2014 ASBC Annual Meeting

Specific detection of bacteria and yeasts in downstream process control of beer and related products

(Gudrun Vogeser, PIKA Weihenstephan GmbH, Pfaffenhofen/Ilm, Germany)

75th ASBC Annual Meeting
June 4–6, 2014
Palmer House, a Hilton Hotel
Chicago, IL

Detection of Beer and Soft-Drink Spoiling Bacteria and Yeast in Different Products

Today, we are seeing a new level of complexity within craft brews and beer mixes. To create novel flavor profiles, brewers are intentionally adding lactic acid bacteria and wild yeasts to their brews, or unintentionally creating new contamination risks through the addition of non-traditional ingredients and flavorings. This brings new challenges to not only monitor the integrity of their yeast/bacteria blends, but also to ensure that these organisms do not contaminate their other products.

By using different media, optimized for a given sample type, we can improve the detection limits for spoilage organisms as well as influence the time-to-result. Through these parameters, the choice of enrichment medium directly influences the safety and overall quality of the product. However, determining the correct medium may result in many parallel analyses.

The optimal method would be ***one unique medium and one method for all types of samples***. PCR analysis supports verification of results and identification of spoilers.

Detection of Spoilage Bacteria

Aim of Study

Evaluation of detection time and usability of different enrichment media to detect typical spoilage organisms in beer and soft drinks

Experimental Design

Two experiments from independent laboratories:

Series I 5 ml sample in tubes*, Series II : 20 ml sample in swing-top bottles (author)

Analysed beverages	Series I: 1: beer 2: Soft Drink pH < 4.4 3: Soft Drink pH > 4.4	Series II: 1: beer 2: Coca Cola 3: Fanta
Dilution	***beverage + broth in equal volumes***	
Replicates	***Tubes: 5 each, Bottles: 2 each***	
Incubation	***25 °C ± 2.0°C, aerobic; tubes anaerobic for lactic acid bacteria***	
Evaluation	***Visual inspection twice per day for turbidity and/or color change***	

Results

Series I: 5 ml sample plus 5 ml broth in tubes

Series I Beer	Spiking conc. cells/ml	FastOrange™ B broth	VLB-S7-S broth	NBB broth
Lactobacillus	25 x10e7	2	3	n.a.
Pediococcus	10 x10e7	2	3	n.a.
Acetobacter	5x10e11	2	2	2
Series I Soft Drink pH < 4.4	**Spiking conc. cells/ml**	**FastOrange™ B broth**	**OFS Agar**	**Orange Serum Agar**
Lactobacillus	3x10e11	2	3	3
Pediococcus	3x10e9	2	3	3
Acetobacter	n.d.	2	2	2
Series I Soft Drink pH > 4.4	**Spiking conc. cells/ml**	**FastOrange™ B broth**	**VLB-S7-S broth**	
Lactobacillus	4x10e11	2	3	
Pediococcus	5x10e9	2	3	
Acetobacter	6x10e11	2	2	

*Data from Report of International Research Center, Beer and beverages XXI Century, Russia
**Life Technologies, USA

Results

Series II: 20 ml sample plus 20 ml FastOrange B™ broth, spiked with 20 µl of turbid culture. Bottles from left to right:
Becks lager beer blank/spiked // Fanta blank/spiked // CocaCola blank/ spiked


L. brevis (B033) 48 h


L. brevis 3 days


Sediments in beer 3 days


Acetobacter (B099) 48 h


Acetobacter 3 days


Sediments in Fanta 3 days

Pichia (H026) and Saccharomyces (H037): no growth in FastOrange B™ broth after 5 days

Conclusions

a) Lactic acid bacteria *Lactobacillus* and *Pediococcus* were detectable one day earlier in FastOrange™ B broth, than in the other tested media.

b) Growth of acetic acid bacteria was similar in all tested media but depends on sample type.

c) FastOrange™ B broth detects growth of spoilers in different beverages by turbidity and a color change.

Non-Brewing Wild Yeasts

Aim of Study

Detection of Non-Saccharomyces Wild Yeasts within Brewer's yeast

Experimental Design

A selective Agar medium based on the EBC method Cu^{2+} Differentiation (4.2.5.1.) is used to distinguish between brewer's yeast and wild yeasts. Different pure yeast cultures were plated both on selective Agar and yeast medium Agar(YPG, used as growth control) .

Results

Growth was monitored for a total number of 50 yeast strains. Plates A and B below show results on copper Agar; plating on YPG showed growth of all strains, data not shown. Size of colonies was rated from A (excellent) via B (medium) to C (poor). Color indicates if growth expectations are met (green: OK, red: Fail).

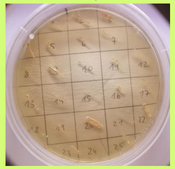
Conclusions

The differentiation between brewer's Saccharomyces yeast and wild Saccharomyces strains can be reliably achieved by use of an optimized copper containing selective agar medium.

Yeast strains on plate A Growth expected for non-Saccharomyces	Position on plate	Growth after days of incubation 5	7
Candida intermedia	1	C	C
Candida intermedia	2	C	C
Candida intermedia	3	C	C
Candida parapsilosis	4	C	C
Candida parapsilosis	5	B	B
Debaryomyces hansenii var. fabryi	6	B	B
Pichia anomala	7	A	A
Pichia anomala	8	B	B
Rhodotorula mucilaginosa	9	B	B
Candida parapsilosis	10	C	C
Saccharomyces kluyveri	11	C	C
Candida haemulonii	12	no	no
Debaryomyces hansenii	13	C	C
Dekkera bruxellensis	14	no	no
Hanseniaspora uvarum	15	C	C
Metschnikowia bicuspidata	16	C	C
Pichia cactophila	17	no	no
Pichia membranefaciens	18	B	B
Dekkera anomala	19	no	no
Issatchenkia orientalis	20	A	A
Pichia novergensis	21	B	B
Saccharomyces bayanus (control strain 1, NO growth expected)	22	no	no
Saccharomyces cerevisiae diastaticus (control strain 2)	23	no	no
Saccharomyces pastorianus (control strain 3)	24	no	no
Torulaspora delbrueckii	25	no	no

Plate A: Selective Agar medium, yeast strains as listed in table left

Plate B: Selective Agar medium, yeast strains as listed in table right



Yeast strains on plate B Inhibiton expected for Saccharomyces	Position on plate	Growth after days of incubation 5	7
S. cerevisiae (bottom fermenting) UG 1001-034	1	no	no
S. cerevisiae WLP 802 Czech Budejovice Lager Yeast	2	no	no
S. cerevisiae WLP 838 Southern Germany Lager Yeast	3	no	no
S. cerevisiae Wyeast 2000-Budvar	4	C	C
S. cerevisiae Wyeast 2001-urquell	5	no	no
S. cerevisiae Wyeast 2112 Californian Lager	6	no	no
S. cerevisiae Wyeast 2124 Bohemian Lager	7	no	no
S. Hefe (top fermenting) brwewer's yeast brewery 8	8	no	no
S. cerevisiae (top fermenting) Safale S04	9	C	C
S. cerevisiae (top fermenting) brewer's yeast 1	10	no	no
S. cerevisiae (top fermenting) brewer's yeast 2	11	no	no
S. cerevisiae (top fermenting) brewer's yeast 3	12	no	no
S. cerevisiae (bottom fermenting) brewer's yeast 4	13	no	no
S. cerevisiae (bottom fermenting) 34/70	14	no	no
S. cerevisiae Wyeast 2278 Czech Pils	15	no	no
S. cerevisiae (top fermenting) brewer's yeast 5	16	no	no
S. cerevisiae (top fermenting) brewer's yeast 6	17	no	no
S. cerevisiae (bottom fermenting) brewer's yeast 7	18	no	no
S. cerevisiae WLP 820 Oktoberfest Lager Yeast	19	no	no
S. cerevisiae WLP833 German Bock Lager Yeast	20	B	B
S. cerevisiae Wyeast 1762 Belgian Abbey II	21	no	no
S. cerevisiae Wyeast 2042 Danish Lager	22	B	B
S. cerevisiae Wyeast 2206 Bavarian Lager	23	no	no
S. cerevisiae Wyeast 2308 Munich Lager	24	no	no
S. cerevisiae Wyeast 3725 PC Bier de Garde	25	no	no

Brettanomyces (Dekkera) and POF Wild Yeasts

Aim of study

Detection and classification of off-flavor Wild yeasts

Experimental Design

Three spoilage yeasts isolated from contaminated soft drink samples, suspected to be Dekkera (anamorph Brettanomyces), were analysed in parallel with

a. Phenolic off Flavor test (POF test, EBC 2.3.9.5):
Dekkera usually reacts POF positive. POF positive yeasts are able to decarboxylate ferulic acid resulting in the formation of 4-vinyl guaiacol which can be detected by a clove-like aroma. Sniff test was done by three persons.

b. Growth on a modified Dekkera medium, FastOrange™ BRETT Agar***
Growth of most non-Dekkera yeasts is inhibited, growth of Dekkera is shown by production of a color change in the Agar from violet to yellow due to acid production.

c. PCR analysis
Genus Brettanomyces yeast is specifically detected with SO detection Kit H Dekkera (Brettanomyces) Screening (Life Technologies™, USA)

All tests were applied as described by Analytica-EBC or by the manufacturer.

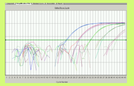
Results

Data are shown in the following table.

Sample number	Color/growth on FastOrange™ ***BRETT*** Agar		PCR Analysis	POF Test NP: non-phenolic; Phen: phenolic
	Day1	Day 2		
F_140303-7	***Deep yellow***	***Deep yellow***	***Dekkera positive***	***NP***
F_140303-8	***No growth***	***No growth***	***Negative***	***Phenolic clove like smell***
F_140303-9	***No growth***	***No growth***	***Negative***	***NP***


FastOrange™ BRETT Agar


PCR Analysis



Conclusions

a. Phenolic Off Flavour test detects Wild yeasts which are capable to produce ferulic acid, a characteristic which is not necessarily expressed by Brettanomyces yeast, and on the other side not only by them. Therefore this test may help in Wild yeast detection, but not in their identification.

b. BRETT Agar can be used both for detection and identification of Dekkera as it shows a clearly visible yellow color due to its growth and acid production.

c. PCR analysis gives most reliable results for both the detection and identification of Dekkera yeast, but needs special laboratory instrumentation.

***PIKA Weihenstephan, Germany, base EBC Dekkera medium 5.1.3.3

Dr. Gudrun Vogeser
PIKA Weihenstephan GmbH
Raiffeisenstrasse 31a
85276 Pfaffenhofen
GERMANY
www.pika-weihenstephan.de

PIKA WEIHENSTEPHAN
PRODUCTS FOR INNOVATIVE CONTROL AND ANALYTICS