FoodTrack

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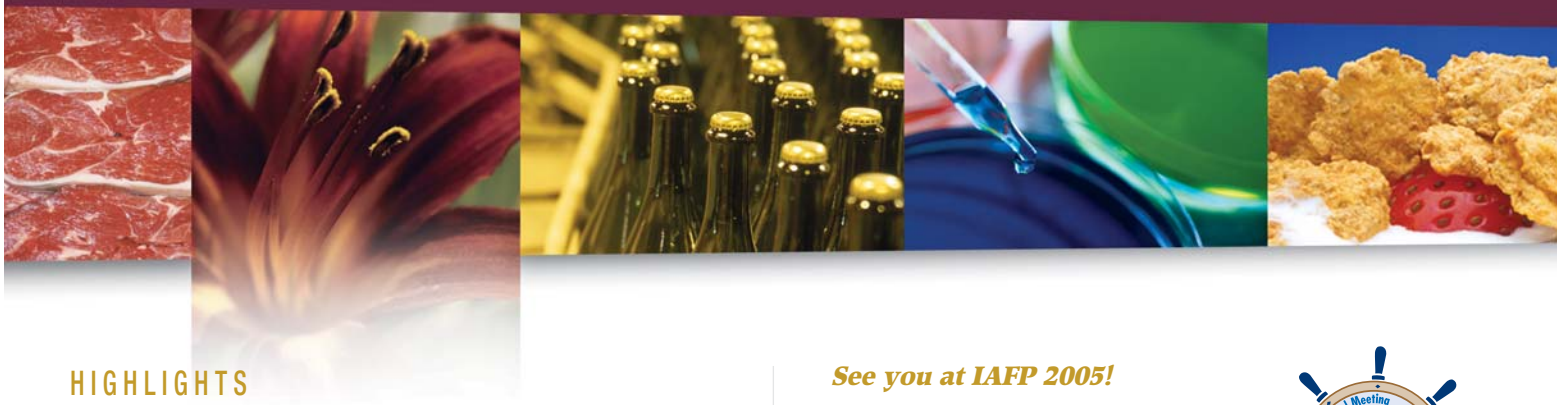

HIGHLIGHTS

See you at IAFP 2005!

Come visit booth #406 to find out how Fisher can be your "one source, infinite solutions" provider for food diagnostics products and services. Be sure to fill out and bring the card inside. You may catch something!

Microbial Forensics: A Solid Solution to Foodborne Illness Cases

Sarah Helber, Ph.D., Richard Jaffe, Ph.D., MT (ASCP) and Thomas Reynolds

It is now commonplace to see litigation of cases involving persons allegedly acquiring foodborne illness or an infectious disease at a public place or food establishment. The evidence presented in these cases is sometimes circumstantial at best and may result in unwarranted awards to plaintiffs because of public sympathy toward the victims. In order for companies to gather the facts and respond to these lawsuits, they will need to fully utilize the power of technology currently available in a modern biology laboratory.

Commonwealth Biotechnologies, Inc. (CBI) of Richmond, VA has addressed the need for determining the origin or presence of a contaminant in food samples or the environment. CBI has put in place a service which follows the basic principles of forensic science when testing microbial samples, applying the best science to the problem.

The unique application of microbial testing techniques to uncover or determine links between outbreaks, or to solve crimes, is a new and burgeoning field.

The Center for Disease Control and Prevention in 1999 estimated that food-borne diseases cause approximately 76 million illnesses, 325,000 hospitalizations and 5,000 deaths in the United States each year.

Food products can be contaminated at many sites in the process of moving food from the "farm to the table." What are the sources? Food may be delivered contaminated (particularly raw foods of animal origin), or the food handler may be infected with the pathogen and transmit it to the food. In addition, the food may be prepared with water contaminated by rodents, insects, or other vermin. The number of cases mistakenly reported as resulting from foodborne illness when they were in fact due to other causes (i.e. the Flu) is not known or recorded.

One of the most serious forms of food-borne illness comes from *E. coli* 0157:H7 contamination of meat products. When a suspected outbreak of *E. coli* 0157:H7 occurs, proper epidemiological assignment of different isolates is necessary for its identification and containment. CBI has the capability to compare the genetic patterns of different *E. coli* 0157:H7 isolates to determine their relatedness and therefore their classification as part of a specific outbreak. This can be an enormous benefit to companies since the vast majority of suspected illnesses are often unrelated to a particular outbreak. Proof of that is paramount in order to address many of the post–outbreak issues and to minimize the potential negative impact to the company. The procedure used by CBI to compare *E. coli* 0157:H7 outbreak samples with suspect samples is known as pulse-field gel electrophoresis (PFGE). This procedure is a well-established, universally accepted method for the identification of *E. coli* 0157:H7 isolates. To perform PFGE, intact chromosomal DNA is purified from E. coli 0157:H7 isolates. The DNA is treated with enzymes that break the DNA at specific sequences, and these large pieces of DNA are separated using agarose gel electrophoresis. The migration patterns of the DNA isolates are then compared, and the identification of identical and non-identical bands between isolates is used to determine their relatedness.

CBI can utilize this method to perform microbial forensics on ground beef suspected of *E. coli* 0157:H7 contamination. In a recent case CBI received 8 *E. coli* 0157:H7 isolates that had been previously confirmed as being related to a recent outbreak. The samples were tested using the PFGE method. Samples run in lanes 2-9 are the 8 related samples from the outbreak and the sample run in lane 10 is unrelated to the outbreak. The PFGE gel shows with two different enzymes the banding pattern is the same on the outbreak samples (lanes 2-9 on the gel Figure 1A and 1B). However, at the same time the sample in lane 10 has a different banding pattern. Since the sample run in lane 10 has a completely different banding pattern from the original isolate, this indicates that the sample isolate could not have been part of the same outbreak as that of the eight related isolates.

Continued on back cover

FOCUS ON FISHER'S FINEST

Mark Mullins

A nine-year Fisher veteran, Mark Mullins assumed the role of Industry Director for Food and Agriculture in February 2005. The University of Tennessee alumnus has previously served Fisher customers as a sales representative and regional sales manager.

Fisher: What is your industry expertise?

Mark: *I've been involved in the food industry for 22 years, including the last nine here at Fisher. I've worked in the lab and sold lab supplies as well.*

Fisher: What issues do you see as being especially challenging to our customers?

Mark: *New ways to manage costs. This goes beyond price, although that's an important factor. Vendor consolidation is a big issue. Customers want a distributor who delivers the best overall value, is reliable, and provides the ultimate error-free buying experience.*

Fisher: How does Fisher meet the needs of our customers in this industry?

Mark: *First we identify their critical issues. Then we offer solutions using Fisher's resources and expertise.*

Fisher: What advice would you give to our sales reps serving this industry?

Mark: *Build the customer relationship starting at the grassroots level. Know the customer and his needs. Be customer-focused in everything. Show the customer how we can help him to meet his objectives, today and in the long-term.*

Fisher: Give us an example of some innovative products in our portfolio.

Mark: *Hygiena is one—a full line of ATP environmental hygiene testing products. This is a national exclusive for Fisher, one of our many differentiated products used in the food industry.*

Fisher: Sum up Fisher's commitment to serving the food industry.

Mark: *Fisher has brought food industry veterans on board who bring the experience and knowledge that will help us develop the tools to meet our customers' needs. From product sourcing, to supply chain, to cost management, we'll be there for our customers.*

Multistage Syringe Filters Clarifiy the Winemaker's Sampling Issue

By Matt Dunleavy Millipore Corporation, Marcia Manix, Kendall Jackson, and Martin Schultz

For connoisseurs, wineries and even the occasional imbiber, enjoying a glass of wine depends entirely on the quality of its taste, scent and color. A good wine should be smooth and gratifying, leaving a pleasant memory of its taste long afterwards. Achieving that level of excellence is the responsibility of the winery, the wine taste-master and more and more, the quality control technician. When winemakers (or vintners) speak of quality, they are ultimately concerned with a wine's acidity and alcohol content. Maintaining these components at the appropriate levels is both an ancient art and a modern science.

The art of winemaking has brought into play the experience, intuition and instincts of master tasters whose discriminatory attributes—their noses, eyes, palates, and tongues—have given them special sensitivity to the aroma, color and taste of a wine. Scientific knowledge, through its improved testing and sampling capabilities, has given wineries better control over the quality of winemaking.

From Grape to Glass: One Step at a Time

Maintaining quality standards by constantly sampling product at each stage of the process is the key to the success of Kendall-Jackson, a northern California vineyard and the 10th largest in the U.S. Not surprisingly, Kendall-Jackson today employs syringe filtering to set up sample batches for analysis. Syringe filtering with microfiltration membranes enhances the control of the quality of the product at each level of production. This starts with the improvement of the clarity of samples in preparation for instrumentation analysis such as spectrophotometric or chromatographic methods.

> The process of clarifying samples consisting of high particulate levels has been markedly improved by augmenting the syringe filter with a graduated prefilter.

These multistage filters eliminate contaminants that could block separation media too early or prejudice analysis results. Exact calculation of the component levels in each sample (including acids, sugar and alcohol) are critical both because of their effects on the taste, flavor and quality of the wine and for calculating their tax consequences. Among the tests Kendall-Jackson performs are mass spectrometry, spectrophotometry and gas chromatography. To protect instruments, prepare samples and maintain productivity, the winery's

quality assurance division employs Millex® HPF syringe filters from Millipore Corporation. HPF syringe filters clarify considerably larger sample quantities without risk of becoming fouled.

Throughout the wine-production process, batches are constantly examined. Following picking and crushing, the liquid components are inspected before subsequent processing. The presence of sugar in the key fermentation stage is checked every day. This is the stage when sugar is converted into alcohol, and this phase of the operation receives particularly close scrutiny since if the alcohol level rises too rapidly it will destroy the yeast. Acidity is also closely observed. As fermentation progresses and the flavor intensifies, bacteria of the malo-lactid strain are introduced and the resultant reduction of L-malic acid monitored.

Millipore Millex HPF Syringe Filters

There are a number of reasons to carry out batch or sample filtration before analysis. For one thing, high-quality analysis requires that any solids present be eliminated beforehand. Also, where additional analysis, such as column chromatography, is needed, filtration improves the results. And especially in the case of spectrophotometric analysis, filtering neutralizes undissolved carbon dioxide, removing a potential source of data inaccuracy. Clarifying wine samples for analysis before the introduction of multilayered filters involved a complicated and costly two-stage operation involving either centrifuging or a two-stage filter with final filtering using a 0.45µm membrane filter. For its own sample clarification process, the Kendall-Jackson quality assurance team was employing an alternative type of multilayered syringe filter. Yet because it would take from two to five filters to process one 25mL sample for volatile acid (VA) testing, this apparatus was considered not cost effective. It was with this experience in mind that other types of filters were evaluated.

Cost and Time Savings

To gain Kendall-Jackson's confidence, trials were held specifically comparing an existing filter and the HPF Millex filter. In order to qualify the HPF Millex filter for these applications, side-by-side chemical analyses were performed. Both devices contain a graduated prefilter and a 0.45µm final membrane filter. The existing device's prefilter included a composite filter rated from 20µm down to 0.7µm. The HPF Millex filter contained a glass fiber prefilter rated from 10µm down to 0.17µm.

> The side-by-side comparison revealed that the HPF Millex filter offered a 300 percent improvement in the precision of the pre-sample process and reduced the need for additional filtering by a factor of four.

Precise % Alcohol Measurements

In addition, both filters were used to clarify alcohol and VA samples prior to analysis. Accurate measurement of the % alcohol (ethanol) content is essential to wine production, and the alcohol content must be listed on every bottle. The Bureau of Alcohol, Tobacco and Firearms sets the tax rate on wine based on this reported percentage, which ranges from below 14 percent to above 17 percent of alcohol. Incorrect reporting could lead to the producer incurring heavy fines. Thus, it is critical that the clarification process not alter the alcohol content of the sample.

Large-capacity vineyards such as Kendall-Jackson demand cost-effective and efficient processes to assess wine quality at each stage of the wine-producing operation. Preparing the appropriate pre-filtered samples for analysis forms a major component of this process.

Determination of Fermentable Sugars in Beer Wort

Randy Benton, Metrohm-Peak, Inc

From the smallest home brewery to the largest commercial ones, all beer is produced in virtually the same manner. In brief, to first create wort, sugars have to be extracted from malted barley in a process called mashing. Next the wort is heated for a period of time, cooled and yeast added. The yeast then consumes the sugars for food and the result is beer production. The sugars released during the production of the malted barley, mashing, are a clear indication about how efficiently the fermentation process will proceed. If the level of fermentable sugars is low, the brewer can then add the appropriate amounts of sugars. Traditionally, in larger breweries, refractive index (R.I.) has been used to quantify fermentable sugars in wort. This method, however, has several disadvantages—one of which is no or poor baseline resolution for the sugars of interest. Another disadvantage of R.I. is the excessive heat, sometimes as high as 900°C, needed for proper identification and quantitation of sugars which may lead to the premature degradation of maltotriose. This degradation and the corresponding lower-than-expected values could potentially be very costly for larger brewing operations.

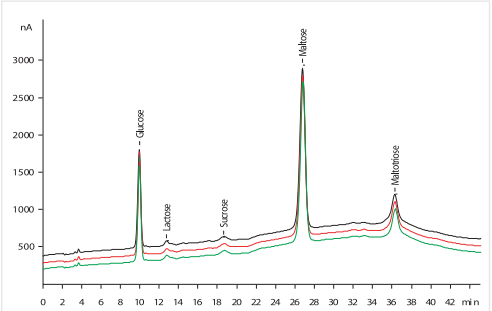

Figure 1

Experimental Conditions

A Metrohm Advanced Ion Chromatography system equipped with 2 Model 818 IC Pumps, Metrohm Spark Triathlon autosampler, 817 Bioscan Pulsed Amperometric Detector and 830 IC interface was used for the analysis. A binary high-pressure gradient system was used for separation of the fermentable sugars in wort. Data acquisition and processing were performed with Metrohm IC-Net 2.3 software. Bioscan temperature was controlled at 320º C. The column employed for analysis was the Metrosep Carb-1 analytical column (250mm x 4mm). The eluent used for the gradient was Eluent A, 180mM sodium hydroxide Eluent B 500 mM sodium hydroxide. The injection volume was 20uL. The gradient employed for the separation was linear starting with 0% "B" and finishing with 75% "B" at 33 minutes. Flowrate used was 1mL/min.

Figure 1. Chromatogram overlay of Fermentable Sugars in Beer Wort

Results and Discussion

Traditonally the brewing industry has used R.I. for the determination of fermentable sugars. This method has a severe drawback, that of very poor resolution of the sugars of interest. Pulsed amperometric detection of carbohydrates has been available for a number of years and has been shown to be an effective tool. Figure 1 is a clear indication of the resolution which can be expected using the instrument, as well as the ability of the instrument to reproduce the analysis.

Conclusion

This application note demonstrates that a qualitative, analytically precise method has been developed for determination of fermentable sugars in beer wort. These sugars are among the many carbohydrates, from monosaccharides to polysaccharides that can be analyzed using the Metrohm Bioscan Pulsed Amperometric Detector.

Metrohm Advanced Ion Chromatography system

metrohm-peak
ion chromatography

To purchase any of the products mentioned in either of these articles, please contact your Fisher Sales Representative or visit ***www. fishersci.com.***

Microbial Forensics: A Solid Solution to Foodborne Illness Cases

Sarah Helber, Ph.D., Richard Jaffe, Ph.D., MT(ASCP) and Thomas Reynolds

Continued from cover

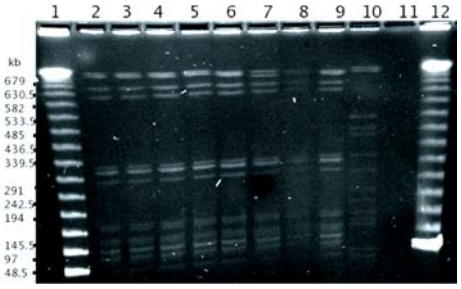

Figure 1A. *PFGE of E. coli O157:H7 isolates with Xba1 digests. E. coli O157:H7 DNA was purified and digested with 60 units of XbaI enzyme and subjected to electrophoresis on a 1% agarose gel using the Pharmacia Biotech Gene Navigator. Lanes 1 and 12 represent lambda DNA molecular weight markers (x1000). The sizes of the markers are indicated on the left. Lanes 2-9 represent eight E. coli O157:H7 isolates that have been previously shown to be related to a single E. coli O157:H7 outbreak. Lane 10 DNA E. coli O157:H7 sample which is complete unrelated to the outbreak and Lane 11 represent a media control to insure purity of the isolates.*

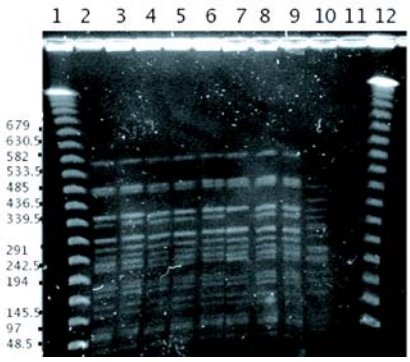

Figure 1B. *PFGE of E. coli O157:H7 isolates with BlnI digests. E. coli O157:H7 DNA was purified and digested with 60 units of XbaI enzyme and subjected to electrophoresis on a 1% agarose gel using the Pharmacia Biotech Gene Navigator. Lanes 1 and 12 represent lambda DNA molecular weight markers (x1000). The sizes of the markers are indicated on the left. Lanes 2-9 represent eight E. coli O157:H7 isolates that have been previously shown to be related to a single E. coli O157:H7 outbreak. Lane 10 represents an sample DNA from an unrelated strain of E. coli O157:H7 and Lane 11 represents a media control to ensure purity of the isolates.*

(see **Figure 1A** and **1B**).

In another client case, CBI was engaged to test environmental samples during a recent outbreak of Norwalk virus. Using PCR and DNA sequence analysis, CBI confirmed the presence of Norovirus in the sample. Performance of DNA sequence analysis allows for isolate identification as well. During 2004 CDC reported received samples from 21 different outbreaks on 17 cruise ships.

Of the 21 outbreaks 9 were confirmed by lab analysis of stools, 3 were attributable to bacterial agents and 9 were unknown etiology. Norwalk virus and its close relatives are the most frequent cause of foodborne illness in this country,—23 million cases each year.

CBI recently worked with Allegheny County (PA) health officials in Pennsylvania to test the efficacy of decontamination of an 800-student, K-12 school in Coraopolis, PA. The school was closed March 23, 2004 after many children were absent or became ill during classes. About 180 people connected to the school came down with the illness. A norovirus, a common cause of such outbreaks, was suspected. After closing the doors the school was disinfected. Subsequently, CBI tested the "hot spots" and confirmed the virus was destroyed and the school reopened.

In summary, CBI has in place validated microbial forensic tools to determine the presence or absence of suspect pathogens in food or food preparation surfaces. The general examples of cases presented in this article show how these principles of microbiology testing and forensic science can be applied to everyday problems of food safety.

Commonwealth Biotechnologies, Inc.

Note on CBI's service. CBI has the unique ability to marry its expertise in microbiology testing with its know-how in Forensic testing. CBI operates a DNA Forensic and Microbial Forensic laboratory. The Fairfax Identity Laboratory (FIL) is a Division of CBI and is certified by the National Forensic Science Technology Council for Human Forensic DNA analysis. CBI brings validated, state-of-the-art molecular testing to the food industry.

Please contact your Fisher Sales Representative for more information.

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❍ Alcoholic Beverages ❍ Poultry
❍ Confectionary ❍ Baked Goods
❍ Cereals/Grains ❍ Commerical Lab
❍ Other _____

2) The most important technical service Fisher can provide as a distributor *(rank them 1-4)*

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____ Food Track newsletter
____ New lab start-up support
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3) I am currently participating in a Frequent Buyer Program

❍ Yes *(go to question 4)* ❍ No (go to question 5)

4) I would be interested in seeing a similar program from Fisher Scientific

❍ Yes ❍ No

5) Do you know who your Fisher sales rep is?

❍ Yes, I know my representative
❍ No, I do not know, please contact me
❍ No, I do not know, please do not contact me

6) Please send me a Fisher Scientific Food Diagnostics & Testing Catalog

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7) Would you like to receive Food Track electronically?

❍ Yes ❍ No

If yes, please be sure to give your e-mail address above.

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