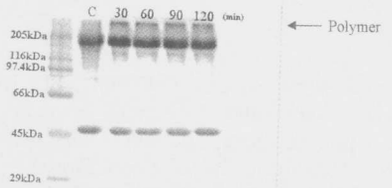

Fig1.SDS-PAGE electrophretogram of chicken myofibrillar protein with 0.3 unit (/mg protein) crude Tgase incubated at 37°C for different times. C:control

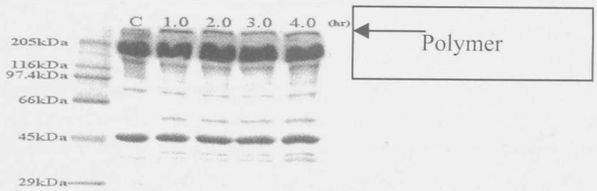

Fig2. SDS-PAGE electrophretogram of chicken myofibrillar Protein with 0.7 unit (/mg protein) crude Tgase incubated at 10°C for different times. C:control

Procedure	Total protein (mg)	Total activity (unit)	Specific activity (unit/mg)	Purification (fold)	Yield (%)
Crude TGase filtrate	1000.73	305.60	0.31	1.00	100
Ammonim sulphate Precipitate (55-75 %)	61.68	99.18	1.61	5.19	32
CM-cellulose	49.02	73.01	1.49	4.81	24

Table1. Changes of Tgase activity and protein concentration from *S. kentuckense* CCRC 12429 by different procedures.