

Colonies of H₂S-negative *Salmonella* strains appear pinkish-yellow.

Most *Citrobacter* colonies that grow on this medium are yellow without evidence of blackening. Growth of *Enterobacter aerogenes* and *Escherichia coli* is markedly inhibited; colonies that do grow appear yellow without evidence of blackening. Growth of *Proteus*, *Pseudomonas*, *Providencia*, *Alteromonas putrefaciens*, *Yersinia enterocolitica* and *Acinetobacter calcoaceticus* is markedly to completely inhibited on XLT4 Agar. *Shigella* species are partially inhibited and colonies appear red.

Limitations of the Procedure

1. XLT4 Agar is intended for detecting and isolating *Salmonella* based on selectivity and colonial characteristics. Presumed *Salmonella* colonies must be confirmed by biochemical and/or immunological methods. Consult appropriate references for further information.⁵⁻⁷
2. Non-*Salmonella* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Salmonella*. Consult appropriate references.⁵⁻⁷

3. Freshly inoculated plates and plates held over several days may develop multicolored, metallic looking crystals/flecks on the surface. These crystals/flecks do not interfere with the performance of the medium.

References

1. Miller and Tate. 1990. The Maryland Poultryman April:2.
2. Miller, Tate, Mallinson and Schemer. 1991. Poultry Science 70:2429.
3. Miller, Tate, Mallinson and Schemer. 1992. Poultry Science 71:398.
4. Tate, Miller and Mallinson. 1992. J. Food Prot. 55:964.
5. U.S. Department of Agriculture. 1998. Microbiology laboratory guidebook, 3rd ed., Food Safety and Inspection Service, USDA, Washington, D.C.
6. Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. Downes and Ito (ed.) 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ XLT4 Agar Base

USDA

Cat. No. 223420 Dehydrated – 500 g

Difco™ XLT4 Agar Supplement

USDA

Cat. No. 235310 Bottle – 100 mL

Xanthine Agar

(See *Nocardia Differentiation Media*)

YM Agar • YM Broth

Intended Use

YM Agar and YM Broth are used for cultivating yeasts, molds and other aciduric microorganisms.

Summary and Explanation

YM Agar and YM Broth (Yeast Mold Agar and Broth) are prepared according to the formulae published by Wickerham.¹⁻³ Wickerham suggested that YM Broth acidified to pH 3.0-4.0 be used as an enrichment medium for yeasts from populations also containing bacteria and molds.

Media selectivity may be enhanced through acidification or through addition of selective agents. YM Broth may be acidified prior to sterilization. YM Agar should be sterilized without pH adjustment and sterile acid added to the sterile molten medium cooled to 45-50°C. Acidified YM Agar should not be heated. Antibiotics may be aseptically added to the sterile media. Other fungistatic materials, such as sodium propionate and diphenyl may be added to YM Agar to eliminate molds and permit the enumeration of yeasts in mixed populations.

Principles of the Procedure

Yeast extract is a source of trace elements, vitamins and amino acids. Malt extract is a source of carbon, protein and nutri-

ents. Peptone is an additional source of carbon and provides nitrogen and amino acids. Dextrose provides carbon. Agar is the solidifying agent.

Formulae

Difco™ YM Agar

Approximate Formula* Per Liter

Yeast Extract	3.0	g
Malt Extract	3.0	g
Peptone	5.0	g
Dextrose	10.0	g
Agar	20.0	g

Difco™ YM Broth

Consists of the same ingredients without the agar.

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
Difco™ YM Agar – 41 g;
Difco™ YM Broth – 21 g.
 Mix thoroughly.
2. Heat the agar medium with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave the agar and broth media at 121°C for 15 minutes.

User Quality Control

Identity Specifications

Difco™ YM Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.1% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.

Reaction of 4.1% Solution at 25°C:	pH 6.2 ± 0.2
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Difco™ YM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in purified water. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent. At pH adjusted to 3.0-4.0, medium becomes slightly opalescent.

Reaction of 2.1% Solution at 25°C:	pH 6.2 ± 0.2
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Cultural Response

Difco™ YM Agar or YM Broth

Prepare two sets of agar plates or broth tubes (one set pH 6.2, one set adjusted to pH 3.0-4.0) per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY pH 3.0-4.0	RECOVERY pH 6.2
<i>Aspergillus niger</i>	16404	10 ² -10 ³	Good	Good
<i>Candida albicans</i>	10231	10 ² -10 ³	Good	Good
<i>Escherichia coli</i>	25922	10 ² -3×10 ²	Marked to complete inhibition	Good
<i>Lactobacillus rhamnosus</i>	7469	10 ² -3×10 ²	Poor to fair	Good
<i>Saccharomyces cerevisiae</i>	9763	10 ² -10 ³	Good	Good

- To increase selectivity, acidify the medium to pH 3.0 to 4.0 (by adding sterile 10% HCl, tartaric acid or 10% citric acid) or add antibiotics (penicillin 20 units per mL final concentration or streptomycin 40 µg per mL final concentration) using aseptic technique. Acidified agar medium should not be reheated.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate YM Agar plates or YM Broth tubes with sample to evaluate for the presence of yeasts, molds, or aciduric microorganisms. Incubate at 30 ± 2°C for 18-72 hours.

To favor isolation of fermentative species, add a layer of sterile paraffin oil 1 cm deep on the surface of the inoculated broth. Incubate the culture until growth appears and then streak onto YM Agar to obtain isolated yeast colonies. To isolate fermentative and oxidative strains, place acidified inoculated YM Broth on a rotary shaker for 1 or 2 days. This favors yeast recovery while preventing the sporulation of molds.

Expected Results

Examine the plates or tubes for growth. Record YM Agar results as colony-forming units (CFU) per volume of sample. Record YM Broth results as growth or no growth.

References

- Wickerham, 1939. J. Tropical Med. Hyg. 42:176.
- U. S. Department of Agriculture. 1951. Tech. Bull. No. 1029.
- Jong and Edwards. 1991. American Type Culture Collection catalog of filamentous fungi, 18th ed. American Type Culture Collection, Rockville, Md.

Availability

Difco™ YM Agar

AOAC **COMP**
Cat. No. 271210 Dehydrated – 500 g

Difco™ YM Broth

Cat. No. 271120 Dehydrated – 500 g
271110 Dehydrated – 10 kg

YPD Agar • YPD Broth

Intended Use

YPD Agar and YPD Broth are used for maintaining and propagating yeasts in molecular microbiology procedures.

Summary and Explanation

General methods in yeast genetics specify using yeast extract-peptone-dextrose (YPD) medium for cultivating *Saccharomyces cerevisiae* and other yeasts.¹ Yeasts grow well on a minimal medium containing only dextrose and salts. The addition of protein and yeast cell extract hydrolysates allows faster growth

so that during exponential or log-phase growth, the cells divide every 90 minutes.¹

Principles of the Procedure

YPD Agar and YPD Broth contain peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. YPD Agar contains agar as the solidifying agent.