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Evaluation of culture media (YM & YNB) and copper sulfate concentrations for wild yeast detection

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INTRODUCTION

Identification of wild yeast contaminants in brewing yeast and in fermentation is of critical importance in maintaining yeast purity and fermentation integrity.

There are numerous media that are commercially available for the detection of wild yeast. This poster will focus on the comparison of YM (Yeast and Mould) and Yeast Nitrogen Base (YNB) media.

Emphasis will be along the nutrient content of the media and how that influences the amount of Copper Sulfate ($CuSO_4$) needed to inhibit brewing yeast while still allowing wild yeast to grow.

DEVELOPMENT

Pure culture strains and real brewery samples were used for this study. Pure culture brewing and wild yeast strains were preserved in 50% glycerol solution and kept frozen in an ultra freezer at -80 ºC.

Where -80 ºC yeast stocks were used, cultures were grown in liquid broth containing yeast extract, peptone and dextrose (YPD broth) for 24 hours with continuous shaking at 150 rpm. 500 µL of cultures were plated on each media. Media formulations are shown in Table #1.

Table #1: Media Formulations (weight per litre)

Yeast Nitrogen Base (YNB) + $CuSO_4$	**Yeast and Mould (YM) + $CuSO_4$**
Yeast Nitrogen Base (w/o amino acids): 6.7 g	Yeast Extract: 3.0 g
Dextrose: 20.0 g	Glucose: 10.0 g
Agar: 20.0 g	Agar: 20.0 g
	Malt extract: 3.0 g
	Peptone: 5.0 g
$CuSO_4$: 50 mg	$CuSO_4$: Variable

TESTING

Concentration of Copper Sulfate in YNB media was kept constant at 50 mg/L, a previously optimized and published concentration.

Recommended values of Copper Sulfate in YM media from previously published methods lead to the conclusion that higher concentrations of Copper Sulfate would be needed to inhibit brewing yeast growth. Copper Sulfate concentrations ranging from 250 – 500 mg/L were tested.

Both the YNBC and the YM plates were incubated aerobically at 28 ºC for 5 days. Observations on the presence or absence of yeast growth was made at 3 and 5 days. Results can be observed in Table #2.

OBSERVATIONS

Brewing yeast strains were not inhibited on either YM or YNB (5 day growth) at 250 mg/L and 50 mg/L of Copper Sulfate respectively (Table #2).

At a minimum Copper Sulfate concentration of 300 mg/L, all brewing yeast growth was inhibited on YM media, YNB media was not tested at levels above 50 mg/L. All levels of Copper Sulfate allowed for the growth of wild yeast on both media (YM and YNB) after 3 days incubation.

DATA

Table #2: Pure cultures plated on YNB and YM with various concentrations of Copper Sulfate.

Media	**YNB**		**YM**		**YM**				
$CuSO_4$ (mg/L)	50		250		300	325	350	375	500
"Cu" (mg/L)	20		100		120	130	140	150	200
Brewing Yeast	3 Days (CFU)	5 Days (CFU)	3 Days (CFU)	5 Days (CFU)	3 and 5 Day (CFU)				
A	0	0	34	100	0	0	0	0	0
B		12	TNTC	TNTC					
C		0	0	0					
D		0							
E		5							
F		0							
G		48	1	11					
Wild Yeast	3 Days (CFU)	5 Days (CFU)	3 Days (CFU)	5 Days (CFU)	3 and 5 Day (CFU)				
Zygosaccharomyces rouxii spp.	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
Pichia anomala spp.									
Saccharomyces diastaticus spp.									

Based on the results obtained from the pure culture tests, samples were collected at the brewery and tested under similar conditions. Brewery samples were collected at various stages of the brewing process.

This study found that the pure culture brewing yeast were able to grow on YM media with Copper Sulfate concentrations below 300 mg/L, therefore we plated brewery samples on YM media containing Copper Sulfate concentrations between 300 - 350 mg/L.

Optimal concentration of $CuSO_4$ added to YM media was found to be 325 mg/L (130 mg/L of Cu2+). See Table #3.

Table #3: Brewery samples plated on YM with various concentrations of Copper Sulfate. Counts shown as colony forming units per 100 mls.

CFU/100 ml										
Samples			**3 days**			**4 days**			**5 days**		
#	Sample #	Wort stream	300ppm	325ppm	350ppm	300ppm	325ppm	350ppm	300ppm	325ppm	350ppm
1	Fermenting beer-1	2500	0	0	0	0	0	0	0	0	0
2	Fermenting beer-2	3600	200	100	100	200	100	100	400	200	200
3	Green beer-1	0400	210	50	0	400	70	0	490	100	0
4	Green beer-2	0100	10	10	10	10	10	10	10	10	10
5	Green beer-3	0600	0	0	0	20	10	0	20	10	0
6	Green beer-4	2500	0	0	0	0	10	0	0	0	0
7	Green beer-5	3600	0	0	0	10	0	0	0	0	0
8	Recovered beer-1	0100	0	0	0	0	0	0	0	0	0
9	Recovered beer-2	0100	190	230	230	230	350	250	240	350	280
10	Recovered beer-3	0100	170	100	10	170	120	40	170	120	50

MEDIA COMPARISON

To evaluate any difference in colony forming units per 100 ml between the YM media (+325 mg/L $CuSO_4$), and YNB (+50mg/L $CuSO_4$), duplicate samples were plated on both media.

Table #4: Brewery samples plated on YM (+ 325 mg/L $CuSO_4$) and YNB media (+ 50 mg/L $CuSO_4$).

			CFU/100 ml at 3 days		**CFU/100 ml at 5 days**	
Sample #	**Process Stage**	**Wort stream**	**YM 325 mg/L**	**YNB 50 mg/L**	**YM 325 mg/L**	**YNB 50 mg/L**
FE26	Fermenting	6400	0	100	0	100
FE27	Fermenting	0900	0	0	0	100
FE28	Fermenting	0400	0	0	0	0
FE30	Fermenting	6400	0	0	100	100
FE31	Fermenting	0400	0	0	0	0
PS408	Aging	0100	0	0	0	0
PS407	Aging	0900	0	0	0	0
PS406	Aging	0600	20	90	20	110
PS401	Aging	0600	0	0	0	0
PS316	Aging	0300	10	0	0	0
PS309	Aging	3600	0	40	0	40
PS308	Aging	0500	10	0	10	10
PS307	Aging	2500	0	0	0	0
PS306	Aging	0600	10	0	20	0
PS303	Aging	7100	0	10	0	0
PS302	Aging	0100	0	0	0	0
PS301	Aging	0400	0	20	0	10
BT94	Bright beer	0100	58	178	62	226
BT221	Bright beer	0900	0	0	0	0
BT222	Bright beer	0400	2	2	2	2
BT233	Bright beer	0600	0	0	0	0
BT236	Bright beer	7149	0	0	0	0

CONCLUSIONS

There are slight differences in colony forming units per 100 ml when comparing YM (325 mg/L $CuSO_4$) and YNB media. This is most likely attributed to different wild yeast strains that were selected on the media.

To effectively inhibit brewing yeast growth, we found that the YM media recipe requires 6.5 times higher concentration of Copper Sulfate compared to YNB, which may be attributed in part to recipe differences/ nutritional requirements.

YM media nitrogen sources include peptone and amino acids present in the yeast extract. YNB is a minimal synthetic medium that lacks some nutrients such as histidine, methionine, tryptophan and a source of carbohydrate.

In general YNB will have a much lower nitrogen content when compared to YM, therefore, the nutrient sources provided are not ideal for either brewing or wild yeast growth.

When using YNB without amino acids, yeast are required to biosynthesise their own amino acids or conduct transamination, which has an energetic cost and may result in reduced growth.

Due to the reduced nutritional composition of YNB media, diminished levels of Copper Sulfate are required to inhibit brewing yeast.