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Comparative Growth of *Listeria monocytogenes* in Commercially Available Tryptic Soy Broth

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Abstract

Purpose: The culture medium must provide the essential growth requirements for bacterial growth to occur. The manufacturing processes and preparation methods of the culture media can affect the culture medium composition and final growth of microorganisms. This study compared the growth of *Listeria monocytogenes* in two different brands of tryptic soy broth (TSB) and investigated which factors are responsible for the different growth response of this organism. **Methods:** Two commercially available TSB products, i.e., BBL and Difco, were compared for their ability to support the growth of *L. monocytogenes*. Commercial TSB and lab-formulated TSB, which was made from individual components, were also compared. Yeast extract (YE), casamino acid, ferric citrate, biotin, choline, folic acid, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, and paraaminobenzoic acid were added individually as growth supplements. The optical density was measured using a spectrophotometer at 600 nm. **Results:** The Difco TSB supported better growth than BBL TSB in all strains of *L. monocytogenes* tested. The addition of YE (0.3% or 0.6%) improved the growth in BBL TSB markedly. The difference in growth responses between BBL and Difco TSB was neutralized by YE supplementation. The Lab-formulated Difco TSB gave a lower growth response than commercial Difco TSB. The Lab-formulated BBL TSB also failed to support the growth of *L. monocytogenes* and gave a similar growth response to commercial BBL TSB. None of the growth supplements, except for riboflavin, improved growth in BBL TSB. **Conclusion:** These results suggest that Difco TSB may contain the essential components in YE or YE itself.

Key words: *Listeria monocytogenes*, growth medium, nutrient requirement

상용되고 있는 Tryptic Soy Broth에서 *Listeria monocytogenes*의 증식 비교

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초 록

목적: 미생물의 증식을 위해서는 배지에 필요한 영양이 충분히 공급되어야 한다. 배지의 제조 과정이나 조성이 미생물의 증식에 영향을 줄 수 있다. 본 연구의 목적은 미생물의 배지로 일반적으로 쓰이고 있는 tryptic soy broth(TSB)를 이용하여 어떤 영양적 요소가 *Listeria monocytogenes*의 증식에 영향을 미치는 지 알아보고자 하였다. **연구방법:** BBL과 Difco의 두 가지 다른 브랜드의 TSB에서 *L. monocytogenes*의 증식 양상을 비교하였다. 또한 실험실에서 TSB의 조성과 똑같이 제조한 배지와 BBL, Difco의 두 배지에서의 *L. monocytogenes*의 증식 양상도 비교하였다. 미생물의 증식은 분광흡도계를 이용하여 혼탁도로 판단하였다. **결과:** 조사에 쓰인 모든 *L. monocytogenes* 아종들이 BBL TSB보다 Difco TSB에서 증식이 빨랐고, yeast extract를 BBL 배지에 첨가하였을 때는 Difco 배지에서의 증식 양상과 거의 같았다. Yeast extract 조성 속의 비타민들을 개별적으로 배지에 첨가하여 증식 양상을 살펴본 결과, riboflavin을 첨가하였을 때 BBL 배지에서의 증식 속도가 증가되었다. **결론:** 이상의 결과로 볼 때, Difco TSB가 yeast extract를 조성 중에 함유하고 있거나, yeast extract 구성 요소 중 *L. monocytogenes* 증식에 필수적인 영양소를 함유하고 있는 것으로 사료된다.

주제어: *Listeria monocytogenes*, 증식 배지, 영양요구성

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Ⅰ. Introduction

Listeria monocytogenes can cause serious public health problems, and has been an important area of research. Methods for the isolation, detection, and enrichment of this organism have been developed in numerous studies in the past several years. The monitoring of growth in bacterial cultures is a basic methodology (Monod J 1949). Pure culture isolation and determination of the growth requirements serves as the basis for the rapid and accurate identification of an organism. The factors and conditions that affect growth include not only the media component, but also environmental influences such as pH, atmosphere, and temperature (Sullivan NM 1993). The growth behavior of *L. monocytogenes* with the effects of pH, atmosphere, temperature, and sodium chloride has been studied (Ahamad N & Marth EH 1989, Buchanan RL et al. 1989, Buchanan RL & Klawitter LA 1990). On the other hand, few studies have examined the effects of the media components on the growth of *L. monocytogenes*. Numerous media are used due to the diversity of microorganisms and their various metabolisms. Even slight differences in the composition of a medium can result in dramatically different growth characteristics of microorganisms (Atlas RM 1993). Although microbiological media with identical compositions from different manufacturers are supposed to be the same, there may be subtle differences between these products. For example, the metal content in commercial media from various manufacturers was found to differ (Bovallius Å & Zacharias B 1971). Despite their significant research implications, systematic examinations of the differences among different commercial brands of media have attracted little attention. This study was, to determine compared the growth of *L. monocytogenes* differs on two brands of tryptic soy broth, i.e., one produced by BBL and the other by Difco, and in the case of any differences, to examine further which growth factors are responsible for the different growth responses of this organism. Furthermore, the nutritional requirements of *L. monocytogenes* in a chemically defined minimal medium were determined to understand the ability of this organism to grow and survive in diverse environments.

Ⅱ. Materials and Methods

1. Bacterial strains

Seven strains of *L. monocytogenes* used in this study were obtained from the FDA Baltimore District Laboratory culture collection. The original sources were as follows. *L. monocytogenes* strains 45A40 and 45A49 were isolated from clinical sources and milk, respectively, and were obtained from the FDA Dallas District Laboratory. Strains 45A90 (serotype 1) and 45A97 (serotype 4b) were isolated from cheese and raw shrimp, respectively, and were obtained from the FDA Seattle District Laboratory. Strain 45A74 (serotype 4) was isolated from cheese by the FDA Baltimore District Laboratory. Strain 45A09 (serotype 1), a cheese isolate, was obtained from the FDA Center for Food Safety and Applied Nutrition, Cincinnati. Strain 45A83 was isolated from cheese and obtained from the Winchester Engineering and Analytical Center, FDA.

2. Culture media

Stock cultures were grown for 24 hr at 35°C on tryptic soy agar (TSA; Difco laboratories, Detroit, MI, USA) slants and maintained at 4°C with monthly transfers. Portions of these cultures were also kept at -80°C in tryptic soy broth with yeast extract (TSBYE, Difco) plus 25% glycerin. Trypticase™ soy broth (BBL, Cockeysville, MD, USA) and Bacto™ tryptic soy broth (Difco) were used and compared throughout the study. Table 1 lists the composition of tryptic soy broth (TSB) from the two brands. Tryptic soy broth was also made from the component ingredients using the two commercial suppliers listed above.

3. Growth supplements

Yeast extract (BBL), at a final concentration of 0.3%, or 0.6%, was added to TSB to determine if the yeast extract stimulated the growth of *L. monocytogenes*. Each component in yeast extract was also added to TSB individually. Several vitamins and cofactors including: biotin, choline chloride, folic acid, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine chloride, paraaminobenzoic acid (PABA), and ferric citrate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The vitamin assay casamino acids were purchasd from Difco. All growth supplements were freshly prepared as a 100 fold stock solutions and filter sterilized using a pre-sterilized filter apparatus containing a membrane (Nalgene Disposable Filter Unit, Nalgene Co., Rochester, NY, USA). Each growth supplement, at an equivalent concentration to the concentration in 0.3% or 0.6% yeast extract, was added to both 10 mL tubes and 100 mL bottles of sterilized medium prior to inoculation.

Table 1. Composition of tryptic soy broth

BBL[1]		Difco[2]	
Component	Conc (g)[3]	Component	Conc (g)
Pancreatic digest of casein	17.0	Bacto tryptone (Pancreatic digest of casein)	17.0
Papaic digest of soybean meal	3.0	Bacto soytone (Papaic digest of soybean meal)	3.0
Sodium chloride	5.0	Sodium chloride	5.0
Dipotassium phosphate	2.5	Dipotassium phosphate	2.5
Dextrose	2.5	Bacto dextrose	2.5

[1] Baltimore Biologicals Ltd., Cockeysville, MD, USA.
[2] Difco Laboratories, Detroit, MI, USA.
[3] Concentration = gram per liter purified water.

4. Growth measurement

To prepare for each experiment, a loopful of inoculum from a stock culture was transferred to a tube containing 10 ml of TSB (BBL, Difco) and incubated for 18-24 hr at 30°C. One milliliter of this "overnight" culture was inoculated into a bottle containing 100 mL of TSB and incubated at 30°C. The growth response was measured by using a spectrophotometer (NOVASPEC II, Pharmacia Biotech, Piscataway, NJ, USA). Every hour, approximately 6.5 mL of the test culture was transferred to a spectrophotometer tube and the optical density determined at 600 nm. For each time period, uninoculated broth with the same composition was used as a reference to blank the spectrophotometer.

5. Statistical analysis

The optical density values (OD_{600}) were analyzed using SPSS Statistics(ver. 6.0, SPSS Inc., Chicago, IL, USA). The relative contributions of different media to the growth of *L. monocytogenes* were examined via single-classification analysis of variance. A t-test was adopted as a statistical tool to test the mean difference between the media.

Ⅲ. Results and Discussion

L. monocytogenes is an important foodborne pathogen. The focus of this study was to compare the growth response of *L. monocytogenes* in the two different commercial brands of tryptic soy broth, i.e., BBL and Difco. The methods for the enrichment, enumeration, and identification of this organism continue to be developed or refined. Initially, the growth aspects of seven strains of *L. monocytogenes* were compared in BBL and Difco TSB. The Difco TSB supported better growth than BBL TSB in all seven of the *L. monocytogenes* strains tested (Fig. 1). This indicates either a possible deficiency of some essential nutrients for growth in BBL broth, which is probably due to the different manufacturing processes or that the Difco broth might contains some additional components that stimulate growth. *L. monocytogenes* is a nonfastidious organism that can grow in common casein-hydrolysate media containing glucose such as tryptic soy broth (Ryser ET & Marth EH 2007). Various growth factors are incorporated in media to enrich or increase the numbers of particular organisms. Acid hydrolysates of casein is commonly used as a source of amino acids. Extracts of yeast cells are also employed as sources of amino acids and vitamins for the cultivation of microorganisms.

One strain of *L. monocytogenes*, 45A49, was selected for further study. The rationale for selecting this particular strain was that 45A49 represents a strain showing a large

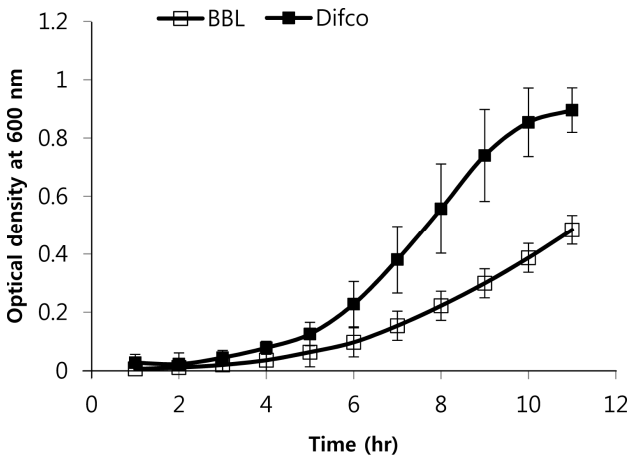

Fig. 1. Average[1] growth response of seven different *Listeria monocytogenes* strains (45A09, 45A40, 45A49, 45A74, 45A83, 45A90, 45A97) in two different brands of tryptic soy broth.
[1] Each point represents the average value of seven determinations (one measurement for each strain).

difference in growth in BBL and Difco TSB. When TSB (BBL and Difco) was supplemented with yeast extract, the observed difference in growth responses between the BBL and Difco product was minimalized. When the 0.3% and 0.6% yeast extracts were added to BBL TSB, the growth response of the *L. monocytogenes* strain 45A49 was similar to that observed in Difco TSB without the yeast extract (Fig. 2). Adding 0.3% and 0.6% yeast extract to BBL TSB minimized the difference between the BBL and Difco broth. Therefore, BBL TSB can be improved or made comparable to Difco TSB by the addition of yeast extract. In contrast, adding yeast extract to Difco TSB had little effect on the growth of *L. monocytogenes* strain 45A49. A similar growth response was observed at all concentrations (0.3%, and 0.6%) of yeast extract and without yeast extract supplementation (Data not shown). This suggests that Difco TSB contains some nutrients in the yeast extract or it contains the yeast extract itself. George SM & Lund BM (1992) also reported that tryptic soy broth was nutritionally limiting for the growth of *L. monocytogenes*. They compared two culture media, Difco TSB and Oxoid TSB plus yeast extract (0.3%), as growth media for *L. monocytogenes*. They reported that the TSB-containing yeast extract resulted in more rapid growth of *L. monocytogenes* than in TSB alone at 2°C and pH 4.5, particularly under aerobic conditions (George SM & Lund BM 1992).

The BBL TSB may be deficient in some nutrient which can be found in yeast extract. Difco TSB might also contain essential nutrients normally found in yeast extract but not in BBL TSB. Although they are from different manufacturers, these two media products are supposed to be the same broth. Table 1 lists the composition of each product, as it appears on the actual label. As indicated in Table 1, TSB is actually a blend of separate component ingredients. Therefore, it is also possible that the separate ingredients used by each brand are different. Both TSB were formulated from separate ingredients obtained from each of the two brands. Fig. 3 shows the growth curve of *L. monocytogenes* strain 45A49 in commercial TSB (BBL, Difco) and lab-formulated TSB (BBL ingredients or Difco ingredients). The best growth was obtained from commercial Difco TSB. Growth in lab-formulated Difco TSB was less than that in commercial Difco TSB but more than that in commercial and lab-formulated BBL TSB. The growth response in lab-formulated BBL TSB was slightly more than that in commercial BBL TSB for the 45A49 strain. This suggests that commercial Difco media contain some additional components. Lab-formulated Difco TSB resulted in a higher growth response than in both commercial and lab-formulated BBL TSB. Moreover separate ingredients such as peptones from different manufacturers are different. Although there may be differences between these products, the precise chemical composition of the components is undetermined due to the heterogeneous nature of its components (Atlas RM 1993).

To clarify the observed stimulatory effects of the yeast extract, the actual separate components found in yeast

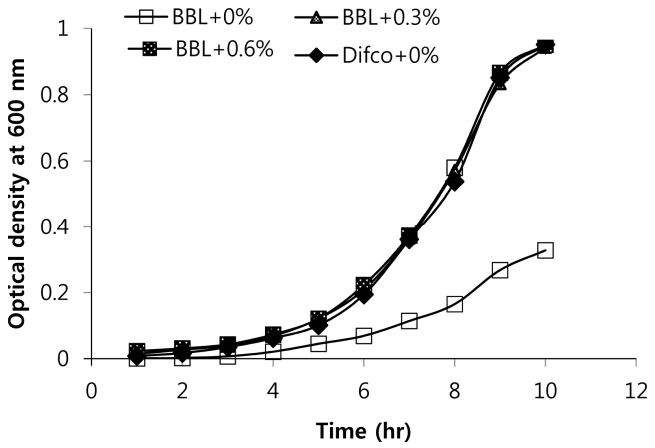

Fig. 2. Growth response of *Listeria monocytogenes* strain 45A49 in BBL tryptic soy broth (TSB) with and without yeast extract and Difco TSB.

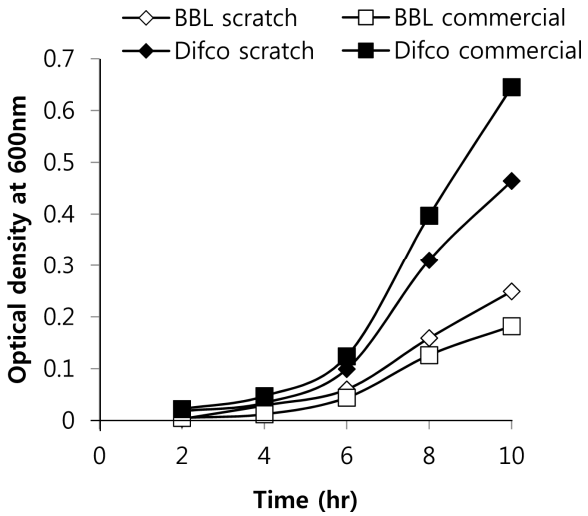

Fig. 3. Growth response of *Listeria monocytogenes* strain 45A49 in two different commercial dehydrated tryptic soy broth (TSB) and two lab-formulated[1)] TSB.

[1)] Made from scratch with separate ingredients contained in commercial dehydrated media; Each composition was presented in Table 1.

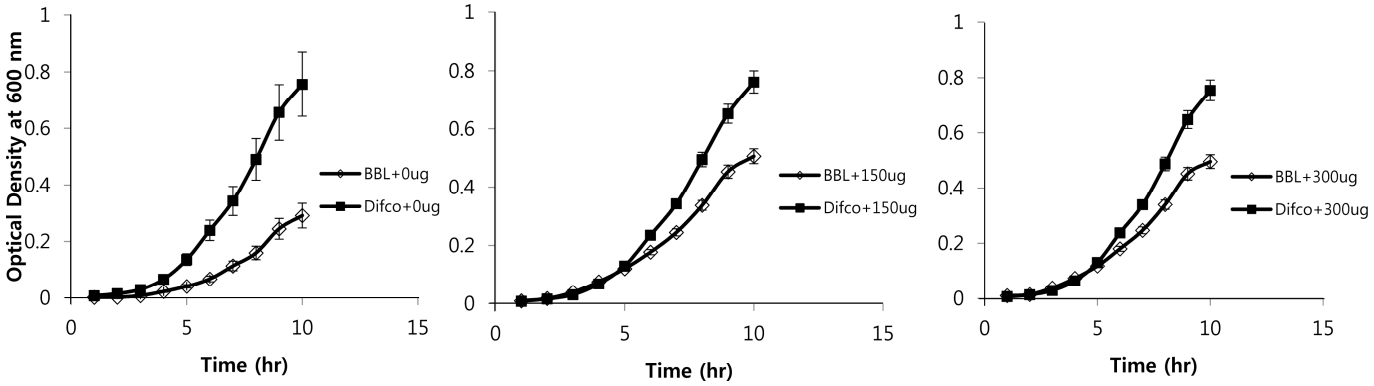

Fig. 4. Growth response of *Listeria monocytogenes* strain 45A49 in two different brands of tryptic soy broth supplemented with riboflavin.

extract were added individually to BBL TSB to determine which factor was responsible for the growth enhancement. None of the individual components in the yeast extract tested except for riboflavin enhanced the growth of *L. monocytogenes* strain 45A49 in BBL TSB (Data not shown). Riboflavin stimulated the growth of *L. monocytogenes* strain 45A49 in BBL TSB, but the level of growth in BBL broth was still lower than that in Difco broth (Fig. 4). The importance of riboflavin was demonstrated by some studies, in which riboflavin alone could stimulate the growth of *L. monocytogenes* (Siddiqi R & Khan MA 1982, Chau MY & Shelef LA 1989). These results confirm the above findings but the addition of riboflavin did not stimulate growth as much as adding yeast extract. Hutner SH (1944) indicated that unidentified factors in commercial "vitamin-free" casein stimulated the growth of *L. monocytogenes*. Cote RJ & Gherna RL (1994) reported that a failure to grow on the defined medium might reflect the requirement for known growth factors supplied by the crude supplements but not by mixtures of the synthetic compounds. An unidentified factor may be present in the yeast extract that may stimulate growth with riboflavin. Patocka F et al. (1960) indicated that thiamine and biotin stimulated growth in the presence of riboflavin. Some combination of nutrients in the yeast extract might have stimulated growth in the presence of riboflavin. Moreover some combination of nutrients in the yeast extract stimulate growth synergistically. In this study, however, the combination of biotin and riboflavin was no more effective than riboflavin alone (Data not shown).

Ⅳ. Summary and Conclusion

These results indicate some factors that may have affected the different growth patterns of *L. monocytogenes*. First, Difco media may contain essential nutrients existing in yeast extract, or yeast extract itself. Second, different manufacturing processes used for commercial media and differences in individual media ingredients used by each manufacturer may have resulted in different growth characteristics of microorganisms. Third, unidentified factors, such as peptones, might exist in the media ingredients and promote growth.

Further research efforts in this area will be necessary. In previous studies, few research attempts have been made to determine the precise growth requirements for *L. monocytogenes*. Further, the agreement in the literature regarding the necessity of each growth factor for *L. monocytogenes* appears weak. Further research along the line of this study will be necessary to achieve a better understanding of the nutritional requirements and metabolism of *L. monocytogenes*.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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