Identification of Bifidobacterium thermophilum RBL67 isolated from baby faeces and partial purification of its bacteriocin

Author(s):
von Ah, Ueli

Publication Date:
2006

Permanent Link:
https://doi.org/10.3929/ethz-a-005349486

Rights / License:
In Copyright - Non-Commercial Use Permitted
Identification of *Bifidobacterium thermophilum* RBL67 isolated from baby faeces and partial purification of its bacteriocin

A dissertation submitted to

SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH
(ETHZ)

For the degree of
Doctor of Technical Sciences

Presented by
UELİ VON AH
dipl. Lm.-Ing. ETH
Born October 3, 1973
Citizen of Emmen LU and Sarnen OW

accepted on the recommendation of
Prof. Dr. Christophe Lacroix, examiner
Prof. Dr. Leo Meile, co-examiner
Prof. Dr. Ismaïl Fliss, co-examiner

Zurich, 2006
# Table of contents

**TABLE OF CONTENTS**

<table>
<thead>
<tr>
<th>Part</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.1</td>
<td>1</td>
</tr>
<tr>
<td>I.2</td>
<td>5</td>
</tr>
<tr>
<td>I.3</td>
<td>12</td>
</tr>
<tr>
<td>I.4</td>
<td>15</td>
</tr>
<tr>
<td>I.5</td>
<td>27</td>
</tr>
</tbody>
</table>

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
</tr>
</tbody>
</table>

**SUMMARY**

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>VII</td>
</tr>
</tbody>
</table>

**ZUSAMMENFASSUNG**

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>IX</td>
</tr>
</tbody>
</table>

**1 INTRODUCTION**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1.1</td>
<td>1</td>
</tr>
<tr>
<td>1.1.2</td>
<td>2</td>
</tr>
<tr>
<td>1.1.3</td>
<td>2</td>
</tr>
<tr>
<td>1.1.4</td>
<td>3</td>
</tr>
<tr>
<td>1.1.5</td>
<td>4</td>
</tr>
<tr>
<td>1.2.1</td>
<td>5</td>
</tr>
<tr>
<td>1.2.2</td>
<td>6</td>
</tr>
<tr>
<td>1.2.4</td>
<td>9</td>
</tr>
<tr>
<td>1.2.5</td>
<td>9</td>
</tr>
<tr>
<td>1.2.6</td>
<td>10</td>
</tr>
<tr>
<td>1.3.1</td>
<td>12</td>
</tr>
<tr>
<td>1.3.2</td>
<td>13</td>
</tr>
<tr>
<td>1.4.1</td>
<td>15</td>
</tr>
<tr>
<td>1.4.2</td>
<td>19</td>
</tr>
<tr>
<td>1.4.3</td>
<td>21</td>
</tr>
<tr>
<td>1.4.4</td>
<td>23</td>
</tr>
<tr>
<td>1.4.5</td>
<td>25</td>
</tr>
<tr>
<td>1.4.6</td>
<td>26</td>
</tr>
<tr>
<td>1.5.1</td>
<td>27</td>
</tr>
<tr>
<td>1.5.2</td>
<td>29</td>
</tr>
<tr>
<td>1.5.3</td>
<td>30</td>
</tr>
</tbody>
</table>
# Table of Contents

1.5.4 IMMOBILIZED CELL CULTURES 31  
1.6 AIMS OF THIS STUDY 36  
1.6.1 HYPOTHESIS 36  
1.6.2 GENERAL OBJECTIVE 36  
1.6.3 SPECIFIC OBJECTIVES 37  

## 2 IDENTIFICATION OF A MODERATELY OXYGEN-TOLERANT ISOLATE FROM BABY FAECES AS *BIFIDOBACTERIUM THERMOPHILUM* 38  

### 2.1 SUMMARY 38  
2.1.1 BACKGROUND 38  
2.1.2 RESULTS 38  
2.1.3 CONCLUSION 39  

### 2.2 BACKGROUND 40  

### 2.3 MATERIAL AND METHODS 42  
2.3.1 BACTERIAL STRAINS AND ROUTINE GROWTH CONDITIONS 42  
2.3.2 GROWTH UNDER OXIDATIVE-, HEAT- AND pH-STRESS CONDITIONS 43  
2.3.3 AMPLIFICATION AND SEQUENCING OF 16S rDNA 43  
2.3.4 PULSED FIELD GEL ELECTROPHORESIS (PFGE) 44  
2.3.5 CLONING AND ANALYSIS OF *groEL* SEQUENCES 45  
2.3.6 DNA-DNA HYBRIDIZATION 46  
2.3.7 CARBOHYDRATE FERMENTATION AND ACID ANALYSIS 46  

### 2.4 RESULTS AND DISCUSSION 47  
2.4.1 PHYLOGENETIC POSITION OF *Bifidobacterium* sp. RBL67 47  
2.4.2 COMPARATIVE ANALYSIS OF *groEL* GENE SEQUENCES 48  
2.4.3 DNA-DNA HYBRIDIZATION 50  
2.4.4 PULSED FIELD GEL ELECTROPHORESIS 52  
2.4.5 PHYSIOLOGICAL PROPERTIES OF *Bifidobacterium* sp. RBL67 53  

### 2.5 CONCLUSIONS 57  

### 2.6 DESCRIPTION OF *Bifidobacterium thermophilum* RBL67 59  

## 3 INCREASED PRODUCTION OF A NEW BACTERIOCIN FROM *BIFIDOBACTERIUM THERMOPHILUM* RBL67 USING REPEATED CYCLE BATCH CULTURE 60  

### 3.1 SUMMARY 60  

### 3.2 INTRODUCTION 61  

### 3.3 MATERIAL AND METHODS 63
3.3.1 STRAINS 63
3.3.2 MEDIUM FOR BACTERIOCIN PRODUCTION 63
3.3.3 FREE CELL BATCH CULTURES 64
3.3.4 CELL IMMOBILIZATION 64
3.3.5 REPEATED CYCLE BATCH CULTURES 65
3.3.6 STORAGE OF BEADS 65
3.3.7 CELL ENUMERATION AND OPTICAL DENSITY DETERMINATION 66
3.3.8 BACTERIOCIN ACTIVITY ASSAY 66
3.3.9 DETERMINATION OF GLUCOSE AND ORGANIC ACID CONCENTRATIONS 67
3.3.10 INFLUENCE OF ORGANIC ACIDS ON ANTIMICROBIAL ACTIVITY 67
3.3.11 STATISTICS 67
3.4 RESULTS 69
3.4.1 COMPARISON OF CELL GROWTH IN MRS-C AND M-PERRIN MEDIUM 69
3.4.2 REPEATED CYCLE BATCH FERMENTATIONS IN MRS-C BROTH AND M-PERRIN MEDIUM 70
3.4.3 TEMPERATURE AND pH EFFECTS ON CELL GROWTH AND BACTERIOCIN PRODUCTION IN REPEATED CYCLE BATCH FERMENTATIONS 71
3.4.4 ORGANIC ACIDS PRODUCTION DURING RCB FERMENTATIONS 73
3.4.5 LONG-TERM STORAGE OF BEADS 74
3.5 DISCUSSION 75
3.6 CONCLUSIONS 78

4 PARTIAL PURIFICATION OF THERMOPHILICIN B67, A BACTERIOCIN PRODUCED BY BIFIDOBACTERIUM THERMOPHILUM RBL67 79

4.1 SUMMARY 79
4.2 INTRODUCTION 80
4.3 MATERIAL AND METHODS 83
4.3.1 BACTERIAL CULTURE AND MEDIA 83
4.3.2 MEDIUM FOR BACTERIOCIN PRODUCTION 83
4.3.3 IMMobilIZATION OF B. THERMOPHILUM RBL67 84
4.3.4 IMMOBILIZED CELL FERMENTATIONS 84
4.3.5 PARTIAL BACTERIOCIN PURIFICATION 85
4.3.6 SDS-PAGE 86
4.3.7 SENSITIVITY OF BACTERIOCIN ACTIVITY TO pH 86
4.3.8 BACTERIOCIN ACTIVITY ASSAY 87
4.3.9 DETERMINATION OF ANTIMICROBIAL INHIBITION SPECTRUM 87
4.3.10 PROTEIN DETERMINATION 88
4.3.11 AMINO ACID SEQUENCE DETERMINATION 88
4.3.12 MOLECULAR MASS DETERMINATION 88

4.4 RESULTS 89
4.4.1 STABILITY OF THERMOPHILICIN B67 PRODUCED BY BIFIDOBACTERIUM THERMOPHILUM RBL67 89
4.4.2 SPECTRUM OF INHIBITORY ACTIVITY 90
4.4.3 PARTIAL PURIFICATION OF THERMOPHILICIN B67 91
4.4.4 SDS-PAGE OF THERMOPHILICIN B67 94

4.5 DISCUSSION 96

4.6 CONCLUSIONS 102

5 IDENTIFICATION AND CHARACTERIZATION OF A NEW BACTERIOCIN-PRODUCER PEDIOCOCCUS ACIDILACTICI UVA1 ISOLATED FROM BABY FAECES 103

5.1 SUMMARY 103
5.2 INTRODUCTION 104
5.3 MATERIAL AND METHODS 107
5.3.1 BACTERIAL STRAINS AND ROUTINE GROWTH CONDITIONS 107
5.3.2 CARBOHYDRATE FERMENTATION PROFILE 108
5.3.3 DETERMINATION OF GROWTH TEMPERATURE RANGE 108
5.3.4 GRAM STAINING AND HAEMOLYSIS TEST 109
5.3.5 16S rDNA SEQUENCING 109
5.3.6 PULSED FIELD GEL ELECTROPHORESIS 110
5.3.7 SCREENING FOR EXTRACHROMOSOMAL ELEMENTS 111

5.4 RESULTS 112
5.4.1 CARBOHYDRATE FERMENTATION PROFILE AND GROWTH TEMPERATURE LIMITS 112
5.4.2 GRAM STAINING AND CATALASE AND HAEMOLYSIS TESTS 112
5.4.3 16S rDNA SEQUENCE ANALYSIS 113
5.4.4 PFGE ANALYSIS OF P. ACIDILACTICI UVA1 114
5.4.5 SCREENING FOR EXTRA-CHROMOSOMAL ELEMENTS 115

5.5 DISCUSSION 117
5.6 CONCLUSIONS 120
5.7 DESCRIPTION OF PEDIOCOCCUS ACIDILACTICI UVA1 121

6 CONCLUSION AND OUTLOOK 122

7 REFERENCES 127
APPENDIX A: PURIFICATION STRATEGIES APPLIED FOR THE
PURIFICATION OF THERMOPHILICIN B67

A.1 CHROMATOGRAPHIC METHODS
A.1.1 ION EXCHANGE CHROMATOGRAPHY
A.1.2 HYDROPHOBIC INTERACTION CHROMATOGRAPHY
A.1.3 REVERSED PHASE (RP) HPLC
A.2 ULTRAFILTRATION
A.3 DIALYSIS
A.4 POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)
A.4.1 SDS-PAGE
A.4.2 NATIVE PAGE
A.5 OTHER EXTRACTION METHODS
A.5.1 2-BUTANOL EXTRACTION
A.5.2 METHANOL-CHLOROFORM EXTRACTION

APPENDIX B: MULTIPLE ALIGNMENTS OF PARTIAL GROEL SEQUENCES
(HSP60) FROM BIFIDOBACTERIUM SP.

ACKNOWLEDGEMENTS

CURRICULUM VITAE