Gluten-Free Labeling: Are Growth Media Containing Wheat, Barley, and Rye Falling through the Cracks?

BACTERIA, MOLD, YEAST, AND enzymes produced by bacteria are used in a variety of products, including probiotics and digestive enzymes. These microorganisms may be grown on media that may include ingredients derived from gluten-containing grain (ie, wheat, barley, and rye). According to the International Probiotics Association, "probiotic bacteria require complex growth media that contain a range of ingredients."[1] According to Deerland Enzymes, the enzymes used in supplements may be produced from fermented bacteria grown on wheat.[2] In addition, the bacteria *Xanthomonas campestris* used to make xanthan gum may be grown on wheat glucose syrup, and the mold *Penicillium roqueforti* used to make blue-veined cheeses may be grown on wheat or barley-derived media.

Historically, some concern has been expressed in the celiac disease community that the use of gluten-derived/gluten-containing growth media may result in residual gluten protein fragments remaining in products containing microorganisms such as bacteria and mold. Regardless, a Canadian Celiac Association analysis of gluten-derived growth media used to grow *Penicillium roqueforti* found gluten to be below the limit of detection using the sandwich and competitive R5 enzyme-linked immunosorbent assays (ELISAs; R-Biopharm R5 ELISA Ridascreen Gliadin and R-Biopharm R5 ELISA Ridascreen Gliadin Competitive, respectively).[3,4] In addition, *Xanthomonas campestris* grown on wheat glucose syrup and tested with both the sandwich and competitive R5 ELISAs tested below the limit of quantification for gluten.[5*] However, an abstract presented at Digestive Disease Week in 2014 further increased concerns about growth media.[8] This study assessed the gluten content of probiotics, including 15 that were labeled as gluten free. Two of the labeled gluten-free products contained more than 20 parts per million (ppm) of gluten based on liquid chromatography-mass spectrometry. In the United States, Food and Drug Administration (FDA)-regulated foods, including supplements, must contain less than 20 ppm gluten to be labeled as gluten free.

One obstacle preventing the resolution of whether residual gluten sometimes remains in products containing bacteria and mold is the general lack of readily available information on the growth media used to cultivate these microorganisms. Although the bacterial or mold strains present in probiotics, enzymes, supplements containing probiotics or enzymes, and some fermented foods is declared, no information on the growth media is typically provided. All of this makes knowing the specific commercial products to target for testing difficult.

CURRENT RULES AND REGULATIONS REGARDING LABELING OF ALLERGENS AND GLUTEN USED IN GROWTH MEDIA

In the United States, under the Food Allergen Labeling and Consumer Protection Act (FALCPA), if an ingredient contains protein from wheat, this must be declared on the food label, either in the ingredients list or in the "Contains" statement.[9] As a result, consumers may occasionally come across a blue cheese that includes in the ingredients list "*Penicillium roqueforti* (wheat)." Barley is not included under FALCPA, so barley protein is not required to be named. In addition, it is up to the manufacturer to declare when wheat protein is present in an ingredient. If the manufacturer is not testing for residual gluten, or is not using an assay designed to detect gluten protein fragments, or is not aware of the potential for residual gluten, then the information provided on the food label may not be accurate.

The US FDA provides very little guidance on whether allergenic protein in growth media should be declared in the ingredients list. In nonbinding recommendations related to manufacturers of enzyme preparations, the FDA states:

> *"If an enzyme preparation contains proteins derived from a major food allergen and is used in foods that normally do not contain this allergen, such an enzyme preparation may fall under the provisions of FALCPA unless it is granted an exemption from allergen labeling as a result of a petition or a notification. For example, such a situation may occur if an enzyme preparation has been formulated with wheat flour and is intended for use in foods that normally do not contain wheat protein."*[10]

In 2015, the FDA published a proposed rule on gluten-free labeling of fermented or hydrolyzed foods.[11] Under the proposed rule, foods fermented or hydrolyzed by the manufacturer (eg, cheese, yogurt) could be labeled as gluten free only if they are considered

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**The sandwich R5 ELISA is a Codex Alimentarius type I method.[6] The competitive R5 ELISA has been accepted as an Official Method of Analysis, First Action status by the Association of Analytical Communities International.[7]*

gluten free before fermentation or hydrolysis. If a food itself is not fermented or hydrolyzed but instead contains fermented or hydrolyzed ingredients from a supplier (eg, Asian-style sauce made using fermented or hydrolyzed soy sauce containing hydrolyzed soy protein or hydrolyzed wheat protein), the manufacturer would have to document from the supplier that the ingredient was gluten free before being fermented or hydrolyzed. Although the proposed rule may appear straightforward, nowhere in the document are growth media discussed. Whether the FDA considers bacteria (as used in probiotics), bacterial enzymes (as used in digestive enzymes), yeast, and mold to be fermented or hydrolyzed ingredients is unclear.

In the United Kingdom, The Food Standards Agency does not require allergenic protein from growth media to be declared on the food label, stating: "Micro-organisms that have been fed on allergenic substrates are not considered to be derived from the allergenic substrates for the purposes of labelling."[12] Both the United States and the United Kingdom may want to consider further study of fermentation media used to produce bacteria and bacterial enzymes, mold, and yeast to determine whether allergenic or gluten protein remain. This suggestion is based on real-world test results of a commercially available probiotic containing a bacterial strain with known growth media: one formulation gluten free and the other not.

GLUTEN ASSESSMENT OF A PROBIOTIC CONTAINING FERMENTED BACTERIA

In June 2016, Gluten Free Watchdog, LLC a gluten test reporting service in Manchester, MA, tested a popular probiotic labeled gluten free. This product was requested for testing by subscribers to this service. Product was shipped unopened to the ISO-certified food testing laboratory Bia Diagnostics, LLC, in Colchester, VT. The probiotic was tested using the R5 ELISA Ridascreen Gliadin Competitive R7021 (R-Biopharm AG). A competitive ELISA is used when assessing food for gluten protein fragments, which occur when gluten protein is hydrolyzed or fermented. Bacteria (and the enzymes they produce) in the probiotic were deactivated before testing to prevent false-positive results.[13] This same probiotic was again tested in July and November 2016. This testing involved different (newer) lots of the probiotic.

As can be seen in the Table, quantifiable gluten protein fragments were found in the probiotic tested in June 2016. The manufacturer informed the test reporting service Gluten Free Watchdog that a bacterial strain grown on yeast peptides derived from spent beer (brewer's) yeast was inadvertently used in the tested lot of probiotics. Spent brewer's yeast is a byproduct of beer brewing. As a result, it may contain malted barley. Publications of the Academy of Nutrition and Dietetics, including the Evidence Analysis Library's Celiac Disease Toolkit[14] and the Pocket Guide to Gluten-Free Strategies for Clients with Multiple Diet Restrictions,[4] recommend against the use of spent brewer's yeast by persons with celiac disease.

As can also be seen in the Table, testing did not indicate any quantifiable gluten peptide fragments in the lots of probiotics tested in July and November 2016. These newer lots contained the same bacterial strain as the older lot; however, the newer lots were grown on a different fermentation medium. Yeast peptides grown in molasses were used vs yeast peptides grown in spent brewer's yeast. As can be seen in the Table, both samples tested below the lower limit of quantification of 10 ppm of gluten.

Table. Gluten analysis of probiotic containing bacterial strain with known growth media

Date of analysis	Growth medium	Extraction 1 (ppm gluten[a])	Extraction 2 (ppm gluten[a])
June 8, 2016	Spent Brewer's yeast	>283.5	>283.5
July 28, 2016	Molasses	<10	<10
November 18, 2016	Molasses	<10	<10

[a]Using the Competitive R5 ELISA Ridascreen Gliadin R7021.

IMPLICATIONS OF FINDINGS ON GLUTEN-FREE LABELING OF PROBIOTICS AND DIGESTIVE ENZYMES

The FDA regulates probiotics and digestive enzymes as foods or supplements (versus drugs). As a result, probiotics and digestive enzymes labeled gluten free must comply with FDA's gluten-free labeling rule and contain less than 20 ppm gluten. As previously stated, probiotics, digestive enzymes, and foods containing microorganisms, such as bacteria, and mold may contain gluten protein fragments vs intact gluten protein. Also, as mentioned previously, the FDA has proposed a rule for gluten-free labeling of fermented or hydrolyzed foods.[11] However, how products containing bacteria and mold cultivated on gluten-derived growth media will be regulated for compliance under these rules remains unclear. The FDA states in the proposed rule:

> *For an ingredient that was fermented or hydrolyzed by a supplier, one way for the manufacturer of a food bearing the "gluten-free" claim to provide adequate assurance that the ingredient is "gluten free" would be to obtain records from that supplier supporting that the ingredient meets the definition of "gluten free," including that the ingredient was manufactured or processed to avoid gluten cross-contact and to contain less than 20 ppm gluten. Adequate assurance regarding the ingredients fermented or hydrolyzed by an ingredient supplier can include documentation regarding the supplier's manufacturing procedures, records of test results from tests conducted by the ingredient supplier on the components of the ingredient before fermentation or hydrolysis, certificate of analysis, or other appropriate documentation provided by the ingredient supplier for the fermented or hydrolyzed ingredient.*

Comments were submitted to the FDA docket during the comment period for the proposed rule, seeking clarification on whether gluten-containing growth media may be used in products labeled as gluten free.[15] The specific questions include:

> *Can FDA please comment on whether wheat- and barley-based*

growth media can be used to "grow" the bacteria that secrete the enzymes used in labeled gluten-free digestive enzymes, probiotics, and similar products? If yes, how is the FDA proposing that manufacturers test these enzymes to ensure that no hydrolyzed gluten fragments remain?

Can the FDA also comment on whether other ingredients produced from fermented bacteria or mold (eg, Xanthomonas campestris*—used to make xanthan gum,* Penicillium roqueforti*—used to make blue cheese, and so forth) grown on wheat- or barley-based growth media are allowed in labeled gluten-free foods? If yes, how is FDA proposing that manufacturers test these bacterial/mold ingredients to ensure that no hydrolyzed gluten fragments remain?*

The FDA also states in the proposed rule that although the FDA recognizes sandwich ELISAs as valid measures of intact gluten (and has stated that the sandwich R5 ELISA will be used when necessary to assess compliance with the gluten-free labeling rule), it does not yet recognize competitive ELISAs or liquid chromatography-mass spectrometry as valid measures to quantify gluten protein fragments into intact gluten. A sandwich ELISA may severely underestimate gluten content when gluten is hydrolyzed (a sandwich ELISA requires two epitopes [ie, antibody-binding sites]; a competitive ELISA requires only one epitope).

FUTURE NEEDS

Based on probiotic test findings, gluten protein fragments sometimes may remain in bacteria grown on gluten-containing media. Knowing the extent of the problem is difficult because when wheat, barley, or rye is used in growth media is generally not known. This is an area that requires much more study.

In the meantime, suppliers of bacteria and bacterial enzymes should be encouraged to disclose the components of growth media to manufacturers. Manufacturers in turn must be aware of the potential for gluten protein fragments to remain in bacteria grown on gluten-containing media. The bacteria should be deactivated and tested with a competitive ELISA. Although not perfect, this would provide some indication of the presence of residual gluten.

In the United States, the FDA should clarify for industry whether growth media for bacteria (and other microorganisms) are considered ingredients, incidental additives, or processing aids, and whether the use of wheat, barley, or rye precludes the food containing the bacteria from being labeled as gluten free. If the use of gluten-containing growth media does not preclude a gluten-free labeling claim, then the FDA should clarify how products containing bacteria and bacterial enzymes should be tested to ensure safety for gluten-free consumers.

PRACTICE IMPLICATIONS

Some individuals with gluten-related disorders use probiotics and digestive enzymes. Consumers should be encouraged to use only those products labeled as gluten free. If consumers have concerns about the growth media used for bacteria and bacterial enzymes, they should be encouraged to contact manufacturers and ask questions. These questions may include:

- Are any of the bacteria or bacterial enzymes used in your product grown on gluten-containing growth media?
- If so, do you test bacterial strains and final products for gluten contamination using a competitive ELISA?

Until more is known about residual gluten from growth media, erring on the side of caution and avoiding products containing ingredients grown on gluten-containing growth media may be best if the product is not tested for residual gluten using a competitive ELISA.

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STATEMENT OF POTENTIAL CONFLICT OF INTEREST

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