### TABLE OF CONTENTS

|  | Page |
|--|------|
| Acknowledgements   | iii  |
| Abstract (English)   | iv   |
| Abstract (Thai)  | vii  |
| List of Tables   | xi   |
| List of Figures  | xii  |
| Abbreviations and Symbols                                    | xiv  |
| Chapter 1 Introduction                                       | 1    |
| Chapter 2 Literature reviews                                 | 3    |
| Chapter 3 Materials and Methods                              | 29   |
| Chapter 4 Results and Discussions                            | 42   |
| Chapter 5 Conclusions  | 69   |
| References   | 70   |
| Appendices   | 87   |
| Appendix A Media   | 88   |
| Appendix B Chemical reagents for agarose gel electrophoresis | 92   |
| Appendix C Nucleotide sequences and GenBank accession        | 94   |
| numbers of 16S rRNA genes of 12 plasmids                     |      |
| isolated from Lactobacillus spp.                             |      |
| Appendix D Nucleotide sequences and GenBank accession        | 106  |
| numbers of pSD11   |      |

Appendix E Biochemical tests of 105 Lactobacillus spp. isolated

109

from fermented foods



# ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright<sup>©</sup> by Chiang Mai University All rights reserved

#### LIST OF TABLES

| Ta  | ble o 9/3/2 19/9  | Page |
|-----|---|------|
| 2.1 | Properties of lactic acid   | 8    |
| 2.2 | Grouping of the genus Lactobacillus                                 | 12   |
| 2.3 | Bacteriocins produced by Lactobacillus plasmids and their activity  | 26   |
|     | spectra   |      |
| 3.1 | Laboratory equipments   | 31   |
| 3.2 | Fermented foods used for bacterial isolation                        | 33   |
| 3.3 | Antibiotics used in this study                                      | 37   |
| 3.4 | Primers used for DNA sequencing                                     | 40   |
| 4.1 | Summary of isolation of <i>Lactobacillus</i> spp.                   | 45   |
| 4.2 | Blast results of 12 plasmid containing <i>Lactobacillus</i> spp.    | 47   |
| 4.3 | Numbers of plasmids in tested <i>Lactobacillus</i> spp.             | 48   |
| 4.4 | Antibiotic susceptibility profiles of tested strains                | 50   |
| 4.5 | Changes of OD660 of 12 plasmid containing <i>Lactobacillus</i> spp. | 53   |
|     | in soluble starch, cmc, colloidal chitin and inulin                 |      |
| 4.6 | Changes of OD660 of 12 plasmid containing <i>Lactobacillus</i> spp. | 54   |
|     | in gelatin, citrate, phenylalanine and tryptophan                   |      |
|     |   |      |

### LIST OF FIGURES

| Figure | e े शिश्वाप्ति १  | Page |  |
|--------|---|------|--|
| 2.1    | Homolactic fermentation   | 5    |  |
| 2.2    | Heterolactic fermentation   |      |  |
| 2.3    | Structural formula of lactic acid   |      |  |
| 2.4    | Chemical synthesis of lactic acid   |      |  |
| 2.5    | Illustration of a bacterium with plasmid enclosed showing                   |      |  |
|        | chromosomal DNA and plasmids  |      |  |
| 2.6    | Forms of plasmids   | 14   |  |
| 2.7    | Theta replication   | 16   |  |
| 2.8    | The rolling circle replication (RCR)  | 17   |  |
| 2.9    | The main steps in using plasmid vectors to clone DNA molecules 2            |      |  |
| 2.10   | Comparison of <i>Lactobacillus</i> plasmids                                 |      |  |
| 4.1    | Neighbour-joining tree based on 16S rRNA gene sequences showing 4           |      |  |
|        | the position of 12 plasmid containing <i>Lactobacillus</i> spp. and related |      |  |
|        | strains 111499 MSI9 8 SI 18 SI 0 17   |      |  |
| 4.2    | Plasmid profiles of <i>Lactobacillus</i> spp.                               | 47   |  |
| 4.3    | The examples of inhibition zones caused by antibiotic susceptibility test   | 50   |  |
| 4.4    | BamHI digestion of 12 plasmids isolated from Lactobacillus spp.             | 56   |  |
| 4.5    | EcoRI digestion of 12 plasmids isolated from Lactobacillus spp.             | 56   |  |
| 4.6    | HindIII digestion of 12 plasmids isolated from Lactobacillus spp.           | 57   |  |

#### LIST OF FIGURES (CONTINUED)

| Figur | e  | Page |  |
|-------|--|------|--|
| 4.7   | Dendrogram of plasmids digested with BamHI                     | 57   |  |
| 4.8   | Dendrogram of plasmids digested with EcoRI                     |      |  |
| 4.9   | Dendrogram of plasmids digested with HindIII                   |      |  |
| 4.10  | Preparation of plasmid pSD11 and cloned into pUC19 vector 60   |      |  |
| 4.11  | Physical map of plasmid pSD11 from Lactobacillus brevis D11    | 61   |  |
| 4.12  | Multiple sequence alignment of the amino acid sequences of Rep | 63   |  |
|       | protein  |      |  |
| 4.13  | Detailed DNA sequence of <i>rep</i> gene from pSD11            | 63   |  |
| 4.14  | Multiple sequence alignment of the amino acid sequences of Mob |      |  |
|       | protein  |      |  |
| 4.15  | Detailed DNA sequence of mob gene from pSD11                   | 66   |  |
|       |  |      |  |

# ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright<sup>©</sup> by Chiang Mai University All rights reserved

### ABBREVIATIONS AND SYMBOLS

| °C   | ปมกาย                             | degree Celsius           |
|------|-----------------------------------|--------------------------|
| bp   | P Q D A                           | base pair                |
| g    |                                   | gram                     |
| kb   | 人                                 | kilobase                 |
| kDa  | Juliu =                           | kilodalton               |
|      | =                                 | liter                    |
| μg   | ₩ <u>₹</u>                        | microgram                |
| μl   |                                   | microliter               |
| mg   | \ <del>-</del>   / <del>*</del> / | milligram                |
| ml   |                                   | milliliter               |
| mm   | 6                                 | millimeter               |
| mM   | AT TOTAL                          | millimolar               |
| M    | UNIV.                             | molar (mol/liter)        |
| MWCO | =                                 | molecular weight cut off |
| ng   | หาวิท <sub>ิ</sub> ยาลั           | nanogram                 |
| OD   | by Chiang A                       | optical density          |
| v/v  | by Chiang A                       | volume by volume         |
| w/v  | ghts re                           | weight by volume         |