Growth Rates of Bacillus Species Probiotics using Various Enrichment Media

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ABSTRACT

Background: Probiotics are well-known as valuable functional foods to promote specific health benefits to consumers. Some Bacillus bacteria have been recently considered as probiotic and food additives. We aimed to investigate the growing rate of probiotic B. subtilis and B. coagulans using several enrichment media incubated at 37 °C for 24 hours.

Methods: Various enrichment media including nutrient broth (NB), tryptic soy broth (TSB), double strength TSB, Mueller Hinton broth (MH), brain-heart infusion broth (BHIB), de Man, Rogosa and Sharpe (MRS), and nutrient yeast extract salt medium (NYSM) were used to enrich the probiotics and they were subsequently incubated for 18 h at 37 °C. The bacteria were then enumerated on TSA medium.

Results: The results showed that B. subtilis ATCC 6633, B. subtilis PY79, and B. coagulans developed in TSB, double strength TSB, TSB yeast extract, BHIB and NYSM, respectively. Moreover, the formulas were achieved based on the optical density curve and the number of bacteria.

Conclusion: Considering that the probiotics are significantly employed as food supplements, it is essential to identify appropriate enrichment media to proliferate these beneficial bacteria.

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Introduction

Probiotics, defined as friendly bacteria, are tremendously attractive for the public because of their effects on improving their hosts’ intestinal microbiota balance. Some Bacillus probiotics have been used as supplemented food additives as they have been applied in some Italian products (1). Among the Bacillus probiotic species, B. subtilis and B. coagulans have been extensively studied. Despite common probiotics (Lactobacilli and Bifidobacteria spp.), spore forming Bacillus probiotics are known as effective bacteria because of being resistant to harsh environmental conditions such as severe heat, various chemicals, and low gastric fluid pH (2).

Various strains of Bacillus probiotics can be effectively grown using suitable enrichment media. These bacteria are able to propagate aerobically at 35-37 °C for 24-48 hours, in common enrichment media such as Tryptic Soy Broth (TSB), Nutrient Broth (NB), Brain-Heart Infusion Broth (BHI), Mueller Hinton Broth (MHB), and Nutrient Yeast Extract Salt Medium (NYSM) (3-9). We aimed to investigate the growth rate of probiotic B. subtilis and B. coagulans in different enrichment media.
incubated at 37 °C for 24 hours and finally select the most appropriate enrichment medium for the best growth.

**Materials and Methods**

**Culture Conditions of Bacterial Strains**

*B. subtilis* ATCC 6633, *B. subtilis* PY79, and *B. coagulans* GBI-30, 6086 were purchased from the Iranian Organizations for Science and Technology (Tehran, Iran). The enrichment media such as BHIB, NB, MHB (Merck, Germany), double strength TSB, TSB complemented with 1% (w/v) yeast extract (TSBYE), TSB, and NYSM, de Man, Rogosa and Sharpe (MRS) (Merck, Germany) were used to enrich the probiotics. They were subsequently incubated for 18 h at 37 °C.

**Counting of Probiotics**

To enumerate the bacteria, serial dilution (10-fold) of each sample was used before being cultured onto the Trypticase soy agar (TSA) medium and the optical density (OD) of each dilution was simultaneously recorded at 600 nm. The cultured media were aerobically incubated at 37 °C for 18 hours and the equations from the curves based on OD and bacterial number were eventually obtained for each sample. Experiments were done in triplicates.

**Statistical Analysis**

SPSS software, version 16, was used for statistical analysis. One-way ANOVA was used for comparisons. The difference of means between groups were also analyzed using Duncan post-test. P<0.05 was considered statistically significant.

**Results**

**Counting of Probiotics in Various Media**

Results of the *Bacillus* probiotics growing in different enrichment media are shown in table 1. According to our results, the highest (1.3×10⁸ CFU/ml) and lowest (8×10⁴ CFU/ml) *B. subtilis* 6633 growth were respectively observed in the double strength TSB and MRS broth. Moreover, the highest growth of *B. subtilis* PY79 was showed in TSBYE (2.2×10⁸ CFU/ml), BHIB (2.1×10⁸ CFU/ml), and TSB (2.1×10⁸ CFU/ml) and the lowest bacterial growth was seen in NB (10⁶ CFU/ml) and MRS (3×10⁶ CFU/ml). The highest and the lowest growth of *B. coagulans* were also observed in NYSM (2.5×10⁸ CFU/ml) and NB (2×10⁴ CFU/ml) (P<0.05), respectively. Furthermore, equations resulted from the curves based on OD and bacterial number are presented in table 2.

**Discussion**

*Bacillus* probiotics have been extensively used in humans as supplemented food additives. They are used in animals and aquaculture as growth promoters, disease-resistance, and competitive exclusion agents. *B. subtilis* and *B. coagulans* are considered as noticeable species among others, which survive through extreme environmental and hosts gastrointestinal conditions (10, 11). The beneficial effects of these probiotics were formerly shown against enteropathogens such as *C. perfringens* (12), *E. coli* (13), *C. jejuni* (14), and *S. enteritidis* (15) which have been studied in both in vivo and in vitro models. According to previous studies, the safety of *B. subtilis* and *B. coagulans* strains has been confirmed and they have also been considered as potential probiotics for public health. Since various strains of *Bacillus* probiotics are able to produce different bacteriocins, proper proliferation of corresponding probiotics in appropriate enrichment media is initially required. It was previously shown that different isolates

<table>
<thead>
<tr>
<th>Strains</th>
<th>Enrichment media</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>NB</td>
</tr>
<tr>
<td><em>B. subtilis</em> ATCC 6633</td>
<td>0.1±0.04a</td>
</tr>
<tr>
<td><em>B. subtilis</em> PY79</td>
<td>1±0.30a</td>
</tr>
<tr>
<td><em>B. coagulans</em> GBI-30</td>
<td>0.02±0.01a</td>
</tr>
</tbody>
</table>

Different letters showed the statistical differences in each rows (P<0.05). Results are reported as mean±SD of three replicate. NB: Nutrient broth; TSB: Tryptic soy broth; MH: Mueller hinton; TSBYE: tryptic soy broth yeast extract medium; BHIB: Brain-heart infusion broth; MRS: Man, rogosha and sharpe; NYSM: Nutrient yeast extract salt medium
of *B. subtilis* from chicken fecal samples revealed antibacterial function against the enteric pathogen. As such, for gaining better and optimal efficiency from such probiotic strains in food industry, the role of enrichment media is substantial. The number of bacteria to reveal such antimicrobial activities was 10^8 CFU/ml. In the previous studies, TSB, BHIB and Trypton yeast media were used to grow *B. subtilis ATCC 6633* (6, 7); while, NB and BHIB were used to grow *B. subtilis PY79* and *B. coagulans* (10). However, in this study BHIB, TSB, double TSB, and NYSM successfully grew in the amount of 10^8 CFU/ml after a proper incubation time for the experimented *Bacillus* species. Thus, we have tried to introduce the best enrichment media to propagate *Bacillus* probiotics to investigate their effective roles against food pathogens for in vivo and in vitro conditions.

**Conclusion**

Identification of appropriate enrichment media to propagate probiotic bacteria which have been tremendously employed as food supplements is necessary. Also, using laboratory enrichment media for growth of probiotics is the first step to combat pathogens in both in vivo and in vitro models.

**Acknowledgement**

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**Conflict of Interest**

None declared.

**References**

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