

X- α -Gal Medium

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CONCLUSIONS

1. Collaborators were able to correctly identify pure ale yeast cultures.
2. Collaborators had difficulty in identifying pure lager yeast cultures or the presence of lager yeast in a mixture.

RECOMMENDATIONS

Repeat the collaborative study with closer attention to incubation time, incubation temperature, and plating procedure.

The subcommittee was charged with evaluating the use of the X- α -gal method for differentiating between ale and lager yeast (1).

PROCEDURE

Each collaborator received five yeast samples on agar slants, labeled A-E. The agar slants contained pure ale yeast, pure lager yeast, or a mixture. Collaborators were instructed to prepare basal medium agar plates, allow the plates to harden and dry, and spread 100 μ l of a 20-mg/ml X- α -gal stock solution. The plates were then stored in the dark for 30-60 min. The five yeast samples were each suspended into sterile distilled water and serially diluted until a 100- μ l aliquot spread onto the prepared agar plates contained 100-200 cells. After four days of incubation at 25-27°C in the dark, the numbers of blue-green and white colonies on the plates were counted. In addition, each collaborator was asked to comment on the method.

RESULTS AND DISCUSSION

Fifteen collaborators submitted results (Tables I-V). Only fourteen responses were used, as one of the collaborators could

not get sufficient yeast growth. Sample A (pure lager yeast) was identified correctly by 10 of the collaborators. The other four collaborators detected white colonies, suggesting the presence of ale yeast (Table I). Samples B and C (Tables II and III, pure ale yeast) were correctly identified by all the collaborators. Sample D (mixture of 98% ale and 2% lager yeasts) was correctly identified

TABLE II
Colony Counts and Collaborator Conclusion for Yeast Sample B^a

Collaborator	Total Plate Count	White Colonies	Blue-Green Colonies	Conclusion
1	88	88	0	Ale
2	257	257	0	Ale
3	136	136	0	Ale
4	98	98	0	Ale
5	202	202	0	Ale
6	150	150	0	Ale
7	141	141	0	Ale
8	109	109	0	Ale
9	608	608	0	Ale
10	67	67	0	Ale
11	243	243	0	Ale
12	183	183	0	Ale
13	236	236	0	Ale
14	180	180	0	Ale

^a Results are the average of duplicate plates.

TABLE III
Colony Counts and Collaborator Conclusion for Yeast Sample C^a

Collaborator	Total Plate Count	White Colonies	Blue-Green Colonies	Conclusion
1	102	102	0	Ale
2	200	200	0	Ale
3	97	97	0	Ale
4	81	81	0	Ale
5	132	132	0	Ale
6	160	160	0	Ale
7	101	101	0	Ale
8	127	127	0	Ale
9	217	217	0	Ale
10	45	45	0	Ale
11	200	200	0	Ale
12	77	77	0	Ale
13	262	262	0	Ale
14	240	240	0	Ale

^a Results are the average of duplicate plates.

TABLE I
Colony Counts and Collaborator Conclusion for Yeast Sample A^a

Collaborator	Total Plate Count	White Colonies	Blue-Green Colonies	Conclusion
1	35	1	34	Lager
2	71	0	71	Lager
3	126	0	126	Lager
4	58	0	58	Lager
5	108	0	108	Lager
6	196	63	133	Mixed
7	123	0	123	Lager
8	157	0	157	Lager
9	491	62	429	Mixed
10	128	10	118	Mixed
11	37	13	24	Mixed
12	130	0	130	Lager
13	148	1	147	Lager
14	100	2	98	Lager

^a Results are the average of duplicate plates.

TABLE IV
Colony Counts and Collaborator Conclusion for Yeast Sample D^a

Collaborator	Total Plate Count	White Colonies	Blue-Green Colonies	Conclusion
1	96	95	1	Mixed
2	306	302	4	Mixed
3	582	581	1	Mixed
4	107	104	3	Mixed
5	182	160	22	Mixed
6	204	202	2	Mixed
7	121	121	0	Ale
8	105	105	0	Ale
9	522	522	0	Ale
10	117	117	0	Ale
11	181	178	3	Mixed
12	286	278	8	Mixed
13	150	150	0	Ale
14	120	116	4	Mixed

^a Results are the average of duplicate plates.

TABLE V
Colony Counts and Collaborator Conclusion for Yeast Sample E^a

Collaborator	Total Plate Count	White Colonies	Blue-Green Colonies	Conclusion
1	71	1	70	Lager
2	158	0	158	Lager
3	118	0	118	Lager
4	100	0	100	Lager
5	284	0	284	Lager
6	155	0	155	Lager
7	106	0	106	Lager
8	95	0	95	Lager
9	640	9	631	Lager
10	148	39	109	Mixed
11	284	0	284	Lager
12	191	0	191	Lager
13	182	0	182	Lager
14	188	0	188	Lager

^a Results are the average of duplicate plates.

by nine of the collaborators (Table IV). The other five collaborators identified the sample as pure ale yeast. Sample E (pure lager yeast) was correctly identified by thirteen collaborators (Table V). The summary of results of the collaborative study is shown in Table VI.

TABLE VI
Summary of Results

Sample	Strain Identity	Verdicts			Percent Correct
		Ale	Lager	Mixed	
A	Lager	0	10	4	71
B	Ale	14	0	0	100
C	Ale	14	0	0	100
D	Ale/Lager	5	0	9	64
E	Lager	0	13	1	93

The above data indicate that some of the collaborators had difficulty reading the plates when lager yeasts were present. Some of this difficulty could have resulted from not allowing sufficient incubation time before analyzing the plates. Some of the collaborators reported that, depending on yeast growth, five or six days were required to achieve the blue-green appearance of all the colonies of a pure lager yeast. In addition, some of the collaborators reported problems with spreading the X- α -gal reagent evenly on the plates.

LITERATURE CITED

1. Tubb, R. S., and Liljestrom, P. L. A colony-colour method which differentiates α -galactosidase positive strains of yeast. *J. Inst. Brew.* 92:588, 1986.