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Growth Rates of Bacillus Species Probiotics using Various Enrichment Media

Maryam Poormontaseri[1], Raheleh Ostovan[1], Enayat Berizi[2], Saeid Hosseinzadeh[1]*

1. Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
2. Nutrition Research Center, Department of Food Hygiene and Quality Control, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran

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*Corresponding author:
Saeid Hosseinzadeh,
Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
Tel: +98-71-36138743
Email: hosseinzadeh@shirazu.ac.ir
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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 TBS, 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 ºC 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, s 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 (1.3×10^9 CFU/ml) and lowest (8×10^4 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 (2.2×10^8 CFU/ml), BHIB (2.1×10^8 CFU/ml), and TSB (2.1×10^8 CFU/ml) and the lowest bacterial growth was seen in NB (10^6 CFU/ml) and MRS (3×10^6 CFU/ml). The highest and the lowest growth of *B. coagulans* were also observed in NYSM (2.5×10^8 CFU/ml) and NB (2×10^4 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 1: Enumeration ($\times10^6$ CFU/ml) of the three stains of *Bacillus* probiotic in different enrichment media

	Enrichment media							
Strains	**NB**	**TSB**	**MH**	**TSBYE**	**Double TSB**	**BHIB**	**MRS**	**NYSM**
B. subtilis ATCC 6633	0.1 ± 0.04^{A}	130 ± 12.64^{B}	0.035 ± 0.01^{C}	10 ± 1.58^{D}	1300 ± 21.24^{E}	450 ± 14.57^{F}	0.08 ± 0.02^{G}	60 ± 6.79^{H}
B. subtilis PY79	1 ± 0.30^{A}	200 ± 14.27^{B}	30 ± 5.61^{C}	220 ± 11.50^{B}	40 ± 6.54^{C}	210 ± 19.29^{B}	3 ± 1.08^{A}	50 ± 8.28^{C}
B. coagulans GBI-30	0.02 ± 0.01^{A}	130 ± 16^{B}	2 ± 0.9^{C}	150 ± 34^{B}	200 ± 29^{D}	70 ± 11^{E}	1 ± 0.05^{C}	255 ± 26^{F}

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, rogosa and sharpe; NYSM: Nutrient yeast extract salt medium

Table 2: Formulas achieved from the curve based on optical density and number of bacteria

Various media	*Bacillus* strains		
	***B. subtilis* 6633**	***B. subtilis* PY79**	***B. coagulans***
NB	y=226.45x-8.0074 R^2=0.9786	y=7066.5x-122.26 R^2=0.9995	y=1928.6x-2.1429 R^2=0.9643
TSB	y=9970.7x+3.749 R^2=0.9996	y=9327.5x-141.92 R^2=0.9954	y=2308.9x-21.644 R^2=0.9978
MH	y=1365.4x-15.404 R^2=0.9994	y=4303.2x+2.4909 R^2=0.9991	y=5021.4x-61.5 R^2=0.9501
TSBYE	y=889.18x-4.0872 R^2=0.9891	y=4301.2x-2.4809 R^2=0.9991	y=5031.4x-62.5 R^2=0.950
TSB double	y=5981x-30.809 R^2=0.955	y=4365.9x-184.66 R^2=0.9851	y=2851.3x-42.714 R^2=0.977
BHIB	y=6209.3x-47.674 R^2=0.9993	y=8737.7x-175.73 R^2=0.9998	y=909.09x-46.455 R^2=0.9709
MRS	y=614.29x-23.771 R^2=0.9856	y=867.17x-8.8114 R^2=0.9764	y=0.0011x+0.0079 R^2=0.99

R^2=Coefficient of determination; NB: Nutrient broth; TSB: Tryptic soy broth; MH: Mueller hinton; TSBYE: tryptic soy broth yeast extract medium; TSB: Tryptic soy broth; BHIB: Brain-heart infusion broth; MRS: Man, rogosa and sharpe

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.

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Conflict of Interest

None declared.

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