USP Probiotic Roundtable Summary

USP Stakeholder Forum

Buffy Stahl – DuPont Nutrition and Health

1 June 2016

Topics discussed

1. Monograph requirements for Dietary Supplements vs. Dietary Ingredients.

2. Reference standards

3. Enumeration challenges. Techniques for enumeration of probiotics and challenges with enumerating probiotic blends

4. Critical to Quality standards. Developing best practices for the industry.

Probiotic Monograph Sponsorship (USP) – to date

Dietary Ingredients – 8 strain-specific monographs published, including the following information

- GRAS package (safety)
- Packaging and storage information (stability)
- Identification tests (strain-specific)**
- Enumeration tests (single strain)**
- Impurities tests
- Validation of 3 lots with CoAs

Dietary Supplements - need a path forward for development of quality standards. What types of information are needed for high-quality standards for finished dosage forms for probiotics;

- Areas of debate: Should monograph development be at the strain-level rather than at the species level?

- What types of accepted enumeration methods should be allowed and is strain-specific enumeration needed in final format?

Strain vs. species specific? DI vs DS

- Opinions were expressed that monograph development should be at the strain-level rather than at the species level, since regulatory filings and safety documentation are typically made at the strain level, also supported by information from clinical studies on specific strains.

- Some concerns around the potential loss of the ability to make general non-strain- specific claims on finished dietary supplement forms, especially when multiple strains of the same species are present and there is a lack of information on strain specificity by the manufacturer..

- USP staff suggested that monographs could be developed at the strain level for ingredients identification, and to the species-level for supplements, if the ingredients within the supplements were already verified to the strain-level.

- Therefore, **Monographs for species-level identification would suffice and meet cGMP requirements for DS when identification to the strain-level was completed via verified DI ingredient material.**

Standards – quality and reference

There is a potential need for reference standards such as genomic DNA for identity testing. This could be sourced from suppliers rather than USP.

Considering the PCR primers used for Identification, a company that does not have the knowledge and capabilities may need to source the primers and/or strains for comparison from an independent source.

USP will ask sponsors to consider this as well as the possibility of supplying materials so that users can verify primer sets or PCR system suitability. USP will need industry help with this.

Contamination could arise from cross-contamination in the fermentation broth. Industry usually relies on information from raw material suppliers, where USP and AOAC methods are already proposed and well-accepted by the panel.

Enumeration

Strain-specific enumeration methods are usually provided by probiotic suppliers. Where the finished format is a single strain, the monograph could contain a specific test for that strain.

- In the case of probiotic blends, enumeration is a challenge and total

counts are usually reported. New molecular methods are being developed to provide proper enumeration of different strains in a blend, however to date perhaps only species can be identified and that is also not routinely done.

Therefore, **total cell count of the blended species of probiotics is current standard in industry, and should be accepted until advanced methods for either species-specific or ultimately strain-specific enumeration have been introduced and accepted.**

USP Verification program for Probiotics –other key messages

- FCC model is to develop monographs at the strain level. USP Dietary Supplement Verification Program for probiotics is new, there are only Food monographs issued at this point, the roundtable focused heavily of this.

- For USP-verified products, the manufacturer has to test 100% of the incoming lots of probiotic ingredients for identity, but not necessarily with the USP methods. Verification could be performed at the level claimed on the label: genus, species or strain.

- Verification of dietary supplements containing a single ingredient are more affordable/quicker than verification of products containing blends. Strain specific enumeration claims are being developed, but currently not accepted industry-wide.

- USP will require compliance with 100% enumeration (CFU) for label claims. It was discussed that an upper cap for overages should not be placed for probiotics because of the decrease in bacteria viability during storage.

Required documents for monograph sponsorship

Genetic Identification

- Required to provide strain identification compared to all other public genomes of the same species (see table).
- Testing comprised of custom PCR assays

Organism	DuPont strains	No. public strains
Bifdobacterium animalis subsp. *lactis*	Bi-07, Bl-04, HN019	n = 14
Lactobacillus acidophilus	NCFM, La-14	n = 12
Lactobacillus paracasei	Lpc-37	n = 63
Lactobacillus rhamnosus	HN001	n = 23

B. lactis comparative genomics

	***B. lactis* strain**														
Genetic target (in DSM 10140)	**ATCC 25527**	**DSM 10140**	**CNCM I-2494**	**Bi-07**	**Bl-04**	**AD011**	**Bl12**	**V9**	**BLC1**	**BB-12**	**B420**	**ATCC 27673**	**BS 01**	**HN019**	**RH**
INDEL2: long-chain fatty acid-CoA ligase	Ins	Ins	Ins	Ins	Del	Ins	Ins	Ins	Ins	Ins	Ins	Ins	Ins	Ins	Ins
igr6	G	G	G	A	A	G	G	G	G	G	G	G	G	G	G
igr9	A	A	A	G	G	G	G	G	G	G	G	G	G	A	G
Balat_0660	C	C	C	T	T	T	T	T	T	T	T	T	T	T	T

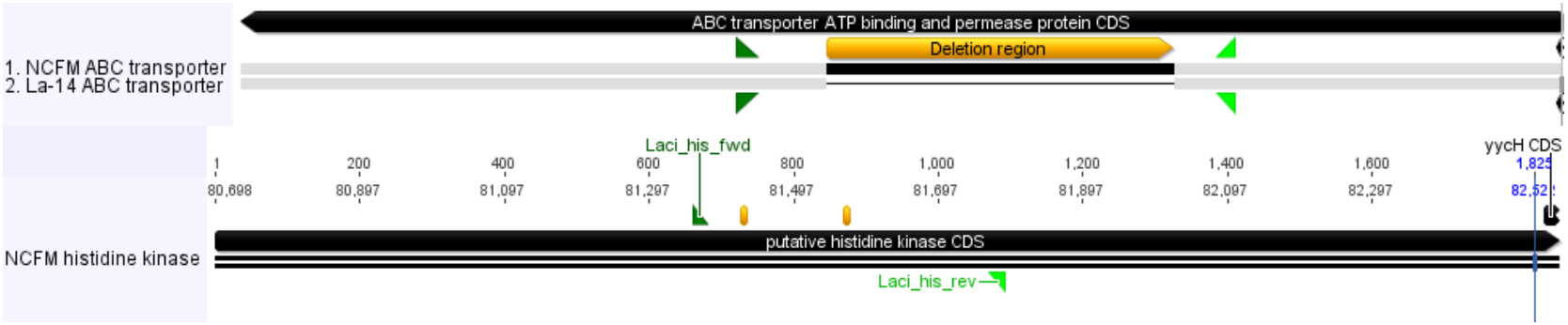
					***L. acidophilus* strain**										
Gene	**Annotation**	**Location in NCFM**	**NCFM identity**	**PCR Identity**	**30SC**	**La-14**	**ATCC 4796**	**CIP 76.13**	**DSM 9126**	**CIRM-BIA 445**	**CFH**	**JCM 1132**	**DSM 20242**	**CIRM-BIA 442**	**VKMV-2020D**
LBA0079	putative histidine kinase	81429	C	C	T	C	A	C	A	A	C	C	na	na	na
		81572	T	T	C	T	C	C	C	C	T	C	na	na	na
LBA1268	uridine monophosphate	1247285	T	T	C	T	C	T	C	C	na	T	na	na	na
		1247477	A	A	G	A	G	A	G	G	na	A	na	na	na
LBA1131	ABC transporter	1110240	Ins	Ins	Ins	Del	Ins	Ins	Ins	Ins	Ins	Ins	na	na	na

L. acidophilus comparative genomics

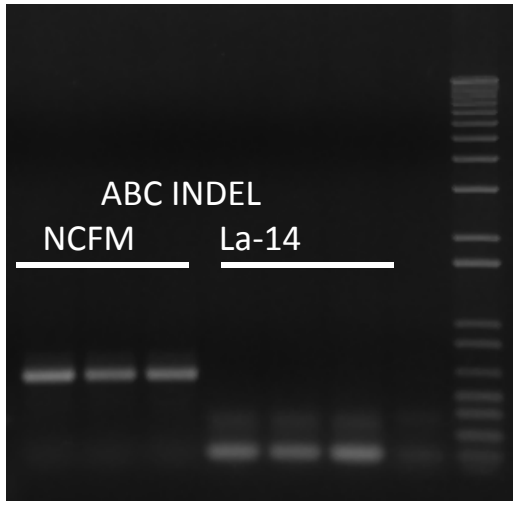
Required documents for monograph sponsorship

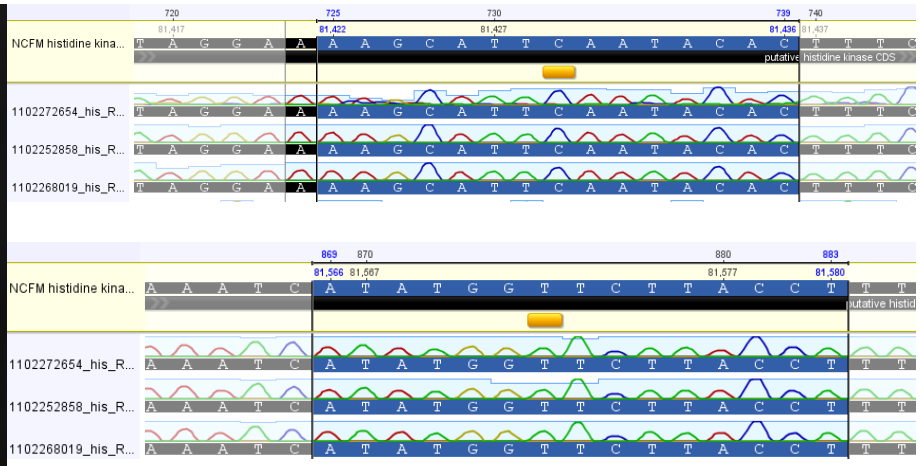
PCR assay

- Primer design



- PCR results of 3 validation lots





Discussion

Thank You