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An aureomycin rose bengal agar for enumeration of yeast and mold in cottage cheese

David James Weakley

To the Graduate Council:

I am submitting herewith a thesis written by David James Weakley entitled "An aureomycin rose bengal agar for enumeration of yeast and mold in cottage cheese." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

W.W. Overcast, Major Professor

We have read this thesis and recommend its acceptance:

J.T. Miles, B.J. Demott

Accepted for the Council:
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(Original signatures are on file with official student records.)

December 16, 1968

To the Graduate Council:

I am submitting herewith a thesis written by David James Weakley entitled "An Aureomycin Rose Bengal Agar for Enumeration of Yeast and Mold in Cottage Cheese." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

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We have read this thesis and recommend its acceptance:

J. T. Miles

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Accepted for the Council:

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Graduate Studies and Research

AN AUREOMYCIN ROSE BENGAL AGAR FOR ENUMERATION OF YEAST AND MOLD IN COTTAGE CHEESE

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

David James Weakley

March 1969

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ABSTRACT

An aureomycin rose bengal agar for the enumeration of yeast and mold in cottage cheese was studied. When mold isolate, yeast isolate, and yeast and mold on cottage cheese counts were compared, preliminary results indicated two of seven levels of aureomycin and rose bengal in peptone dextrose agar gave comparable or higher counts than potato dextrose agar. The two levels of aureomycin and rose bengal in peptone dextrose agar were given more detailed study. The first level contained 20 parts per million aureomycin and 20 parts per million rose bengal in peptone dextrose agar. The results of mold isolate, yeast isolate, and yeast and mold on cottage cheese counts with this agar were compared to counts on potato dextrose agar. The second level contained 30 parts per million aureomycin and 10 parts per million rose bengal in peptone dextrose agar. The mold counts with this level were compared with mold counts on the other aureomycin rose bengal agar and potato dextrose agar. The growth of five bacterial organisms on the 20 parts per million aureomycin and 20 parts per million rose bengal in peptone dextrose agar was studied.

The results of the study show no statistical significant difference for yeast isolate, mold isolate, and yeast and mold on cottage cheese counts between the 20 parts per million aureomycin and 20 parts per million rose bengal in peptone dextrose agar and potato dextrose agar. Also, there was no statistical significant difference between the two

levels of aureomycin and rose bengal in peptone dextrose agar and potato dextrose agar for mold counts. Tests for bacterial growth indicate there was no bacterial growth on aureomycin rose bengal agar.

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CHAPTER I

INTRODUCTION

Cottage cheese is one of the more popular cheeses in the United States. The per capita consumption is approximately 4.5 pounds (7). Cottage cheese is also a perishable dairy food, which, with its high moisture and porous texture, is ideal for growth of bacteria, yeast, and molds. The presence of yeast and mold in cottage cheese imparts stale, musty, moldy, and yeasty flavors to the product. Also, discoloration appears when growth is extensive. Yeast and mold occurrence is indicative of poor sanitation practices in manufacturing and packaging. Therefore, yeast and mold counts may be used as an index of product storability. The presence of yeast and mold becomes an even greater problem for the dairy industry as it becomes more important to lengthen the time interval between production and consumption of cottage cheese.

Much of the literature on the enumeration of yeast and mold has dealt with their detection in butter and soil. The problem of enumeration of yeast and mold in cottage cheese has been given very little attention. At present there is no definite standard or limit for yeast and mold counts but the limit of 10 per gram of cottage cheese is gaining widespread usage. The limit of 10 per gram would seem to be too high since the presence of one mold colony on the surface of the cottage cheese will cause the consumers to discard the carton.

Potato dextrose agar, acidified with tartaric acid to a pH of 3.5

is recommended by American Public Health Association (2) for the enumeration of yeast and mold in cottage cheese. There are several problems encountered with potato dextrose agar in enumerating yeast and mold in cottage cheese. Some of these problems are: excessive spreading of mold colonies, distinguishing yeast colonies from curd particles, lack of growth by some species of yeast and mold at the low pH, and the use of acid causes a precipitation of casein which makes counting difficult at the dilutions used for low count cheese.

The objective of the present study was to determine if a more suitable medium could be developed for the enumeration of yeast and mold in cottage cheese.

CHAPTER II

REVIEW OF LITERATURE

A desirable medium for the enumeration of yeast and mold in cottage cheese should allow the development of maximum numbers and kinds of yeast and mold while limiting spreading radial growth of mold colonies and excluding bacterial growth. Also, the medium should not cause a re-precipitation of casein in cottage cheese.

Definition

Yeast and bacteria are similar in gross colony morphology, methods of cultivation, and biochemical activity (23, 44). Yeasts are unicellular, usually ovoid, or elliptical cells that are large by comparison to bacteria. Some yeasts form ascospores and are known as "true" yeasts; these yeasts have only a limited ability to resist adverse conditions (13). Yeasts in which ascospores are not present are called "false" yeasts. The "false" yeasts reproduce by budding (35). Reproduction by budding consists of an outgrowth from the wall of the parent cell, and then later breaking loose as an individual cell (35). The "false" yeasts are the yeasts of primary importance in dairy products because they ferment lactose with the production of alcohol and carbon dioxide (13, 35). Among the common genera of yeasts that have been found in dairy products are: Candida, Mycoderma, Pichia, Debaryomyces, Sporobolomyces, Torulopsis, and Saccharomyces (23).

Molds are multicellular organisms that are more complex than either

yeast or bacteria. However, the methods of cultivation and biochemical activities of molds are similar to yeast and bacteria (13). Molds reproduce mainly by spore formation (35). Most mold colonies generally appear white, cream, green, black, or brownish because of the pigmentation in the hyphae, collectively called mycelium, or large numbers of asexual spores (13).

Common mold genera that have been found in dairy products are Rhizopus, Mucor, Penicillium, Aspergillus, Cladosporium, Alternaria, and Geotrichum (13, 25). Geotrichum candidum is the most common mold found in dairy products and grows best on products soured by lactic acid production (13).

Both yeasts and molds grow over wide ranges of pH, osmotic pressure, and temperature. However, studies by Thom and Ayers (48) and Macy (25) have shown that pasteurization destroys yeasts and molds. Therefore, the presence of yeasts and molds in products which are pasteurized may be interpreted as an indication of poor manufacturing practices.

Sources and Effects of Yeast and Mold in Cottage Cheese

As long as air is available yeasts and molds can grow on moist acid foods even at temperatures near freezing (13). Marcus (26) reported that molds and yeasts are present in the air floating on dust particles. Heldman et al. (15) used a Casella slit sampler to quantitatively sample the air in a cottage cheese production area. These workers reported the yeast and mold mean counts to be 9 and 111 per 5 cubic feet, respectively. The mean mold count of the air in the cottage cheese production area

was significantly high and detrimental to the shelf life of cottage cheese.

Other sources of yeasts and molds are the floors, ceilings, and walls of all cheese plants. Yeasts and molds are also present in all samples of raw milk (26). However, yeast and mold are destroyed by pasteurization (25, 48). Martin et al. (28) reported contamination of cottage cheese from the makers' hands, impure wash water, or added in improperly pasteurized cream.

To exclude spoilage organisms from cottage cheese, careful personnel, proper pasteurization, sanitation of equipment, clean working areas, cooking temperatures over 120° F, and microbiologically clean wash water are essential (12). Kester (20) found ultra violet light effective in destroying yeasts and molds in the air. There are many sources whereby yeasts and molds may contaminate cottage cheese. However, high quality cottage cheese can be made under carefully controlled plant conditions with a shelf life of at least 15 days, with development of little or no microbial off flavors or visual defects (38).

One of the most difficult problems in marketing cottage cheese is maintaining its freshness and desirable qualities. Cottage cheese with its high moisture and porous texture is ideal for growth of yeasts and molds. Growth of yeasts and molds imports stale, musty, moldy, and yeasty flavors to cottage cheese (13, 34). Discoloration occurs when growth is extensive (34).

The results of surveys made in Iowa (9), Michigan (24), Kansas (28), Connecticut (32), Tennessee (38), and Illinois (49) indicate

the widespread occurrence of yeasts and molds in cottage cheese. The presence of yeasts and molds in cottage cheese causes losses by spoilage (28). Marcus (26) stated that the presence of yeasts and molds in cottage cheese caused losses by forcing producers to take losses in return and dumped cheese. Also, the poor quality of cheeses would cause losses with reduced sales.

Methods of Enumeration

Many of the problems encountered in soil fungi plating methods are similar to problems found in yeast and mold enumeration in cottage cheese. The main problems encountered are: separating bacteria from soil fungi, restricting spreading fungal growth, and poor fungal growth from acid sensitive fungi when an acid medium is used. Therefore, the findings in the detection of soil fungi sould be beneficial in the development of a more suitable medium for yeast and mold detection. Waksman (51) in 1922 developed an acid medium in the dilution plate technique. The medium was peptone dextrose agar with enough sulfuric acid added to lower the pH to 4.0. With this medium most bacteria are inhibited while fungi are allowed to grow due to the fact fungi can grow at a higher acidity than bacteria.

The principle of separating yeast and mold from bacteria by use of an acid media has been used for the detection of yeasts and molds in dairy products. Thom and Church (47) used malt agar in their studies of the aspergilli. Fullmer and Grimes (14) also used malt agar in their studies on yeast growth on synthetic agar media. Hood and White (17) in

their studies of media used for counting yeasts and molds in butter found malt agar acidified with lactic acid to pH 3.5 just prior to pouring of plates to be the most suitable and reliable media. Malt agar was recommended by the committee of the American Dairy Science Association (3, 4) in their 1930 and 1933 reports for the microbiological analysis of butter. Parfitt (42) recommended malt agar for use in obtaining comparative yeast and mold counts in butter. American Public Health Association (1) recommended the use of malt agar for mold counts of dry milk, however, the latest edition (2) has dropped this recommendation.

Potato dextrose agar is presently recommended by American Public Health Association (2) for determinations of yeast and mold in dairy products, including cottage cheese. Shadwich (43) in a study of media found potato dextrose agar gave the most consistent and highest yeast and mold counts in butter.

Malt agar and potato dextrose agar both rely on the addition of an acid to permit detection of yeast and mold without bacteria. However, as is the case with soil fungi an acid medium for detection of yeasts and molds in cottage cheese has certain limitations. Miller (31) reported that on potato dextrose agar some fungi sporulate poorly if at all due to the acid condition of the medium. Studies by Martin (7) and Paharia and Kommedahl (39) give similar evidence that an acid medium generally depresses the numbers and kinds of soil fungi. An acid medium has little effect on restricting the radial colony size of fungi (33, 50). Also, not all bacteria are eliminated by the use of an acid medium (6).

Littman (22) reported molds and yeasts were permitted to grow as non-spreading colonies with the use of crystal violet, streptomycin, and oxgall in potato dextrose agar. Martin (27) reported that oxgall agar suggested by Littman (22) retarded the growth of some fungi and the intense color development of the agar made identification difficult.

Martin (27) suggested the use of rose bengal and streptomycin in peptone dextrose agar recommended by Waksman (51). Martin (27) used rose bengal at the rate of 1:30,000 and 30 micrograms streptomycin per milliliter in his medium. He reported very little bacterial growth with this medium. Also, fungal counts on this medium were higher than when acid was used in the peptone dextrose agar. Disadvantages to this agar were the fact that not all bacteria were eliminated and the reduction in colony size would make identification difficult (27).

Miller and Webb (30) in the isolation of yeasts from soil used lactic acid, rose bengal, and oxgall in potato dextrose agar singly and in combination. The results indicated that rose bengal and oxgall restricted the spreading of colonies thus aiding detection and counting. Also rose bengal and oxgall gave more reliable counts than when lactic acid was used in potato dextrose agar (30). The effectiveness of oxgall depended on the concentration used, on the composition of basal medium, and on the number of potential colony sites on the plates (41).

Dulaney et al. (11) reported that an antibiotic combination in nutrient agar adequately inhibited bacterial growth thus allowing fungal colonies to be identified. The antibiotic combination consisted of penicillin 5 micrograms per milliliter, streptomycin 10 micrograms per

milliliter, polymyxin 40 micrograms per milliliter, bacitracin 20 micrograms per milliliter, and aureomycin 20 micrograms per milliliter. To restrict rapid spreading of fungal colonies 0.03 percent sodium desoxycholate was added to the medium prior to sterilization.

From the substances that investigators have used only oxgall, rose bengal, and sodium desoxycholate have the ability to reduce radial growth of fungus colonies, but none of the three substances are dependable bacterial inhibitors alone (27, 29).

Butler and Hine (6) suggested the use of the antibiotic novobiocin for isolation of fungi from soil. Novobiocin was used at the concentration of 100 micrograms per milliliter of potato dextrose agar. Comparison of novobiocin agar and rose bengal streptomycin agar (27) indicated novobiocin agar to be less effective in preventing the radial growth of spreading colonies (6).

Papavizas and Davey (41) in their evaluation of 41 various media and antimicrobial agents for isolation of soil fungi reported that a modified Warcup medium (53), V-8 juice dextrose yeast extract agar, and dextrose peptone yeast extract agar were the more satisfactory media for isolation of soil fungi. A modification of peptone dextrose agar with rose bengal and streptomycin was found to be satisfactory for isolating high numbers of fungi from soil (41).

Johnson and Manka (19) in later work on isolating soil fungi found that peptone dextrose agar with rose bengal as suggested by Martin (27) and modified by substituting 2 micrograms aureomycin per milliliter for streptomycin, was superior for soil fungi detection. Dextrose

peptone yeast extract agar with 0.25 percent oxgall and 2 micrograms aureomycin per milliliter gave similar results to the modified peptone dextrose agar as suggested by Martin (27).

Aureomycin was substituted for streptomycin in the medium suggested by Martin (27) due to work with antibiotics by Johnson (18). Johnson (18) reported that significantly more fungal colonies developed on media containing 2, 0.5, or 0.25 micrograms aureomycin per milliliter than on media containing streptomycin. Martin (27) reported that streptomycin retarded the growth of a few fungi in pure culture, this fact may account for the difference in fungal colonies. Studies by Hesseltine et al. (16) show that aureomycin can be used as a selective agent against bacteria in the isolation of yeasts from corn steep liquor or from soil. Their studies show that a number of yeasts were not completely inhibited by aureomycin concentrations as high as 500 micrograms per milliliter.

Olson (36) found that yeasts and molds were able to grow well in 2,000 parts per million concentrations of aureomycin while bacteria were inhibited by concentrations of 10 parts per million or less. Olson and Bonner (37) recommended the use of 100 parts per million aureomycin in potato dextrose agar for enumeration of molds and yeasts in cottage cheese. Olson and Bonner (37) reported higher counts with aureomycin agar than with acidified potato dextrose agar. A problem with aureomycin agar may be the spreading of mold colonies.

In summary, the literature suggests that the modification of Johnson and Manka (19) to the medium suggested by Martin (27) would offer the

most promising results for yeast and mold counts in cottage cheese. Peptone dextrose agar with rose bengal and aureomycin added fulfills the nutritional requirements for molds and yeasts (52). The rose bengal is known to restrict spreading colonies (8, 19, 41, 45) and aureomycin has been proven to selectively inhibit both gram-negative and gram-positive bacteria, while not influencing yeast and mold counts (16, 36, 37, 40).

CHAPTER III

EXPERIMENTAL METHODS

Preparation of Media

The essential elements for growth of yeast and mold are supplied by the experimental basal medium. The constituents of this basal medium are listed below with the concentration of each constituent.

Agar	20.0 grams
Potassium Dihydrogen Phosphate	1.0 gram
Magnesium Sulfate	0.5 gram
Peptone	5.0 grams
Dextrose	10.0 grams
Distilled water	1,000.0 milliliters

The amount of each constituent of basal medium was weighed and placed in distilled water. The constituents were brought into solution by heating in a steam cabinet. One hundred milliliters of the solution was dispensed into screw-cap glass bottles and sterilized by autoclaving at 15 pounds pressure for 15 minutes.

Ten grams of rose bengal were added to 1,000 milliliters of distilled water to give a 1 percent solution. The solution was sterilized by autoclaving at 15 pounds pressure for 15 minutes. A chlortetracycline (aureomycin) capsule containing 100 milligrams aureomycin was split open and the contents shaken into sterile distilled water. The rose bengal and aureomycin solutions were stored at 4° C. Fresh

aureomycin solutions were made up weekly. Rose bengal and aureomycin were added by means of a sterile pipette at the desired level to the basal medium prior to pouring of plates.

Potato dextrose (PD) agar acidified with tartaric acid to a pH of 3.5 was used as the comparable medium (2). A commercially prepared potato dextrose agar* (10) was used with the following composition.

Infusion from white potatoes	200 milliliters
Glucose	20 grams
Agar	15 grams
Distilled water	800 milliliters

Preparation of Mold and Yeast Isolates

Preparation of yeast isolates was accomplished by picking, with a wire loop, colonies of yeast from PD agar plates made from old cottage cheese. The colonies were examined microscopically and found to be yeast. The yeast colonies were placed into tryptose phosphate broth and incubated at 21° C for five days.

Mold isolates were prepared by picking, with a wire loop, mold colonies from PD agar plates made from old cottage cheese. The mold colonies were then streaked on nutrient agar slants and incubated at 21° C for five days. After growth of the mold, nutrient broth was poured over the slant. A sterile glass rod was used to scrape the mold growth into the nutrient broth.

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*Bacto-Potato Dextrose Agar. Difco Laboratories, Inc., Detroit 1, Michigan.

Species of bacteria used in this study were: Escherichia coli, Streptococcus lactis, Aerobacter aerogenes, Lactobacillus casei, and Pseudomonas fragi. Lyophilized cultures of these bacteria were obtained from the Microbiological Laboratories of the Department of Dairying and activated by the addition of one milliliter of litmus milk and incubated for twenty-four hours at the optimum temperature for each organism.

Sampling Procedure

Sampling of the yeast suspension and the nutrient broth mold suspension was accomplished by making serial dilutions using 99 milliliter water blanks (2). Duplicate platings were made using aureomycin rose bengal (AR) agar and PD agar. Approximately 15 milliliters of each of the agars were mixed with a gentle rotary motion and allowed to solidify. After solidification the plates were inverted and incubated at 21° C for five days. Counts were then made using a Quebec Colony Counter.

Sampling procedure for the litmus milk, containing the five previously mentioned organisms was conducted by plating directly from the litmus milk to the petri plates. AR agar was immediately poured into the plates. After mixing and solidification, the plates were inverted and incubated for 48 hours at optimum temperature of each organism. Comparison counts of the organisms present in the litmus milk were made on Plate Count agar (2).

Cottage cheese was sampled by weighing on a torsion balance 10 grams of the sample into a sterile Virtis Homogenizer container and covered. Ten milliliters of sterile 2 percent sodium citrate solution were

added to the weighed samples with sterile pipettes. The samples were then homogenized for approximately one minute. Duplicate platings were made from the samples using AR agar and PD agar. Approximately 15 milliliters of the agars were poured into each plate. The plates were mixed and allowed to solidify at which time the plates were inverted and incubated at 21° C for five days. Yeast and mold counts were made at this time using a Quebec Colony Counter.

Statistical Analysis

Statistical analysis of the data were based on the methods outlined by Steel and Torrie (46).

CHAPTER IV

RESULTS AND DISCUSSION

The objective of this study was to develop a more suitable medium for the enumeration of yeast and mold in cottage cheese. Research by Johnson and Manka (19) indicated that peptone dextrose basal medium with rose bengal and aureomycin added gave good results in the isolation of soil fungi. The basal medium with the concentrations of rose bengal and aureomycin used by Johnson and Manka (19) was used in counting yeast and mold in cottage cheese. The counts are shown in Table I.

In Table I the results of the yeast and mold counts on cottage cheese show that at the level of aureomycin and rose bengal used in the basal medium by Johnson and Manka (19) the counts were lower than with PD agar. Also, yeast and mold colonies were considerably smaller in radial growth with AR agar than PD agar.

The data in Tables II and III are preliminary yeast and mold isolate counts. The counts were a comparison of PD agar and six combinations of rose bengal and aureomycin in basal medium. All counts in Table II were made from a yeast isolate suspension and counts in Table III were made from a mold isolate suspension.

The results of the preliminary counts of Table II and Table III indicated that 20 parts per million rose bengal and 20 parts per million aureomycin in basal medium gave the most consistent and comparable results of all combinations in comparison with PD agar. The level of

TABLE I

COMPARISON OF YEAST AND MOLD COUNTS IN COTTAGE CHEESE WITH AR AGAR AND PD AGAR

Trial	ARA*	PDA
	per gram	
1	66,000	82,000
2	62,000	75,000
3	55,000	87,000
4	50,000	66,000
5	57,000	75,000
6	61,000	85,000
7	6	15
8	6	25
9	9	20

*ARA = 20 ppm aureomycin + 33 ppm rose bengal in basal medium.

TABLE II

COMPARISON OF YEAST COUNTS WITH PD AGAR AND SIX COMBINATIONS OF AR AGAR

Replications	ARA[1]	ARA[2]	ARA[3]	ARA[4]	ARA[5]	ARA[6]	PDA
				x10[3]			
1	2,000	9,000	9,000	3,400	2,300	1,400	2,700
2	6,000	5,000	10,000	2,600	1,900	2,100	2,500
3	7,000	4,000	6,000	3,700	2,400	1,700	1,700
4	2,000	6,000	8,000	2,800	2,600	1,300	2,400
5	3,000	8,000	8,000	2,500	2,700	1,100	2,200
Average	4,000	6,000	8,000	3,000	2,400	1,500	2,300

ARA[1] = 10 ppm aueromycin + 10 ppm rose bengal in basal medium.

ARA[2] = 20 ppm aueromycin + 10 ppm rose bengal in basal medium.

ARA[3] = 30 ppm aueromycin + 10 ppm rose bengal in basal medium.

ARA[4] = 10 ppm aueromycin + 20 ppm rose bengal in basal medium.

ARA[5] = 20 ppm aueromycin + 20 ppm rose bengal in basal medium.

ARA[6] = 30 ppm aueromycin + 20 ppm rose bengal in basal medium.

TABLE III

COMPARISON OF MOLD COUNTS WITH PD AGAR AND SIX COMBINATIONS OF AR AGAR

Replications	ARA[1]	ARA[2]	ARA[3]	ARA[4]	ARA[5]	ARA[6]	PDA
	x10[3]						
1	3,200	2,900	2,800	3,400	2,000	2,200	2,300
2	3,600	3,300	3,400	2,500	1,600	2,000	1,900
3	2,400	2,400	1,800	1,600	1,900	1,300	1,600
4	3,800	2,200	3,400	3,800	2,500	1,700	2,600
5	2,700	2,500	3,900	2,700	2,100	1,500	2,500
Average	3,100	2,600	3,100	2,800	2,000	1,700	2,200

ARA[1] = 10 ppm aureomycin + 10 ppm rose bengal in basal medium.

ARA[2] = 20 ppm aueromycin + 10 ppm rose bengal in basal medium.

ARA[3] = 30 ppm aueromycin + 10 ppm rose bengal in basal medium.

ARA[4] = 10 ppm aueromycin + 20 ppm rose bengal in basal medium.

ARA[5] = 20 ppm aueromycin + 20 ppm rose bengal in basal medium.

ARA[6] = 30 ppm aueromycin + 20 ppm rose bengal in basal medium.

10 parts per million rose bengal and 30 parts per million aureomycin in basal medium gave the highest counts in both Table II and III. The radial growth of yeast and mold colonies using aureomycin and rose bengal in basal medium, although not measured, were observed to be smaller than the radial growth of colonies on PD agar.

The data in Table IV compares the yeast counts of 20 parts per million aureomycin and 20 parts per million rose bengal in basal medium agar with those counts on PD agar. A comparison of the means shows very little difference between the yeast counts on AR agar and PD agar. However, it may be noted that the mean counts were slightly higher with AR agar. This difference was not statistically significant as is indicated in Table X in the appendix. Also, the yeast colonies were smaller in size with AR agar than with PD agar.

Table V shows the yeast and mold counts from cottage cheese using AR agar and PD agar. The data in Table V indicate little difference in the yeast and mold counts between AR agar and PD agar. Again it may be noted that the mean counts were slightly higher with AR agar. However, this difference was not statistically significant as indicated in Table XI in the appendix.

Table VI shows additional yeast and mold counts of 17 samples of cottage cheese using AR agar and PD agar. The 17 samples of cottage cheese were from 17 different manufacturing plants across the state of Tennessee. While there was no statistical analyses made on the data in Table VI, one may observe that the counts were similar for both AR and PD agar.

TABLE IV

COMPARISON OF YEAST COUNTS WITH
AR AGAR AND PD AGAR

Replications	ARA*	PDA
	x10^4	
1	32	28
2	28	29
3	34	33
4	31	30
5	29	25
6	25	38
7	29	34
8	37	30
9	34	35
10	40	36
11	31	27
12	38	32
13	44	30
14	28	28
15	32	33
16	26	39
17	30	24
18	34	31
19	46	34
20	29	30
Total	656	626
$\bar{x}$**	32.4	31.2

*ARA = 20 ppm aureomycin + 20 ppm rose bengal in basal medium.

**$\bar{x}$ = Mean.

TABLE V

COMPARISON OF YEAST AND MOLD COUNTS ON COTTAGE CHEESE WITH AR AGAR AND PD AGAR

Trial	ARA*	PDA
	per gram	
1	50	50
2	60	50
3	60	70
4	50	40
5	80	70
6	70	70
7	90	90
8	80	60
9	40	30
10	50	50
11	30	40
12	40	30
13	110	90
14	100	70
15	80	90
Total	990	900
$\bar{x}$**	66.0	60.0

*ARA = 20 ppm aureomycin + 20 ppm rose bengal in basal medium.

**$\bar{x}$ = Mean.

TABLE VI

COMPARISON OF SEVENTEEN COTTAGE CHEESE YEAST AND MOLD COUNTS WITH AR AGAR AND PD AGAR

Samples	ARA*	PDA
	per gram	
1	870	830
2	7	5
3	2,500	2,800
4	4,700	3,800
5	3,800	3,300
6	2	4
7	5	3
8	TNC**	TNC
9	400	400
10	2	3
11	12	10
12	TNC	TNC
13	13,000	13,000
14	2,600	2,600
15	TNC	TNC
16	2	4
17	TNC	TNC

*ARA = 20 ppm aureomycin + 20 ppm rose bengal in basal medium.

**TNC = Too numerous to count.

The data in Table VII show the comparison of mold counts with AR agar and PD agar. The data in Table VII indicate little difference in mold counts using AR agar or PD agar. Once again it may be noted that the mean count was slightly higher with AR agar. However, there was no statistical difference between the mold counts with AR agar and PD agar as indicated in Table XII in the appendix.

Table VIII gives an additional comparison of mold counts with AR agar and PD agar. While no statistical analysis was made on the data in Table VIII, the counts on AR agar and PD agar, but for a few exceptions, were similar. However, these few exceptions indicated a higher mold count with AR agar than with PD agar.

The data in Table IX show only a small difference in the mold counts with 30 parts per million aureomycin and 10 parts per million rose bengal in basal medium, 20 parts per million aureomycin and 20 parts per million rose bengal, and PD agar. It should be noted that the mean counts were slightly higher with the two different levels of aureomycin and rose bengal in basal medium than with PD agar. However, as Table XIII in the appendix indicates there was no statistical difference between the two levels of aureomycin and rose bengal in basal medium and PD agar. The radial growth of the mold colonies for both levels of aureomycin and rose bengal in basal medium were observed to be smaller than colonies on PD agar.

In order to test for bacterial growth on AR agar, 20 replications were made with each of the organisms mentioned earlier. There was no visible bacterial growth on any of the plates after incubating for 48

TABLE VII

COMPARISON OF MOLD COUNTS WITH
AR AGAR AND PD AGAR

Replications	ARA*	PDA
	x10^5	
1	48	35
2	45	39
3	42	45
4	41	42
5	43	46
6	35	41
7	39	48
8	47	45
9	41	43
10	36	66
11	55	46
12	47	43
13	51	42
14	57	52
15	54	49
16	84	48
17	62	55
18	49	65
19	57	53
Total	983	968
$\bar{x}$**	49.15	48.40

*ARA = 20 ppm aueromycin + 20 ppm rose bengal in basal medium.

**$\bar{x}$ = Mean.

TABLE VIII

COMPARISON OF ADDITIONAL MOLD COUNTS
WITH AR AGAR AND PD AGAR

Replications	ARA*	PDA
	x10^5	
1**	15	5
2	12	6
3	9	9
4	11	4
5	9	11
6	7	10
7	8	8
8	18	19
9	17	14
10	23	24
11	17	14
12	15	16
13	15	15

*ARA = 20 ppm aueromycin + 10 ppm rose bengal in basal medium.

**Replications 1-7 were made from a different mold suspension than replications 8-13.

TABLE IX

COMPARISON OF MOLD COUNTS WITH PD AGAR AND TWO LEVELS OF AR AGAR

Replications	ARA*	ARA**	PDA
		$x10^4$	
1	1	2	1
2	3	1	0
3	0	1	1
4	2	0	2
5	1	2	2
6	2	2	2
7	2	1	1
8	1	3	2
9	2	1	1
10	1	0	1
11	2	2	0
12	1	2	3
13	1	1	1
14	2	1	2
15	2	2	2
16	1	1	1
17	3	1	1
18	2	2	4
19	1	3	1
20	3	2	0
Total	33	30	28
$\bar{x}$***	1.65	1.50	1.40

*ARA = 30 ppm aueromycin + 10 ppm rose bengal in basal medium.

**ARA = 20 ppm aueromycin + 20 ppm rose bengal in basal medium.

***$\bar{x}$ = Mean.

hours at each organism's optimum temperature. From these results one may conclude that AR agar sufficiently inhibits bacterial growth to the extent that yeast and mold counts can confidently be reported without interference of bacterial colonies.

In summary, while no statistical difference can be reported between the mean counts on AR agar and PD agar, in all instances slightly higher mean counts were reported with AR agar. This could be of importance when the presence of one mold colony on the surface of cottage cheese could cause the consumer to discard it.

Assuming the medium to have no statistical significant difference in counts, the AR agar would have several definite advantages. One, the problem of spreading colonies is virtually eliminated by the use of rose bengal in the medium. Secondly, there is no precipitate of casein with AR agar to interfere with distinguishing colonies from curd particles. Thirdly, the problem of some yeast and mold species not growing due to a low pH is eliminated. Fourthly, the red color imparted to the AR agar by rose bengal gives a better color background for detecting mold and yeast colonies.

However, there are some limitations with AR agar. For one, aureomycin has to be added to the medium just prior to pouring. This is due to aureomycin not being heat stable (40). However, tartaric acid must also be added to PD agar just prior to pouring. Also, fresh solutions of aureomycin must be made frequently since aureomycin loses its bactericidal properties rapidly at room temperature (40). However, in a study by Johnson (18) solutions of aureomycin made with distilled

water containing not more than 1 milligram per milliliter may be dispensed in screw-cap tubes and stored in a frozen state. Solutions stored in this manner may be used for periods up to two months. Another problem may be that the reduced size of the colonies may cause problems when the medium is used for isolating species of yeasts and molds. The reduced colony size would only be a problem in isolation of species until the researcher becomes familiar with the growth patterns exhibited on AR agar.

The results of this study indicate that aureomycin rose bengal agar can be used for detecting yeast and mold in cottage cheese. From this study it may be assumed the AR agar could be used for enumerating yeast and mold in other dairy products such as butter and cream. However, further study on these products with AR agars may be helpful.

CHAPTER V

SUMMARY AND CONCLUSION

An aureomycin rose bengal agar for the enumeration of yeast and mold in cottage cheese was studied. Various combinations of aureomycin and rose bengal in peptone dextrose basal medium were compared to potato dextrose agar acidified with tartaric acid. Mold isolates, yeast isolates, and yeast and mold counts of cottage cheese were compared using AR agar and PD agar. Tests for bacterial growth on AR agar were also made.

Two levels of aureomycin and rose bengal in basal medium were found to give no statistical significant difference in mold counts when compared to PD agar. There was no statistical significant difference in AR agar and PD agar in counts of yeast isolates, and yeast and mold in cottage cheese. Tests for bacterial growth indicate there was no bacterial growth on AR agar.

The results of the study show no statistical significant differences in counts on AR agar and PD agar. With no significant difference in counts the AR agar would offer several advantages. The problem of spreading colonies is virtually eliminated. There is no precipitation of casein with AR agar to interfere with distinguishing colonies from curd particles. The pH is high enough with AR agar for most species of yeast and mold to grow. Also, the red color of AR agar gives a better color background for detecting yeast and mold colonies. However, there are several disadvantages with AR agar. One,

aureomycin has to be added to the medium just prior to pouring. Secondly, fresh solutions of aureomycin must be made up frequently. Thirdly, the reduced size of the colonies may cause problems when AR agar is used in isolating species of yeast and mold.

Since there is no significant difference in counts between AR agar and PD agar and the advantages offered by AR agar outweigh its disadvantages, it would appear that AR agar could be used in every instance that PD agar is used for enumerating yeast and mold.

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APPENDIX

TABLE X

ANALYSIS OF VARIANCE FOR YEAST COUNTS

Source of Error	DF	SS	MS	F
Total	39	981		
Among Groups	1	22	22	.85NS*
Error	38	959	25.82	

*NS = Not significant.

TABLE XI

ANALYSIS OF VARIANCE FOR YEAST AND MOLD COUNTS ON COTTAGE CHEESE

Source of Error	DF	SS	MS	F
Total	39	437.98		
Among Groups	1	11.45	11.45	1.02NS*
Error	38	426.53	11.22	

*NS = Not significant.

TABLE XII

ANALYSIS OF VARIANCE FOR MOLD COUNTS

Source of Error	DF	SS	MS	F
Total	39	3,733		
Among Groups	1	5	5	.005NS*
Error	38	3,728	981	

*NS = Not significant.

TABLE XIII

ANALYSIS OF VARIANCE FOR MOLD COUNTS WITH PD AGAR AND TWO LEVELS OF AR AGAR

Source of Error	DF	SS	MS	F
Total	59	45		
Among Groups	2	.64	.32	.41NS*
Error	57	44.36	.778	

*NS = Not significant.

VITA

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