

Content Analysis of Commercially Available Probiotics

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ABSTRACT

We carried out content analysis of 4 batches each of 3 commercially available probiotic formulations proposed to be containing *Bacillus clausii*. Species identification was done using MALDI-TOF-MS technique while bacterial count was done using plate colony count. Only one of the three probiotic formulation analyzed was found to have homogeneous population of *B. clausii* while none was found to have the exact viable bacterial count as suggested on the label.

Keywords: *Bacillus clausii*.

World Health Organization (WHO) defines probiotics as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [1]. The pharmaceutical market is flooded with numerous probiotic products with very few effective checks-and-balances to regulate their quality. We intended to culture probiotic samples said to contain *Bacillus clausii*, and determine the probiotic species and their count and see if they match with what has been mentioned on the label.

The study was proposed and carried out between September – 2016 to January 2017. After hospital’s ethical committee approval, we tested a total of 12 samples (4 each – 4 different batches) of 3 popular probiotics containing *B. clausii* namely – Product 1 (Enterogermina – Expiry range: 05/18 – 08/18), Product 2 (Tufpro – Expiry range: 01/18 – 08/18) and Product 3 (Darolac Aqua – Expiry range: 12/17 – 09/18). The expiry date of products as mentioned by manufacturer was 2 years for all 3 products. MALDI-TOF – MS identification method (Matrix Assisted Laser Desorption Ionization Method Time of Flight– Mass Spectrometry) was used to identify bacterial species. Plate count method was used and colonies were counted as Colony Forming Units (CFU)/sample using Miles and Mishra method [2,3].

Only Product 1 was found to contain a homogenous population of *Bacillus clausii*, whereas Product 2 and Product 3 showed growth of *Bacillus subtilis* species in the samples. None of these samples had uniform viable bacterial counts across all samples as mentioned on the labels (**Table I**).

Our results are in agreement with some of the previously done studies. In a study by Elliot, *et al.* [4], only 3 out of 9 tested probiotic supplements from South Africa were found to be containing the same bacteria as mentioned on the label [4], while studies by Berman, *et al.* [5] and Temmerman, *et al.* [6] found only 1/20 and 6/55 of the different probiotic supplements tested

to be consistent with the product label, respectively. Patrone, *et al.* [7] analyzed 5 probiotics containing only *Bacillus clausii*, and found that bacterial species and counts varied among the samples.

MALDI-TOF-MS technique used by us is a cost-effective method, which determines species as well as strains with fair amount of accuracy [8-10]. The main advantage of colony counting method is that it can be used to count large number of organisms, and also that it counts only viable organisms. The limitation of our study is the small sample size with limited number of products analyzed.

Future research needs to focus on analyzing more probiotic products both from the pharmaceutical as well as the dairy industry and tested at various time points of their viability including tests close to their expiry dates.

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REFERENCES

1. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, *et al.* Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol.* 2014;11:506-14.
2. Miles AA, Mishra SS, Irvin JO. The estimation of bactericidal power of the blood. *J Hyg (Lond).* 1938;38:732-49.
3. Hedges AJ. Estimating the precision of serial dilutions and viable bacterial counts. *Int J Food Microbiol.* 2002;76:207-14.
4. Elliot E, Teversham K. An evaluation of nine probiotics available in South Africa. *S Afr Med J.* 2004;94:121-4.
5. Berman S, Spicer D. Safety and reliability of lactobacillus supplements in seattle, Washington (a pilot study). *International J Alternative Med.* 2003;1:2.
6. Temmerman R, Pot B, Huys G, Swings J. Identification and antibiotic susceptibility of bacterial isolates from probiotic products. *Int J Food Microbiol.* 2003;81:1-10.
7. Patrone V, Molinari P, Morelli L. Microbiological and molecular characterization of commercially available probiotics containing *Bacillus clausii* from India and Pakistan. *Int J Food Microbiol.* 2016;237:92-7.

8. Won SY, Kyeong ML, Kyu JH. Taxonomic identification of bacillus species using matrix-assisted laser desorption ionization time of flight mass spectrometry. *Ann Clin Microbiol.* 2016;19:110-20.
9. Bizzini A, Durussel C, Bille J, Greub J, Prod'hom G. Performance of matrix-assisted laser desorption ionization time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. *J Clin Microbiol.* 2010;48:1549-54.
10. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, *et al.* Ongoing revolution in bacteriology: Routine identification of bacteria by matrix-assisted laser desorption ionization time of flight mass spectrometry. *Clin Infect Dis.* 2009;49:543-51.

TABLE I RESULTS OF CONTENT ANALYSIS OF 3 COMMERCIALY AVAILABLE PROBIOTICS CONTAINING *BACILLUS CLAUSII*

<i>Parameter</i>	<i>Product 1</i>	<i>Product 2</i>	<i>Product 3</i>
Species on Label	<i>Bacillus clausii</i>	<i>Bacillus clausii</i>	<i>Bacillus clausii</i>
Isolated Species	<i>Bacillus clausii</i>	<i>Bacillus subtilis</i>	<i>Bacillus subtilis</i>
Label Count	2×10^9	2×10^9	2×10^9
Isolated species count			
Batch 1			
Batch 2	1×10^9	4×10^6	5×10^9
Batch 3	2.5×10^8	2.5×10^6	2×10^8
Batch 4	1×10^9	1.6×10^6	7×10^8
	2×10^9	3.4×10^6	1.3×10^8