学位論文審査の要旨

博士の専攻分野の名称　博士（農学）　氏　名　Jian Chen

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学位論文題名

Antibacterial activity of D-Tryptophan against food-borne pathogenic bacteria:
Application to food processing and investigation of the mechanism
（D-トリプトファンによる食品有害細菌の増殖抑制：
食品への応用と増殖抑制メカニズムの解明）

This PhD thesis comprises 87 pages, 19 Figures, and 5 Chapters with 1 reference thesis.

This study found that adding essential amino acid D-Tryptophan (D-Trp) inhibited the growths of some foodborne bacterial pathogens under osmotic stress or chilling stress. We confirmed the D-Trp treatment conditions for its optimal growth inhibitory effect *in vitro*. In addition, application of this novel nature food preservative in controlling *Vibrio* spp. in oyster under high-salt environment and controlling *Listeria monocytogenes* in diary production at refrigerated temperature. Furthermore, we investigated the mechanism of D-Trp by amino acid and metabolome analysis.

1. Growth inhibitory effect of D-Tryptophan under osmotic stress and its application in oysters.

Oysters are the primary transmission vehicles for human *Vibrio* infections. Raw oyster consumption is frequently associated with gastroenteritis. This is because *Vibrio* spp. are osmolarity-resistant bacteria that grow and survive under high-salt environment (seawater). We found that adding a small amount of D-Trp (< 40 mM) inhibits the growths of *V. parahaemolyticus* and *V. vulnificus* under a high-salt environment at even ambient temperature. We further investigated the D-Trp treatment conditions and clarified the relationship between sodium chloride and D-Trp concentrations for optimal growth inhibitory effect of *Vibrio* spp. The results will be useful for enhancing the effectiveness of D-Trp by increasing salinity levels. Furthermore, in nutrients-less environment (artificial seawater), a stronger inhibitory effect could be observed at relatively lower salinity levels, indicating that D-Trp may be regarded as effective food preservation in terms of salinity reduction.

2. Growth inhibitory effect of D-Tryptophan under chilling stress and its application in milk

Refrigeration is the primary method for food preservation. However, normal refrigeration alone is inadequate to restrain psychrophilic bacteria that are able to survive and grow at chilled temperatures. In particular, *Listeria monocytogenes* is strongly resistant to chill stress and tend to be more pathogenic during long-term refrigeration period. Adding a small amount of D-Trp (< 40 mM) could reduce and delay the psychrotrophic growth of *L. monocytogenes* at 4 °C for 30 days. Increasing the level of D-Trp further decreased the growth rate and extended the lag phase duration of *L. monocytogenes*. Milk and dairy products are implicated as the vehicle of transmission for listeriosis that commonly occurs as consequence of post-pasteurization contamination with *L. monocytogenes*. The addition of 40 mM D-Trp significantly ($P < 0.05$) inhibited the growth of *L. monocytogenes* in artificially pasteurized milk throughout one-month refrigeration period. These results indicated that D-Trp could significantly retard the psychrotrophic growth of *L. monocytogenes* by decreasing chill tolerance of *L. monocytogenes* at refrigeration temperatures and may serve as a novel food preservative to extending the shelf-life of milk production.

3. The mechanism of antibacterial activity of D-Tryptophan

Under osmotic stress, bacteria exhibit D-Trp-dependent growth inhibition. To confirm whether D-Trp actually was taken-up into bacterial cell and consequently cause metabolic disorder and growth inhibition under osmotic stress, we examined the changes of intracellular D-Trp levels. Changes in the D-Trp content in *Escherichia coli* (ATCC 25922) cells during 12 h incubation at 37°C was determined in different culture media containing 40 mM D-Trp with or without osmotic stress of 3% sodium chloride. High-performance liquid-chromatography (HPLC) based amino acid analysis of cell lysates revealed that the uptake and accumulation of D-Trp at high level do not directly contribute to the bacterial growth inhibition. Without osmotic stress, *E. coli* growth was not affected by 40-mM D-Trp and maintained a relative higher intracellular D-Trp contents than that in *E. coli* cells with osmotic stress. We further examined if the presence of extracellular D-Trp is a prerequisite for bacterial growth inhibition. After removed D-Trp from cell culture, a bacterial growth recovery was observed under osmotic stress, indicating that the bacterial inhibition requires the presence of in extracellular D-Trp. In conclusion, we supposed that D-Trp itself is not toxic for bacterial cell and its antibacterial mechanism is most likely taken place outside of bacterial cell instead of intracellular matrix.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.