**Supplementary Table S1.** Effects of different bactericidal treatments on the number of *Bacillus coagulans* spores.

|  |  |
| --- | --- |
| Treatments | Spore counts of *B. coagulans* (CFU/g powder) |
| Before treatment | After treatment |
| Sodium hypochlorite (500 ppm, 30 sec) | 5.25 × 108a | 5.37 × 108a |
| Ethanol (75%, 10 min) | 5.25 × 108a | 7.41 × 108b |
| Heating (75°C, 30 min) | 5.25 × 108a | 1.58 × 109b |
| Ultraviolet radiation (15 min) | 5.25 × 108 | ND1 |

1 Not detected: colony numbers < 1 ×102 CFU/g were the limit of quantification and were regarded as ND.

a-b Values in a line followed by different superscripts are significantly different (*p* < 0.05).

**Description of Table S1**

 Among different bactericidal treatments, only sodium hypochlorite (NaOCl) did not cause an apparent change in the spore number of *Bacillus coagulans*, showing that NaOCl treatment would be an appropriate approach for enumerating the spore number of *B. coagulans* in our study. Unlike NaOCl, treating the bacterial samples with ethanol and heating would result in significant increases in the spore numbers while ultraviolet radiation would kill the bacteria. Hence, the use of NaOCl would avoid the influence of “heat activation” on the comparison between the spore count and the total viable count in this study.

**Supplementary Table S2.** Bactericidal activity of sodium hypochlorite treatment (500 ppm, 30 sec) on different bacterial samples.

|  |  |  |
| --- | --- | --- |
| Samples | Total viable counts (CFU/g) | Colony numbers determined by the “two-step process” |
| Before treatment | After treatment |
| Fecal samples from rats without *B. coagulans* | 1.95 × 109 | 1.74 × 102 | ND1 |
| Fecal solution2 mixed with *B. coagulans* spores | 2.47 × 109 | 5.60× 108 | 5.57 × 108 |
| *B. coagulans* (vegetative form) | 2.75 × 106 | 0 | 0 |
| *Lactobacillus plantarum*(vegetative form) | 1.75 × 107 | 0 | 0 |
| *B. subtilis* (vegetative form) | 2.30 × 108 | 0 | 0 |

1 Not detected: colony numbers < 1 × 102 CFU/g were the limit of quantification and were regarded as ND.

2 Approximately 5.25 × 108 CFU/g of *B. coagulans* spores was added into the bacterial mixture.

**Description of Table S2**

 Treating the fecal samples with sodium hypochlorite could effectively eliminate most of the unwanted microorganisms in different samples including fecal sample without *B. coagulans*, vegetative *B. coagulans*, vegetative *B. subtillis and Lactobacillus plantarum*.There were no detectable counts of *B. coagulans* in fecal samples from rats after the two-step process. It was confirmed that the number of *B. coagulans* spores was not affected in this experiment*.*

**Supplementary Table S3.** Primers for the PCR amplification of bacterial 16S rDNA in *B. coagulans.*

|  |  |
| --- | --- |
| Primer | Sequence (5’ to 3’) |
| fD1 | AGAGTTTGATCCTGGCTCAG |
| rP1 | ACGGTTACCTTGTTACGACTT |

**Supplementary Figure S1.** Electrophoretic analysis of bacterial 16S rDNA from the target *B. coagulans* colony discerned by the two-step process*.*



M: Bio 100 Mass DNA ladder, BC: 16S rDNA of *B. coagulans*

**Supplementary Figure S2.** Sequence of 16S rDNA and blasting results.

GTCGTGCGGACCTTTTAAAAGCTTGCTTTTAAAAGGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGATCGGGATAACGCCGGGAAACCGGGGCTAATACCGGATAGTTTTTTCCTCCGCATGGAGGAAAAAGGAAAGACGGCTTTTGCTGTCACTTACAGATGGGCCCGCGGCGCATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGAAGAAGGCCTTCGGGTCGTAAAACTCTGTTGCCGGGGAAGAACAAGTGCCGTTCGAACAGGGCGGCGCCTTGACGGTACCCGGCCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCTTCTTAAGTCTGATGTGAAATCTTGCGGCTCAACCGCAAGCGGTCATTGGAAACTGGGAGGCTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACCTCCCTGGAGACAGGGGCCTCCCCTTCGGGG



**Supplementary Table S4.** The numbers (log CFU/g) of *Bifidobacterium* spp. and *Lactobacillus* spp. between the control and BC groups.

|  |  |  |
| --- | --- | --- |
| Groups | *Bifidobacterium* spp. | *Lactobacillus* spp. |
| Control | 6.75a | 7.95a |
| BC | 7.22b | 9.29b |

a-b Values in a column followed by different superscripts are significantly different (*p* < 0.05).

**Description of Table S4**

 At the end of the feeding period, the numbers of *Bifidobacterium* spp. and *Lactobacillus* spp. in the BC group were found to be significantly higher than those in the control group. It was inferred that the germinated *B. coagulans* might promote the growth and acid-production of other intrinsic acid-producing bacteria in digestive tract.