Response To The Letter To The Editor: Safety And Reliability Of Lactobacillus Supplements

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Citation


Abstract

January 30th, 2004

The authors responses and explanations for the points made in the letter to the editor.

The authors will address each point in the order it appears in the letter.

Concern: Appropriate conditions for the growth of Bifidobacteria:

Answer: Both the BHI (Brain heart infusion) broth, which was selected for its growth of fastidious organisms, and the MRS media were kept under anaerobic conditions both prior to and immediately after plating. Media was chosen after examining the literature for isolation of Lactobacillus and other species and consultation with microbiologists who specialize in anaerobic isolations (1,2,3,4). The authors acknowledge that different microbiologists often favor different media for isolation of identical bacteria (4,5,6). As Bifidobacteria and other anaerobes often take 48 hours to appear, our initial incubations were 72 hours from initial plating to examination and subcultures of isolated organisms were 48 hours from plating until examination. In addition, to confirm that appropriate conditions for growth of Bifidobacteria, Lactobacillus and other anaerobic species were present, the media was tested in the same laboratory under identical conditions with a variety of organisms (with different growth requirements) before each set of inoculations. Growth of various Bifidobacteria species on MRS media was successful with each effort.

Concern: Isolation of Bifidobacteria.

Answer: In the letter to the editor, Drs. Leyer and Russell indicated that Bifidobacteria were not isolated in 7 of 9 products in our pilot study. That is correct. In our follow-up study examining 74 new products and using the same techniques and media (manuscript in preparation), Bifidobacteria was isolated 14 times with 30 products listed as having Bifidobacteria on the label. These isolated species included B. lactis, B. animalis, B. bifidum, B. infantis and B. longum and B. species.

A point to consider in the isolation percentages is the supplier of the micro-organisms to the companies. We saw interesting trends with our larger study in which multiple products from the same company had identical contaminants and also the absence of certain bacteria, including Bifidobacteria. In addition, we noticed that products from one company also had recovery patterns similar to the products of other companies. After speaking with microbiologists from several of our product companies, it appears that many companies may be supplied by a fairly small number of suppliers.

Concern: Protocol description:

Answer: The authors acknowledge that the protocol description may not be as detailed as a microbiologist might like and although it is optimum to obtain all details of a protocol, prior to repetition, from the Materials and Methods, this is not always possible. It is the decision of the authors and the editor of the journal how detailed to make the protocol description in part due to the journal and anticipated readership. Being research scientists, we have often found that consultation with the authors of an original paper to be necessary before repetition of that protocol is attempted as the protocol description may be limited by choice or necessity in a journal.

Concern: Dilution of samples and Selection criteria:

Answer: As stated in the paper, one capsule was added to 7
mL of BHI broth at 37 degrees until dissolution. The broth was mixed vigorously and 0.1 mL was then used to plate. Plating was done by two protocols. One was complete plate streak (initially) and also streaking for isolation to best examine colony morphology. Colonies were examined visually and using magnifying lenses. All colony types that appeared unique were gram stained and subcultured for isolation and purity. Since the product labels were blinded to us and we had no idea how many organisms might be in each sample, all colony types were isolated, examined, and sent in pure culture for nucleic acid analysis. In addition, since we used different media and at different growing conditions, we examined colonies on all media and sent in samples from all media. Also, as stated in the paper, two microbiologists worked on each product. Two samples were examined by one microbiologist and two samples were examined by another microbiologist. (For example sample 9 A and 9 B were done by one microbiologist and 9 C and 9 D by another). The microbiologists then compared colony morphologies, isolations, etc. with each other.

We, the authors, agree that there is always a possibility that the numbers of a specific organism were too small to show up with the initial dilution and plating. There is also the possibility that the Bifidobacteria in a product is very fastidious and will not grow on our media despite the fact that control organisms and other Bifidobacteria were isolated. However, that raises a very important question from two clinical microbiologists. What are the chances of organisms in small number or those that have very fastidious growth requirements being able to survive HCl, peristalsis, an onslaught of pancreatic enzymes, bile and still be present to temporarily colonize either the gastro-intestinal tract or the genitourinary tract.

If you have additional questions, please contact the authors.

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