Evaluation of Multinitrogen Source Media for Wild Yeast Detection in Brewing Culture Yeast[1]

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ABSTRACT

A multinitrogen source medium called CLEN (employing cadaverine, lysine, ethylamine, and nitrate as the sole nitrogen sources) was developed based on published data. Theoretically, CLEN would support the growth of a greater number of wild yeast species (415 of 469 or 88%) than would lysine medium (355 or 76%). A comparison of means of detecting wild yeast (growth on CLEN, lysine agar, XMACS [xylose, mannitol, adonitol, cellobiose, and sorbitol] agar, Lin's agar, yeast-maltose agar with cycloheximide, copper sulfate agar, and yeast-maltose agar at 37°C) was made with known pure cultures of a variety of yeast species and with brewery production yeast cultures. CLEN supported the growth of more yeast species and the growth of larger numbers of wild yeast in brewery samples than did lysine medium. Growth on CLEN was more rapid than that on lysine agar. None of the media tested is capable of detecting all wild yeast; several in combination are needed for a thorough examination.

Keywords: Media, Microbiology, Nitrogen source, Plating, Wild yeast, *Saccharomyces*

Wild yeasts have long presented a problem in breweries. Contamination of pitching yeast with wild yeast is often more difficult to detect than contamination of yeast with bacteria because the biochemical and physiological characteristics of wild and culture yeasts are often almost identical (3). Wild yeast can lead to flavor and other problems, which can cause spoiled or poor-quality products (5). A range of procedures have been developed for wild yeast detection. These include the addition of cycloheximide to media (6), the use of lysine as sole nitrogen source (9), and the use of other specialized media such as Lin's medium (7), copper sulfate medium (8,10), and XMACS (xylose, mannitol, adonitol, cellobiose, and sorbitol) medium (4). Growth at 37°C also has been used (11,12).

Two basic mechanisms are used in these means of detecting wild yeast. Cycloheximide, copper sulfate, and Lin's media and 37°C incubation techniques all prevent growth of culture yeast (at least lager yeast) but support growth of some wild yeast species. Lysine and XMACS media supply nutrients that brewing culture yeasts are not able to utilize but that are available to other yeast species. In the case of lysine medium, the nitrogen source provides the selectivity. XMACS is based on carbon source selectivity.

The object of the work reported here was to develop a medium that would detect additional wild yeast species by inclusion of additional nitrogen sources, as well as maintain detection of those wild yeasts detected by lysine agar. A similar approach was used previously with carbon sources to design the XMACS medium (4), and an improved nitrogen source medium was suggested in that article. Data from Barnett et al (2) indicate that *Saccharomyces cerevisiae* cannot utilize lysine, nitrite, nitrate, ethylamine, cadaverine, creatine, or creatinine as sole nitrogen sources. Therefore, the use of any of these compounds or a combination of them as the sole source of nitrogen in a growth medium would theoretically detect yeasts other than *S. cerevisiae*. In fact, lysine agar represents one application of this concept.

Attempts were made to determine whether the above hypothesis could be applied practically. Two approaches were chosen. In one, pure cultures of known yeast species were tested. In the other, an examination of actual brewery yeast samples was conducted.

EXPERIMENTAL

Media

Lysine agar (9) contained yeast carbon base (YCB, Difco Laboratories, Detroit, MI) at 11.75 g/L, lysine·HCl at 2.5 g/L (Sigma Chemical, St. Louis, MO), and Difco purified agar at 20 g/L. YCB and lysine were dissolved at 10× the final medium concentration and filter-sterilized (0.22-μm pore size). The filter-sterilized solutions were added to the sterile molten agar just before the plates were poured.

Lin's agar (7) contained Difco yeast extract at 4.0 g/L, malt extract at 2.0 g/L (Difco), peptone at 2.0 g/L (Difco), dextrose at 10.0 g/L (Sigma), K_2HPO_4 at 1.0 g/L (Sigma), NaCl at 0.5 g/L (Mallinckrodt, St. Louis, MO), crystal violet at 0.4 ppm (Mallinckrodt), fuchsin-sulfite mixture at 0.1 g/L (Mallinckrodt), and Difco agar at 20 g/L.

Copper sulfate agar (10) was prepared by aseptically adding a filter-sterilized solution of $CuSO_4$ (Sigma) to yeast-maltose (YM) agar (Difco) just before plates were poured. The final copper sulfate concentration was 200 mg/L.

Cycloheximide agar was prepared by aseptically adding 5.0 ml of 1.0% (w/v) filter-sterilized cycloheximide (Sigma) to 500 ml of YM agar just before plates were poured.

XMACS agar (4) contained xylose (10 g/L), mannitol (10 g/L), adonitol (10 g/L), cellobiose (10 g/L), sorbitol (10 g/L) (all from Sigma), yeast nitrogen base without amino acids (7 g/L) (YNB-AA, Difco), and purified Difco agar (20 g/L). The YNB-AA and the five sugars were made up in 10× solutions and filter-sterilized. The filter-sterilized solutions were added to the sterile molten agar just before plates were poured.

Multinitrogen source agars were prepared by combining the various nitrogen sources with 10× solutions of YCB, filter-sterilizing, and aseptically adding them to molten agar just before plates were poured. All nitrogen sources were obtained from Sigma. When ethylamine or cadaverine was present, the source solution of YCB and nitrogen was adjusted to pH 5.8 ± 0.2 with concentrated HCl. The following concentrations of nitrogen compounds were used, both when the nitrogen compounds were used alone or in combination: lysine·HCl (2.5 g/L), ethylamine (0.9 g of 70% solution per liter), KNO_3 (1.4 g/L), KNO_2 (1.2 g/L), cadaverine (1.4 g/L [free base] or 2.4 g/L [diHCl]), creatine (1.8 g/L), and creatinine (1.5 g/L).

Brewing yeasts were obtained from the yeast brinks of eight different breweries. Samples were obtained at one-month intervals over a period of three months.

Pure cultures (Table I) were obtained from either the Stroh culture collection or the American Type Culture Collection (ATCC, Rockville, MD). Cultures were maintained on YM agar slants (Difco).

Plating of pure cultures was done by streaking on the various media. Production yeast samples were diluted to a total count of 100 million cells per milliliter, and a 0.1-ml aliquot of that dilution was spread over the surface of the various media with a glass "hockey" stick. The plates were incubated at 27°C (or 37°C in the case of YM agar) for seven days. After incubation, plates were inspected for colonies of wild yeast or bacteria with a Quebec colony counter (Reichert-Jung, Inc., Buffalo, NY).

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Colonies more than 1 mm in diameter under given incubation conditions were scored as positive for growth. Suspect colonies were picked, restreaked on the same medium, and reincubated before they were transferred to YM agar slants for storage and future work. Differentiation between wild yeast and bacteria was accomplished with direct microscopy.

Medium Design

Hypothesis development was accomplished with the aid of a computer data base developed from data contained in Barnett et al (2) and described previously (4).

Statistical Analysis

The statistical significance of differences in growth results on different media were determined using the Kruskal-Wallis test (1) on results grouped by month.

RESULTS AND DISCUSSION

The theoretical effectiveness of various nitrogen source combinations were assessed using a computer data base management program (4) into which data from Barnett et al had been entered. The objective was to select nitrogen sources that cannot support the growth of brewing culture yeast but that can support the growth of additional nonbrewing species not detected by components already in a medium. What could not readily be predicted was whether the addition of one of the nitrogen sources could have an inhibitory effect on the growth of some yeast species. There was also uncertainty because some species are listed as "unknown" or "weak" or "variable" for growth on some nitrogen sources. Thus, the theoretical predictions had to be tested in practice. Two major approaches to this testing could be taken. In the first case, the growth of known pure strains on various selective media could be compared. In the second, actual production yeast samples could be examined with several different wild yeast media and the results could be compared. Both types of testing were carried out.

TABLE I
Yeast Cultures Used in Pure Culture Growth Support Studies

Code	Culture[a]	Designation[b]
1	*Rhodotorula* sp.	Y-8
2	*Saccharomyces cerevisiae*	Y-10
3	*S. diastaticus (S. cerevisiae)*	Y-78
4	*S. willianus (S. cerevisiae)*	Y-80
5	*S. pastorianus (S. cerevisiae)*	Y-81
6	*S. ellipsoideus (S. cerevisiae)*	Y-83
7	*S. c.* var. *ellipsoideus (S. cerevisiae)*	Y-93
8	*S. bayanus* var. *pastorianus (S. cerevisiae)*	Y-94
9	*S. bayanus (S. cerevisiae)*	ATCC 26250
10	Stroh B (*S. cerevisiae*)	NA
11	*Candida mycoderma (C. vini)*	Y-85
12	*Torulaspora fermentati (T. delbrueckii)*	Y-87
13	*C. lambica (Pichia fermentans)*	ATCC 38617
14	*S. bailii (Zygosaccharomyces bailii)*	ATCC 8766
15	*Kluyveromyces marxianus*	ATCC 10606
16	*P. farinosa*	ATCC 2252
17	*Torulopsis versatilis (C. versatilis)*	ATCC 20191
18	*K. bulgaricus (K. marxianus)*	ATCC 16045
19	*C. utilis (Hansenula jadinii)*	ATCC 9950
20	*Hanseniaspora uvarum*	ATCC 32369
21	*Schizosaccharomyces pombe*	ATCC 2476
22	*C. intermedia*	ATCC 9442
23	*T. delbrueckii*	ATCC 10662
24	*P. membranaefaciens*	ATCC 10653
25	*S. rouxii (Z. rouxii)*	ATCC 10687
26	*C. humulis*	ATCC 22992
27	*Brettanomyces claussenii*	ATCC 10562
28	*B. lambicus*	ATCC 10563
29	*Dekkera bruxillensis*	ATCC 36234
30	*B. anomalus*	ATCC 10559
31	*S. exiguus*	ATCC 10599
32	*D. intermedia*	ATCC 24196
33	*B. custersianus*	ATCC 34446
34	*Citeromyces matritensis*	ATCC 34087
35	*K. lodderii*	ATCC 24206
36	*P. mucosa*	ATCC 22541
37	*R. aurantiaca*	ATCC 10655
38	*S. japonicus*	ATCC 10660
39	*Sporobolomyces roseus*	ATCC 24257
40	*Trichosporon pullulans*	ATCC 9331
41	*S. malidevorans*	ATCC 46954
42	*Trigonopsis variabilis*	ATCC 10679
43	*Torulopsis cantarelli (C. cantarelli)*	ATCC 36588
44	*Torulaspora melissophila (Debaryomyces melissophilus)*	ATCC 24614

[a] Name in parentheses is species as it appears in Barnett et al (2).
[b] Species from the Stroh culture collection (designated Y) or the American Type Culture Collection.

Theoretical Considerations and Preliminary Laboratory Work

Data in Barnett et al (2) indicated that the incorporation of creatine and/or creatinine into a multinitrogen source medium containing cadaverine, lysine, ethylamine, nitrite, and nitrate would, at most, increase the number of yeast species that would grow on that medium by one. Therefore, creatine and creatinine were not used in the multinitrogen source media.

The remaining nitrogen sources of interest were each tested individually at several concentrations in single nitrogen source media using a battery of test yeast cultures. Results indicated that the presence of nitrite in a medium resulted in growth inhibition of several of the pure cultures of yeast. Media containing nitrite were excluded from further investigation.

The nitrogen sources of the medium were thus limited to cadaverine, lysine, ethylamine, and nitrate (CLEN). Data in Barnett et al indicated that 355 of the 469 yeast species (76%) should grow when lysine alone is used as the sole source of nitrogen. With the addition of cadaverine, ethylamine, and nitrate to the lysine medium, an additional 60 yeast species were expected to grow, for a total of 415 or 88%. Among these 60 additional species are several that have been reported as brewery wild yeast: *E. nana, R. araucariae, D. melissophila* (previously known as *Torulaspora melissophila*), *R. mucilaginosa, P. farinosa, T. variabilis, C. albidus*, and *T. pullulans*.

The species listed as lysine negative and NO_3 positive included *Candida bacarum, C. buffonii, C. sonckii, Citeromyces matritensis, Leucosporidium nivalis, Rhodosporidium malvinellum, Rhodotorula araucariae, R. aurantiaca, R. diffluens, R. pilatii, Sporidiobolus johnsonii, Sporobolomyces holsaticus*, and *S. puniceus*.

The species listed as lysine negative, ethylamine positive, and NO_3 negative or variable included *C. auriculariae, C. karawaiewii, C. philyla, C. susciphila, Debaryomyces melissophila, Pichia mucosa*, and *S. singularis*. The species listed as lysine variable and NO_3 positive included *Bullera tsugae, C. foliarum, C. lacticondensi, Cryptococcus albidus, C. kuetzingii, C. lupi, C. macerans, L. antarcticum, L. frigidum, L. scottii, R. diobovatum, R. infirmo-miniatum, R. paludigenum, R. toruloides, R. glutinis, R. graminis, R. javanica, S. salmonicolor, Sterigmatomyces halophilus, S. nectairii*, and *Trichosporon pullulans*.

The species listed as lysine variable, ethylamine positive, and NO_3 negative or variable included *C. cantarellii, C. meliciosophila, C. membranaefaciens, Clavispora lusitaniae, C. ater, Kluyveromyces lodderii, Lipomyces tetrasporus, Phaffia rhodozyma, P. farinosa, R. mucilaginosa, R. pilimanae, S. indicus*, and *Trigonopsis variabilis*. The species listed as lysine variable, weak, or unknown; cadaverine positive; NO_3 negative or variable; and ethylamine negative or variable included *C. gastricus, Eeniella nana, P. abadieae, and Schizosaccharomyces malidevorans*.

Of the 469 yeast species described in Barnett et al, 16 had maximum growth temperatures below 25°C, and six more species were questionable for growth at temperatures above 25°C. No

wild yeast medium could detect these species if the incubation was conducted at 27° C. Therefore, excluding *S. cerevisiae*, only 452 species were considered in this study. Lysine agar would theoretically support the growth of 347 of these (77%), and CLEN agar would theoretically support the growth of 401 of the 452 species (89%). Indications of "variable" or "unknown" in Barnett et al were assumed to be negative responses in this study.

Testing of Pure Culture Strains

The pure yeast cultures listed in Table I were plated on the various test media and growth was observed (Table II). Three of the pure yeast cultures listed in Table I that were, according to data in Barnett et al (2), capable of utilizing lysine as a sole source of nitrogen did not produce colonies greater than 1 mm in diameter on lysine agar. Those yeasts were *C. cantarellii* (previously known as *T. cantarellii*), *Zygosaccharomyces rouxii,* and *S. malidevorans.* All three of these yeasts grew on CLEN agar.

Of the *Saccharomyces* species, only *S. exiguus* grew on either lysine agar or CLEN agar. Only one culture listed as variable for growth on lysine in Barnett et al (2) grew on lysine agar (*Brettanomyces custersianus*). Of those yeasts listed as negative or variable for growth on lysine medium, the following grew on CLEN agar: *C. matritensis, K. lodderii, P. mucosa, R. aurantiaca, D. melissophila,* and *T. variabilis.*

At least one of the other wild yeast media supported growth of the cultures that grew on CLEN except for *S. malidevorans* and *D. melissophila. T. pullulans,* although theoretically capable of growth on CLEN, grew only on the control medium (YM agar).

Testing of Brewery Samples

The results from plating yeast brink samples from eight breweries on the various wild yeast media each month for three months appear in Table III. No colonies were detected on XMACS after seven days of growth, but by 14 days, more colonies were

TABLE II
Growth of Pure Yeast Cultures Observed on Various Wild Yeast Media

Culture Code[a]	Medium or Condition					
	CLEN[b]	Lysine	XMACS[c]	Cycloheximide	$CuSO_4$	YM[d] at 37° C
1	+	+	+	+	+	+
2	–	–	–	–	+	+
3	–	–	–	–	+	+
4	–	–	–	–	+	+
5	–	–	–	–	–	–
6	–	–	–	–	+	+
7	–	–	–	–	+	+
8	–	–	–	–	+	+
9	–	–	–	–	–	+
10	–	–	–	–	–	+
11	+	+	+	–	+	+
12	+	+	+	–	+	–
13	+	+	–	–	+	+
14	+	+	+	–	+	–
15	+	+	+	+	+	+
16	+	+	–	–	–	+
17	+	+	–	–	+	+
18	+	+	–	+	+	+
19	+	+	+	–	+	+
20	+	+	+	+	+	–
21	–	–	–	–	+	+
22	+	+	+	–	+	–
23	+	+	+	–	+	–
24	+	+	–	–	+	–
25	+	–	+	–	+	–
26	–	–	–	–	–	–
27	+	+	–	+	+	+
28	+	+	–	–	+	+
29	+	+	–	+	+	–
30	+	+	+	+	+	+
31	+	+	–	+	+	–
32	+	+	–	+	+	–
33	+	+	–	+	+	+
34	+	–	+	–	+	–
35	+	–	–	–	+	+
36	+	–	+	–	–	–
37	+	–	+	–	–	–
38	–	–	–	–	–	+
39	–	–	–	–	–	–
40	–	–	–	–	–	–
41	+	–	–	–	–	–
42	+	–	–	+	+	+
43	+	–	–	+	+	–
44	+	–	–	–	–	–

[a] Cultures 1-8, 11, and 12 are from the Stroh culture collection. The rest, with the exception of culture 10, are from the American Type Culture Collection.
[b] Cadaverine, lysine, ethylamine, and nitrate.
[c] Xylose, mannitol, adonitol, cellobiose, and sorbitol.
[d] Yeast-maltose agar.

seen than on any other medium (virtually no change between 7 and 14 days was noted on the other media). Although each of the five carbon sources in XMACS was present in a concentration similar to that of the main carbon source (usually glucose) in each of the other media, it appeared that yeasts were unable to use the carbon sources in XMACS as readily.

The results in Table III were grouped by month and tested for significant differences using the Kruskal-Wallis test, which has been recommended for such comparisons (1). After seven days of incubation, the XMACS and cycloheximide results were similar and significantly lower than results from Lin's and lysine media. Results from growth at 37°C and on CLEN were similar but higher. CLEN and copper sulfate medium gave the highest results. Although the CLEN results were not different from either copper sulfate medium or growth at 37°C, results from the latter two do differ significantly. The results after 14 days of growth follow a pattern very similar to that of the seven-day results, except that XMACS changed from the lowest result to the highest.

CLEN is clearly an improvement over lysine medium. It detected approximately twice as many wild yeast colonies in the brewery samples. The colonies also grew faster; they were detectable in three to five days versus five to seven days. After seven days of incubation, the wild yeast colony diameters were 30–50% larger on CLEN agar. The explanation is twofold. CLEN is not only able to support the growth of some yeast species that cannot grow on lysine medium (Table II), it also provides additional nutrients to many species that can grow on lysine agar.

The wild yeasts found with the various media were picked and placed in pure culture. Each isolate was streaked onto each of the wild yeast media to determine the uniqueness, if any, of each

TABLE III
Number of Wild Yeast Colonies Detected in Brewery Brink Yeast Samples After Growth at 27°C for 7 Days on Various Wild Yeast Media

		Medium or Condition						
Month	Plant	CLEN[a]	Lysine	XMACS[b]	Cycloheximide	$CuSO_4$	YM at 37°C[c]	Lin's
1	1	5	3	0 (14)	1	1	3	0
	2	2	1	0 (7)	0	1	2	1
	3	0	0	0 (6)	0	4	1	0
	4	2	1	0 (9)	0	1	2	0
	5	9	3	0 (9)	0	2	0	0
	6	0	0	0 (3)	0	0	0	3
	7	0	0	0 (1)	1	4	1	0
	8	4	1	0 (5)	0	6	3	1
Month 1 total		22	9	0 (54)	2	19	12	5
2	1	5	4	0 (12)	1	8	7	0
	2	3	0	0 (14)	0	7	4	1
	3	0	0	0 (7)	0	6	0	0
	4	2	0	0 (10)	0	2	5	0
	5	6	3	0 (7)	0	3	0	0
	6	0	0	0 (1)	0	2	4	6
	7	1	0	0 (4)	2	4	2	0
	8	2	1	0 (8)	0	4	3	2
Month 2 total		19	8	0 (63)	3	36	25	9
3	1	5	2	0 (5)	0	7	4	0
	2	1	0	0 (7)	0	5	4	0
	3	1	1	0 (5)	0	3	2	0
	4	4	2	0 (8)	0	5	3	0
	5	6	4	0 (9)	0	1	0	0
	6	0	0	0 (7)	0	5	0	4
	7	1	1	0 (5)	1	5	0	0
	8	3	1	0 (7)	0	3	0	1
Month 3 total		21	11	0 (53)	1	34	13	5
7-day total		62 cd[d]	28 b	0 a	6 a	89 d	50 c	19 b
14-day total		62 c	28 b	170 d	6 a	89 c	50 c	19 b

[a] Cadaverine, lysine, ethylamine, and nitrate.
[b] Xylose, mannitol, adonitol, cellobiose, and sorbitol. Number in parentheses is number of wild yeast colonies detected after 14 days of incubation at 27°C. The number of wild yeast colonies on media other than XMACS after 14 days of incubation were approximately the same as the seven-day count.
[c] Yeast-maltose agar. Colonies were detected after seven days at 37°C.
[d] Totals in each row with the same letter are not significantly different at the $P = 0.05$ confidence level by the Kruskal-Wallis test.

TABLE IV
Number of Isolates Positive or Exclusive for Growth After Seven Days When Isolates From Brewery Samples Were Replated on All Media

Result	CLEN[a]	Lysine	XMACS[b]	Cycloheximide	$CuSO_4$	YM[c] at 37°C	Lin's
Positives[d]	49	37	82	18	119	68	53
Exclusive[d]	2	0	20	2	19	6	0

[a] Cadaverine, lysine, ethylamine, and nitrate.
[b] Xylose, mannitol, adonitol, cellobiose, and sorbitol.
[c] Yeast-maltose agar.
[d] Total after seven days.

medium's ability to detect wild yeast. The results are summarized in Table IV. Care must be taken in interpreting these results because the particular organisms found in the breweries sampled may not be typical or representative of the situation elsewhere. In fact, the pattern of results for each brewery tends to be similar for all three months, suggesting that the results for each plant are likely repeated findings of the same wild yeasts rather than different contaminants in each sampling. Most of the isolates grew on more than one medium, although growth on a single medium was the most frequent individual result. Forty-nine isolates grew on one media, 42 grew on two, eight grew on three of the media, 23 grew on four, 21 grew on five, 11 grew on six, and 0 grew on all seven media. The results indicate a certain amount of redundancy among some of the media tested. Copper sulfate and XMACS media and 37°C incubation supported the growth of the largest numbers of isolates. In terms of isolates that grew only on single media, indicated in the table as exclusive growth, XMACS and copper sulfate media had similar numbers that were noticeably larger than those of the other media. The fact that neither lysine nor Lin's medium produced exclusive growth was examined. As in the case with the pure yeast cultures, all of the isolates that grew on lysine medium also grew on CLEN. All of the isolates that grew on Lin's medium also grew on copper sulfate medium. The lysine and CLEN results are not at all surprising because anything that grows on lysine medium should also grow on CLEN (which contains lysine) unless it is inhibited by another component. At least for these samples, CLEN was clearly a complete (and superior) replacement for lysine medium.

CONCLUSIONS

CLEN agar performed significantly better than lysine agar in supporting the growth of wild yeast in brewery samples. Not only were more colonies detected, but growth was more rapid. The greatest number of wild yeast colonies in brewery samples were seen on XMACS, but growth on this medium took longer than on the other media. With the brewery samples, the isolates that grew on only one of the test media were noted. CLEN supported the growth of a small number of brewery isolates that did not grow on any of the other media tested. Twenty isolates grew only on XMACS, and 19 grew only on copper sulfate media; the other media had much smaller numbers of isolates that grew on them exclusively. No one medium detected all of the wild yeast.

LITERATURE CITED

1. American Society of Brewing Chemists. Report of the Subcommittee on Statistical Analysis. *Journal* 45:106-109, 1987.
2. Barnett, J .A., Payne, R. W., and Yarrow, Y., *Yeasts: Characteristics and Identification*. Cambridge University Press, New York, pp. 39-59, 1983.
3. Campbell, I. Detection and identification of yeasts. *Tech. Q. Master Brew. Assoc. Am.* 8:129-133, 1971.
4. De Angelo, J., and Siebert, K. J. A new medium for the detection of wild yeast in brewing culture yeast. *J. Am Soc. Brew. Chem.* 45:135-140, 1987.
5. Gilliland, R. B. Deterioration and Improvement of Brewing Yeast. European Brewery Convention Murograph V. Fermentation and Storage Symposium. Zoeterwoede, The Netherlands, pp. 51-65, 1978.
6. Harris, J. O., and Watson, W. The use of controlled levels of actidione for brewing and non-brewing yeast strain differentiation. *J. Inst. Brew.* 74:286-290, 1968.
7. Lin, Y. Detection of wild yeasts in the brewery. III. A New Differential Medium. *Proc. Am. Soc. Brew. Chem.* pp. 69-76, 1974.
8. Lin, Y. Formulation and testing of cupric sulfate medium for wild yeast. *J. Inst. Brew.* 87:151-154, 1981.
9. Morris, E. O., and Eddy, A. A. Method for measurement of wild yeast infection in pitching yeast. *J. Inst. Brew.* 63:34-35, 1857.
10. Taylor, G. T., and Marsh, A. S., MYGP + copper, a medium that detects both *Saccharomyces* and non-*Saccharomyces* wild yeast in the presence of culture yeast. *J. Inst. Brew.* 90:134-145, 1984.
11. Walsh, R. M., and Martin, P. A. Growth of *Saccharomyces cerevisiae* and *Saccharomyces uvarum* in a temperature gradient incubator. *J. Inst. Brew.* 83:169-172, 1977.
12. Walsh, R. M., and Martin, P. A. Detection of wild yeast in *Saccharomyces carlsbergensis*. *J. Inst. Brew.* 84:79, 1978.

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