# Medium for Producing Cells of Lactic Acid Bacteria[1]

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The selective medium for lactobacilli devised by Rogosa, Mitchell, and Wiseman (J. Bacteriol. **62**:132, 1951; J. Dental Res. **30**:682, 1951), purchased by us in dehydrated form under the name "LBS medium" from BBL, proved to be quite inhibitory for the growth of *Leuconostoc mesenteroides*, and some strains of *Pediococcus cerevisiae* and *Lactobacillus plantarum*. However, when the amount of glacial acetic acid added was reduced from 1.32 ml per liter to an amount sufficient to adjust the pH to 5.6 ± 0.05, this inhibition was reversed, and the medium retained a high degree of selectivity. The modified medium, plus 0.0075% bromcresol green dye (to aid colony counting), were successfully used for separating relatively low populations of lactic acid bacteria occurring on pickling cucumbers from exceedingly high populations of other microbial groups.

Because the colonies of different species of lactics grown on the modified LBS agar were very large as compared with those on Trypticase Sugar Agar (BBL) with 0.5% yeast extract (TSA), it occurred to us that a LBS broth might prove useful in the production of cells of these fastidious organisms. Experiments were conducted in which cell yields from LBS broth (pH 5.8) prepared from the individual ingredients and brought to a boiling temperature were compared with yields from a broth having the following composition: Trypticase, 1%; yeast extract, 1%; dextrose, 1%; and $K_2HPO_4$, 0.5%; at pH 6.8 to 7.0 (TYE).

Tubes containing 10 ml of each medium were inoculated from stock cultures grown in TSA stabs, and were incubated 18 to 24 hr at the optimal temperature for the species used (32 or 45 C); the entire culture was used to inoculate 190 ml of the respective media. Cell yields were determined after 24- and 48-hr incubation. Plate counts were determined by use of LBS medium, and total cell counts by use of a Petroff-Hausser counting chamber. For dry-weight yields, cells were centrifuged from 200 ml of culture; the pellet was resuspended in a small volume of water, and was transferred to a tared bottle, dried at 110 C for 48 hr, and weighed.

A summary of typical results is given in Table 1. Cell yields after 24- and 48-hr incubation were comparable; therefore, only the 48-hr data are presented. The LBS broth produced higher cell yields with all species tested than did the TYE broth. However, its most pronounced effect was observed with *P. cerevisiae*, *Lactobacillus plantarum*, and certain strains of *L. delbrueckii*; the cell yields of these species in LBS broth were four to six times higher than in TYE. However, four of seven strains of *L. delbrueckii* tested grew poorly in both media.

TABLE 1. *Cell yields of various species of lactic acid bacteria grown in LBS and TYE broths*[a]

| Organism | Strain[b] | No. of g (dry wt) of cells per liter | | No. of cells per ml × $10^{-6}$ | |
|---|---|---|---|---|---|
| | | LBS | TYE | LBS | TYE |
| *Pediococcus cerevisiae* | FBB-39[c] | 2.5 | 0.4 | 1,523 | 112 |
| *Lactobacillus plantarum* | FBB-12[c] | 2.8 | 0.4 | 1,850 | 273 |
| *L. brevis* | FBB-70[c] | 1.4 | 0.3 | 194 | 58 |
| *L. fermenti* | NRRL B-585[c] | 1.3 | 0.3 | 300 | 110 |
| *L. delbrueckii* | NRRL B-443[d] | 0.8 | 0.3 | 130 | —[e] |
| | NRRL B-445 | 4.3 | 1.0 | 3,580 | 670 |
| *L. thermophilus* | NRRL B-1952 | 1.5 | 0.5 | 60 | 23 |
| *L. lactis* | NRRL B-736 | 1.5 | 0.8 | 960 | 300 |
| *L. helveticus* | NRRL B-1842 | 1.3 | 0.5 | 100 | —[e] |
| *L. bulgaricus* | NRRL B-734 | 1.0 | 0.5 | 200 | 40 |
| *Leuconostoc mesenteroides* | FBB B-41[c] | 1.0 | 0.3 | 705 | 340 |

[a] LBS broth (BBL); TYE broth: Trypticase, 1%; yeast extract, 1%; dextrose, 1%; and $K_2HPO_4$, 0.5%; at pH 6.8 to 7.0.

[b] FBB cultures were originally isolated from cucumber fermentations by one of the authors. The NRRL cultures were obtained from the Northern Utilization Research Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Ill.

[c] Two strains of each species were tested; the results were comparable.

[d] Seven strains were tested; the results with four strains are represented by NRRL B-443, and three by NRRL B-445.

[e] Cell populations too low to be counted.

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The plate count data are not presented, since they corresponded closely with the total counts. There was no rapid loss in cell viability in any of these cultures within 48 hr resulting from LBS.

LBS broth is easily prepared, and does not have to be sterilized, only brought to a boiling temperature. Plating studies demonstrated that some microbial groups other than lactic acid bacteria (e.g., coliform bacteria) will grow in LBS agar when the pH is raised above that specified herein. However, when lactic acid bacteria are inoculated into the broth medium, the initial pH (5.8) is rapidly lowered by acid development to a level that precludes development of endospores or incidental contaminants. We experienced no contamination of any of our cultures produced in the medium. However, there are, undoubtedly, bacterial endospores present in the boiled medium, so that the method would not be suitable for producing cell preparations wherein these residual spores would constitute a problem.

Resting cells of *L. plantarum* harvested from LBS broth cultures fermented glucose to lactic acid rapidly.

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