

Probiotic Authentication at Purity-IQ

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Probiota Americas

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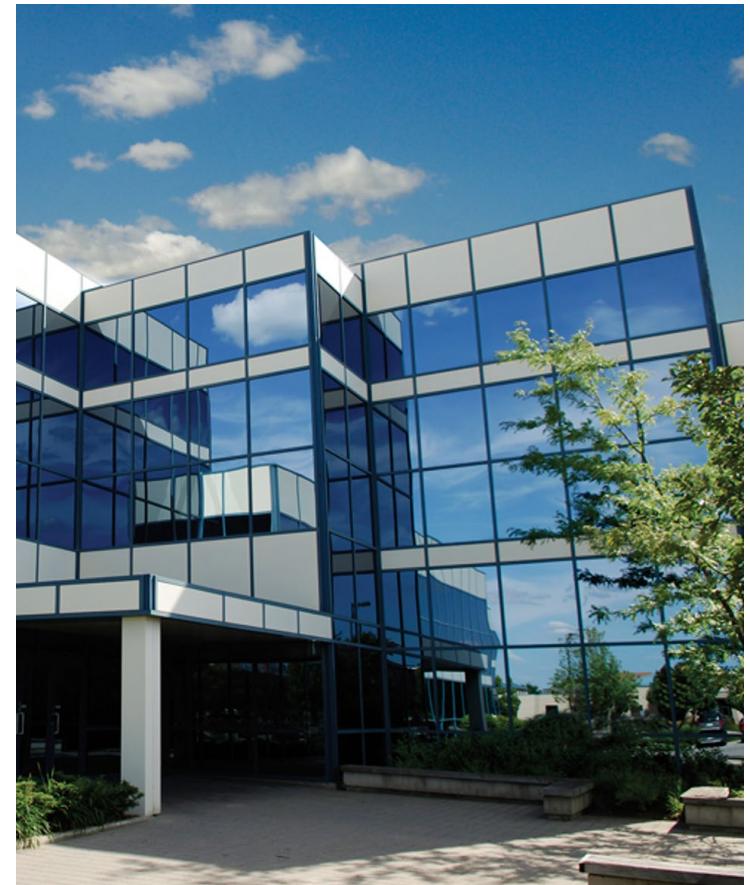
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Purity-IQ

- Purity-IQ is a biotechnology company working with leading-edge genomic and nuclear magnetic resonance (NMR) technologies to support companies in regulatory compliance, quality assurance management system requirements, and brand protection.
- Purity-IQ offers:
 - A range of analytical tools including identity and consistency testing of natural health products, and probiotic viable count enumeration.
 - Research and development services, designed to address research questions pertaining to probiotic products and provide advanced analytics and insights that support the industry needs.
 - A certification for companies that can adhere to the highest levels of quality assurance.



Purity-IQ Strain-Specific Authentication

- Probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.”
- Probiotic health benefits are diverse and are:
 - Strain specific
 - Dose-dependent
- Probiotic Authentication Program at Purity-IQ:
 - Probiotic Identity Testing
 - Probiotic Viable Count Determination



Probiotic Strain-Specific Identity Testing

- Targeted strain-specific identification via qPCR
- High taxonomic resolution
- Validated according to AOAC validation guidelines

SHEHATA ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 102, No. ■, 2019 1

SPECIAL SECTION

Guidelines for Validation of Qualitative Real-Time PCR Methods for Molecular Diagnostic Identification of Probiotics

Probiotic Strain-Specific Identity Testing - Assay Design

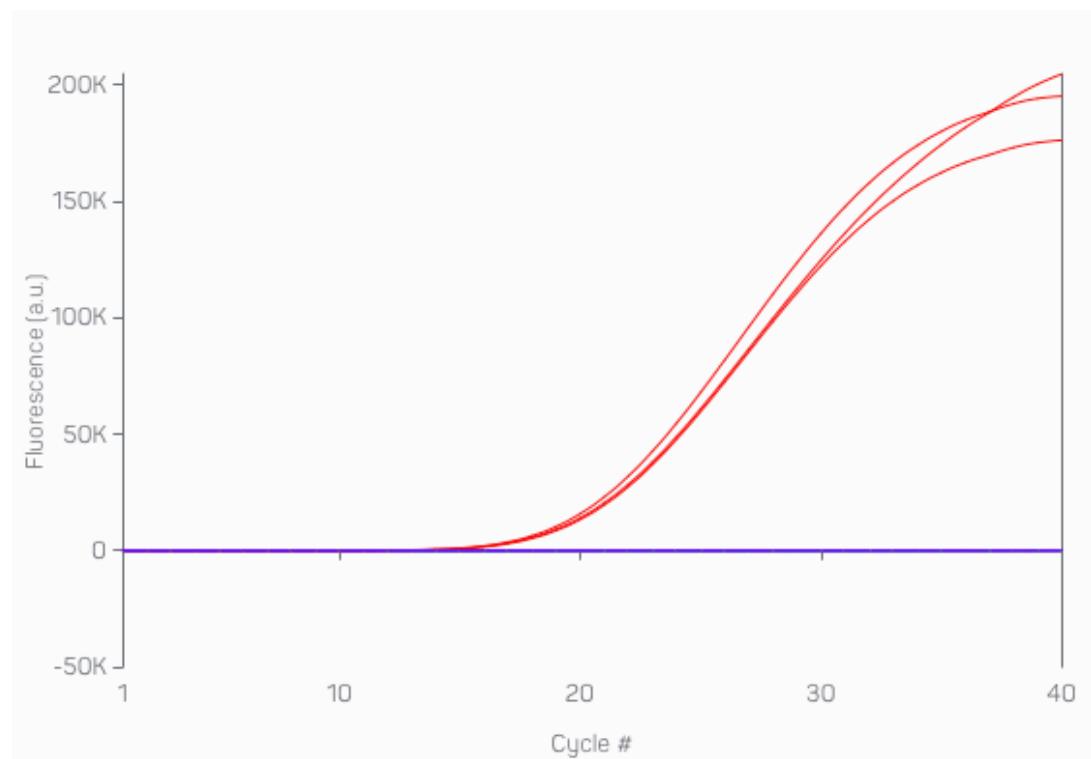
- Bioinformatic tools are used to identify a unique sequence region in the genome sequence of the target strain.

Sequence ID	66	68	70	72	74	76	78	80	82	84	86	88	90	92	Organism	Identity
Query_225... (+)	T	T	G	G	A	C	C	T	C	G	T	G	C	C	A	100.00
CP003498.1 (+)	Bifidobacterium animalis subsp. lactis Bi-07	100.00
CP047190.1 (+)	C	Bifidobacterium animalis	99.19
CP042940.1 (+)	C	Bifidobacterium animalis	99.19
CP028460.1 (+)	C	Bifidobacterium animalis subsp. animalis	99.19
CP031154.1 (+)	C	Bifidobacterium animalis subsp. lactis	99.19
CP031703.1 (-)	C	Bifidobacterium animalis subsp. lactis	99.19
CP035497.1 (+)	C	Bifidobacterium animalis	99.19
CP022724.1 (+)	C	Bifidobacterium animalis subsp. lactis	99.19
CP017098.1 (+)	C	Bifidobacterium animalis	99.19
CP009045.1 (+)	C	Bifidobacterium animalis subsp. lactis	99.19
LR699002.1 (+)	C	Bifidobacterium animalis	99.19
CP007522.1 (+)	C	Bifidobacterium animalis subsp. lactis KLDS2.0603	99.19
CP007255.1 (+)	C	Bifidobacterium animalis	99.19
CP0080571.1 (+)	C	Bifidobacterium animalis subsp. lactis	99.19
CP0033941.1 (+)	C	Bifidobacterium animalis subsp. lactis ATCC 27673	99.19
CP004053.1 (+)	C	Bifidobacterium animalis subsp. lactis Bi12	99.19
CP0069249.1 (+)	C	Bifidobacterium animalis subsp. lactis	99.19
CP0069248.1 (+)	C	Bifidobacterium animalis subsp. lactis	99.19
CP003039.2 (+)	C	Bifidobacterium animalis subsp. lactis BLCl	99.19
CP001853.2 (+)	C	Bifidobacterium animalis subsp. lactis BB-12	99.19
CP045589.1 (+)	C	Bifidobacterium animalis	99.19
CP003497.1 (+)	C	Bifidobacterium animalis subsp. lactis B420	99.19
CP002567.1 (+)	C	Bifidobacterium animalis subsp. animalis ATCC 25527	99.19
CP002915.1 (+)	C	Bifidobacterium animalis subsp. lactis CNCM I-2494	99.19
CP001892.1 (+)	C	Bifidobacterium animalis subsp. lactis V9	99.19
CP001606.1 (+)	C	Bifidobacterium animalis subsp. lactis DSM 10140	99.19
CP001515.1 (+)	C	Bifidobacterium animalis subsp. lactis Bi-04	99.19
CP001213.1 (+)	C	Bifidobacterium animalis subsp. lactis AD011	99.19
CP015407.2 (-)	C	Bifidobacterium animalis subsp. animalis	98.37
CP065311.1 (-)	C	Bifidobacterium animalis	98.37

Probiotic Strain-Specific Identity Testing - Assay Validation

Validation of real-time PCR methods for strain identification

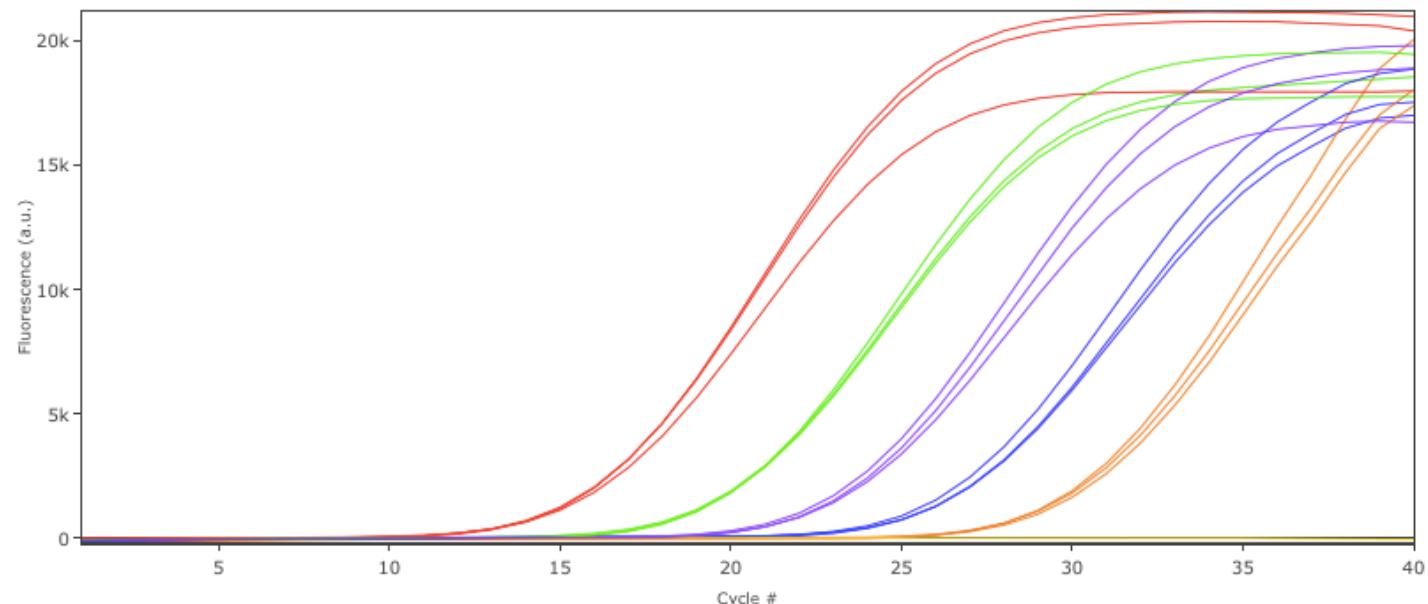
- Specificity
- Sensitivity
- Repeatability
- Reproducibility
- Applicability



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Probiotic Strain-Specific Identity Testing - Assay Validation

Validation of real-time PCR methods for strain identification

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 - Applicability
- The methods are applicable to:
 - Different dosage forms with different additives
 - Different food matrices
 - Mono-strain and multi-strain blends



Probiotic Strain-Specific Identity Testing - Available Assays

Strain-specific methods are available for > 80 strains:

<i>Bacillus coagulans</i> R0449	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> Bi-26	<i>Lactobacillus gasseri</i> HA-275	<i>Lactiplantibacillus plantarum</i> UALp-05
<i>Bacillus subtilis</i> R0179	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> HA-116	<i>Lactobacillus gasseri</i> Lg-36	<i>Limosilactobacillus reuteri</i> HA-188
<i>Bacillus subtilis</i> DE111	<i>Bifidobacterium longum</i> UABI-14	<i>Lactobacillus helveticus</i> HA-122	<i>Limosilactobacillus reuteri</i> 1E1
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> B94	<i>Enterococcus faecium</i> R0026	<i>Lactobacillus helveticus</i> R0052	<i>Limosilactobacillus reuteri</i> Lrc NCIMB 30242
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> Bb-12	<i>Lactobacillus acidophilus</i> DDS-1	<i>Lactobacillus helveticus</i> L10	<i>Limosilactobacillus reuteri</i> R0444
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> Bi-07	<i>Lactobacillus acidophilus</i> La-14	<i>Lacticaseibacillus paracasei</i> 8700:2	<i>Lacticaseibacillus rhamnosus</i> GG (ATCC 53103)
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> Bl04	<i>Lactobacillus acidophilus</i> LA-5	<i>Lacticaseibacillus paracasei</i> HA-108	<i>Lacticaseibacillus rhamnosus</i> HA111
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> UABla12	<i>Lactobacillus acidophilus</i> NCFM	<i>Lacticaseibacillus paracasei</i> HA-196	<i>Lacticaseibacillus rhamnosus</i> HA114
<i>Bifidobacterium bifidum</i> Bb-06	<i>Lactobacillus acidophilus</i> R0418	<i>Lacticaseibacillus paracasei</i> HA-274	<i>Lacticaseibacillus rhamnosus</i> HN001
<i>Bifidobacterium bifidum</i> HA-132	<i>Lactobacillus acidophilus</i> UALA-01	<i>Lacticaseibacillus paracasei</i> R0215	<i>Lacticaseibacillus rhamnosus</i> Lr-32
<i>Bifidobacterium bifidum</i> R0071	<i>Levilactobacillus brevis</i> HA-112	<i>Lacticaseibacillus paracasei</i> Lpc-37	<i>Lacticaseibacillus rhamnosus</i> R0011
<i>Bifidobacterium bifidum</i> UABb-10	<i>Levilactobacillus brevis</i> Lbr-35	<i>Lacticaseibacillus paracasei</i> L26	<i>Lacticaseibacillus rhamnosus</i> SD-5839
<i>Bifidobacterium bifidum/lactis</i> Bb-02	<i>Lacticaseibacillus casei</i> Lc-11	<i>Lacticaseibacillus paracasei</i> UALpc-04	<i>Ligilactobacillus salivarius</i> HA-118
<i>Bifidobacterium breve</i> Bb-03	<i>Lacticaseibacillus casei</i> UALc-03	<i>Lactiplantibacillus plantarum</i> 299v	<i>Ligilactobacillus salivarius</i> Ls-33
<i>Bifidobacterium breve</i> HA-129	<i>Lactobacillus crispatus</i> UALcr-35	<i>Lactiplantibacillus plantarum</i> HA-119	<i>Lactococcus lactis</i> HA-136
<i>Bifidobacterium breve</i> R0070	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> Lb-87	<i>Lactiplantibacillus plantarum</i> Heal 9	<i>Lactococcus lactis</i> LI-23
<i>Bifidobacterium longum</i> BI-05	<i>Limosilactobacillus fermentum</i> HA-179	<i>Lactiplantibacillus plantarum</i> Lp-115	<i>Saccharomyces boulardii</i> CNCM I-1079
<i>Bifidobacterium longum</i> HA-135	<i>Limosilactobacillus fermentum</i> SBS-1	<i>Lactiplantibacillus plantarum</i> R1012	<i>Streptococcus thermophilus</i> HA-110
<i>Bifidobacterium longum</i> R0175	<i>Limosilactobacillus fermentum</i> UALf32	<i>Lactiplantibacillus plantarum</i> Rosella	<i>Streptococcus thermophilus</i> St-09
<i>Bifidobacterium longum</i> subsp <i>infantis</i> R0033	<i>Lactobacillus gasseri</i> BNR17	<i>Lactiplantibacillus plantarum</i> SD-5870	<i>Streptococcus thermophilus</i> St-21

Probiotic Strain-Specific Enumeration

- Strain-specific enumeration via targeted qPCR methods
- Can be used for mono-strain or multi-strain products
- Suitable for stability assessments throughout the shelf life
- Applicable for heat-killed, and next-generation probiotics

ORIGINAL RESEARCH article

Front. Microbiol., 18 January 2023

Sec. Food Microbiology

Volume 13 - 2022 | <https://doi.org/10.3389/fmicb.2022.1076631>

Real-time polymerase chain reaction methods for strain specific identification and enumeration of strain

Lactocaseibacillus paracasei 8700:2



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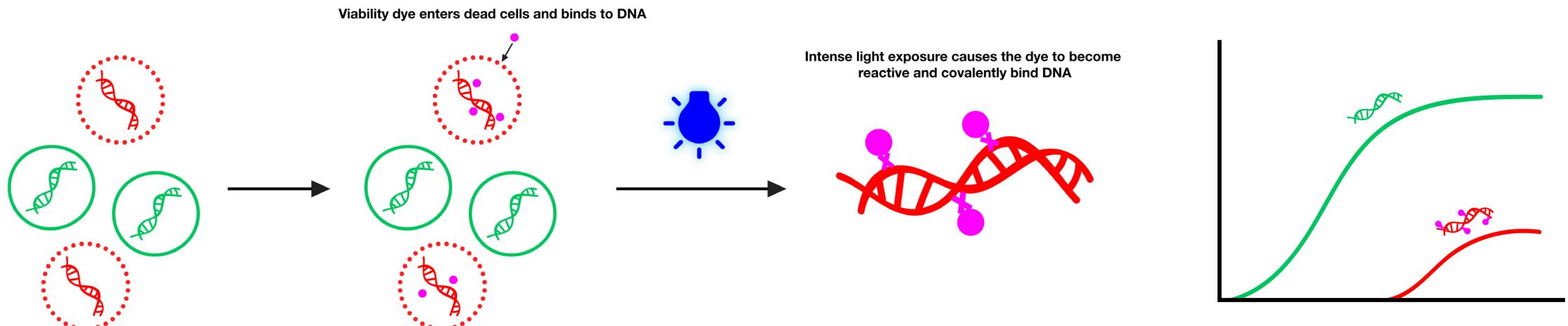
Basma Hassane³ and



Steven G. Newmaster¹

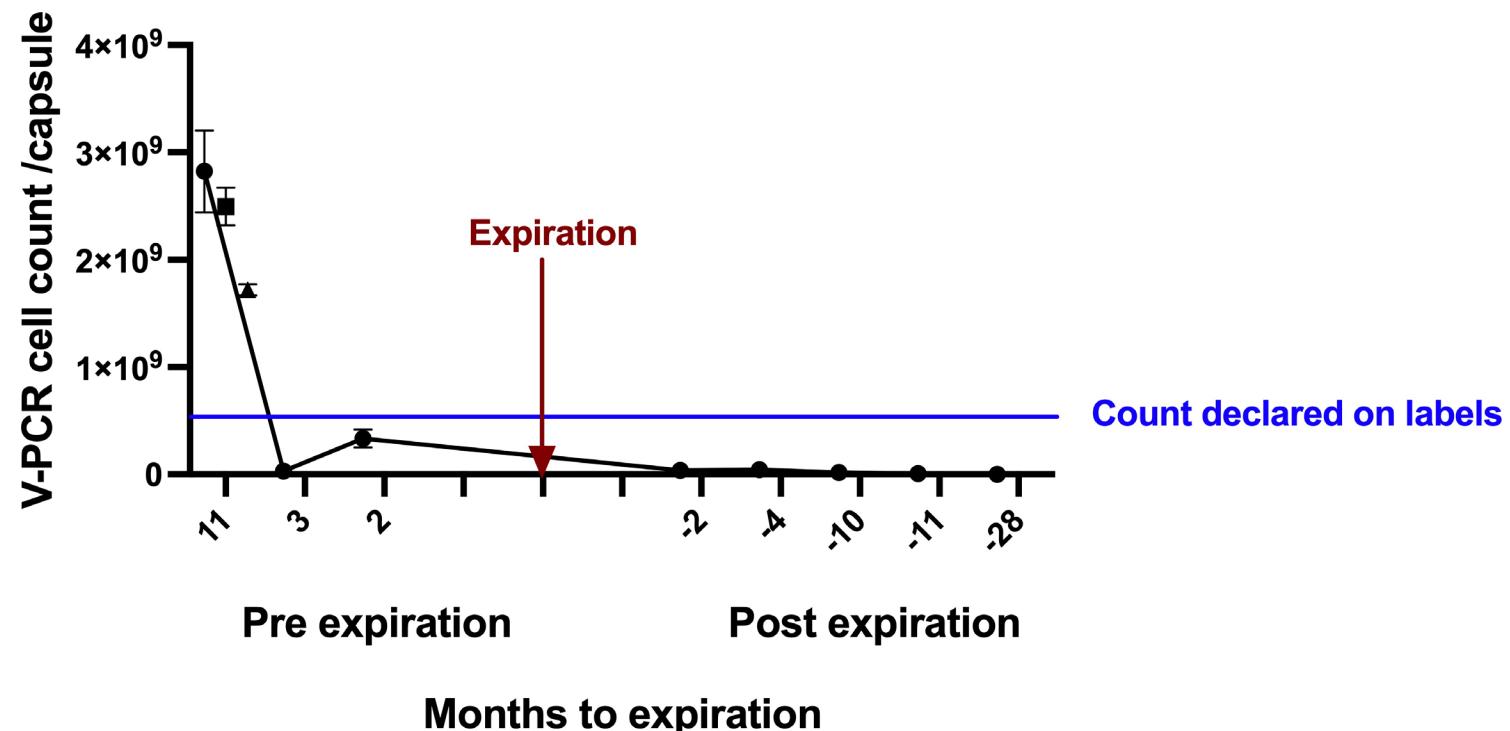
Strain-Specific Enumeration - Principle

- Based on viability qPCR which is a technique that relies on intercalating dyes to inactivate DNA from dead cells so only DNA from viable cells can react in PCR.



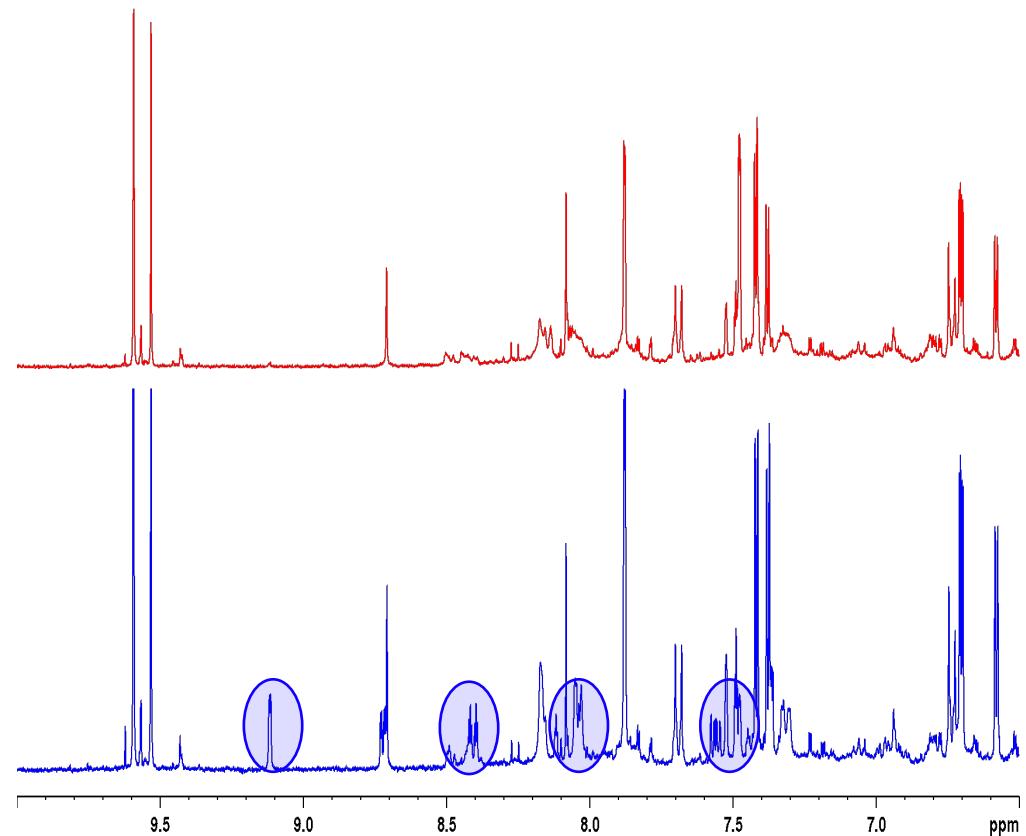
Strain-Specific Enumeration – Stability Monitoring

- Ten multi-strain finished products tested at different expiration dates using viability qPCR



Product Consistency Via Nuclear Magnetic Resonance (NMR) Spectroscopy

- Ensure label compliance
- Ensure the safety of consumers
- No changes to cryo-protectants, stabilizers, excipients
- Monitor for the presence of adulterants (voluntarily added or not)



Conclusions

- The methods developed offer reliable tools for strain-specific identification and enumeration in support of high-quality and efficacious probiotic products.
- The enumeration methods enable stability monitoring of individual strains in finished multi-strain products, which cannot be achieved using conventional plate count methods.
- NMR metabolomics monitors batch-to-batch consistency to ensure product compliance.

Thank You - Our Team



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Probiotics Specialist

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