THE INHIBITORY ACTION OF VARIOUS ANTIBIOTICS ON MICROORGANISMS IMPORTANT IN DAIRY PRODUCTS

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MICROORGANISMS IMPORTANT IN DAIRY PRODUCTS

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INTRODUCTION

The treatment of infectious mastitis with antibiotics has caused trouble for the dairy industry by the inhibition of starter cultures used in fermented dairy products. Previous work has shown that antibiotics, when used as a treatment for mastitis, are given off in the milk in sufficient quanities to inhibit the growth of the lactic acid producers used in the production of various fermented dairy products. Very little work has been done on the inhibitory action of antibiotics on other organisms important to the dairy industry, including those generally causing defects.

The work herein reported was initiated to show the effect of various antibiotics on microorganisms commonly associated with dairy products, both those organisms that are commonly used in fermented dairy products and those organisms that are associated with flavor defects.

REVIEW OF LITERATURE

Penicillin was one of the first antibiotic to come into use in the dairy industry as a means of combating mastitis. Bryan, et al (4) found penicillin to be an effective treatment for acute local and acute systemic mastitis caused by streptococci or staphylocci. Two hundred thousand units of penicillin given extravenously and 100,000 units into the involved quarter or quarters, repeated at 12- or 24 intervals, usually resulted in prompt clinical improvement. To obtain bacteriological recovery, the infections remaining were treated during the chronic stage of the disease.

Soon other antibiotics were making their appearance. Bryan, et al (5) reported a bougie containing 50,000 units each of penicillin and streptomycin to be an effective treatment for nine cases of acute coliform mastitis, one of staphylococcic mastitis and six of acute strepotoccic mastitis.

Bryan, et al (6) found when 157 cows, having streptococcic mastitis, were treated by infusing each quarter with 150 mg. of tyrothricin, 142 or 90 per cent became free from the infection of the udder. Comparisons of the standard plate counts on milk produced by 10 cows before treatment with those on the milk produced after treatment showed reductions in bacterial counts varying from 30 to 90 per cent.

With the treatment of mastitis with antibiotics, trouble soon appeared for the industry. Jackson and Bryan (11) reported that

when 25,000 units or more of penicillin of any medication form were administered to cows in all stages of lactation, measurable milk levels of penicillin were obtained at the 24-hour sampling period in each case and for longer periods with those treated during the latter part of the lactation.

Bryan (3) found that of 27 samples of herd milk collected at random at several milk plants and checked for penicillin, 26 had varying levels of penicillin and the other was suspected of having penicillin present. This presents a problem to the industry as penicillin is not destroyed by proper pasteurization.

Hansen (10) found penicillin, streptomycin, aureomycin, sulfanilamide and sufamerazine, when used as a treatment for mastitis, are given off in the milk in sufficient amounts to restrict growth of lactic acid producers. In reconstituted milk penicillin and streptomycin are not destroyed by the process of condensing and drying, whereas sulfanilamide, sulfamerzaine and aureomycin are changed and they appeared to have a stimulating effect on acid production.

Wilkowski and Krienke (21) found that a concentration of one unit of penicillin per ml. in raw mixed milk held at 10 degrees C. significantly retarded microbial growth during a three day storage period. Coliform organisms that were inoculated into sterilized milk were not significantly influenced by as much as 10 units of penicillin per ml. of milk. The activity of the penicillin was reduced in the milk after a storage of 2 days at 10 degrees C. and this was thought to be due to "penicillinase" produced by E. coli with which the milk was inoculated.

Katznelson and Hood (13) found that complete inhibition of acid production by six starter cultures was obtained with 0.5 units of penicillin per ml. of milk, strong inhibition with 0.1 unit and moderate with 0.05 units. Of six antibiotics tested against starter culture they found that penicillin was the most inhibitory, with subtilin next and chloromycetin was the least potent. Of 45 strains of lactic streptococci isolated from started cultures, all were completely inhibited in sterile skim milk by penicillin at a concentration of 0.2 to 0.4 units per ml.

Doan (8) reported very serious inhibition of starter activity is caused by 0.1 unit of penicillin per ml. in milk. Partial arrest or slow acid development results from 0.05 unit per ml. of milk. Doan found that pasteurization has practically no effect on any of the antibiotics studied, while autoclaving reduces potency to a detectable degree.

Krienke (15) found there was practically no acid production at the end of 18 hours when the milk was inoculated with a culture and the milk contained 0.0005 mg. of aureomycin hydrochloride per ml. of milk and the "drug containing" milk had been pasteurized at 145 degrees F. for 30 minutes. When the concentration of aureomycin was reduced to 0.00005 mg. per ml. the acid production was nearly normal as compared to the control.

Calbert (7) reported that the presence of antibiotics in milk will cause a sudden drop in the plate counts on milk or will cause an increase in the time required for milk to reduce in the methylene blue test.

Johns and Katznelson (12) found that one part of penicillin in 167,000,000 parts of milk may retard the dye reduction test on raw milk.

Stoltz and Hankinson (19) found that when the standard plate count was run on raw milk, penicillin in concentrations of 1.0, 0.1 and 0.01 unit per ml. of milk was effective in controlling the bacterial population. Streptomycin when added to the raw milk in concentrations of 5.0, 10. and 0.1 mg. per ml. showed an immediate lowering of the count that lasted 12 hours before an increase started. Penicillin in concentrations of 1.0 and 0.1 mgs. was effective for a 24 hour period before an increase started and 48 hours was required to gain the count of the control. Tyrothricin, which is made up of gramicidin and tyocidin, at concentrations of 5.0, 1.0 and 0.1 mg was effective in lowering the bacteria counts in raw milk. Aureomycin at levels of 0.5, 0.25 and 0.1 mcgm. per ml. of raw milk was effective in lowering the counts for a period of 36 hours and had not gained the counts of the controls at the end of 48 hours.

Drury (9) says that less antibiotics should be used for mastitis treatment and more good sound dairy practices. Antibiotics are only tools to be used when other means fail.

Merck and Co. (16) in their brochure on penicillin show that 15 ppm. of penicillin when added to agar completely stopped S. lactis. At .5 ppm. penicillin apparently had no effect on S. lactis. At a concentration of 500 ppm., E. coli and A. aerogenes grew well.

Shiveler and Weiser (18) found that with dry streptomycin added to raw milk to give a concentration of 10 ppm. there was no reduction of the bacterial population. When the milk containing the 10 ppm. was

pasteruized the bacterial population was restricted in its growth for 24 hours. Aureomycin at a concentration of 10 ppm. was effective in controlling the bacterial population of raw milk. The aureomycin is much more effective when the raw milk is pasteruized. This shows that the aureomycin was more effective on thermoduric organisms usually surviving pasteurization than on the organisms in raw milk. When 5 units of penicillin were added to milk and the milk then pasteurized at 145 degrees for 30 minutes the penicillin is much more effective in controlling the bacterial population than when the penicillin is added to the pasteurized milk. Raw milk with penicillin added showed a reduction of bacterial population up to 48 hours. With 5 units of penicillin added to raw milk and the milk then pasteruized and the pasteurized milk held at 8 to 10 degrees C. the standard plate count, on an average of five samples, increased from 100 to 16,400 in 48 hours.

Antibiotics have been tried as a means of preservation of foods. Anderson and Michenor (1) state that peas and asparagus showed no apoilage when from 5 to 20 ppm. of subtilin was added and the mixtures heated for 5 to 10 minutes at 212 degrees F. or below. This process is based on two principles. The destruction of non-spore forming bacteria, yeasts, fungi and the destruction of spore forming bacteria with milk heat and subtilin or other antibiotics, singly, or in combination. Aureomycin, and chloromycetin have also shown good results.

Tarr et al (20) have shown aureomycin, terramycin and chloromycetin, in the order named, to be the most effective inhibitors of growth of the natural mixed bacterial flora of flesh meats at temperatures between 0 degrees C. and 21 degrees C., while rimocidin inhibited yeast

growth. Aureomycin caused marked inhibition of spoilage in 0.5 and 2.0 units per gram concentration when incorporated in minced flesh and was equally effective when applied by immersing steaks in solutions containing the anitbiotic in 5 or 10 units per ml. concentrations.

III. METHODS

A. Source and Preparation of Antibiotics Used.

The antibiotics used in this work were obtained from various sources as follows:

1. Achromycin Hydrochloride
   Lederle Laboratories Division
   New York City, N. Y.

2. Aureomycin Hydrochloride
   Lederle Laboratories Division
   New York City, N. Y.

3. Bacitracin
   53.2 Units Per mgm.
   Wyeth Incorporated
   West Chester, Pa.

4. Dibenzylethylenediamine Dipenicillin G
   1170 Units per mg.
   Wyeth Incorporated
   West Chester, Pa.

5. Potassium Penicillin G.
   1580 Units per mgm.
   Wyeth Incorporated
   West Chester, Pa.

6. Procaine Penicillin G
   980 Units per mgm.
   Wyeth Incorporated
   West Chester, Pa.

7. Dihydrostreptomycin
   Wyeth Incorporated
   West Chester, Pa.

8. Sulfathiazole
   Local Drug Store
   Stillwater, Oklahoma

9. Tyrothricin
   S. B. Penick and Company
   New York City, N. Y.

10. Magnamycin
    995 Units per mg.
    Chas Pfizer and Co.
    New York City, N. Y.

11. Terramycin
    910 Units Per mg.
    Chas Pfizer and Co.
    New York City, N. Y.

12. Viocin
    Chas Pfizer and Co.
    New York City, N. Y.

A fresh solution of each antibiotics was prepared for each trial. This was done by weighing .4 gram of antibiotics into a sterile screw cap test tube and adding 19.6 ml. of sterile distilled water. From this two per cent stock solution the required concentration could be made by diluting with sterile distilled water.

B. The Double Dilution Method.

The double dilution method was used when rather wide variations in concentrations of antibiotics were desired, such as in preliminary screening tests. The procedure for each antibiotic was as follows: Enough of the antibiotic was added to a measured 20 ml. portion of the medium to produce the maximum concentration required. The contents of the tube were mixed by inverting several times and about one-half of the mixture was added to a tube containing 10 ml. of the medium. The concentration in the second tube would be about one-half of that in the first tube. This procedure was repeated twice more so that, for example, you could have approximate concentrations of 400, 200, 100 and 50 ppm.

C. Source and Propagation of Cultures Used.

Cultures for this work were those carried in the stock culture supply of the Dairy Department of the Oklahoma Agriculture and Mechanical College and consisted of: Alcaligenes viscosus, Aerobacter aerogenes, Escherichia coli. Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Pseudomonas fragi, Pseudomonas mucidolens, Pseudomonas putrefaciens, Streptococcus faecalis, Streptococcus liquefaciens, Streptococcus lactis, Torula eremoris and Torula sphaerica.

The cultures were transferred into sterile litmus milk a day or two before each trial to give a fresh culture. The cultures were carried in sterile litmus milk with a small amount of calcium carbonate added to enhance the keeping quality.

D. Agar Plate Technique.

The agar plate technique was used to determine the growth of organisms on agar containing various concentrations of antibiotics. Dehydrated trytone-glucose-extract agar (Difco) was prepared according to Standard methods (17). The agar was dispensed in 10 ml. quantities into screw cap test tubes, and if the antibiotics were to be sterilized in the agar they were added at this time. The tubes containing the agar were sterilized at 15 pounds pressure for 20 minutes, cooled up to 45 degrees C. and poured into sterile petri plates. The plates were allowed to solidify, inverted, and placed in an incubator at 37 degrees C., usually overnight, to allow the surface of the agar to dry. The plates were marked into sections and one drop of a 1 to 100 dilution of each test organism in sterile water was placed on

the plate. The plates were incubated at room temperature (21 to 28 degrees C.) or at 37 degrees C., depending on the test organisms, and noted for rate and extent of growth.

EXPERIMENTAL

A. The Inhibitory Action of Antibiotics added to the Media Before Sterilization on the Growth of Various Microorganisms.

1. Growth of Organisms on agar containing various concentrations of antibiotics.

In the agar plate technique used in these trials the antibiotics were added to the agar and the mixture then sterilized. The double dilution method was employed to give concentrations of 100, 200, 400 and 800 ppm. This was the screening process and was used to determine rather wide levels of antibiotic tolerance by the organisms.

The next step in the screening process was to determine the range in tolerance of the organisms failing to grow at a concentration of 100 ppm. Using the agar plate technique and the double dilution method concentrations of 12.5, 25, 50 and 100 ppm. were prepared. The 100 ppm. was duplicated from the last run to give an overlapping negative result.

The final step in the process of screening the organisms was to determine tolerances for antibiotics of the organisms which grew in concentrations of 800 ppm. The agar plate method was again used but it was found that about 1600 ppm. was about the maximum concentrations that could be obtained by using aqueous solutions. If the organisms required more than 1600 ppm. to inhibit growth, then the antibiotic was considered to be ineffective against that organism. Enough aqueous solution

of the antibiotic was added to 10 ml. quantities of agar contained in screw cap test tubes to produce 1000, 1200, 1400 and 1600 ppm. and the mixture then sterlized. When the work was first started it was decided to use the agar plate technique as a screening method only. Litmus milk would be used to give narrower ranges of tolerances.

Table I shows the ranges of concentration for the antibiotics required to inhibit the growth of the test organisms when the antibiotics were added to the agar before sterilization.

1. Achromycin.

A. aerogenes, Ps. fragi, T. cremoris and T. sphaerica were able to grow on agar with 1600 ppm. of achromycin added. E. coli and Ps. putrefaciens were able to grow with 400 ppm. but were not able to grow with 800 ppm. Alc. viscosus was able to grow with 300 ppm. but was unable to grow with 400 ppm. Ps. mucidolens was able to grow with 200 ppm. but unable to grow with 400 ppm. L. acidophilus and S. faecalis grew with 50 ppm. but were stopped with 100 ppm of achromycin. L. casei, S. liquefaciens and S. lactis grew with 12.5 ppm. but were stopped with 25 ppm. L. bulgaricus was negative with 12.5 ppm. of achromycin.

2. Aureomycin.

T. cremoris and T. sphaeirca were the only two organisms able to grow on agar with 1600 ppm. of aureomycin added. L. bulgaricus was able to grow with 200 ppm. of

aureomycin but was stopped with 400 ppm. Alc. viscosus, A. aerogenes, E. coli and Ps. fragi were able to grow with 100 ppm. but was stopped with 200 ppm. L. casei grew with 50 ppm. but was stopped with 100 ppm. L. acidophilus and Ps. mucidolens were able to grow with 25 ppm. but stopped with 50 ppm. Ps. putrefaciens, S. faecalis, S. liquefaciens and S. lactis were all stopped by 12.5 ppm. of aureomycin.

3. Bacitracin.

Ps. fragi, Ps. mucidolens, Ps. putrefaciens, T. cremoris and T. sphaeirca were able to grow on agar with 1600 ppm. of bacitracin added. Alc. viscosus, E. coli and L. casei were were able to grow with 400 ppm. but were stopped by 800 ppm. A. aerogenes and S. liquefaciens grew with 100 ppm. but were stopped by 200 ppm. L. acidophilus, S. faecalis and S. lactis were able to grow with 12.5 ppm. but were stopped by 25 ppm. L. bulgaricus was negative with 12.5 ppm. of bacitracin.

4. Di Penicillin G

Ps. fragi, Ps. mucidolens, T. cremoris and T. sphaerica were able to grow on agar with a concentration of 1600 ppm. added. A. aerogenes was able to grow with 400 ppm. but was stopped by a concentration of 800 ppm. E. coli grew with 100 ppm. but was stopped by 200 ppm. Alc. viscosus and Ps. putrefaciens grew with 50 ppm. but were stopped by 100 ppm. L. acidophilus, L. bulgaricus, S. faecalis, S. liquefaciens and S. lactis grew with 12.5 ppm. but were stopped by 25 ppm. L. casei was stopped by 12.5 ppm. concentration of di penicillin G.

TABLE I - THE GROWTH OF MICROORGANISMS ON AGAR CONTAINING VARIOUS AMOUNTS OF ANTIBIOTICS
(Antibiotics added before sterilization)

	Achromycin		Aureomycin		Bacitracin		Di Penicillin G.		Potassium Penicillin G.		Procain Penicillin G.		Sulfathiazole		Streptomycin		Tryothricin	
	x	-	x	-	x	-	x	-	x	-	x	-	x	-	x	-	x	-
	ppm. of antibiotics added to the agar																	
Alc. viscosus	300	400	100	200	400	800	50	100	100	200	50	100	12.5	25	12.5	25	1600	**
A. aerogenes	1600	**	100	200	100	200	400	800	200	400	400	800	800	1000	25	50	1600	**
E. coli	400	800	100	200	400	800	100	200	50	100	200	400	1600	**	25	50	1600	**
L. acidophilus	50	100	25	50	12.5	25	12.5	25	*	12.5	*	12.5	800	1000	25	50	100	200
L. bulgaricus	*	12.5	200	400	*	12.5	12.5	25	*	12.5	*	12.5	800	1000	12.5	25	12.5	25
L. casei	12.5	25	50	100	400	800	*	12.5	*	12.5	12.5	25	800	1000	25	50	100	200
Ps. fragi	1600	**	100	200	1600	**	1600	**	800	1000	1600	**	1600	**	100	200	1600	**
Ps. mucidolens	200	400	25	50	1600	**	1600	**	1600	**	1600	**	400	800	50	100	1600	**
Ps. putrefaciens	400	800	*	12.5	1600	**	50	100	100	200	100	200	400	800	25	50	1600	**
S. faecalis	50	100	*	12.5	12.5	25	12.5	25	50	100	50	100	1600	**	50	100	100	200
S. liquefaciens	12.5	25	*	12.5	100	200	12.5	25	*	12.5	*	12.5	1600	**	400	800	100	200
S. lactis	12.5	25	*	12.5	12.5	25	12.5	25	*	12.5	*	12.5	12.5	25	*	12.5	50	100
T. cremoris	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**
T. sphaerica	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**

x equals growth
- equals no growth
* no growth on agar containing 12.5 ppm.
** grew on agar containing 1600 ppm.

5. Potassium Penicillin G.

Ps. mucidolens, T. cremoris and T. sphaerica were able to grow on agar with 1600 ppm. of potassium penicillin added. Ps. fragi was able to grow with 800 ppm. but was stopped by 1000 ppm. A. aerogenes was able to grow with 200 ppm. but was stopped by 400 ppm. Alc. viscosus, and Ps. putrefaciens were able to grow with 100 ppm but were stoppod by 200 ppm. concentration. E. coli and S. faecalis could grow with 50 ppm. but were stopped by a concentration of 100 ppm. L. acidophilus, L. bulgaricus, L. casei, S. liquefaciens and S. lactis were all stopped by 12.5 concentration.

6. Procain Penicillin G.

Ps. fragi, Ps. mucidolens, T. cremoris and T. sphaerica were able to grow on agar with 1600 ppm. of procain penicillin added. A. aerogenes was able to grow with 400 ppm. but was stopped by 800 ppm. E. coli grew with 200 ppm. but was stopped by 400 ppm. Ps. putrefaciens was able to grow with 100 ppm. but was stopped by a concentration of 200 ppm. Alc. viscosus and S. faecalis was able to grow with 50 ppm. but were stopped with 100 ppm. L. casei could grow with 12.5 ppm. but was stopped by a concentration of 25 ppm. L. acidophilus, L. bulgaricus, S. liquefaciens and S. lactis were stopped by a concentration of 12.5 ppm.

7. Sulfathiazole.

E. coli, Ps. fragi, S. faecalis, S. liquefaciens, T. cremoris and T. sphaerica were able to grow on agar

with 1600 sulfathiazole added. A. aerogenes, L. acidophilus, L. bulgaricus and L. casei were growing with a concentration of 800 ppm. but were stopped with 1000 ppm. Ps. mucidolens and Ps. putrefaciens were able to grow with 400 ppm. but were stopped by 800 ppm. Alc. viscosus and S. lactis were able to grow with 12.5 ppm. but were stopped by 25 ppm.

8. Streptomycin.

T. cremoris and T. sphaerica were the only two organisms able to grow on agar with 1600 ppm. of streptomycin added. S. liquefaciens was able to grow with 400 ppm. but was stopped by 800 ppm. Ps. fragi grew with 50 ppm but not with 100 ppm. A. aerogenes, E. coli, L. acidophilus, L. casei and Ps. putrefaciens grew with 25 ppm. but not with 50 ppm. Alc. viscosus and L. bulgaricus grew with 12.5 ppm. but not with 25 ppm. S. lactis was stopped by 12.5 concentration.

9. Tyrothricin.

Alc. viscosus, A. aerogenes, E. coli, Ps. fragi, Ps. mucidolens, Ps. putrefaciens, T. cremoris and T. sphaerica were able to grow on agar with 1600 ppm. of tyrothricin added. L. acidophilus, L. casei, S. faecalis and S. liquefaciens were able to grow with 100 ppm. but not with 200 ppm. S. lactis was able to grow with 50 ppm. but not with 100 ppm. L. bulgaricus was the least resistant growing with 12.5 ppm. but not with 25 ppm. It should be noted, especially the results in the Table for any concentration of tyrothricin above 100 ppm., may be inaccurate as it

was observed that tyrothricin had a rather low solubility in water and probably the concentrations obtained were no greater than 200 ppm.

The results show that aureomycin, streptomycin, and the penicillins, in the order named, had the greatest inhibitory effect against the test organisms. Tyrothricin and sulfathiazole were the least effective. The yeasts were the most resistant organisms followed by the coliform group and Alc. viscosus. The results shown here are probable partly inaccurate as later work showed that some of the antibiotics are at least partly destroyed by the sterilization exposures used. No attempt was made to determine the amount of destruction by the sterilizing process.

2. The Growth of Organisms in Litmus Milk Containing Various Concentrations of Antibiotics.

The work with litmus milk was an attempt to bring the tolerances of the test organisms toward the antibiotics to narrower concentrations.

A 2 per cent solution, in sterile water, of each antibiotic was used. The antibiotic was prepared fresh for each trial. Litmus milk was dispensed into test tubes in 10 ml. quanities using a burette with a capacity of 50 ml. The concentrations of antibiotics were added with a 1 ml. pipette graduated in tenths. The amounts of antibiotic used for each organism were determined from the results of the screening test with the agar plate technique. Four concentrations of

each antibiotic were used for each test organism. The tubes of litmus milk with the antibiotics added were plugged with cotton and sterilized in the autoclave at 15 pounds pressure for 15 minutes. One control for each organism was prepared in a like manner with the exception no antibiotic was added to that tube. The fresh cultures of the test organisms were diluted 1 to 100 in sterile water and one drop of the dilution was added to each tube of the four concentrations of antibiotics and one drop to the control. The tubes were incubated at room temperature (21 to 28 degrees C.) or at 37 degrees C., depending on the test organism, and noted for rate and extent of growth. The tubes were observed for several days if the growth was very slow or negative.

Table II shows the ranges of concentration for the antibiotics required to inhibit the growth of the test organism when the antibiotics were added to the litmus milk before sterilization.

1. Achromycin.

T. cremoris and T. sphaerica were able to grow in litmus milk with 1600 ppm. of achromycin added. A. aerogenes was able to grow with 1800 ppm. but not with 2000 ppm. Ps. fragi was able to grow with 1000 ppm. but not with 1200 ppm. E. coli was able to grow with 400 ppm. but not with 800 ppm. Alc. viscosus and Ps. mucidolens were able to grow with 300 ppm. but not with 400 ppm. Ps. putrefaciens was able to grow with 250 ppm. but not with 300 ppm. L. acidophilus and S. faecalis were able to grow with 75 ppm. but not with 100

TABLE II - THE GROWTH OF MICROORGANISMS IN LITMUS MILK CONTAINING VARIOUS AMOUNT OF ANTIBIOTICS
(Antibiotics added before sterilization)

Test organisms	Achromycin		Aureomycin		Bacitracin		Di Penicillin G.		Potassium Penicillin G.		Procain Penicillin G.		Sulfathiazole		Streptomycin		Tryothricin	
	x	-	x	-	x	-	x	-	x	-	x	-	x	-	x	-	x	-
	ppm. of antibiotics added to the litmus milk																	
Alc. viscosus	300	400	65	75	300	400	25	50	50	75	25	50	15	20	10	20	1600	**
A. aerogenes	1800	2000	100	200	100	200	600	700	200	250	500	1000	1900	1100	25	50	1600	**
E. coli	400	800	100	200	800	1000	100	200	50	75	300	400	1600	**	50	100	1600	**
L. acidophilus	75	100	50	100	25	50	5	10	6	8	8	12	900	1000	10	20	100	200
L. bulgaricus	10	15	100	200	5	10	.50	1	2	4	.5	1	900	1000	20	25	8	12
L. casei	20	25	50	100	800	1000	1	2	1	2	20	25	900	1000	20	25	100	200
Ps. fragi	1000	1200	100	125	1600	**	1600	**	900	1000	1200	1400	1600	**	400	600	1600	**
Ps. mucidolens	300	400	50	100	1600	**	1600	**	1600	**	1600	**	400	800	75	100	1600	**
Ps. putrefaciens	250	400	10	20	1600	**	50	100	75	100	50	100	400	800	25	50	1600	**
L. faecalis	75	100	50	100	10	15	15	20	50	75	50	75	400	800	50	100	100	125
S. liquefaciens	20	25	4	10	75-	100	15	20	10	15	10	15	1600	**	100	200	50	75
S. lactis	10	15	.25	2	10	15	10	15	1	2	*	.25	8	12	1	2	25	50
T. cremoris	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**
T. sphaerica	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**	1600	**

* no growth in litmus milk containing .25 ppm.
** grew in litmus milk containing 1600 ppm.
x equals growth
- equals no growth

ppm. L. casei and S. liquefaciens were able to grow with 20 ppm. but not with 25 ppm. L. bulgaricus and S. lactis were able to grow with 10 ppm. but not with 15 ppm.

2. Aureomycin.

T. cremoris and T. sphaerica were able to grow in milk with 1600 ppm. of aureomycin added. A. aerogenes, E. coli and L. bulgaricus were able to grow with 100 ppm. but were not able to grow with 200 ppm. Ps. fragi was able to grow with 100 ppm. but was not able to grow with 125 ppm. Alc. viscosus was able to grow with 65 ppm. but was not able to grow with 75 ppm. L. acidophilus, L. casei, Ps. mucidolens and S. faecalis were able to grow with 50 ppm. but not with 100 ppm. Ps. putrefaciens was able to grow with 10 ppm. but not with 20 ppm. S. liquefaciens was able to grow with 4 ppm. but not with 10 ppm. S. lactis was able to grow with .25 ppm. but not with 2 ppm.

3. Bacitracin.

Ps. fragi, Ps. mucidolens, Ps. putrefaciens. T. cremoris and T. sphaerica were able to grow in milk with 1600 ppm. of bacitracin added. E. coli and L. casei were able to grow with 800 ppm. but were not able to grow with 1,000 ppm. Alc. viscosus was able to grow with 300 ppm. but not with 400 ppm. A. aerogenes was able to grow with 100 ppm. but not with 200 ppm. S. liquefaciens was able to grow with 75 ppm. but not with 100 ppm. L. acidophilus was able to grow with 25 ppm. but not with 50 ppm. S.

lactis and S. faecalis were able to grow with 10 ppm. but not with 15 ppm. L. bulgaricus was able to grow with 5 ppm. but not with 10 ppm.

4. Di Penicillin G.

Ps. fragi, Ps. mucidolens, T. cremoris and T. sphaerica were able to grow in milk with 1600 ppm. of di penicillin G added. A. aerogenes was able to grow with 600 ppm. but not with 700 ppm. E. coli was able to grow with 100 ppm. but not with 200 ppm. Ps. putrefaciens was able to grow with 50 ppm. but not with 100 ppm. Alc. viscosus was able to grow with 25 ppm. but not with 50 ppm. S. faecalis and S. liquefaciens were able to grow with 15 ppm. but not with 20 ppm. S. lactis was able to grow with 10 ppm. but not with 15 ppm. L. acidophilus was able to grow with 5 ppm. but not with 10 ppm. L. casei was able to grow with 1 ppm. but not with 2 ppm. L. bulgaricus was able to grow with .5 ppm. but not with 1 ppm.

5. Potassium Penicillin G.

Ps. mucidolens, T. cremoris and T. sphaerica were able to grow in milk with 1600 ppm. of potassium penicillin added. Ps. fragi was able to grow with 900 ppm. but not with 1000 ppm. A. aerogenes was able to grow with 200 ppm. but not with 250 ppm. Ps. putrefaciens was able to grow with 75 ppm. but not with 100 ppm. Alc. viscosus, E. coli and S. faecalis were able to grow with 50 ppm. but not with 75 ppm. S. liquefaciens was able

to grow with 10 ppm. but not with 15 ppm. L. acidophilus was able to grow with 6 ppm. but not with 8 ppm. L. bulgaricus was able to grow with 2 ppm. but not with 4 ppm. L. casei and S. lactis were able to grow with 1 ppm. but not with 2 ppm.

6. Procain Penicillin G.

Ps. mucidolens, T. cremoris and T. sphaerica were able to grow in milk with 1600 ppm. of procain penicillin added. Ps. fragi was able to grow with 1200 ppm. but not with 1400 ppm. A. aerogenes was able to grow with 500 ppm. but not with 1000 ppm. E. coli was able to grow with 300 ppm. but not with 400 ppm. Ps. putrefaciens was able to grow with 50 ppm. but not with 100 ppm. S. faecalis was able to grow with 50 ppm. but not with 75 ppm. Alc. viscosus was able to grow with 25 ppm. but not with 50 ppm. L. casei was able to grow with 20 ppm. but not with 25 ppm. S. liquefaciens was able to grow with 10 ppm. but not with 15 ppm. L. acidophilus was able to grow with 8 ppm. but not with 12 ppm. L. bulgaricus was able to grow with .5 ppm. but not with 1 ppm. S. lactis was negative with .25 ppm.

7. Sulfathiazole.

E. coli, Ps. fragi, S. liquefaciens, T. cremoris and T. sphaerica were able to grow in milk with 1600 ppm. of sulfathiazole added. A. aerogenes was able to grow with 1000 ppm. but not with 1100 ppm. L. acidophilus, L. bulgaricus and L. casei were able to grow with 900 ppm.

but not with 1,000 ppm. Ps. mucidolens, Ps. putrefaciens and S. faecalis were able to grow with 400 ppm. but not with 800 ppm. Alc. viscosus was able to grow with 15 ppm. but not with 20 ppm. S. lactis was able to grow with 8 ppm. but not with 12 ppm.

8. Streptomycin.

T. cremoris and T. sphaerica were able to grow in milk with 1600 ppm. of streptomycin added. Ps. fragi was able to grow with 400 ppm. but not with 600 ppm. S. liquefaciens was able to grow with 100 ppm. but not with 200 ppm. Ps. mucidolens was able to grow with 75 ppm. but not with 100 ppm. A. aerogenes and Ps. putrefaciens were able to grow with 25 ppm. but not with 50 ppm. L. bulgaricus and L. casei were able to grow with 20 ppm. but not with 25 ppm. Alc. viscosus and L. acidophilus were able to grow with 10 ppm. but not with 20 ppm. S. lactis was able to grow with 1 ppm. but not with 2 ppm.

9. Tyrothricin.

Alc. viscosus, A. aerogenes, E. coli, Ps. fragi, Ps. mucidolens, Ps. putrefaciens, T. cremoris and T. sphaerica were able to grow in milk with 1600 ppm. of tyrothricin added. L. casei and L. acidophilus were able to grow with 100 ppm. but not with 200 ppm. S. faecalis was able to grow with 100 ppm. but not with 125 ppm. L. liquefaciens was able to grow with 50 ppm. but not with 75 ppm. S. lactis was able to grow with 25 ppm. but not with 50 ppm. L. bulgaricus was able to grow with 8 ppm. but not with 12 ppm.

It should be noted, especially the results in the Table for any concentration of tyrothricin above 100 ppm., may be inaccurate as it was observed that tyrothricin had a rather low solubility in water and probably the concentrations obtained were no greater than 200 ppm.

The results show that streptomycin, aureomycin and the penicillins, in the order named, had the greatest inhibitory effect against the test organisms. Tyrothricin and sulfathiazole were the least effective. The two yeasts were the most resistant organisms followed by the coliform group, the Pseudomonas group and Alc. viscosus. S. lactis was the least resistant of the organisms tested. The results shown here are probably partly inaccurate as later work showed that some of the antibiotics are at least partly destroyed by the sterilization exposures.

In another phase of the work on antibiotics, samples of raw milk from the dairy herd and of pasteurized milk from the dairy plant at Oklahoma Agriculture and Mechanical College, were plated on tryptone glucose agar containing concentrations of 50, 100, 200 and 400 ppm. of aureomycin. Several trials were run in which the concentrations of aureomycin were added to the agar before sterilization at 15 pounds pressure for 20 minutes and several more in which the concentrations of aureomycin were added to sterilized agar after cooling to 49 degrees C. A control was used which had no aureomycin added.

The results show that the plates containing the aureomycin added before sterilization had several bacterial

colonies per plate, even on the plates containing 400 ppm. of aureomycin. However, when the aureomycin was added to the agar after sterilization, no growth occurred on the plates, even with only 50 ppm. of aureomycin added, except for an occasional yeast colony. These results indicate there was apparently considerable destruction of the aureomycin by the sterilization exposures of 15 pounds pressure for 20 minutes.

B. The Inhibitory Action of Antibiotics Added to Media after Sterilization on the Growth of Various Microorganisms.

1. Growth of Organisms on agar containing various concentrations of antibiotics added after sterilization.

The results of the preceding work suggest that autoclaving antibiotics in agar or litmus milk reduced the ability of some of them to inhibit the growth of the test organisms. Additional Trials were run in which the aqueous solution of the antibiotics were added to the media after sterilization.

In the agar plate technique used in these trials solutions of the antibiotics in sterile water were added to the agar after sterilization and cooling to about 49 degrees C. Concentrations of 50, 100, 200 and 400 ppm. of the antibiotics were added with pipettes to the measured 10 ml. quanities of the sterile agar contained in screw capped test tubes. This was the screening process and was used to determine rather wide levels of antibiotic tolerances by the organisms.

The next step in the screening process was to determine the range of tolerance of the organisms failing to grow at a

concentration of 50 ppm. using concentrations of 5, 10, 20 and 40 ppm. of the various antibiotics.

The next step in the screening process was to determine the range of tolerance of the organisms failing to grow at a concentration of 5 ppm. using concentrations of 1, 2, 3 and 5 ppm. of the various antibiotics.

The final step in the process of screening the organisms was to determine tolerances for antibiotics of the organisms which grew in concentrations of 400 ppm. Enough aqueous solution of the antibiotics were added to 10 ml. quantities of sterile agar to give concentration of 400, 600, 800 and 1,000 ppm.

Table III shows the concentrations of the antibiotics required to inhibit the growth of the test organisms when the antibiotics were added to agar after sterilization and cooling to about 49 degrees C.

1. Aureomycin.

T. cremoris and T. sphaerica were the only two organisms able to grow on agar with 1,000 ppm. of aureomycin added. A. aerogenes was the next resistant, growing with a concentration of 3 ppm. but not with 5 ppm. E. coli and L. bulgaricus were able to grow with 1 ppm. but were not able to grow with 2 ppm. Alc. viscosus, L. acidophilus, L. casei, Ps. fragi, Ps. mucidolens, Ps. putrefaciens, S. faecalis, S. liquefaciens and S. lactis were negative with 1 ppm. of aureomycin.

2. Bacitracin.

A. aerogenes, E. coli, Ps. fragi, Ps. mucidolens, T. cremoris and T. sphaerica were able to grow on agar with 1,000 ppm of bacitracin added. Ps. putrefaciens was able to grow with 200 ppm. but not with 400 ppm. Alc. viscosus was able to grow with 50 ppm. but not with 100 ppm. S. liquefaciens was able to grow with 5 ppm. but not with 10 ppm. L. casei was able to grow with 3 ppm. but not with 5 ppm. L. acidophilus, L. bulgaricus, S. faecalis and S. lactis were negative with 1 ppm. concentration of bacitracin.

3. Dipenicillin G.

Ps. fragi, Ps. mucidolens and T. sphaerica were able to grow on agar with 1,000 ppm. of di penicillin added. The other yeast, T. cremoris, and A. aerogenes, were able to grow with 100 ppm. but not with 200 ppm. E. coli was able to grow with 50 ppm. but not with 100 ppm. Ps. putrefaciens was able to grow with 10 ppm. but not with 20 ppm. Alc. viscosus was able to grow with 3 ppm. but not with 5 ppm. S. faecalis and S. liquefaciens are able to grow with 1 ppm. but not with 2 ppm. L. acidophilus, L. bulgaricus, L. casei and S. lactis were all negative with 1 ppm. concentration of dipenicillin G.

4. Potassium Penicillin G.

Ps. mucidolens, T. cremoris and T. sphaerica were able to grow on agar with 1,000 ppm. of potassium penicillin added. Ps. fragi was able to grow with 100 ppm. but not

with 200 ppm. A. aerogenes was able to grow with 50 ppm. but not with 100 ppm. E. coli was able to grow with 20 ppm. but not with 40 ppm. Ps. putrefaciens was able to grow with 5 ppm. but not with 10 ppm. Alc. viscosus was able to grow with 2 ppm. but not with 3 ppm. S. liquefaciens was able to grow with 1 ppm. but not with 2 ppm. L. acidophilus, L. bulgaricus, L. casei, S. faecalis and S. lactis were all negative with 1 ppm. concentration of potassium penicillin G.

5. Procain Penicillin G.

Ps. mucidolens, T. cremoris and T. sphaerica were able to grow on agar with 1000 ppm. of procain penicillin added. A. aerogenes and Ps. fragi were able to grow with 200 ppm. but not with 400 ppm. E. coli and Ps. putrefaciens were able to grow with 20 ppm. but not with 40 ppm. Alc. viscosus was able to grow with 2 ppm. but not with 3 ppm. S. liquefaciens was able to grow with 1 ppm. but not with 2 ppm. L. acidophilus, L. bulgaricus, L. casei, S. faecalis and S. lactis were all negative at 1 ppm. concentration of procain penicillin.

6. Sulfathiazole.

E. coli, T. cremoris and T. sphaerica were able to grow on agar with 1000 ppm of sulfathiazole added. A. aerogenes was able to grow with 800 ppm. but not with 1,000 ppm. Ps. putrefaciens and S. liquefaciens were able to grow with 600 ppm. but not with 800 ppm. Ps. fragi, Ps. mucidolens and S. faecalis were able to grow with 200

ppm. but not with 400 ppm. S. lactis was able to grow with 100 ppm. but not with 200 ppm. L. casei was able to grow with 20 ppm. but not with 40 ppm. L. acidophilus was able to grow with 3 ppm. but not with 5 ppm. Alc. viscosus and L. bulgaricus were able to grow with 1 ppm. but not with 2 ppm.

7. Streptomycin.

T. cremoris and T. sphaerica were able to grow on agar with 1,000 ppm of streptomycin added. Ps. fragi, Ps. mucidolens and S. liquefaciens were able to grow with 20 ppm. but not with 40 ppm. Ps. putrefaciens and S. faecalis were able to grow with 3 ppm. but not with 5 ppm. Alc. viscosus, E. coli and A. aerogenes were able to grow with 1 ppm. but not with 2 ppm. L. acidophilus, L. bulgaricus, L. casei and S. lactis were all negative with 1 ppm. concentration of streptomycin.

8. Tyrothricin.

A. aerogenes, E. coli, Ps. fragi, Ps. mucidolens, Ps. putrefaciens, T. cremoris and T. sphaerica were able to grow on agar with 1,000 ppm. of tyrothricin added. Alc. viscosus was able to grow with 200 ppm. but not with 400 ppm. S. faecalis, S. liquefaciens and S. lactis were able to grow with 3 ppm. but not with 5 ppm. L. acidophilus, L. bulgaricus and L. casei were negative with 1 ppm. concentration of tyrothricin.

TABLE III - THE GROWTH OF MICROORGANISMS ON AGAR CONTINING VARIOUS AMOUNTS OF ANITBIOTICS
(Antibiotics added to the agar after sterilization)

Test organism	Aureomycin		Bacitracin		Di-Penicillin G.		Potassium Penicillin G.		Procain Penicill-G.		Sulfathiazole		Streptomycin		Tyrothricin		Magnamycin		Terramycin		Viocin	
ppm.	x	-	x	-	x	-	x	-	x	-	x	-	x	-	x	-	x	-	x	-	x	-
	ppm. of antibiotics added to the agar																					
Alc. viscosus	*	1	50	100	3	5	2	3	2	3	1	2	1	2	200	400	1	2	*	1	20	40
A. aerogenes	3	5	1000	**	100	200	50	100	200	400	800	1000	1	2	1000	**	1000	**	5	10	20	40
E. coli	1	2	1000	**	50	100	20	40	20	40	1000	**	1	2	1000	**	1000	**	5	10	10	20
L. acidophilus	*	1	*	1	*	1	*	1	*	1	3	5	*	1	*	1	*	1	*	1	10	20
L. bulgaricus	1	2	*	1	*	1	*	1	*	1	1	2	*	1	*	1	*	1	3	5	5	10
L. casei	*	1	3	5	*	1	*	1	*	1	20	40	*	1	*	1	*	1	*	1	5	10
Ps. fragi	*	1	1000	**	1000	**	100	200	200	400	200	400	20	40	1000	**	100	200	3	5	50	100
Ps. mucidolens	*	1	1000	**	1000	**	1000	**	1000	**	200	400	20	40	1000	**	1000	**	3	5	400	600
Ps. putrefaciens	*	1	200	400	10	20	5	10	20	40	600	800	3	5	1000	**	5	10	2	3	20	40
S. faecalis	*	1	*	1	1	2	*	1	*	1	200	400	3	5	3	5	*	1	1	2	100	200
S. liquefaciens	*	1	5	10	1	2	1	2	1	2	600	800	20	40	3	5	5	10	1	2	400	600
S. lactis	*	1	*	1	*	1	*	1	*	1	100	200	*	1	3	5	*	1	*	1	5	10
T. cremoris	1000	**	1000	**	100	200	1000	**	1000	**	1000	**	1000	**	1000	**	800	1000	1000	**	1000	**
T. sphaerica	1000	**	1000	**	1000	**	1000	**	1000	**	1000	**	1000	**	1000	**	1000	**	1000	**	1000	**

x equals growth
- equals no growth
* no growth on agar containing 1 ppm.
** grew on agar containing 1000 ppm.

9. Magnamycin.

A. aerogenes, E. coli, Ps. mucidolens and T. sphaerica were able to grow on agar with 1,000 ppm. of magnamycin added. One of the yeasts, T. cremoris, grew with 800 ppm. but not with 1000 ppm. Ps. fragi was able to grow with 100 ppm. but not with 200 ppm. Ps. putrefaciens and S. liquefaciens were able to grow with 5 ppm. but not with 10 ppm. Alc. viscosus was able to grow with 1 ppm. but not with 2 ppm. L. acidophilus, L. bulgaricus, L. casei, S. faecalis and S. lactis were negative with 1 ppm. concentration of magnamycin.

10. Terramycin.

T. cremoris and T. sphaerica were able to grow on agar with 1,000 ppm. of terramycin added. A. aerogenes and E. coli were able to grow with 5 ppm. but not with 10 ppm. L. bulgaricus, Ps. fragi and Ps. mucidolens were able to grow with 3 ppm. but not with 5 ppm. Ps. putrefaciens was able to grow with 2 ppm. but not with 3 ppm. S. faecalis and S. liquefaciens were able to grow with 1 ppm. but not with 2 ppm. Alc. viscosus, L. acidophilus, L. casei and S. lactis were negative with 1 ppm concentration of terramycin.

11. Viocin.

T. cremoris and T. sphaerica were able to grow on agar with 1,000 ppm. of viocin added. Ps. mucidolens and S. liquefaciens were able to grow with 400 ppm. but not with 600 ppm. S. faecalis was able to grow with 100 ppm. but not with 200 ppm. Ps. fragi was able to grow with 50 ppm. but not with 100 ppm. Alc. viscosus, A. aerogenes and Ps.

putrefaciens to grow with 20 ppm. but not with 40 ppm. E. coli and L. acidophilus were able to grow with 10 ppm. but not with 20 ppm. L. bulgaricus, L. casei and S. lactis were able to grow with 5 ppm. but not with 10 ppm.

The results show that aureomycin, terramycin, streptomycin, viocin, magnamycin and the penicillins, in the order named, had the greatest inhibitory effect against the test organisms. Tyrothricin, bacitracin and sulfathiazole were the least effective. The yeasts were the most resistant organisms followed by the coliform group, the pseudomonas group and Alc. viscosus. Di penicillin and magnamycin were the only antibiotics showing any inhibitory effect toward the yeasts. T. cremoris was stopped by 200 ppm. of dipenicillin and 1000 ppm. of magnamycin. S. lactis was the least resistant growing with 100 ppm. of sulfathiazole, 3 ppm. of tyrothricin and 5 ppm. of viocin but was negative with 1 ppm. concentration of all of the other antibiotics. The Lactobacillus group was next in susceptability followed by the Streptococcus group.

2. The Growth of Organisms in Litmus Milk containing various concentrations of antibiotics added after sterilization.

In the litmus milk method used in these trials solutions of antibiotics in sterile water were added to sterile litmus milk. The amounts of antibiotics used for each organism were determined from the results of the screening test with the agar plate technique. Four concentrations of each antibiotic was used with each test organisms.

Litmus milk was dispensed into test tubes in 10 ml. quantities using a burette with a capacity of 50 ml. The tubes were plugged with cotton and sterilized at 15 pounds pressure for 15 minutes. The milk was cooled and the four concentrations of antibiotics were aseptically added with a 1 ml. pipette graduated in tenths. One control for each organism was prepared in a like manner with the exception no antibiotic was added to that tube. The fresh cultures of the test organisms were diluted 1 to 100 with sterile water and one drop of the dilution was added to each tube of the four concentrations of antibiotics and one drop to the control. The tubes were incubated at room temperature (21 to 28 degrees C.), or at 37 degrees C., depending on the test organism, and noted for rate and extent of fermentation as an indication of degree of inhibition of growth. The rate and extent of growth of the test organisms was designated as follows: Good (XXXX); Fair (XXX); Poor (XX); Very Poor (X); and no growth (0). The growth designated as very poor usually appeared after several days incubation and was not considered as being of significance in determing the concentration required to inhibit growth.

When there was some doubt as to whether growth actually existed the designation "very poor" was used but the designation "poor" was used in the analysis of the results to indicate the highest levels of antibiotics tolerated by the test organisms. The cultures were incubated for about 5 days before the results would be reported as no growth.

Table IV shows the concentrations of antibiotics required to inhibit the growth of the test organisms when the antibiotics were added to sterilized and cooled litmus milk. Table IV gives a range of four concentrations of antibiotics and the rate or extent of growth for the test organisms over that range.

1. Aureomycin.

T. cremoris and T. sphaerica were able to grow in litmus milk with 2200 ppm. or more of aureomycin added. A. aerogenes, E. coli and L. bulgaricus were able to grow with 6 ppm. Alc. viscosus grew with 3 ppm. Ps. putrefaciens grew with .75 ppm. L. acidophilus, L. casei and S. liquefaciens grew with .50 ppm. S. faecalis grew very poorly over a range of .25 ppm. to .75 ppm. while S. lactis was able to grow with .25 ppm. concentration of aureomycin added.

2. Bacitracin.

A. aerogenes, E. coli, T. cremoris and T. sphaerica were able to grow in litmus milk with 2200 ppm. or more of bacitracin added. Ps. putrefaciens was able to grow with 1000 ppm. Alc. viscosus was able to grow with 140

TABLE IV - THE GROWTH OF ORGANISMS IN LITMUS MILK CONTAINING VARIOUS CONCENTRATION OF ANTIBIOTICS
(Antibiotics added after sterilization)

Test organisms used	Alc. viscosus		A. aerogenes		E. coli		L. acidophilus		L. bulgaricus		L. casei		Ps. putrefacien		S. faecalis		S. lactis		S. liquefaciens		T. cremoris		T. sphaerica	
Antibiotics	ppm	grth	ppm	grth	ppm	grth	ppm	grth	ppm	grth	ppm	grth	ppm	grth	ppm	grth	ppm	grth	ppm	grth	ppm	grth	ppm	grth
Aureomycin	2	xxx	5	xx	5	xx	.25	xxx	5	xx	.25	xxx	.50	xx	.25	x	.25	x	.25	xxxx	1600	xxxx	1600	xxxx
	3	xx	6	xx	6	xx	.50	xxx	6	xx	.50	xx	.75	xx	.50	x	.50	o	.50	xxx	1800	xxxx	1800	xxxx
	4	o	7	x	7	x	.75	x	7	x	.75	x	1	x	.75	x	.75	o	.75	x	2000	xxxx	2000	xxxx
	5	o	8	x	8	x	1	o	8	o	1	o	2	o	1	o	1	o	1	o	2200	xxxx	2200	xxxx
Bacitracin	100	xxxx	1600	xxxx	1600	xxxx	5	xx	5	x	5	xxxx	400	xxxx	5	xxxx	5	xx	5	xxxx	1600	xxxx	1600	xxxx
	120	xxx	1800	xxxx	1800	xxxx	6	xx	6	o	6	xxx	600	xxxx	6	xxxx	6	xx	6	xxxx	1800	xxxx	1800	xxxx
	140	xx	2000	xxxx	2000	xxxx	7	x	7	o	7	xx	800	xxxx	7	xx	7	x	7	xxx	2000	xxxx	2000	xxxx
	160	x	2200	xxxx	2200	xx	8	o	8	o	8	x	1000	xx	8	x	8	x	8	x	2200	xxxx	2200	xxxx
Dipenicillin G.	5	xxxx	200	xxxx	100	xxxx	.25	xxxx	.25	xxxx	2	xxxx	20	xxxx	3	xxxx	.25	xxxx	5	xxxx	1600	xxxx	1600	xxxx
	6	xxxx	300	xxxx	200	xxxx	.50	xxxx	.50	xxxx	3	xxx	30	xxxx	4	xxxx	.50	xx	6	xx	1800	xxxx	1800	xxxx
	7	xxx	400	xx	300	xx	.75	xx	.75	xx	4	xx	40	xxx	5	xxx	.75	x	7	xx	2000	xxxx	2000	xxxx
	8	xx	500	x	400	x	1	x	1	x	5	x	50	xx	6	xx	1	o	8	o	2200	xxxx	2200	xxxx
Potassium Penicillin G.	5	xxxx	100	xxxx	40	xx	.25	x	.25	o	.25	x	2	xxxx	.25	xx	.25	x	5	x	1600	xxxx	1600	xxxx
	6	xxx	120	xxx	50	xx	.50	o	.50	o	.50	o	3	xxxx	.50	xx	.50	o	6	o	1800	xxxx	1800	xxxx
	7	xx	140	xx	60	xx	.75	o	.75	o	.75	o	4	xxxx	.75	x	.75	o	7	o	2000	xxxx	2000	xxxx
	8	x	160	x	70	xx	1	o	1	o	1	o	5	x	1	x	1	o	8	o	2200	xxxx	2200	xxxx
Procain Penicillin G.	5	xxx	200	xxxx	40	x	.25	xxxx	.25	xxxx	.25	xxxx	40	xx	.25	xxxx	.25	xxxx	5	xxx	1600	xxxx	1600	xxxx
	6	xxx	300	xx	50	o	.50	xxxx	.50	xxxx	.50	xxxx	50	xx	.50	xxxx	.50	xxxx	6	xx	1800	xxxx	1800	xxxx
	7	xx	400	x	60	o	.75	xxxx	.75	xxx	.75	xxx	60	xx	.75	xxx	.75	xxx	7	x	2000	xxxx	2000	xxxx
	8	xx	500	x	70	o	1	xx	1	xx	1	xx	70	xx	1	xx	1	xx	8	x	2200	xxxx	2200	xxxx
Sulfathiazole	5	xxxx	1000	x	1000	x	5	xxxx	5	xxxx	50	x	1000	x	400	xx	200	xx	1600	xx	1600	xxxx	1600	xxxx
	6	xxxx	1200	o	1200	o	6	xxx	6	o	60	o	1200	o	600	o	220	o	1800	o	1800	xxxx	1800	xxxx
	7	xxxx	1400	o	1400	o	7	o	7	o	70	o	1400	o	800	o	240	o	2000	o	2000	xxxx	2000	xxxx
	8	xx	1600	o	1600	o	8	o	8	o	80	o	1600	o	1000	o	260	o	2200	o	2200	xxxx	2200	xxxx
Streptomycin	5	x	5	x	5	x	5	x	5	x	5	x	5	x	5	x	5	x	40	xxxx	1600	xxxx	1600	xxxx
	6	o	6	o	6	o	6	o	6	o	6	x	6	o	6	o	6	o	50	xxxx	1800	xxxx	1800	xxxx
	7	o	7	o	7	o	7	o	7	o	7	o	7	o	7	o	7	o	60	xxx	2000	xxxx	2000	xxxx
	8	o	8	o	8	o	8	o	8	o	8	o	8	o	8	o	8	o	70	xx	2200	xxxx	2200	xxxx
Tyrothricin	400	xxxx	1600	xxxx	1600	xxxx	5	xxxx	5	xxxx	5	xxxx	1600	xxxx	5	xxxx	5	xxxx	5	xxxx	1600	xxxx	1600	xxxx
	500	xxxx	1800	xxxx	1800	xxxx	10	xxxx	10	xxxx	10	xxxx	1800	xxxx	10	xxxx	6	xxxx	10	xxxx	1800	xxxx	1800	xxxx
	600	xxx	2000	xxxx	2000	xxxx	15	xxx	15	xxx	15	xxx	2000	xxx	15	xxxx	7	xxxx	15	xxxx	2000	xxxx	2000	xxxx
	700	xx	2200	xxxx	2200	xxxx	20	xx	20	xx	20	xx	2200	xx	20	xx	8	x	20	xx	2200	xxxx	2200	xxxx
Magnamycin	5	xxxx	1400	xxxx	1600	xxx	.25	xx	.25	o	.25	xx	1	xx	2	x	3	x	1	xxxx	1000	xxxx	1200	xxxx
	10	xxx	1500	xxxx	1800	xxx	.50	x	.50	o	.50	x	2	xx	25	o	4	o	2	xx	1200	xx	1300	xxxx
	15	xx	1600	xxxx	2000	xxx	.75	o	.75	o	.75	o	3	x	3	o	5	o	3	x	1400	xxx	1400	xxxx
	20	x	1700	xxx	2200	xxx	1	o	1	o	1	o	5	o	4	o	6	o	5	x	1600	xxx	1600	xxxx
Terramycin	1	xxxx	.5	x	1	xx	.25	xxxx	2	xx	.25	xxxx	.25	x	.25	xx	.25	xxx	.25	xxxx	1600	xxxx	1600	x
	1.25	xx	.6	x	2	xx	.50	xxx	2,25	x	.50	xxx	.50	o	.50	x	.50	xx	.50	xx	1800	xxxx	1800	o
	1.5	o	.7	o	3	o	.75	xx	2.5	x	.75	xx	.75	o	.75	o	.75	xx	.75	xx	2000	xxxx	2000	o
	1.7	o	.8	o	5	o	1	x	3	x	1	x	1	o	1	o	1	x	1	x	2200	xxxx	2200	o
Viacin	40	xxxx	40	xxxx	30	xxxx	20	xxxx	5	xxxx	5	xxxx	40	xxx	200	xxxx	10	xxxx	1600	xxxx	1600	xxxx	1600	xxxx
	50	xxxx	50	xxxx	40	xxx	30	xxx	6	xxxx	6	xxxx	45	xxx	300	xxxx	20	xxxx	1800	xxxx	1800	xxxx	1800	xxxx
	60	xxx	60	xxx	50	xx	40	xx	7	xxx	7	xxx	50	xx	400	xxx	30	xxx	2000	xxxx	2000	xxxx	2000	xxxx
	70	xx	70	xx	70	x	50	x	8	x	8	x	60	o	500	x	40	x	2200	xxxx	2200	xxxx	2200	xxxx

Grth---Abrev. for growth
xxxx -- good
xxx -- fair
xx -- poor
x -- very poor
o -- none

L. casei, S. faecalis and S. liquefaciens were able to grow with 7 ppm. L. acidophilus and S. lactis were able to grow with 6 ppm. L. bulgaricus was able to grow very poorly with 5 ppm of bacitracin added.

3. Dipenicillin G.

T. cremoris and T. sphaerica were able to grow in litmus milk with 2200 ppm. or more of dipenicillin added. A. aerogenes was able to grow with 400 ppm. E. coli was was able to grow with 300 ppm. Ps. putrefaciens was able to grow with 5 ppm. Alc. viscosus was able to grow with 8 ppm. S. liquefaciens was able to grow with 7 ppm. S. faecalis was able to grow with 6 ppm. L. casei was able to grow with 4 ppm. L. acidophilus and L. bulgaricus were able to grow with .75 ppm. S. lactis was able to grow with .5 ppm. concentration of dipencillin added.

4. Potassium Penicillin G.

T. cremoris and T. sphaerica were able to grow in litmus milk with 2200 ppm or more or potassium penicillin added. A. aerogenes was able to grow with 140 ppm. E. coli grew poorly over a range of 40 to 70 ppm. Alc. viscosus was able to grow with 7 ppm. S. liquefaciens was able to grow poorly with 5 ppm. Ps. putrefaiens was was able to grow with 4 ppm. S. faecalis was able to grow with .50 ppm. L. acidophilus, L. casei and S. lactis grew very poorly with .25 ppm. L. bulgaricus was negative with .25 ppm. of potassium penicillin added.

5. Procain Penicillin G.

T. cremoris and T. sphaerica were able to grow in litmus milk with 2200 or more of procain penicillin added. A. aerogenes was able to grow with 300 ppm. Ps. putrefaciens was able to grow over a range of 40 to 70 ppm. E. coli was able to grow very poorly with 40 ppm. Alc. viscosus was able to grow with 8 ppm. S. liquefaciens was able to grow with 6 ppm. L. acidophilus, L. bulgaricus, L. casei, S. faecalis and S. lactis were able to grow with 1 ppm. concentration of procain penicillin added.

6. Sulfathiazole.

T. cremoris and T. sphaerica were able to grow in litmus milk with 2200 ppm or more of sulfathiazole added. S. liquefaciens was able to grow with 1600 ppm. A. aerogenes, E. coli and Ps. putrefaciens grew poorly with 1000 ppm. S. faecalis was able to grow with 400 ppm. S. lactis grew very poorly with 200 ppm. L. casei grew very poorly with 50 ppm. Alc. viscosus was able to grow good with 8 ppm. L. acidophilus was able to grow with 6 ppm. L. bulgaricus was able to grow with 5 ppm. of sulfathiazole added.

7. Streptomycin.

T. cremoris and T. sphaerica were able to grow in litmus milk with 2200 ppm. or more of streptomycin added. S. liquefaciens was able to grow with 70 ppm. Alc. viscosus, A. aerogenes, E. coli, L. acidophilus, L. bulgaricus, L. casei, Ps. putrefaciens. S. faecalis and

S. lactis were all able to grow very poorly with 5 ppm. concentration of streptomycin added.

8. Tyrothricin.

A. aerogenes, E. coli, Ps. putrefaciens, T. cremoris and T. sphaerica were able to grow in litmus milk with 2200 ppm. or more of tyrothricin added. Alc. viscosus was able to grow in 7 ppm. L. acidophilus, L. bulgaricus, L. casei, S. faecalis and S. liquefaciens were able to grow with 20 ppm. S. lactis was able to grow with 7 ppm. of tryrothricin added.

9. Magnamycin.

E. coli was able to grow in litmus milk with 2200 ppm. or more of magnamycin added. A. aerogenes was able to grow with 1700 ppm. or more. T. cremoris and T. sphaerica were able to grow with 1600 ppm. or more Alc. viscosus was able to grow with 15 ppm. Ps. putrefaciens was able to grow with 2 ppm. S. lactis grew very poorly with 3 ppm. S. faecalis grew very poorly with 2 ppm. S. liquefaciens was able to grow with 2 ppm. L. acidophilus and L. casei were able to grow with .25 ppm. L. bulgaricus was negative with .25 ppm. of magnamycin added.

10. Terramycin.

T. cremoris was able to grow in litmus milk with 2200 ppm. or more of terramycin added. T. sphaerica grew very poorly with 1600 ppm. A. aerogenes grew very poorly with 5 ppm. E. coli and L. bulgaricus were able to grow with 2 ppm. Alc. viscosus was able to grow with 1.25

L. acidophilus, L. casei, S. lactis and S. liquefaciens were able to grow with .75 ppm. S. faecalis was able to grow with .25 ppm. Ps. putrefaciens grew very poorly with .25 ppm. of terramycin added.

11. Viocin.

S. liquefaciens, T. cremoris and T. sphaerica were able to grow in litmus milk with 2200 ppm. or more of viocin added. S. faecalis was able to grow with 400 ppm. Alc. viscosus and A. aerogenes were able to grow with 70 ppm. E. coli and Ps. putrefaciens with 50 ppm. L. acidophilus was able to grow with 40 ppm. S. lactis was able to grow with 30 ppm. L. bulgaricus and L. casei were able to grow with 7 ppm. of viocin added.

The results show that aureomycin, terramycin, streptomycin, the penicillins, viocin, magnamycin and bacitracin, in the order named, had the greatest inhibitory effect against the test organisms. Tyrothricin and sulfathiazole were the least effective. The yeasts were the most resistant organisms, followed by the coliform group and Alc. viscosus or Ps. putrefaciens, Magnamycin and terramycin were the only antibiotics showing any inhibition toward the two yeasts. S. lactis was the least resistant organism being stopped with .25 ppm of concentration of some of the antibiotics. The Lactobacillus was the next in susceptibility toward the antibiotics.

C. The Use of Aureomycin Agar for Yeast and Mold Counts.

The results of the work reported herein show that the two yeasts were very resistant to the various antibiotics tested. The yeasts were able to grow with concentrations of 2200 ppm. or more of all but three of the antibiotics used. Dipenicillin, terramycin and magnamycin were the only antibiotics showing any inhibitory action toward the yeasts and then only in high concentrations. The resistance of the yeasts indicate the possibility of adding antibiotics to agar to inhibit the growth of bacteria in yeast and mold counts on various dairy products. Since aureomycin was very effective in inhibiting the test cultures of bacteria, this antibiotic was selected for use in an attempt to determine the effectiveness of it in inhibiting bacteria in yeast and mold counts. Samples of butter and cottage cheese were plated on acidified potato dextrose agar according to Standard Methods (17) and on potato dextrose agar with 50 ppm and 100 ppm. of aureomycin added.

1. Yeast and mold counts on butter.

Table V shows the results obtained from plating butter for yeast and mold counts using acidified potato dextrose agar and potato agar with 50 ppm and 100 ppm. of aureomycin added. The acidified agar averaged 45 colonies per plate while the agar containing the 50 ppm. of aureomycin averaged 56 colonies and the agar containing the 100 ppm. of aureomycin averaged 61 colonies per plate. The plates containing the potato dextrose agar with the 50 ppm of aureomycin added had higher numbers of colonies per plate than the acidified potato dextrose agar plates with 15 samples, the same number with 2 and lower with 7, whereas the plates containing the potato

TABLE V - COMPARISON OF COUNTS ON AUREOMYCIN AGAR WITH STANDARD PLATE COUNTS FOR YEAST AND MOLDS IN BUTTER

Sample	Number of colonies per plate on potato dextrose agar with Tartaric acid added to p H 3.5	Aureomycin added 50 ppm	100 ppm
1	0	3	4
2	11	15	19
3	94	90	96
4	52	44	44
5	8	5	4
6	11	3	8
7	160	227	293
8	69	82	67
9	11	5	3
10	84	137	172
11	230	253	256
12	4	4	2
13	131	177	185
14	29	28	28
15	11	24	27
16	21	21	31
17	23	29	31
18	5	12	11
19	30	42	47
20	26	41	29
21	8	10	17
22	14	20	20
23	58	69	74
24	1	0	3
Average	45.45	55.87	61.25

dextrose agar with 100 ppm. of aureomycin added had higher numbers of colonies per plate than the acidified potato dextrose agar plates with 17 samples and lower with 7. The colonies growing on potato dextrose agar with aureomycin added were usually larger than those growing on the acidified potato dextrose agar.

2. Yeast and mold counts on cottage cheese.

The samples of cottage cheese were prepared for plating by placing 1 gram of the cottage cheese in a Sterile mortar, one ml. of sterile 20 per cent sodium citrate was added and the mixture throughly ground with a sterile pestle. Eight ml. of sterile distilled water, contained in a screw capped test tube, were added and the mixture then poured back into the tube. If further dilutions were needed they were made from this 1 to 10 dilution.

Table VI shows the results obtained from plating cottage cheese for yeast and mold counts using acidified potato dextrose agar and potato dextrose agar with 50 ppm. and 100 ppm. of aureomycin added. The acidified agar averaged 37 colonies per plate while the agar containing the 50 ppm. of aureomycin averaged 41 colonies and the agar containing the 100 ppm. of aureomycin averaged 41 colonies per plate. The plates containing the potato dextrose agar with the 50 ppm. of aureomycin added had higher numbers of colonies per plate than the acidified agar plates with samples and lower with 4, whereas the plates containing the potato dextrose agar with 100 ppm. of aureomycin added had higher numbers of colonies per plate than the acidified agar plates with 7 samples and lower with 4 samples. The colonies growing on the potato dextrose agar with the 50 ppm. and the 100 ppm. of aureomycin added were usually larger than those growing on the acidified potato dextrose agar.

TABLE VI - COMPARISON OF COUNTS ON AUREOMYCIN AGAR WITH STANDARD PLATE COUNTS FOR YEAST AND MOLDS IN COTTAGE CHEESE

Sample	Number of colonies per plate on potato dextrose agar with		
	Tartaric acid added pH 3.5	Aureomycin added 50 ppm.	100 ppm.
1	1	16	13
2	39	68	52
3	6	1	1
4	60	73	78
5	136	67	71
6	12	10	11
7	27	30	33
8	21	27	37
9	26	37	37
10	58	39	56
11	21	73	63
Average	37	41	41

The plates containing the potato dextrose agar with 50 ppm. and 100 ppm. of aureomycin added were much easier to count than those acidified with tartaric acid because there was no precipitated milk solids present, whereas the plates poured from the lower dilutions (1 to 10 and 1 to 100) with acidified potato dextrose agar contained considerable amounts of precipitated milk solids which made counting very difficult. All of the colonies appearing on the plates containing the agar with aureomycin added that might possibly be bacterial colonies, were picked and stained with the Gram stain. A total of approximately 75 colonies were examined. All colonies proved to be yeasts and no bacteria were present in any of the colonies picked.

3. The influence of aureomycin on the rate of growth of molds.

In order to determine the influence of aureomycin on the growth of molds, pure cultures of molds were picked from plates poured from samples of butter and of cottage cheese and inoculated onto acidified potato dextrose agar and onto potato dextrose agar with 50 ppm. and 100 ppm. of aureomycin added by stabbing the plate with an inoculating needle that had been exposed to the mold culture. The plates containing the molds were incubated at 21 degrees C. and the rate of growth determined by measuring the diameter of each mold colony every day for four days.

Table VII shows the average growth rate of 10 cultures of molds on acidified potato dextrose agar and on potato dextrose agar containing 50 ppm. and 100 ppm. of aureomycin during a four day period. While 12 cultures were inoculated on the plates, the average size of the colonies is given for only 10 of the cultures because two of the cultures failed to grow on the acidified agar, whereas all the cultures grew well on the agar with aureomycin added.

TABLE VII - THE RATE OF GROWTH OF MOLD COLONIES ON ACIDIFIED POTATO DEXTROSE AGAR AND ON AGAR WITH AUREOMYCIN ADDED

Period of incubation at 21°C.	Average size in cm. of mold colonies on potato dextrose agar with		
	Tartaric acid added to pH 3.5	Aureomycin added 50 ppm.	100 ppm.
1 day	.27	.37	.31
2 days	.83	1.06	.93
3 days	1.74	1.92	1.84
4 days	2.33	2.63	2.52

The results show the average growth rate of the molds on the potato dextrose agar with 50 ppm. and 100 ppm. of aureomycin added was significantly greater than the average growth rate of the molds on the acidified potato dextrose agar. The mold colonies developed faster on the agar containing 50 ppm. of aureomycin than they did on the agar containing 100 ppm. but the difference was not significant. From the results it appears that potato dextrose agar with aureomycin added is superior to acidified potato dextrose agar as a growth medium for molds.

SUMMARY

The inhibitory action of various antibiotics on microorganisms important in dairy products was determined by adding different concentrations of the antibiotics to agar and to litmus milk and then determining the rate or extent of growth of the test organisms. The agar plate technique was used to determine rather wide ranges of tolerances whereas narrower ranges of tolerances were determined by growing the test cultures in litmus milk with the antibiotics added. In the initial trials, where the antibiotics were added to the media before sterilization, there was apparently partial destruction of some of the antibiotics by the sterilization exposures and the results probably do not represent a good measure of the effectiveness of the antibiotics in inhibiting bacterial growth. No attempt was made to determine the amount of destruction by the sterilization exposures.

Additional trials were run in which the various concentrations of antibiotics were added to the media after sterilization. In general, the lactic acid producing organisms commonly used in fermented dairy products were much less resistant to the various antibiotics than those usually causing defects in dairy products.

Alc. viscosus, A. aerogenes, E. coli, L. acidophilus, L. bulgaricus, L. casei, Ps. putrefaciens, S. faecalis, S. lactis and S. liquefaciens were inhibited with 6 ppm. or less of aureomycin. T. cremoris and T. sphaerica grew in litmus milk with 2200 ppm. or more of aureomycin added.

L. acidophilus, L. bulgaricus, L. casei, S. faecalis, S. liquefaciens and S. lactis were inhibited with 10 ppm. or less of bacitracin. Alc. viscosus was able to grow within a range of 50 ppm. to 140 ppm. while Ps. putrefaciens was able to grow with a range of 20 ppm to 1000 ppm. A. aerogenes, E. coli, T. cremoris and T. sphaerica were the most resistant and grew in litmus milk with 2200 ppm. or more of bacitracin added.

Alc. viscosus, L. acidophilus, L. bulgaricus, L. casei, S. faecalis, S. liquefaciens and S. lactis were inhibited with 10 ppm. or less less of dipenicillin, potassium penicillin and procain penicillin. Ps. putrefaciens was able to grow within a range of 10 ppm. to 300 ppm. of potassium penicillin and procain penicillin but was inhibited with 10 ppm or less of dipenicillin. A. aerogenes and E. coli were able to grow within a range of 50 ppm. to 300 ppm. of all three of the penicillin's. T. cremoris and T. sphaerica were the most resistant and grew in litmus milk with 2200 ppm. or more of dipenicillin, potassium penicillin and procain penicillin added. T. cremoris was stopped with 200 ppm. concentration of dipenicillin when grown on the agar plate technique.

Alc. viscosus, L. acidophilus and L. bulgaricus were inhibited with 100 ppm. or less of sulfathiazole. L. casei was able to grow within a range of 100 ppm. to 200 ppm. A. aerogenes, E. coli, Ps. putrefaciens, S. faecalis and S. liquefaciens were able to grow within a range of 1000 ppm. to 1600 ppm. T. cremoris and T. sphaerica were were the most resistant and grew in litmus milk with 2200 ppm. or more of sulfathiazole added.

Alc. viscosus, A. aerogenes, E. coli, L. acidophilus, L bulgaricus, L. casei, Ps. putrefaciens, S. faecalis and S. lactis were

inhibited with 10 ppm. or less of streptomycin. S. liquefaciens was able to grow within a range of 20 ppm. to 70 ppm. T. cremoris and T. sphaerica, were the most resistant and grew in litmus milk with 2200 ppm. or more of streptomycin added.

L. acidophilus, L. bulgaricus, L. casei, S. faecalis, S. liquefaciens and S. lactis were inhibited with 20 ppm. or less of tyrothricin. Alc. viscosus was able to grow within a range of 400 to 700 ppm. A. aerogenes, E. coli, Ps. putrefaciens, T. cremoris and T. sphaerica were the most resistant and grew in litmus milk with 2200 ppm. or more of tyrothricin added.

Alc. vicosus, L. acidophilus, L. bulgaricus, L. casei, Ps. putrefaciens, S. faecalis, S. lactis and S. liquefaciens were inhibited with 15 ppm. or less of magnamycin. A. aerogenes, T. cremoris and T. sphaerica were able to grow within a range of 1000 ppm. to 1600 ppm. E. coli was the most resistant and grew in litmus milk with 2200 ppm. or more of magnamycin added.

Alc. viscosus, A. aerogenes, E. coli, L. acidophilus, L. bulgaricus, L. casei, Ps. putrefaciens, S. faecalis, S. lactis and S. liquefaciens were inhibited with 10 ppm. or less of terramycin. T. sphaerica was able to grow within a range of 1600 ppm. to 1800 ppm. T. cremoris was the most resistant and grew in litmus milk with 2200 ppm. or more of terramycin added.

L. bulgaricus and L. casei were inhibited with 10 ppm. or less of viocin. Alc. viscosus, A. aerogenes, E. coli, L. acidophilus, Ps. putrefaciens and S. lactis were able to grow within a range of 20 ppm. to 70 ppm. while S. faecalis was able to grow within a range of 400 ppm. to 500 ppm. S. liquefaciens, T. cremoris and T. sphaerica were the most resistant and grew in litmus milk with 2200 ppm. or more of viocin added.

The high resistance of the yeasts to the various antibiotics suggested the use of antibiotics to inhibit the growth of bacteria in yeast and mold counts. Several comparisons of yeast and mold counts were made in which butter and cottage cheese were plated on acidified potato dextrose agar and on potato dextrose agar containing 50 ppm. and 100 ppm. of aureomycin. The results indicated that the aureomycin agar was superior to the acidified agar for yeast and mold counts because it gave higher counts, the colonies were larger and there was no precipitated milk solids in the plates poured from cottage cheese. The concentrations of aureomycin needed to effectively inhibit bacteria growth apparently did not inhibit the growth of yeasts and molds, in fact, the growth of both yeasts and molds appeared to be more rapid and extensive on aureomycin agar than on the acidified agar.

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