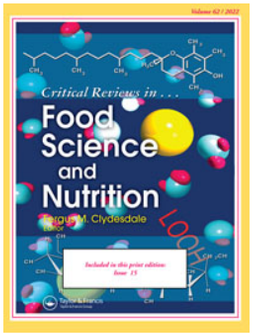

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Correction

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Correction

Article title: Authenticity of probiotic foods and dietary supplements: A pivotal issue to address
Authors: Vincenzina Fusco, Francesca Fanelli, and Daniele Chieffi
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When the article was first published online, Table 1 had been typeset incorrectly.

Now, these errors have been corrected and republished online as below:

Table 1. Excerpt of studies reporting compliance with the labels of probiotic foods and supplements.

Country	Number of products	Type of product	Method	Result	Reference
USA	13	Capsules, powder and tablets	Cultivation and phenotypic identification (API kits)	Two out of 13 matched the quali/quantitative attributes mentioned in their labels. The remaining contained extra species, did not contain *L. acidophilus*, or lacked a listed species, or contained less than a tenth of expected viable cells.	Hamilton-Miller, Shah, and Smith 1996
Italy	15	Pharmaceutical products	Cultivation and phenotypic identification (API kits)	In 10 out of 15 products the expected species was absent or other species than those expected were present. 11/15 had a level of viable cells lower than expected.	Canganella et al. 1997
Britain and other EU countries	52	Fermented functional foods, dietary supplements, health products	Cultivation and phenotypic identification (API kits)	46 out of 52 products did not contain the expected species or contained species other than those expected or contained extra species or the labeled nomenclature was not corrected. Only 6 products contained the expected levels of probiotics.	Hamilton-Miller, Shah, and Winkler 1999
Italy	13	7 probiotic yogurts and 6 commercial probiotic lyophilized products	Cultivation, PCR-DGGE and species-specific PCR	Probiotic yogurts lacked one of the expected species or contained a species different than the expected. Lyophilized products did not contain all the expected species or contained extra species or species different than those expected. Viable cells ranged from <10 to 10^7 CFU/mL in probiotic yogurts and from <10 to 10^8 CFU/g in 4 out of 6 lyophilized products whereas in the remaining ranged from $<10^5$ to 10^{10} CFU/g.	Fasoli et al. 2003
Belgium	55	30 European probiotic supplements in tablets, powder or capsules and 25 dairy products	Cultivation and identification by SDS-PAGE of whole cell proteins	Only two out of 30 probiotic food supplements were compliant with the label whereas the remaining did not contain all the species mentioned in the label or contained extra species or species other than those mentioned in the label or no viable cells could be isolated. Only 2 out of 25 dairy products were compliant with the label whereas the remaining lacked one or more species or contained extra species or were incorrectly labeled (*L. casei* in the label in place of *L. paracasei*). In food supplements viable cells ranged from <1 to 10^6 CFU/g whereas in dairy products ranged from 10^5 to 10^9 CFU/mL.	Temmerman, Pot, et al. 2003

(*Continued*)

Table 1. (*Continued*)

Country	Number of products	Type of product	Method	Result	Reference
Belgium	10	5 freeze-dried products, 4 dairy products, 1 fruit drink	Culture-independent approach by PCR-DGGE and culture dependent approach by cultivation and identification of isolates by SDS-PAGE of whole cell proteins	For six products some of the species claimed in the labels could not be isolated and two products contained a strain of species other than those mentioned in the label. Freeze-dried products contained from 10^5 to 10^7 CFU/g while dairy products contained between 10^7 and 10^9 CFU/mL. Mostly, the PCR-DGGE approach confirmed and was in some cases able to detect more claimed species than the culture-dependent approach.	Temmerman, Scheirlinck, et al. 2003
Poland	64	5 products licensed for medicinal purposes (in capsules or lyophilized)	Cultivation and phenotypic identification (API kits) and, for bifidobacteria, PCR and sequence analysis	Three of the five products contained the expected bacterial strains, while the remaining contained strains of species of the claimed genus but other than that claimed on the label. 57 of the 64 samples contained bacterial counts consistent with those claimed on the labels.	Szajewska et al. 2004
Belgium	58	28 food supplements, 22 yogurts, 5 dairy fruit drinks and 3 pharmaceuticals	Culture-dependent approach: cultivation, genus-specific PCR; BOX-PCR and PFGE; phenotypic characterization (Biolog). Culture-independent approach: PCR-DGGE.	The culture-dependent and independent approaches overlapped as concerns the bifidobacterial composition for all dairy fruit drinks and yogurts but for one yogurt no viable bifidobacteria were found, while by DGGE two *Bifidobacterium* species were revealed. For the remaining products discrepancies between the culture-dependent and –independent approaches were found. High genomic homogeneity among the *Bifidobacterium* strains was found by PFGE. Yogurts contained between 10^3 and 10^8 CFU/mL of viable cells, while dairy fruit drinks contained between 10^4 and 10^6 CFU/mL of viable bacterial cells. 10^2-10^9 and 10^7 CFU/g of viable bacterial cells were present in food supplements and pharmaceutical preparations, respectively.	Masco et al. 2005
USA	14	Tablets and capsules	T-RFLP and species-specific PCR	Five products missed one claimed species. 12 products contained extra species.	Marcobal, Underwood, and Mills 2008
Italy	72	Food supplements (41 samples from 29 processing plants and 31 of the same brand from retailers at timed intervals)	Culture dependent approach: selective plate counting with phenotypic and genotypic identification by API kits and genus- and species-specific PCRs of the isolates, respectively. Culture-independent approach: species-specific and genus-specific PCRs of the DNA directly isolated from the probiotic samples	Viability of all species reported in the labels was confirmed in only 5 of the 41 supplements. 25 of the 41 samples contained viable cells in the same number as in the labels. 20 of 41 supplements contained the claimed species whereas 21/41 species were incorrectly named, 9/41 had obsolete names and 4/41 contained non-declared species. After 3 months 7/24 samples had the same composition as those from the manufacturer while after 8 and 13 months only 2 and 1, respectively, had the same composition.	Aureli et al. 2010
Belgium	15	Probiotic products containing lyophilized *Saccharomyces boulardii*	Cultivation, solid phase cytometry (SPC) and microsatellite typing.	Species were compliant with those mentioned in the labels. Viable cells ranged from 9.15 to 10.57 log CFU/g of product.	Vanhee et al. 2010
USA	15	Three separate samples of five different pharmaceutical probiotics products	Cultivation	Only one of the five probiotic products did not specified the number of viable cells on the label that was found to be about 10^9-10^{10} CFU/g. The remaining 4 products had viable colony counts similar to those claimed on their labels.	Goldstein et al. 2014
Bangladesh	4	Probiotic dietary supplements	Cultivation	All samples contained less viable cells than claimed in their labels.	Begum et al. 2015

(*Continued*)

Table 1. (*Continued*)

Country	Number of products	Type of product	Method	Result	Reference
Italy	8	Two probiotic cheeses and 6 probiotic drinks	Culture dependent approach: cultivation, species-specific PCRs, REP-PCR and RAPD-PCR	50% of the probiotic products resulted mislabeled i.e. in the label it was claimed the presence of *L. casei* but they resulted to contain *L. paracasei* strains. The other species mentioned in the label were found in the probiotic products. The two probiotic cheeses contained less viable cells than claimed in the label.	Di Lena et al. 2015
Italy	9	Five oral and four vaginal commercial products containing probiotic strains of *Lactobacillus* species	Culture dependent approach: cultivation, phenotypic (API kits) and genotypic (PCR-RFLP, 2-step multiplex species-specific PCR; species-specific PCR of the *tuf* gene) identification	One of the nine products was mislabeled containing *L. paracasei* instead of the claimed *L. casei*. Another product used an obsolete name (bacillo di Döderlein) on the label in place of *Lactobacillus acidophilus*. The remaining 7 products conteined the species claimed on their labels. Seven (all oral products and 2 out of 4 vaginal products) contained the number of viable cells claimed in their labels whereas the number of viable cells was not specified in the labels of the remaining two vaginal products.	Blandino et al. 2016
UK	7	Commercial products liquid or lyophilized and packed in capsules or sachet	Culture dependent approach: cultivation and phenotypic identification (API kits)	Four out of 7 products contained less viable cells than claimed in the labels. Only three products contained all the species claimed in the labels, whereas the remaining 4 missed some species. However, the API kit used misidentified some bacteria	Fredua-Agyeman, Parab, and Gaisford 2016
USA	16	Not specified but defined as bifidobacterial probiotic products	Culture-dependent approach: cultivation, subspecies-specific multiplex PCR; MALDI-TOF MS and genome sequencing. Culture-independent approach: bifidobacterial terminal RFLP	Only one matched the bifidobacterial species claimed in the label, while the remaining 15 missed the labeled species or contained non-bifidobacterial species. Pill-to-pill and lot-to-lot variations were found.	Lewis et al. 2016
Canada	52	Commercial dietary supplements	Culture-dependent approach: cultivation, multiplex species-specific PCRs and strain specific PCR. Culture-independent approach: metagenomics	17 products contained significantly less viable cells of probiotics than claimed in the labels, whereas the remaining 35 contained above the label claim. 30/52 products contained a correctly labeled classification, whereas, for the remaining 22 one or more species claimed in the label were missing or the products contained extra species not listed in the labels or there was a mislabeling (for examples in the label "*B. infantis* (*B. lactis*)" or "*L. casei*" in the label when it actually was "*B. longum*" or "*L. paracasei*")	Morovic et al. 2016
USA	10	Dietary supplements (most in capsules)	Culture-dependent approach: cultivation and species-specific PCR. Culture-independent approach: metagenomics, microarray	1/10 contained less than claimed viable cells. Different lots of 4 products did not contain a claimed species, but contained a non claimed species. In two lots of a product it was found *Enterococcus faecium* as an extra species. Sequence analyses revealed mislabeling in 4 products.	Patro et al. 2016
Poland	25	16 dietary supplements, 7 foods for special medical purposes and 2 medicinal products	Culture-dependent approach: cultivation, phenotypical identification (API kits) and MALDI-TOF MS	12/25 were non compliant with the claimed species because some claimed species were missing or there were species diverse from those claimed in the labels. Not all the products contained the claimed number of viable probiotics.	Zawistowska-Rojek et al. 2016

(*Continued*)

Table 1. (*Continued*)

Country	Number of products	Type of product	Method	Result	Reference
China	28	20 commercial probiotic yoghurts and 8 probiotic supplements (capsules, tablets and sachets) purchased from Chinese supermarkets	Cultivation, 16S rRNA gene sequencing and PCR-DGGE	Viable cells were not recovered from 2/8 probiotic supplements while in the others the total viable cell counts were higher (and in one case equal to) than declared on the labels. For 15/20 probiotic yoghurts the amount of viable cells was not reported on the label and in 5/20 products no viable cells were detected although for one of them the number of cells was stated on the label. In 15/20 probiotic yoghurts the viable cell counts were higher then 10^6 CFU/mL. In 4/20 yoghurts labeled as containing lactic acid bacteria, *L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus* were detected. One yoghurt, reporting to contain *L. casei*, was found to contain *L. delbrueckii* subsp. *bulgaricus*. *L. acidophilus* and *L. casei* were found in 4/20 and 5/20 yoghurts, respectively, although these species were not listed on the label. *L. helveticus* was detected in 7/8 probiotic supplements even it was not labeled. Moreover, not all the bacterial species identified by DGGE were label-claimed on the probiotic supplements. Additionally, although *Bifidobacterium* species were labeled in several yoghurts and supplements, *B. animalis* subsp. *lactis* and *B. longum* subsp. *suis* were detected in only 2 supplements.	Chen et al. 2017
Italy	10	Probiotic products purchased in pharmacies (vials, capsules, bottles, drops and sachets)	Cultivation, identification by biochemical tests (Vitek® II test cards) and MALDI-TOF MS	4/10 products showed CFU counts compliant with the labels. 1/10 had lower CFU count than the label claim and 5/10 had CFU counts 1-3 log higher than those reported on the labels. Likely due to the difficulty to recognize the *Bifidobacterium* colonies and to the genus complexity, the authors did not identify the three *Bifidobacterium* species labeled on 2 products and in the same 2 products they identified the claimed *Lactobacillus delbrueckii* subsp. *bulgaricus* only at the genus level. No microorganism contamination was found in any product. Nomenclature inaccuracies were detected on the label of four products: *Bifidobacterium* BB-12 and *B. lactis* HN019 instead of *B. animalis* subsp. *lactis*; *S. faecium* instead of *E. faecium* and *S. thermophilus* St-21 and *S. thermophilus* BT-01 instead of *S. salivarius* subsp. *thermophilus*.	Vecchione et al. 2018
USA	21	15 probiotic supplements and six beverages	Cultivation, 16S rRNA gene sequencing, MALDI-TOF MS, and Biolog microbial identification	82% of the species consistent with those claimed on the label by 16S rRNA gene sequencing, with 71% of isolates identified by MALDI-TOF MS and 60% identified correctly by Biolog.	Ansari et al. 2019
Italy	10	Pharmaceutical products (capsules, lyophilized preparation and liquid formulations)	Cultivation, BCL Vitek2, MALDI-TOF MS and 16S rRNA gene sequencing	4/10 products contained spores of contaminant species in addition to those of the *Bacillus* claimed in the labels. 5/10 products contained lower levels of viable cells than those claimed on the labels, while 3/10 contained higher amounts than those labeled.	Celandroni et al. 2019
Italy	10	Powder-based probiotic supplements	Culture-dependent approach: cultivation, genus-specific PCR, fungal and bifidobacterial ITS, and 16S rRNA gene sequencing. Culture-independent approach: metagenomics and quantitative real time PCR	By culture-independent approach, 2/10 contained extra species while the remaining 8 were compliant with the claimed species. The culture-dependent approach confirmed these results but found also additional species in another product. The quantitative real time PCR revealed low levels of some probiotics but the expected amount of each probiotic is not listed and results of the enumeration both by cultivation and real time PCR are missing.	Lugli et al. 2019

(*Continued*)

Table 1. (*Continued*)

Country	Number of products	Type of product	Method	Result	Reference
Bulgaria	26	Dietary supplements (16 imported commercial products and 10 powdered products from local manufacturers)	Cultivation, genus- and species- specific PCRs.	21/26 products were not compliant with the label as concerns the content of viable probiotic cells. In most cases, no viable cell growth was observed on the media utilized to enumerate probiotics while in the remaining cases lower levels of viable cells than claimed were counted. Most products did not contain all the claimed species or contained extra species.	Marinova et al. 2019
Italy	1	Multi-strain probiotic product VSL#3 (different lots of sachets containing freeze-dried bacterial cells)	Culture-independent approach: shotgun metagenomic sequencing, quantification by species-specific qPCR and counting by flow cytometry. Metaproteomic analysis.	The metagenomic analysis confirmed the identity of the eight strains blended in the VSL#3 product apart from a low contamination (0.0068% of the total reads) considered as originating from the production, laboratory or sequencing process. The relative abundance estimated from metagenomic data were confirmed by the species specific qPCR with *Streptococcus thermophilus* as the most abundand species while *Lactobacillus helveticus* and *Bifidobacterium breve* were the least abundant, although these results did not differentiate viable and death cells in the formulation. Live cells enumerated by flow cytometry were above the amount declared on the label.	Mora et al. 2019
China	19	Probiotic products from local and multinational healthcare industries	Culture-dependent approach: cultivation, species-specific and strain-specific PCRs, and high-throughput 16S rRNA gene sequencing of the pool of DNA isolated from each bacterial content found during culturing. Culture-independent approach: high-throughput 16S rRNA gene sequencing of DNA isolated directly from the products and species-specific PCRs.	Three products had a CFU count of viable probiotic cells lower than the label claim; one product contained no viable cells at all, while one product had 6/15 probiotic bacterial contents not viable. The species of the latter two products were found to be present by the culture-independent metagenomic approach, thus they were died or non-culturable. 3/17 (19 minus 2 that were excluded from the sequence analysis due to low-quality reads) products analyzed by sequencing were found non compliant with the label due to missing claimed species or to the presence of extra species not listed in the labels.	Ullah et al. 2019
Canada	6	Two pharmaceutical formulations claimed to contain *E. coli* strains (liquid formulation and capsules) (two samples from three different lot numbers)	Cultivation	In both products the number of viable probiotic cells was 2 orders of magnitudes under those claimed in the labels.	Zimmer and Dorea 2019
Republic of the Philippines (PK) and Republic of Korea (SK)	10	Probiotic products intended for children consumption (5 obtained from pharmacies in PK and 5 bought in pharmacies and online stores in SK)	Culture-dependent approach: cultivation, 16S rRNA gene sequencing. Culture-independent approach: metagenomic analysis of the 16S rRNA V3-V4 region.	1/10 products contained viable number of the microorganisms lower than claimed on the label. 4/10 products contained *Enterococcus* strains not reported on the labels. Metagenomic analysis revealed that 1/2 of the analyzed products did not contain most of the declared microorganisms.	Dioso et al. 2020
India	20	Probiotic products bought from a local pharmacy (9 single strain probiotic products and 11 multiple strains probiotic products)	Cultivation, identification by biochemical tests (Vitek® II test cards), MALDI-TOF MS, metagenomic analysis of the V3-V4 regions of 16S rRNA gene. Species-specific PCR and ITS region sequencing for *Saccharomyces cerevisiae*.	One product showed no amplification products of the 16S rRNA gene (as well as no viable bacterial content, in accordance with the manufacturer's declaration stating it was a heat killed probiotic product). Results confirmed the presence of *S. cerevisiae* in all products declaring its presence but in one product no viable yeast cells were detected. 12/20 products contained different species, strains or extra species.	Kesavelu et al. 2020

(*Continued*)

Table 1. (*Continued*)

Country	Number of products	Type of product	Method	Result	Reference
South Korea, USA and Canada	19	10 pharmaceuticals in capsules and 9 powdered supplements obtained from various markets	Culture independent approach: total genomic DNA extraction and species-specific PCRs	4/19 products contained *Lactobacillus helveticus* instead of *L. acidophilus* claimed on the labels. 3/19 products contained *L. paracasei* instead of the label-claimed *L. casei*. In one product additional *Lactobacillus* species, not reported on the label, were detected.	Kim et al. 2020
United States and Canada	182	Probiotic products collected from various manufacturers (capsules, powder, powder sticks, chewable tablets, drops, gummies)	Cultivation by pour plate technique using MRS agar supplemented with 0.05% L-cysteine hydrochloride monohydrate, species- and strain-specific PCRs, high-throughput sequencing of V3 and V4 region of 16S rRNA gene	11 species were not detected in 10 samples. Missing species were *L. casei* in 7 products, *B. longum* and *B. bifidum* in 1 product and *B. longum* in another one. *B. longum* subsp. *longum* was reported as *B. longum* subsp. *infantis* in one product. *B. animalis* subsp. *lactis* was undeclared in 1 product. In 5/72 samples tested, viable count was lower than declared and in 1 product no viable cells were found. Non-compliance was reported for 15/182 products and undeclared species in relative abundance of ca. 1-2% were detected in 14 samples.	Shehata and Newmaster 2020
The Netherlands	15	Capsules from five different strips, originating from three different lots of a probiotic product for neonatal care	Cultivation and MALDI-TOF MS	Only 1 lot was compliant with the label as concerns the probiotic species contained. The other two lots missed one or both claimed probiotic strains and contained contaminants such as *Streptococcus oralis*, *Lactococcus garvieae*, *Enterococcus faecalis*, *Enterococcus faecium* and *Lactococcus lactis*.	Vermeulen et al. 2020