

Bacillus cereus, *B. licheniformis* 與 *B. subtilis* 在醃漬食品中的分佈

*林旭陽 朱淑儀 方紹威 施養志

行政院衛生署藥物食品檢驗局

摘 要

自民國79年10月至80年6月間,在台北縣市各超級市場、傳統市場及食品商店等,抽購醃漬食品120件(包括30件葉菜類製品,38件根莖類製品,39件瓜果類製品,13件黃豆製品),進行 *Bacillus cereus* 病原菌、*B. subtilis* 及 *B. licheniformis* 機會性病原菌檢驗,計有47件檢體被檢出至少含有 *B. cereus*、*B. subtilis* 及 *B. licheniformis* 等三株細菌之一,其中葉菜類製品之檢出率為30.0%,根莖類為31.6%,瓜果類為48.7%,黃豆製品為53.8%;以細菌的種類而言,則以 *B. subtilis* 之檢出率最高,共計26件(21.7%)。

前 言

醃漬物(Pickle)的種類很多,依原料、配料的種類,食鹽濃度,加工程度,微生物作用的方法等,大致上可分為糖漬、醬油漬、粕漬、醋漬、鹽漬、味噌漬、芥末漬、麴漬和醪漬等九種。在製造過程中,有微生物作用顯著,和微生物幾乎不作用的兩種。前者包括長期鹽漬、米糠漬、米糠味噌漬、醃酵醃漬物(Pickle)和酸泡菜等。後者包括短期鹽漬、粕漬、味噌漬、醬油漬、麴漬、醋漬、芥末漬、山葵漬等,利用蔬菜原料與配料之間成分的轉移以及酵素作用而製成產品⁽¹⁾。

在醃漬物中,由於微生物的作用,會產生有機酸可以防腐,並會產生良好風味。但不良的微生物作用則產生酸敗、膨脹、酪酸臭、組織軟化、退化、變色等之變質或腐敗,有時也會有黴菌及病原菌的生長⁽¹⁾。一般醃漬物的最終pH值為4.6或更低,水活性(A_w) >0.85 ⁽²⁾。大部分的病原菌,無法在其中生長⁽¹⁾。但中毒菌 *Bacillus cereus* 生長的最低pH值範

圍為4.35~4.90,最高pH值則為9.30,水活性介於0.912~0.950之間⁽³⁾,故有可能在醃漬物中生長也不一定。在國外並曾發生過由 *B. subtilis* 所引起的食品中毒案,其涉及食品為醃漬洋蔥⁽⁶⁾。同時,醃漬物若污染 *B. licheniformis* 和 *B. subtilis* 時,這些菌會分泌蛋白質分解酵素和糖類分解酵素,引起食品加工上的變質和腐敗問題,事實上這兩株菌亦曾造成一些酸性食品(pH4.1~4.6)的腐敗^(1,2,3)。這些長桿菌屬細菌主要是從土壤和植物而,可能會跟隨醃漬原料一起被醃漬,且它們在不良環境下能形成孢子而存活,因此很可能繼續存在於醃漬最終產品中。綜合以上幾點的論述,醃漬物中若污染有 *B. cereus* 病原菌或 *B. licheniformis* 和 *B. subtilis* 等機會性病原菌(Opportunistic pathogens),並且大量繁殖,則極可能造成食品的腐敗,甚至引起食品中毒案的發生。所以有必要進行市面上醃漬物中 *B. cereus*、*B. licheniformis* 和 *B. subtilis* 之調查,以了解它們在醃漬食品中的分布情形。本計畫欲針對市售醃漬食品,如酸菜、筍干和醬油漬的瓜類等製品,抽購120件,依據相關資料進行上述三株細菌之檢驗。

材料與方法

一、檢體來源

*現任職行政院衛生署檢疫總所基隆分所

於79年10月至80年6月,至台北縣、市各超級市場、傳統市場及食品商店等,抽購市售各種醃漬品,包括酸菜、醬瓜、醃筍、榨菜、醃蘿蔔、醃薑、醃蒜、落蕎、豆腐乳等合計120件。檢體分別包裝冷藏後,攜回本局實驗室予以檢驗。

二、微生物檢驗

檢體適當切碎,均勻混合後,取25 g加至225 ml 0.85%生理食鹽水中均勻混合為檢液,依據中國國家標準中有關食品中*Bacillus cereus*檢驗法進行檢驗⁽⁴⁾,生化試驗部份以產孢菌屬生化測定卡片(Bacillus Biochemical Card, BAC-VITEK System, U. S. A)配合VITEK微生物自動鑑定儀(VITEK JR. Systems, McDOMEILL DOUGLAS HEALTH SYSTEMS company, U. S. A)檢測分析。

三、水活性、酸鹼度與鹽度測定

將檢體均勻細切後,置於測定皿內,直接以水活性測定儀(Model WA-14 TH, Rotronic Hygro-skop DT, Swiss),以恆溫25°C測定。以酸鹼度計(691 pH meter, Metrohm AG, Herisau, Swiss)進行pH值測定。以鹽度計(No.12350, BINKO, Japan)進行鹽度測定。

結果與討論

由市面上抽購120件醃漬食品,進行*B. cereus*, *B. subtilis*和*B. licheniformis*之檢驗,發現有8件檢出*B. cereus* (6.7%); 26件檢出*B. subtilis* (21.7%); 13件檢出*B. licheniformis* (10.8%),共計47件檢出

以上這三株細菌(39.2%)(表一),表示超過三成以上的醃漬食品檢體污染有三株*Bacillus*屬細菌。蔡等⁽⁵⁾曾抽購253件的真空包裝食品桿菌屬細菌之污染情形,共計有89件(35.2%)檢體檢出含*Bacillus* spp.,此與本調查報告之檢出率大約相同。若將抽購的醃漬食品種類分成蔬菜類、根莖類、瓜果類及黃豆製品等四大類,則此四類醃漬食品檢出*B. cereus*, *B. subtilis*和*B. licheniformis*等三株長桿菌屬細菌之檢出率分別為30.0%, 31.6%, 48.7%和53.8%

(表一)。在各類醃漬食品中以*B. subtilis*檢出最高。

能檢出*Bacillus* spp.的各類檢體最低之Aw值和pH值,與鹽度示如表二。根莖類食品中的薑,其pH值雖低至3.4,但仍可檢出*B. cereus*。Raevuori et al.⁽⁷⁾發現*B. cereus*的營養細胞,在肉類食品中能生長的之最低pH值為4.35,最低Aw值為0.95; Goepfert et al.⁽⁸⁾則表示在一般實驗室培養基中,*B. cereus*營養細胞能生長之最低pH值為4.9;但根據游等⁽⁹⁾實驗顯示:在2%鹽度下,醃漬泡菜之醃漬液之pH值降至3.8時,*B. cereus*仍有生長情形。在蔬菜類醃漬食品中的淡榨菜,其pH值雖低至4.1,仍可檢出*B. subtilis*。另外,除了有一件辣蘿蔔檢體的鹽度高達14.6%,仍可檢出*B. subtilis*和*B. licheniformis*外;尚有一件良京檢體的pH值低至4.1,鹽度高達11.0%,Aw為0.88,猶可檢出*B. subtilis*。依據Kramer et al.⁽⁶⁾統計英國地區媒介*B. subtilis*和*B. licheniformis*而引起食品中毒的食物,蔬菜類被認為是其中一項重要的媒介食物。因此在製造這一類醃漬產品時,仍須注意*Bacillus* spp.的污染問題。在瓜果類食品中的玫瑰李製品,其pH值低至2.8,鹽度更高達33.2%,Aw值為0.73仍可檢出*B. subtilis*;另外有一件青辣椒的製品其pH值為4.0,鹽度為19.2%,Aw值為0.88,尚可檢出*B. subtilis*和*B.*

Table 1. Incidence of *B. cereus*, *B. subtilis* and *B. licheniformis* in pickles

Species	NO. of positive samples (%)				
	Leaf	Earth	Fruit	Soybean	Total(%)
	vegetables n=30	vegetables n=38	vegetables n=39	products n=13	n=120
<i>B. cereus</i>	6.7	5.3	5.1	15.4	6.7
<i>B. subtilis</i>	20.0	15.8	25.6	30.8	21.7
<i>B. licheniformis</i>	3.3	10.5	17.9	7.7	10.8
Total	30.0	31.6	48.6	53.9	39.2

n, number of samples examined.

Table 2. The highest salt content, the lowest water activity (Aw) and pH of pickles with *B. cereus*, *B. subtilis* and *B. licheniformis*

Species	Leaf vegetables			Earth vegetables			Fruit vegetables			Soybean products		
	Salt			Salt			Salt			Salt		
	Aw	content	pH	Aw	content	pH	Aw	content	pH	Aw	content	pH
<i>B. cereus</i>	0.95	4.8	4.6	0.94	10.8	3.4	0.91	11.6	4.2	0.81	46.0	4.8
	(Szuchuan cabbage)			(Paper preserved radish)			(Salted peper) (Pickled cucumber) (Salted peper)			(Soybean curd with miso)		
<i>B. subtilis</i>	0.94	5.6	4.1	0.88	14.6	4.1	0.73	33.2	2.8	0.71	46.0	4.8
	(Szuchuan cabbage)			(Sweet (Peper (Sweet (Preserved plum) (Gluten) (Soybean curd with miso)								
				scallion) preserved scallion) radish)								
<i>B. licheniformis</i>	0.96	4.7	4.6	0.92	14.6	3.9	0.85	19.2	4.0	0.95	12.0	5.7
	(Szuchuan cabbage)			(Sweet (Peper (Sweet (Pickled (Green peper) (Soybean curd with sesame oil)								
				garlic) preserved garlic) radish)			cucumber)					

licheniformis。黃豆製品中，有一件米醬豆腐乳的鹽度高達46%，Aw爲0.81，也可檢出*B. cereus*和*B. subtilis*。

參考文獻

1. 李福臨.1983.醃漬物的微生物.食品工業.15(2):31-38.

2. Speck, M. L.(ed.). 1984. Compendium of methods for the microbiological examination of foods, 2nd ed. American Public Health Association, Washington, D. C., pp. 664-665.

3. Doyle, M. P. 1989. Foodborne bacterial pathogens. Marcel Dekker, Inc., N. Y., pp.58-63.

4. 經濟部中央標準.1989. 食品微生物之檢驗法—仙人掌桿菌之檢驗.中國國家標準12540.N6212.

5. 蔡淑貞,張睿昇,施養志,陳陸宏.1992. 真空包裝食品中長桿菌屬細菌之污染.食品科學.19:68.

6. Kramer, J. H. and Gilbert, R. J. 1989. *Bacillus cereus* and other *bacillus* species. In “Foodborne Bacterial Pathogens” . p. 21. Ed. Doyle, M. P. Marcel Dekker, Inc. New York and Basel.

7. Raevuori, M. and Genigeorgis C. 1975. Effect of pH and sodium choloride on growth of *Bacillus cereus* in laboratory medium and certain foods. Appl. Microbiol. 29 : 68.

8. Goepfert, J. M., Spira W.M. and Kim H. U. 1972. *Bacillus cereus* : food poisoning organism. A review. J. Milk Food Technol. 35 : 231.

9. 游淑玲,周正俊,1987. 不同鹽度下醃漬泡菜時一些食品中毒菌之存活.食品科學.14:119.

Distribution of *Bacillus cereus*, *B. licheniformis* and *B. subtilis* in Marketed Pickles

HSU-YANG LIN, SU-YI CHU, SHAO-WEI FANG AND DANIEL YANG-CHIH SHIH

National Laboratories of Foods and Drugs, Department of Health, Executive Yuan

ABSTRACT

From Oct. 1990 to Jun. 1991, 120 samples of pickles(including 30 leaf vegetables, 38 earth vegetables, 39 fruit vegetables and 13 soybean products) were purchased from supermarkets, traditional markets and food retail stores in Taipei area and examined for the presence of *Bacillus cereus* (pathogen), *B. subtilis* and *B. licheniformis* (opportunistic pathogen). A total of 47

samples contained at least one of the three *Bacillus* species. *Bacillus* spp. was detected in 30.0% of leaf vegetables, 31.6% of earth vegetables, 48.7% of fruit vegetable and 53.8% of soybean products. The detection rate *B. subtilis* was 21.7%, which was the highest one among the three *Bacillus* species.

Key words : Pickles, Pathogen, *Bacillus* spp.

Title Index to Volume 1 (1993)

Title	Author	Vol. (No.) : Page
DRUG		
• The Validation of Analytical Assays for Biopharmaceutical Studies	Gerald K. Shiu	1 (1) : 1
• Determination of ML-1035 Enantiomers in Plasma by Chiral High Performance Liquid Chromatography	Kin-Kai Hwang and Arun K. Mendagere	1 (1) : 5
• Nucleophilic Substitution and Racemization of 3-Ethoxy-N-desmethyldiazepam Enantiomers in Acidic Ethanol	Shen K. Yang and Xiang-Lin Lu	1 (1) : 23
• Determination of Ondansetron and Metabolites in Plasma and its Pharmacokinetics in Cancer Patients	Oliver Yoa-Pu Hu, Tsang-Wu Liu, Pei-Shan Chiang, Li-Ling Huang and Wei-Shou Huang	1 (1) : 35
• Preparation of Hepatitis B Vaccine Reference Standard for Potency Test	Chia Po Lin, Tso-Ling Chen, Ruoh-Ing Yang, Chin-Jung Chen and Jung-Tian Hsieh	1 (1) : 43
• Stability of Geniposide, Berberine and Paeoniflorin in Uen-Ching-Yiin, San-Hwang-Shieh-Shin-Tang and Sheau-Ching-Long-Tang	Shiow-Jane Lin, Fang-Su Liu, Fen-Ling Lu, Cheng-Yu Hwang and Kuo-Ching Wen	1 (1) : 49
• Immunity and Control Analysis of <i>H. Influenzae</i> Type b Polysaccharide-Protein Conjugate Vaccine	Chi-Jen Lee	1 (2) : 133
• Study on the $\Delta^3 \rightarrow \Delta^2$ Isomerization During Preparation of Cefuroxime Double Ester Prodrugs	Hui-Po Wang and Jia-Shuai Lee	1 (2) : 145
• Identification and Characterization of Endothelin Receptors in Rat Cerebellum	Hwei-Fang Cheng, Jing-Ruey Yeh and Kwen-Jen Chang	1 (2) : 165
• Quantitative Analysis of Cardamon and Fennel Combination	Lih-Ching Chang and Shuenn-Jyi Sheu	1 (2) : 183
• Determination of Paeoniflorin and Geniposide in Traditional Chinese Medicinal Preparation by High Performance Liquid Chromatography	Fang-Su Liu, Long-Dar Lin and Kuo-Ching Wen	1 (2) : 191
• The Intrapulmonary Airway Smooth Muscle of Mice-An Ultrastructural Study	Chieng-Hsun Chiang	1 (2) : 227
• Pharmacoscintigraphy : Techniques for Studying Drug Release And Absorption in Humans	Keith Kwei-Hang Chan	1 (3) : 235
• The Rapid Determination of Ethanol in Postmortem Brain Samples by Solvent Extraction and Gas Chromatography	Steven J. Moore, Ann C. Porter, Anna E. Ezell, Arthur S. Hume and I. K. Ho	1 (3) : 245
• HPLC Analysis of Emodin in Serum, Herbs and Chinese Herbal Prescriptions	J-Wen Liang, Su-Lan Hsiu, Huei-Chen Huang and Pei-Dawn Lee Chao	1 (3) : 251

• Studies on Tannins of the Bark of <i>Macaranga tanarius</i> (L.) Muell. et Arg.	Jer-Huei Lin	1 (3) : 275
• Application of HPLC Method for Quality Control of Cefadroxil	Su-I Chou and Shu-Cheng You	1 (3) : 305
• Sedative-Hypnotics : Addiction and Current Research Status	Yi-Tang Tseng, Susan E. Wellman and I. K. Ho	1 (4) : 311
• The Application of Capillary Electrophoresis in Analysis of Chinese-Herb Drugs	Ying-Mei Liu and Shuenn-Jyi Sheu	1 (4) : 327
• A Simple and Easy to Prepare Synthetic Multiple-Interaction Chiral Stationary Phase Column for Chiral Drug Analysis	Gerald K. Shiu	1 (4) : 335
• Conversion of Quazepam to 2-Oxoquazepam in Alkaline Solution	Shen K. Yang and Michael S. Yang	1 (4) : 341
• Simultaneous Quantitation of Caffeine, Ethoxybenzamide and Propyphenazone in Oral Analgesic Tablets by High-Pressure Liquid Chromatography	An-Rong Lee, Kuang-Sen Sung and Wen-Hsin Huang	1 (4) : 351
• Survey of Arsenic Contents in Shell-medicament as Chinese Medicine	Tzu-Ming Pan, Yen-Yu Zhen, Chien-Chih Chen and Ching-Shing Wang	1 (4) : 365

FOOD

• Studies of Rapid Determination of Vitamin E and Its Application on Natural Foodstuffs	Jeng-De Su	1 (1) : 61
• Establishment of Standard Analytical Methods for Determination of Pesticide Residues in Crops-Tests of Terbutos, Diflubenzuron, Aldicarb and Oxamyl	Chia-Fen Tsai, Jy-Mei Yan, Bih-Chiou Chang and Shin-Shou Chou	1 (1) : 71
• Investigation on the Safety of Plastic Food Containers for Use in Microwave Oven	Meei-Jiuan Bai, Hwa Chu, Chieu-Chen Cheng and Shin-Shou Chou	1 (1) : 81
• Studies on Analytical Methods of Robenidine Residues in Chicken meat and Viscera	Mai-Ling Jao, Ruey-Jub Lin, Chen-Chung Lee, Shu-Chi Lee and Shin-Shou Chou	1 (1) : 97
• Survey of <i>Staphylococcus aureus</i> in Vegetable Salad	Wei-Hsia Kao and Daniel Yang-Chih Shih	1 (1) : 107
• Rapid Methods for the Selection of <i>Salmonella</i> in Foods	Peter Feng	1 (1) : 119
• Influence of Dietary Vitamin E on the Content of Vitamin E in Blood Plasma, Ovary Tissue and on Hemolysis of Cultured Female Grey Mullet (<i>Mugil cephalus</i>)	Jiin-Fung Shyu and Bonnie Sun Pan	1 (2) : 155
• Analysis of Carotenoids in Grass Prawn Heads by High Performance Liquid Chromatography	Pai-Wen Wu and Lucy Sun Hwang	1 (2) : 175

• Microbial Sanitary Properties of Cup Soft Drinks and Hot Beverages from Automatic Vending Machines in Taiwan	Yueh-Jong Chung-Wang, Li-Ping Kuo, A-Yang Lin, Jason Dalang Hong and Yu-Yun Tsai	1 (2) : 199
• Studies on the Analysis of Ethopabate in Chicken Meat and Livers	Yea-Min Kao and Pi-Chiou Chang	1 (2) : 207
• Studies on Analytical Method of Thiamine Dilaurylsulfate in Soy Sauce	Shu-Ju Su, Chun-Kuo Yu, Shu-Chi Lee, Chao-Hong Liu and Shin-Shou Chou	1 (2) : 215
• Production and Characterization of Cholera toxin-like Enterotoxin by a Non-01 <i>Vibrio cholerae</i> Isolated from Food	Hin-Chung Wong and Jui-Hsi Weng	1 (3) : 259
• Survey of β -Lactam Antibiotics and Heavy Metals in Infant Milk Powder and Human Milk	Hwia-Cheng Ding, Haw-Jang Liu, Yea-Lian Sheu and Tsung-Chain Chang	1 (3) : 265
• Incidence of <i>Salmonella</i> in Egg Yolks of Chicken, Duck and Processed Eggs in Taiwan	You-Min Fu, Ting Su and Ling-Mei Chou	1 (3) : 281
• Simultaneous Determination of Five Synthetic Antioxidants in Fats and Oils	Shyh-Shii Yang, Mei-Ju Lee, Shu-Chi Lee, Shu-Ju Su and Shin-Shou Chou	1 (3) : 287
• Detection of Staphylococcal Enterotoxins in Rice Using Enzyme Immunoassay Kits	Shu-Shen Hung, Tsuey-Ping Huang and Hsi-Chin Ko	1 (3) : 297
• Garlic as Anti-Oxidants and Free Radical Scavengers	George C. Yang, Parvin M. Yasaei and Samuel W. Page	1 (4) : 357
• Comparison between HPLC and HPIC for Determination of Acesulfame Potassium in Food	Pai-Wen Wu, Chieu-Chen Cheng and Shin-Shou Chou	1 (4) : 373
• Studies on the Benzene Formation in Beverages	Pi-Chiou Chang and Ken Ku	
• Distribution of <i>Bacillus cereus</i> , <i>B. licheniformis</i> and <i>B. subtilis</i> in Marketed Pickles	Hsu-Yang Lin, Su-Yi Chu, Shao-Wei Fang and Daniel Yang-Chih Shih	1 (4) : 395

OTHERS

• Biomarkers : An Important Strategy for Human Health Risk Assessment	Peter P. Fu, Yuh Shen Wu, Feng-Shiow Lai, Mei In Chung and Ching-Cheng Lai	1 (1) : 7
---	--	-----------

『藥物食品分析期刊』徵稿稿約

- 一、凡有關藥物或食品檢驗分析之學術研究論著，未曾在其他刊物刊載者，皆為本期刊徵求之對象，本刊原則上每季出版一期。
- 二、稿件如為原著須按摘要(Abstract)、前言(Introduction)、材料與方法(Materials and Methods)、結果(Results)、討論(Discussion)或結果與討論(Results and Discussion)、誌謝(Acknowledgments)、參考文獻(References)及對照用中文或英文摘要(Abstract, 論著本文如為中文請附英文摘要, 如為英文請附中文摘要)之順序撰寫繕打, 如為案例報告(Case report)或短訊(Research note)則按摘要、前言、材料與方法(案例)、結果與討論、參考文獻及對照用中文或英文摘要之順序撰寫繕打。
- 三、稿件中文或英文不拘。中文稿件請用標準稿紙(20×25)從左到右橫寫, 或直接列印於電腦報表紙, 文末並須附對照用英文摘要。英文稿件請用打字機繕打, 文中行間應空兩行, 或直接列印於電腦報表紙, 文末須附對照用中文摘要。表格(tables)及圖(figures)之標題應以英文列於表格上方或圖下方, 其內容則以英文為主, 必要時加註中文, 英文第一字母除介詞、連接詞和冠詞外, 均須大寫。
- 四、稿件應按下列順序分頁書寫(來稿請編頁數):
 - 首 頁(Title Page): 包括題目(中文20字以內, 英文40字以內, 英文每一字首大寫)、作者姓名、服務單位(英文稿則附英文全名)、連絡人、姓名、通訊處、電話號碼及傳真號碼。
 - 第二頁: 包括摘要(Abstract), 約300字, 英文鍵語(Key words)。
 - 第三頁以後: 本文、誌謝、參考文獻及對照用中文或英文摘要(800字左右)。
- 五、文內如需分段或分條敘述者, 其符號中文稿請依一、(一)、1、(1)、A、(A)、a、(a)……次序採用, 英文稿請依I、(I)、1、(1)、A、(A)、a、(a)……次序採用; 須加註腳處以指定符號註明之, 依次為*、**、***、****、……等。
- 六、數字請用阿拉伯字(英文句首除外), 單位一律用國際公認之標準符號, 如: cm、mm、 μ m、ml、kg、g、mg、 μ g、ng、pH、ppm、 $^{\circ}$ C……等。
- 七、插圖必須原圖(3×4吋以上)三套。如係照片, 必須光面黑白清晰, 每張背面並以軟鉛筆註明號碼、題目及著者姓名。如係圖表, 應以白紙墨繪, 光學和電子顯微鏡照片, 請註明擴大倍率或比率。
- 八、參考文獻:
 - A. 文獻依文內引用順序排列, 文內引用之處, 請以(1)、(2)、……數字於文字右上角標明。
 - B. 寫法依作者姓名、年代、篇名、期刊名或書名、卷數(期數)及起迄頁數之順序。如係書籍, 請加註編者、版別、出版社名及出版地城市名。範例:
 1. Cole, M.T., Marilyn, B.K. and Camerion, R.H. 1986. Evaluation of Methods for Extraction of Enteric Virus from Louisiana Oyster. J. Food Prot. 49 : 592-602.
 2. Boeck, L.D. and Wetzel, R.W. 1990. A New Lipopeptide Antibiotic Complex: Factor Control Through Precursor Directed Biosynthesis. The J. of Antibiotics. 43(6) : 607-615.
 3. Miller, J.C. and Miller, J.N. 1986. Statistics for Analytical Chemistry. pp.100-102. Ellis Horwood Ltd. England.