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Application Note
Version 1

Strain-Specific Identification of PROBIOTICS

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Background

The global probiotic market has been growing rapidly in recent years where it was valued at USD 58.17 billion in 2021 and is expected to reach USD 111.21 billion in 2030 (Grand-View-Research-Inc, 2022). Consumers are taking probiotics for their various health benefits and are choosing products based on the health benefits claimed on the labels. Looking at product labels, probiotic nomenclature typically includes a genus name (e.g., *Bifidobacterium*), a species name (e.g., *animalis*) and a strain name (e.g., BB-12, Bi-07 or Bl-04) (Figure 1). The health benefits of probiotics are strain specific (Klein et al., 2010; McFarland et al., 2018), for example, strains Lafti B94 and BB-12 are both *Bifidobacterium animalis* subsp. *lactis* species but their genetic differences confer different health benefits to the host. *B. lactis* Lafti B94 at a daily concentration of 10B, helps children and adolescents with bloating and constipation in irritable bowel syndrome (IBS), while BB-12 at a concentration of 1B per day helps in reducing crying and fussing in colicky infants.

Because the health benefits of probiotics are strain specific, detection/identification at the species level (e.g., *animalis, bifidum* or *longum*) is not sufficient and identification at the strain level (e.g., BB-12, Bi-07 or Bl-04) is essential. Reliable, and highly specific strain identification methods are important for probiotic product authentication and quality assurance. DNA based methods such as qPCR can detect small genetic variances between strains belonging to the same species and can achieve strain-specific identification when carefully designed and validated. Here we show how strain-specific identification methods can differentiate closely related strains from the same species.

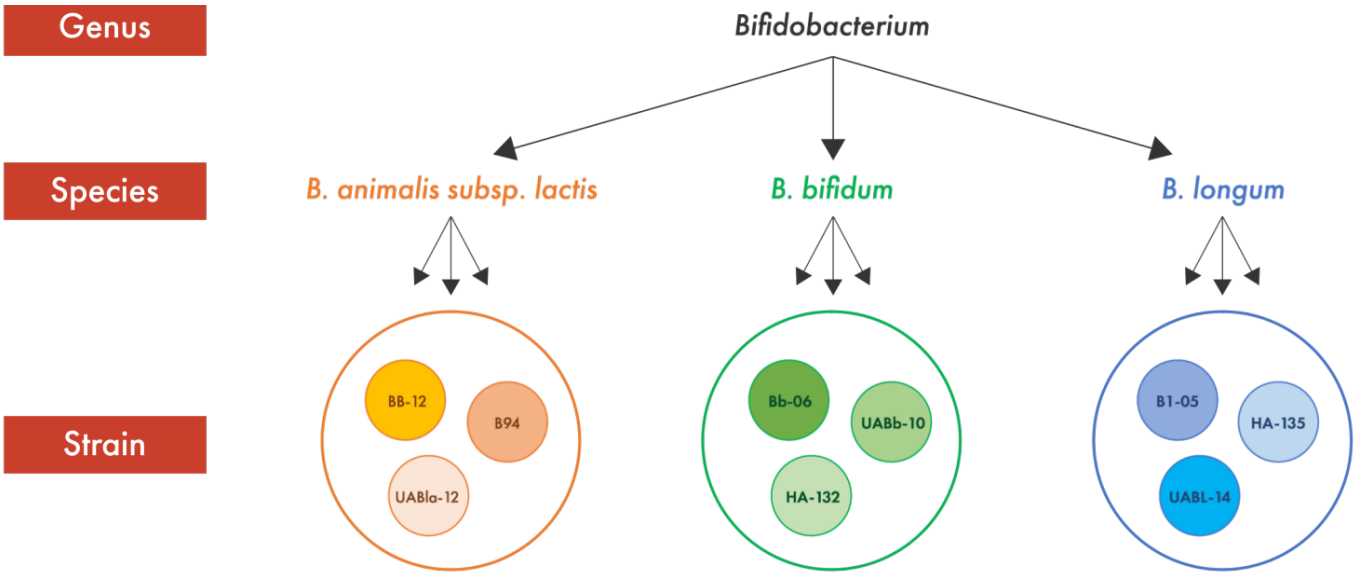

Figure 1. Explaining probiotic nomenclature. Probiotic nomenclature typically includes a genus name (e.g., *Bifidobacterium*), a species name (e.g., *animalis*) and a strain name (e.g., BB-12, Bi-07 or Bl-04).

Materials and Methods

qPCR assays were designed and validated for species-specific identification of *Bifidobacterium animalis* subsp. *lactis*. Additionally, qPCR assays were designed and validated for strain-specific identification of *Bifidobacterium animalis* subsp. *lactis* strains Bl-04, Bi-07, and BB-12 (Shehata et al., 2021). Similarly, species-specific identification of *Lacticaseibacillus rhamnosus* (*L. rhamnosus*) and strain-specific identification of *L. rhamnosus* strains GG, Lr-32, HN001, HA-111 and HA-114 were designed and validated. The strain-specific identification methods were designed to target the genetic differences between strains to achieve strain level specificity. The assays were validated following the AOAC guidelines for validation of qualitative real-time PCR methods for molecular diagnostic identification of probiotics (Shehata et al., 2019), which includes validation for specificity, sensitivity, and precision (Broeders et al., 2014; Shehata et al., 2019).

Results and Discussion

The species-specific identification method of *Bifidobacterium animalis* subsp. *lactis* gave a positive result with all tested strains (Bl-04, Bi-07, BB-12, HN019, B420 and HA-194) (Table 1). On the other hand, Bl-04 strain-specific method gave a positive result with strain Bl0-04 and was negative with strains Bi-07, BB-12, HN019, B420 and HA-194. Bi-07 strain-specific method gave a positive result with strain Bi-07 and was negative with strains Bl-04, BB-12, HN019, B420 and HA-194. BB-12 strain-specific method gave a positive result with strain BB-12 and was negative with strains Bl-04, Bi-07, HN019, B420 and HA-194 (Table 1).

Similarly, the species-specific identification method of *L. rhamnosus* gave a positive result with all tested strains (GG, Lr-32, HN001, HA-111 and HA-114) (Table 2). On the other hand, GG strain-specific method gave a positive result with strain GG and was negative with strains Lr-32, HN001, HA-111 and HA-114. Lr-32 strain-specific method gave a positive result with strain Lr-32 and was negative with strains GG, HN001, HA-111 and HA-114. HN001 strain-specific method gave a positive result with strain HN001 and was negative with strains GG, Lr-32, HA-111 and HA-114. HA-111 strain-specific method gave a positive result with strain HA-111 and was negative with strains GG, Lr-32, HN001 and HA-114. HA-114 strain-specific method gave a positive result with strain HA-114 and was negative with strains GG, Lr-32, HN001 and HA-111 (Table 2).

The results show that strain-specific identification methods could differentiate closely related strains. The methods are specific, simple, quick and offer reliable tools for strain-specific identification of probiotics.

Identification Methods	Strain Bl-04	Strain Bi-07	Strain BB-12	Strain HN019	Strain B420	Strain HA-194
Species-Specific	+	+	+	+	+	+
Bl-04 Strain-Specific	+	-	-	-	-	-
Bi-07 Strain-Specific	-	+	-	-	-	-
BB-12 Strain-Specific	-	-	+	-	-	-

Table 1: Using species-specific and strain-specific identification methods to identify multiple strains of *Bifidobacterium animalis* subsp. *lactis*.

Identification Methods	Strain GG	Strain Lr-32	Strain HN001	Strain HA-111	Strain HA-114
Species-Specific	+	+	+	+	+
GG Strain-Specific	+	-	-	-	-
Lr-32 Strain-Specific	-	+	-	-	-
HN001 Strain-Specific	-	-	+	-	-
HA-111 Strain-Specific	-	-	-	+	-
HA-114 Strain-Specific	-	-	-	-	+

Table 2: Using species-specific and strain-specific identification methods to identify multiple strains of *L. rhamnosus*.

Conclusions

Carefully designed and validated strain-specific identification methods can achieve strain specific detection/identification of probiotics. Confirming strain identity is vital for an efficacious product. Additionally, availability of these methods is of paramount importance to authenticate finished probiotic products that contain multiple strains of the same species, which cannot be achieved using species-specific methods. The methods have low limits of detection (can be as low as 0.5 picograms of DNA), which means high sensitivity to detect target strains even when they are present in small ratios in multi-strain blends. Thus, strain-specific identification methods are reliable and valuable tools for probiotic authentication.

References

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