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A COMPARATIVE INVESTIGATION OF CERTAIN FILM-FORMING FUNGI[1]

M. A. JOSLYN[2] AND W. V. CRUESS[3]

Aerobic, film-forming microorganisms of yeast-like appearance occur very commonly on brines used in the storage of olives and vegetables used for pickles and on fermented liquors such as cider, wine, and beer. They are commonly known as *Mycoderma vini* or 'wine flowers' when they occur on fruit products, as *Mycoderma cerevisiae* on cereal products, and as 'film yeast' or 'scum' on pickle brines. These microorganisms are of considerable economic importance, for they bring about certain undesirable conditions in the flavor, odor, and composition of food products on which they grow.

Although certain forms of *Mycoderma cerevisiae* and *Mycoderma vini* have been extensively studied,[4] the forms occurring on the surface of vegetable and olive brines have received relatively scant attention. The object of this investigation has been to study *Mycodermas*[5] from these sources in comparison with *Mycoderma vini* and certain molds found in association with this and other *Mycodermas.*

[1] This paper is an extension of a Master's thesis submitted by the senior author.

[2] Research Assistant in Fruit Products.

[3] Associate Professor of Fruit Products and Chemist in the Experiment Station.

[4] An excellent review of the earlier investigations will be found in reference 1 in the bibliography.

[5] The *Mycodermas* investigated here are by some writers referred to as 'false yeasts' or *Mycoderma* yeasts.

EXPERIMENTAL PROCEDURE

Certain of the characteristics of twenty-one microorganisms were determined as follows:

Morphological Characteristics.—The macroscopical appearance of pure cultures of the various microorganisms growing in cucumber juice, malt-extract medium, nutrient solutions of sucrose, dextrose lactose, maltose, mannite, and glycerin, cucumber-juice agar and cucumber-juice gelatin was observed.

The size, form, and general microscopical appearance of cells from seven-day old growth on cucumber juice and the microscopical appearance of agar colonies were also observed.

The cultures were tested for spore formation by culturing on moist gypsum blocks.

Cultural Characteristics.—The more important cultural characteristics were studied. These investigations may be classified as follows:

Fermentation Tests.—The rates of fermentation in nutrient solutions of sucrose, dextrose, lactose, maltose, mannite and glycerin were determined by noting the loss in weight of the nutrient solutions at regular intervals, after inoculation with pure cultures of the various microorganisms.

Biochemical Characteristics.—The destruction of added lactic acid in cucumber-juice medium and of natural acids, mostly lactic acid, in dill brine was determined by noting the loss in acid content by titration with sodium hydroxid. The tolerance of the organisms studied to acetic, citric, lactic, malic, oxalic, and tartaric acids was determined by noting the highest concentration of acid at which pellicle formation occurred in synthetic carbohydrate-free medium. The effect of the organisms on these acids was also determined.

The salt tolerance of these organisms was determined by noting the highest concentration of salt at which pellicle formation occurred in various media. The effect of salt on the rate of oxidation of lactic acid in cucumber juice was also observed.

The effect of disturbing the pellicle and of changing the ratio of surface to volume of the nutrient medium on the activity of certain *Mycodermas* was determined by noting the loss in acid content due to oxidation.

The effect of a layer of neutral mineral oil, of pH value of the medium, of oxygen supply, of light and of certain antiseptics and germicides on the formation of the pellicle was determined.

A study was made of the nitrogen fixation by these organisms in a medium low in, but not devoid of, nitrogen was made by determining the nitrogen content of cultures and dilute cucumber juice in which they were grown.

The effect of salt concentration and of pH value of the medium on the death temperatures of certain *Mycodermas* and a *Penicillium* mold was determined.

All experiments were carried out in duplicate, unless otherwise stated in the text, and only pure cultures derived by single-cell isolations from the sources listed elsewhere were used. The cultures used were purified by repeated plating on cucumber juice agar medium. Freshly grown cultures were used for each experiment and the organisms were generally previously cultured in the medium to be used for the particular experiment. This was done to avoid irregularities in behavior due to change of culture media and to secure active cultures.

Preparation of Media.—The various media used in the tests were prepared as follows:

1. Cucumber-juice medium. Fresh cucumbers were crushed and the juice extracted by pressure in a hydraulic press. The juice was boiled in an open kettle with infusorial earth and filtered brilliantly clear. This furnished a medium of amber or straw-yellow color and was found to be suitable for the growth of all the organisms studied. The composition of the medium was varied to suit the nature of the experiment. However, the cucumber juice used in preparing most of the special media was of the following composition:

Balling degree—4.65
Acidity expressed as lactic—0.1 grams per 100 cc
pH (determined electrometrically)—5.7

Cucumber juice was used as the basic culture medium because most of the microorganisms studied were isolated from cucumber-pickle brines. The natural juice and the media made from it were sterilized by auto-claving at 15 pounds pressure for 30 minutes.

2. Dill-pickle brine medium. Brine from fermented dill pickles was boiled in an open kettle with infusorial earth and then filtered clear. The pH value of this medium was 3.2, the density 6° Baumé, and its total acid content expressed as lactic was 1.20 grams per 100 cc. This medium was sterilized by heating in live steam at approximately 100° C for one hour. Later incubation tests proved this method of sterilization satisfactory. This medium was found suitable for the growth of the majority but not all of the microorganisms studied.

3. Malt-extract medium. 'Spra-malt,' a dried preparation of malt extract, was dissolved in water and the solution brought to about 15° Balling. It was sterilized by the usual method of discontinuous sterilization in live steam at approximately 100° C. All of the microorganisms studied grew well in this medium.

4. Synthetic medium. The synthetic nutrient solution used for determining the action of the microorganisms on the various sugars and acids consisted of 0.01 grams of magnesium sulfate, 0.5 grams of dipotassium phosphate and 10 grams of bacto-peptone dissolved in 1,000 cc of distilled water. This solution furnished a satisfactory medium for the tests in which it was used.

The sugar solutions used in the fermentation tests were sterilized by the usual method of discontinuous sterilization at 100° C.

All media were tested for sterility before use and the methods of sterilization used were found adequate.

TABLE 1

SOURCES OF THE MICROORGANISMS STUDIED

Laboratory number of organism	Kind of microorganism and its source
1	*Mycoderma* from surface of brine from green olives prepared at Visalia, 1925.
2	*Mycoderma* from surface of brine used for storage of ripe olives at Lindsay, 1926.
3	*Mycoderma*. Source similar to that of No. 2.
4	*Mycoderma* from dill pickle brine from H. J. Heinz Co., Berkeley, 1927.
5	*Mycoderma* from dill pickle brine from Mueller Bros., Oakland, 1927.
6	*Mycoderma*. Same source as No. 4.
7	*Mycoderma* from cucumber storage brine ("salt stock" brine), California Conserving Co., Hayward, 1927.
8	*Mycoderma* from "salt stock" brine, Mueller Brothers Co., Oakland, 1927.
9	*Mycoderma* from same source as No. 7.
10	*Mycoderma* from "salt stock" brine. H. J. Heinz Co., Watsonville, 1927.
11	*Mycoderma* from fermented apple juice (vinegar stock) from H. J. Heinz Co., Watsonville, 1927.
12	*Mycoderma* from fermenting apple juice, H. J. Heinz Co., Watsonville, 1927.
13	*Mycoderma* from fermented apple juice from Jones Vinegar Co., Watsonville, 1927.
14	*Mycoderma* isolated from California grapes in 1912 by W. V. Cruess.
15	*Mycoderma*. Same source as No. 14.
16	*Mycoderma*. Same source as No. 14.
17	*Torula*, pink. From soil in cucumber field near Hayward, 1927.
18	*Penicillium* mold. Forms a red pigment. From dill-pickle brine from H. J. Heinz Co., Berkeley, 1927.
19	*Penicillium* mold. Forms brown conidia. From olive-storage brine from Visalia, 1926.
20	*Mucor* mold from moldy cucumbers.
21	*Penicillium* mold. Forms dark green conidia. From "salt stock" brine from California Conserving Co., Hayward, 1927.

SOURCES OF MICROORGANISMS STUDIED

Twenty-one organisms were studied. Of these, 16 are *Mycodermas,* isolated from the several different sources given in the following list: one is a pink *Torula* which grows in the form of a ring on liquid media in test tubes; four are molds of which 3 are *Penicillium* species and 1 is a *Mucor.* All of the organisms described in this report were isolated by us from the sources indicated in table 1, which also gives the laboratory number of each.

Since the principal object of this investigation was to compare the principal morphological and cultural characteristics of the various organisms, they will not be described individually but rather collectively and in relation to one another.

The fresh-olive storage brines from which some of the organisms were isolated were of approximately 9° Baumé and contained 0.2–0.5 per cent acid expressed as lactic. The green-olive brines mentioned in the list were from 50-gallon casks of green Manzanillo olives prepared by the Spanish fermentation process. The dill brines were from commercially prepared 50-gallon casks of dill pickles prepared by the usual fermentation process. These brines were of 5–7° Baumé and contained from 1.1 to 1.5 per cent acid expressed as lactic. The salt stock brines were of 16–18° Baumé and contained less than 0.5 per cent acid expressed as lactic at the time of isolation of the organisms. The fermented apple juice from which some of the organisms were isolated was stored in large, partially open vats, and the film growth on the liquid was used for plating. The *Mycoderma* cultures from grapes were obtained by washing the grapes with sterile water and making culture plates from the washings.

MORPHOLOGICAL CHARACTERISTICS

Growth on Liquid Media.—All of the organisms are strongly aerobic and formed films on quiescent culture liquids. In cultures of organisms 1 to 16, a pellicle appeared at the beginning of development, generally white but changing to a light gray in old cultures. The pellicles were not viscous but were quite fragile and very easily broken. Growth took place also along the sides of the tube to a height of more than three centimeters and the upper portions of this growth invariably turned brown. Some sediment was formed by all.

TABLE 2

MACROSCOPICAL APPEARANCE OF ORGANISMS INVESTIGATED

Medium	Organism	Macroscopical appearance
Cucumber juice	1, 2, 4, 5, 6, 8	Fragile but rather thick, wrinkled, chalky white film, spreading upward 2–3 cm on walls of tube, where it in time becomes brown. Medium becomes turbid. Numerous granules form below the film, some of which settle to the bottom of the tube forming a loose, granular sediment. Very little gas.
	3, 7, 13	Thin, only lightly wrinkled film; considerable growth on walls of tube; turbid medium; much fine sediment. Little to no gas.
	9, 10	Fragile, wrinkled, rather thick film; turbid medium; compact sediment. Little to no gas.
	11	Similar to No. 3 except film more wrinkled and heavier.
	12	Appearance similar to No. 1. Forms much gas.
	14	Thick, slightly wrinkled film, slight sediment. Liquid like No. 1. Much gas.
	15	Smooth, slight film growth, heavy sediment. Medium turbid with small suspended granules. Noticeable gas formation.
	16	Appearance similar to No. 15; but less gas.
	17	Principally in the medium; decided ring formation at surface; much sediment. Ring and sediment pink. No gas.
	18	Typical *Penicillium* mycelium and conidia. In later stages of growth, surface growth dark green to yellow; medium and under portion of growth become deep red. Pigment does not change in color with reaction of medium.
	19	Typical *Penicillium* mycelium and brown conidia.
	21	Typical *Penicillium* mycelium and dark green conidia.
	20	Typical, gray, hair-like *Mucor* mycelium with typical spherical sporangiophores.
Cucumber juice diluted 1 to 3 with water	1 to 17	Similar to growth in undiluted juice.
	18, 19, 20, 21	Growth much less vigorous than in undiluted juice.
Diluted malt syrup	1 to 21	Similar to growth in undiluted cucumber juice.
Dill-pickle-brine	1, 2, 4, 5, 6, 14	Growth less luxuriant; films thinner; more granular and more gray and less sediment than in cucumber juice.
	3	Film whiter and growth less vigorous than Nos. 1, 2, 4, 5, and 6.
Dill-pickle-brine	7 to 13	Observations similar to but growth less vigorous than Nos. 1, 2, 4, 5, 14.
	19	Growth scant.
	9, 17, 18, 20, 21	No growth.
Nutrient sucrose solution	1 to 21	Growth similar to that in cucumber juice, but no visible gas formation; Nos. 4, 5, and 10 grew more luxuriantly than others.
Nutrient dextrose solution	1 to 21	Growth similar to that in cucumber juice, but no visible gas formation; Nos. 4, 5, and 10 grew more luxuriantly than others.
Nutrient lactose solution	1 to 17	Growth slow and slight. No gas.
	18 to 21	Growth fairly vigorous.
Nutrient maltose solution	1 to 21	Growth vigorous and characteristic. No visible gas formation.
Nutrient mannite solution	1, 2, 7, 9, 10, 13	Moderate growth. No gas.
	17, 18, 19, 20, 21	Vigorous growth. No gas.
	3, 4, 5, 6, 8, 11, 12, 14, 15, 16	Scant growth. No gas.
Nutrient glycerin solution	1, 4, 5, 8, 10, 11, 13, 15, 16	Faint growth. No gas.
	2, 3, 6, 7, 9, 12, 14, 17	Good growth. No gas.
	18, 19, 20, 21	Vigorous growth. No gas.

They differed among themselves in the general appearance of the film, the amount and character of sediment, and in the appearance of the substrate, whether clouded or clear.

The macroscopical appearance of the microorganisms in the various media studied is shown in table 2.

TABLE 3

MICROSCOPICAL APPEARANCE OF CELLS OF ORGANISMS 1 TO 17

Organism	Microscopical appearance of cells	Size in microns		
		Average	Maximum	Minimum
1	Sausage-shaped to filamentous. Chain formation common	2 x 10	2 x 12	3 x 4
2	Spherical to long rod-shape. Sausage-shaped predominates	4 x 3	4 x 8	3 x 3
3	Spherical to oval	4 x 4	4 x 6	2 x 2
4	Sausage-shaped to filamentous. Branched chains common	2 x 8	4 x 3	2 x 4
5	Short sausage-shaped	4 x 6	4 x 10	2 x 3
6	Sausage-shaped to filamentous. Tendency to form a mycelium	2 x 8	2 x 15	2 x 7
7	Spherical to ellipsoidal; some sausage-shaped	3 x 4	2 x 6	2 x 2
8	Spherical to sausage-shaped	4 x 5	3 x 8	2 x 2
9	Spherical to short sausage-shaped	4 x 4	4 x 8	2 x 2
10	Spherical to short ellipsoidal	3 x 3	4 x 4	2 x 2
11	Spherical to sausage-shaped	3 x 4	4 x 6	2 x 3
12	Spherical to sausage-shaped. Much variation in size	4 x 8	4 x 14	2 x 2
13	Short sausage-shaped cells predominate	4 x 6	4 x 8	2 x 3
14	Sausage-shaped. Branched chain growth common	4 x 9	4 x 16	4 x 5
15	Spherical to ellipsoidal	3 x 5	4 x 6	1.5 x 1.5
16	Spherical to sausage-shaped. Forms branched chains	4 x 4	6 x 6	2 x 2
17	Spherical	4 x 4	6 x 6	2 x 2

TABLE 4

APPEARANCE OF COLONIES ON SOLID MEDIA

Organism	Growth on agar media	Growth on gelatin media
1, 2, 4	Small, irregular, convex, rugose, cretaceous colonies. Edges entire under microscope.	Small, irregular, flat (but slightly raised), rugose, gray, opaque colonies. Edges entire under microscope.
3	Small, irregular, convex, rugose, opaque colonies. Edges entire under microscope.	Similar to No. 1.
5	Similar to No. 1.	Small, irregular, concave, rugose, gray, opaque colonies. Edges entire under microscope.
6	Similar to No. 3.	Similar to No. 1.
7	Similar to No. 1.	Similar to No. 5.
8	Similar to No. 3, but slightly brown.	Similar to No. 1.
9, 10	Similar to No. 1.	Small, irregular, raised, rugose, cretaceous colonies. Edges entire under microscope.
11, 12, 13, 14, 15, 16	Similar to No. 1.	Small, punctiform, opaque gray colonies. Edges entire under microscope.
17	Small, irregular, convex to palomate, smooth, glistening, pink colonies. Edges entire under microscope.	Small, round, concave, smooth, glistening pink colonies. Edges entire under microscope.
18, 19, 20, 21	Typical mycelial mold colonies.	Typical mycelial mold colonies.

It is evident from these observations that the organisms differ in their growth on and in various media. Relative vigor of growth among the twenty-one organisms varied considerably according to the medium.

The size, form, and general appearance of organisms 1 to 17 after culture for seven days at room temperature are given in table 3.

Growth on Cucumber-Juice Agar and Gelatin.—The appearance of seven-day-old colonies on cucumber-juice agar and on cucumber-juice gelatin are given according to the terminology adopted by Chester[2] in table 4.

Spore Formation.—Using the usual methods of causing spore formation[1, 3]—by growth in moist gypsum blocks or in saturated gypsum solutions—no spore formation could be observed with cultures 1 to 17, although under the same conditions yeasts which were known to form spores (*Saccharomyces ellipsoideus*) sporulated satisfactorily.

FERMENTATION TESTS

A synthetic nutrient medium was prepared as described on page 204. Sucrose, dextrose, lactose, and maltose were added in concentration of 10 grams per 100 cc, and glycerin and mannite in concentration of 5 grams per 100 cc. Seventy cc portions of sucrose and dextrose were placed in 125 cc Erlenmeyer flasks, 80 cc portions of lactose and maltose and 70 cc portions of glycerin and mannite were placed in 4-ounce bottles.

After sterilization the solutions were inoculated with the different organisms, and loss in weight during the incubation period at room temperature was determined. This loss corrected for loss in weight from evaporation was used as a measure of fermentation. A lower concentration of glycerin was used because of its greater osmotic pressure. The loss in weight of the inoculated flasks after correction for losses by evaporation at the end of 30 days is given in table 5.

An examination of table 5 will show that most of the organisms studied caused slight to moderate destruction of dextrose, sucrose and maltose but with the exception of the molds did not destroy any appreciable proportion of the lactose or mannite. However, organism No. 3 fermented mannite feebly.

The organisms in general grew best in the maltose medium, with dextrose a close second, and sucrose, glycerin, mannite, and lactose in the order stated. They grew very poorly in lactose. At the end of two months the pH value of the solutions given in table 5 was determined electrometrically and the data are shown in table 6

TABLE 5

LOSS IN WEIGHT IN GRAMS IN 30 DAYS

Organism	Sucrose* *grams*	Dextrose* *grams*	Lactose† *grams*	Maltose† *grams*	Glycerin‡ *grams*	Mannite‡ *grams*
1	0.42	2.31	0.00	0.45	0.25	0.00
2	0.19	2.40	0.00	0.23	0.50	0.00
3	0.18	1.25	0.20	0.50	0.21	1.31
4	0.54	3.16	0.15	0.38	0.54	0.00
5	0.30	3.08	0.23	0.28	0.38	0.00
6	0.26	4.16	0.00	0.50	0.24	0.00
7	0.90	1.16	0.14	0.63	0.26	0.00
8	0.25	1.61	0.00	0.29	0.60	0.04
9	0.71	0.97	0.00	0.50	0.51	0.00
10	0.39	0.71	0.00	0.50	0.37	0.00
11	0.04	0.57	0.00	0.77	0.09	0.00
12	1.11	0.43	0.52	0.20	0.08	0.00
13	0.41	0.27	0.77	1.00	0.33	0.00
14	0.70	3.75	0.00	0.28	0.32	0.00
15	0.20	3.66	0.00	0.19	0.48	0.00
16	0.18	0.72	0.00	0.19	0.00	0.00
17	0.19	0.43	0.00	0.00	0.44	0.00
18	1.16	.....	0.00	1.53	0.40	0.00
19	0.60	2.47	1.20	1.48	0.72	0.65
20	0.06	3.16	0.00	1.68	0.57	0.02
21	1.61	0.64	1.02	1.49	0.35	0.34

* Concentration 10 grams per 100 cc; 70 cc of solution used in 125-cc Erlenmeyer flask.
† Concentration 10 grams per 100 cc; 80 cc of solution used in 4-oz. bottles.
‡ Concentration 5 grams per 100 cc; 70 cc of solution used in 4-oz. bottles.

TABLE 6

CHANGE IN pH VALUE PRODUCED BY GROWTH OF ORGANISMS 1 TO 21 AFTER 60 DAYS

Organism	Sucrose *pH*	Dextrose *pH*	Maltose *pH*	Glycerin *pH*	Mannite *pH*
Control	5.60	4.90	5.10	6.4	6.8
1	5.35	4.00	5.00	.....	.....
2	6.20	4.00	4.75	5.3	6.8
3	4.70	3.60	4.50	6.4	6.8
4	4.50	3.65	4.10	6.9	8.2
5	5.35	3.80	5.10	5.8	6.8
6	6.35	4.30	4.80	6.4	7.5
7	4.35	4.10	4.75	7.0	7.4
8	3.65	3.80	4.80	6.1	6.4
9	4.15	4.00	4.35	7.0	7.1
10	4.35	4.05	4.50	7.5	7.1
11	5.50	2.85	3.60	6.2	7.1
12	2.00	2.10	4.30	5.1	7.2
13	4.30	2.40	4.40	6.8	7.0
14	5.30	5.00	5.10	6.7	6.9
15	5.10	4.10	5.40	6.2	7.7
16	5.05	3.90	4.70	6.4	6.6
17	4.10	3.50	4.40	.....	7.0
18	3.20	4.00	3.60	5.5	5.1
19	7.00	6.10	4.90	.....	6.7
20	3.75	3.15	2.80	3.3	7.3
21	6.70	6.15	5.90	6.5	6.6

It had been noted previously by various workers that certain fungi are capable of changing the reaction of the media which they ferment, that is, are capable of producing acids or of destroying them.

It is evident that the majority of these organisms were capable of producing acid especially in sucrose, dextrose, and maltose solutions. The nature of the acid was not determined. Titration of total acid with N/10 sodium hydroxid confirmed pH determinations and the total acidities were found to be in the inverse order of the pH values.

DESTRUCTION OF AND TOLERANCE FOR CERTAIN ORGANIC ACIDS

It has long been known that the *Mycodermas* reduce the acidity of pickle brines by oxidation, as reported in publications such as those of Round and Lang,[4] Le Fevre,[5] Shinkle,[6] Cruess,[7] and others. It is commonly recommended that the 'scum' forming on brines be skimmed frequently to avoid loss of lactic acid on which the keeping quality of the product depends.

Previous to the preliminary report of Joslyn[8] no data on the rate of destruction of the acid could be found in the literature. Joslyn found that the mixed culture of *Mycoderma* used by him destroyed acid more rapidly than the pure culture; both, however, destroyed more than half of the acid originally present (1.13 per cent) in one month at 80° F. Destruction was somewhat slower at room temperature than at 80° F. Cruess[9] determined the rate of destruction of lactic acid in olive brines by three pure cultures of *Mycoderma* a culture of *Penicillium glaucum,* and two mixed cultures. He found that the *Penicillium* mold destroyed lactic acid about as rapidly as the *Mycoderma* cultures.

Lactic Acid in Cucumber Medium.—In our studies the lactic-acid-destroying activity of organisms 1 to 21 in a cucumber medium was determined in the following manner: The organisms were cultured in cucumber juice for one week prior to the test; one cc of the well-shaken culture was pipetted into 150 cc of sterile cucumber juice containing 0.9 gram of added lactic acid per 100 cc. The rate of oxidation of lactic acid was determined by noting the loss in total acid as shown by titration with N/10 sodium hydroxid at regular intervals. The data is given in table 7.

In order to avoid an error from the carbon dioxid formed, especially in cultures 3, 6, 15, and 20, the samples to be titrated were

either heated slightly below the boiling temperature for several minutes or were diluted with boiling distilled water to volatilize carbon dioxid before titration. The pellicle in each culture was unavoidably disturbed upon shaking before removal of the sample. Such disturbance interfered with the activity of the organisms, as will be shown later.

The organisms differed from each other in their rate of oxidation of lactic acid. Organisms 1, 4, 8, 9, 14, 16, and 21 oxidized lactic acid rather rapidly; organisms 2, 7, 10, 13, 19, and 20 not so rapidly; organisms 3, 17, and 18, slowly.

TABLE 7

PERCENTAGE OF LACTIC ACID OXIDIZED

Organism No.	Time in days			
	7 days *per cent*	14 days *per cent*	21 days *per cent*	35 days *per cent*
1	31.9	49.0	.....	72.9
2	30.9	42.3	.....	67.2
3	.....	.....	.....	38.5
4	22.4	28.8	53.8	78.8
5	28.5	38.4	.....	81.7
6	0	33.6	52.0	71.1
7	14.0	25.0	29.8	60.6
8	26.5	38.5	48.0	88.5
6	18.8	42.2	44.2	76.8
10	9.7	11.5	32.7	63.5
13	18.7	.....	29.8	62.5
14	9.2	38.5	51.9	80.7
15	.....	15.4	23.1	71.2
16	18.8	32.7	51.9	74.0
17	3.4	3.8	7.7	35.7
18	13.1	.....	32.7	43.0
19	23.7	49.0	.....	59.6
20	.....	13.5	19.3	66.3
21	25.1	28.8	38.5	74.0

It would have been desirable to note whether or not the *Mycodermas* were capable of oxidizing all the lactic acid and producing an alkaline reaction in the medium. Unfortunately in the later stages of the experiment, cultures 1 to 17 inclusive became contaminated, chiefly with mold. However, the cultures were retained for three months. Molds 19, 20, and 21 had produced an alkaline reaction in the medium; of the infected *Mycoderma* cultures, only Nos. 16 and 17 developed an alkaline reaction. In experiments reported by Cruess[9] only one culture (a mixture of *Mycoderma* and mold) produced an alkaline reaction in 7 months' incubation of inoculated olive brines.

In Dill Brine.—We also determined the rate of oxidation by these organisms of the acid naturally formed in dill-pickle brine in the following manner. Ten-cubic-centimeter portions of dill brine were placed in plugged test tubes. These were sterilized and then inoculated with loopfuls of organisms 1 to 21. A sufficient number of test tubes was prepared so that at each examination an undisturbed 10 cc sample of each could be used for titration.

Organisms 17, 18, 20, and 21 failed to grow in the dill brine. The rate of oxidation of the natural acid in the dill brine is shown in table 8.

TABLE 8

PERCENTAGE OF ACID OXIDIZED IN DILL BRINE

Organism	Time in days					
	8 days *per cent*	13 days *per cent*	18 days *per cent*	23 days *per cent*	39 days *per cent*	81 days *per cent*
1	53.3	71.7	92.3	96.2	98.5	99.0
2	53.7	71.5	91.0	94.0	97.0	97.5
3	15.0	42.4	54.5	60.6	95.5	97.5
4	54.5	82.5	92.5	94.0	98.5	98.5
5	55.3	75.8	89.6	94.7	97.0	97.5
6	59.0	72.0	89.4	96.2	98.5	99.0
7	34.8	74.2	91.0	98.5	Basic	Basic
8	43.2	57.6	85.0	91.0	97.0	97.0
9	18.2	50.8	79.5	96.2	Basic	Basic
10	49.2	82.0	91.0	98.0	Basic	Basic
11	50.0	75.0	77.2	79.5	82.6	91.0
12	61.3	77.3	94.0	97.0	98.5	98.5
13	30.3	47.0	73.4	77.3	80.2	80.2
14	62.8	83.5	96.2	97.5	99.3	99.3
15	50.0	54.5	76.0	94.0	98.5	99.5
16	22.7	34.8	43.2	47.0	60.6	91.5
19	18.2	59.0	89.5	97.8	Basic	Basic

The organisms did not differ as much from each other in their rate of oxidation of acid in this test as they did in cucumber-juice medium, but the loss of acid is more rapid. Organisms 1, 2, 4, 5, 6, 11, 12, 14, and 15 exhibited similar rates of oxidation of acid. The initial rate was rapid but decreased with decrease in concentration of acid. Organisms 3, 7, 8, 9, 10, 16, and 19 oxidized the acid slowly at first but more rapidly as the concentration of the acid decreased. Organism 13 oxidized the acid least rapidly. Organisms 7, 9, 10, and 19 produced an alkaline reaction in the dill brine. Typical rates of acid-destruction are shown by the curves in fiugre 1.

In both the cucumber juice and the dill-pickle brine, darkening of the medium occurred when low total acid was reached. This dark color persisted even after acidifying the medium. It may have been caused by oxidation of some coloring material of the brine.

Destruction of Various Organic Acids in a Sugar-Free Medium.— It is evident that these organisms were able to destroy lactic acid. However, sugars and other carbon compounds were present in the above media and these may have furnished some of the carbon and energy for growth.

In order to eliminate sugars, nutrient-acid solutions were prepared by adding to the sugar-free nutrient media previously described

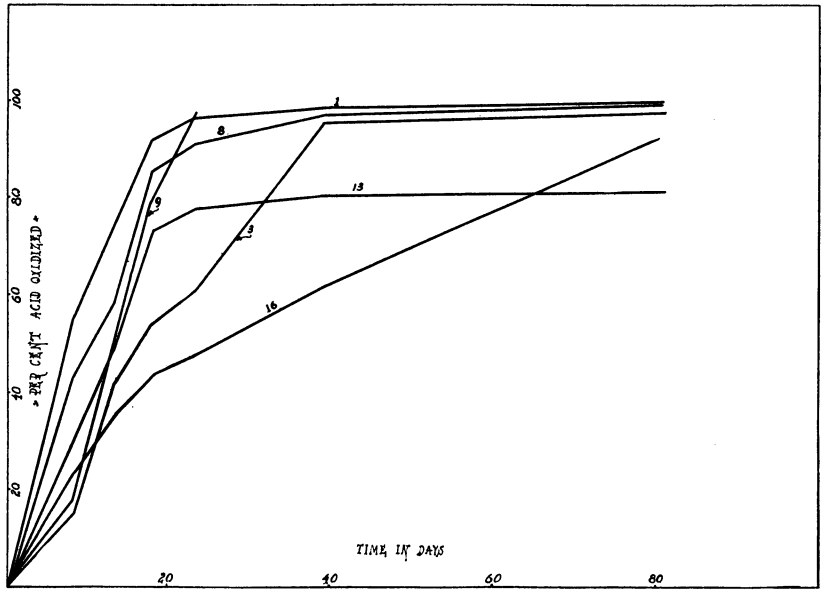

Fig. 1. Rate of oxidation of acid in dill brine.

(page 204) various amounts of acetic acid, citric acid, lactic acid, malic acid, oxalic acid, and tartaric acid. The sterile, tubed acid media were inoculated with loopfuls of the organisms which had been grown for two weeks in malt extract medium. These tubes were then stored at room temperature and growth or its absence in each tube determined periodically. It was found that film formation occurred sooner in the media containing the smaller amounts of acid and sooner in solutions of citric, lactic, and malic acids than in those of the other acids. The examinations were continued over a period of from 3 to 4 weeks incubation. The results were as follows:

In acetic acid, organisms 1 to 21 inclusive grew in 0.024 N (0.14 per cent) solution, all except Nos. 18, 20, and 21 grew in 0.042 N (0.25 per cent) solution; all except Nos. 7, 9, 10, 17, 18, 20, and 21 grew in 0.060 N (0.36 per cent), 0.76 N (0.46 per cent and pH of 4.5),

and 0.095 N (0.57 per cent); all except the aforementioned and No. 19 grew in 0.140 N (0.84 per cent and pH of 3.8); only Nos 1, 2, 3, and 11 grew in 0.28 N (1.68 per cent, pH 3.5) and none grew in 0.67 N (4.0 per cent pH 3.35).

In citric acid all except Nos. 7 and 9 grew in the solution of 0.2 N (1.28 per cent pH 2.75); all except Nos. 4, 7, 9, and 10 in 0.38 N (2.43 per cent, pH 2.5); and in 0.6 N (3.84 per cent, pH 2.3) all except Nos. 4, 7, 9, 10, 11, and 19; in 0.82 N (5.25 per cent pH 2.1); and in 1.0 N (6.4 per cent, pH 1.9) only Nos. 6, 12, 14, 15, 16, 17, and 18 grew.

In malic acid Nos. 7, 9, 10, 13, and 16 failed to grow at 0.2 N (1.34 per cent, pH 2.65); Nos. 7, 9, 10, 11, 13, 16, 19, 20, and 21 failed to grow at 0.42 N (2.81 per cent, pH 2.3); Nos. 7, 8, 9, 10, 11, 13, 16, 17, 19, 20, and 21 failed to grow at 0.62 N (4.15 per cent, pH 2.1); Nos. 5, 7 to 13 inclusive, 15, 16, 17, 19, 20, and 21 failed to grow at 0.95 N (6.36 per cent, pH 1.85); and only Nos. 2, 6, and 18 grew in 1.02 N (6.7 per cent, pH 1.8).

In oxalic acid Nos. 3, 11, and 13 failed to grow at 0.016 N (0.072 per cent); Nos. 3, 11, 13, and 16 at 0.027 N (0.122 per cent), and Nos. 1 to 5 inclusive, 7 to 11 inclusive, 13, and 16 failed to grow at 0.06 N (0.27 per cent).

In tartaric acid Nos. 1, 4, 5, and 7 to 11 inclusive, and 13 did not grow at 0.2 N (1.50 per cent, pH 2.6); Nos. 1, 2, 4, 5, 7 to 11 inclusive, 13, 15, 16, and 17 failed to grow at 0.37 N (2.78 per cent, pH 2.35); Nos. 1, 2, 4 to 13 inclusive, 15, 16, 17, and 20 failed to grow at 0.55 N (4.13 per cent, pH 2.10); Nos. 1, 2, 3 to 13 inclusive, 15, 16, 17, 19, 20, and 21 failed to grow at 0.77 N (5.78 per cent, pH 1.95), and also at 0.94 N (7.05 per cent, pH 1.85).

Most of the organisms studied were able to utilize the acids investigated for growth when the concentration was not too high. Of the acids investigated lactic acid was more suitable to the growth of yeast and citric acid to the growth of molds. Reference to figure 2 will show that the acids studied can be arranged as follows in order of their suitability for the growth of organisms studied at the concentrations investigated: citric, lactic, malic, tartaric, acetic, and oxalic. The organisms studied had a lower tolerance for oxalic acid than for any of the other acids studied. Of the molds studied No. 18 was most tolerant of acid. Of the *Mycodermas* Nos. 1, 2, 3, 4, 5, 6, 12, and 14 were most tolerant. While there appeared to be considerable correlation between growth and pH value for lactic, tartaric, and malic acids,

yet these acids apparently also exerted a considerable specific effect; that is, at equal pH values the acids varied in toxicity. For acetic and oxalic acid the specific action was particularly marked.

The culture media were titrated at the end of the incubation period and it was found that invariably reduction in the total acid content accompanied growth. This loss in acid was less in the more acid solutions owing probably to the fact that the amount of growth was less. It is remarkable that the organisms studied were able to obtain food

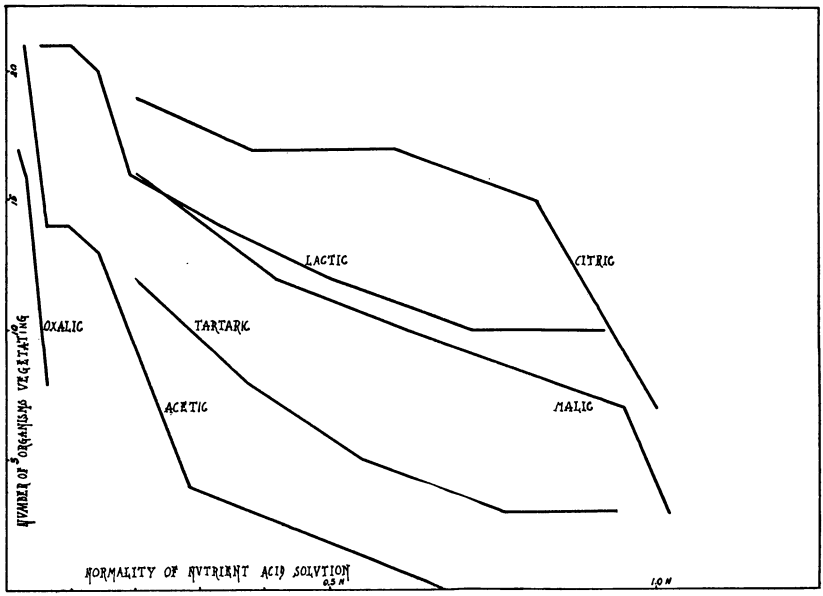

Fig. 2. Acid tolerance in synthetic nutrient medium.

and energy for growth from the oxidation of various organic acids which in the concentrations employed are normally toxic to culture yeasts such as *Saccharomyces ellipsoideus.*

Tolerance for Acids in Brines.—The effect of salt on the acid tolerance of a few of the organisms under investigation was studied. Cucumber juice was diluted 1:3 with distilled water and 10 per cent of sodium chloride was added. To this medium various amounts of acetic, citric, and lactic acids were added. Flasks of the medium were then inoculated with loopfuls of organisms 3, 6, 7, 10, 18, and 20, which were previously cultured in the diluted cucumber-juice medium. The inoculated bottles were stored at room temperature and at the end of 30 days were examined for growth and titrated for acid. At this

time solutions in which no growth was evident were re-inoculated with 1 cc portions of cultures of the organisms mentioned above and incubated for another month. The results obtained are shown in table 9.

The addition of as little as 0.5 per cent acetic acid inhibited the growth of all the organisms in the presence of 10 per cent salt. All of the acids in the concentrations used inhibited the growth of organism No. 20. Organism 18 was especially tolerant of citric acid and fairly tolerant of lactic acid. However, its growth was slight in acid concentrations higher than 1.0 per cent. It grew better than the other organisms in the citric-acid brine. In lactic-acid brines organism 6 grew best with organisms 15 a close second.

TABLE 9

GROWTH IN ACIDIFIED BRINES

Organism No.	No acid	Per cent acetic acid		Per cent citric acid						Per cent lactic acid			
		0.5	1.0	1	2	4	5	6	7	1	2	4	5
3	+	.....	.....	+	.....	.....	.....	.....	.....	+	+	.....	.....
6	+	.....	.....	+	+	+	.....	.....	.....	+	+	+	.....
7	+	.....	.....	+	.....	.....	.....	.....	.....	+	+	.....	.....
10	+	.....	.....	+	+	.....	.....	.....	.....	+	+	.....	.....
18	+	.....	.....	+	+	+	+	+	.....	+	+	.....	.....
20	+	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

The acid in each culture was titrated at the close of the experiment. In the 1 per cent citric-acid brine the greatest loss of acid was caused by organism 18 and the least by organism 3 and 6. Organisms 7 and 10 caused but little loss in acid. In solutions of higher citric-acid content the loss in acid was but slight, even with organism 18.

In the 1 per cent lactic-acid brine, organisms 7 and 10 caused the greatest loss, organisms 3 and 18 were next, and organism 6 last. The loss in acid in 2 per cent lactic-acid solutions was less but considerable, and in the 4 per cent lactic acid still less, the loss caused by organism 6 being slight.

The presence of 10 per cent salt greatly decreased the tolerance of these organisms for acid, as shown both by growth and by destruction of the acid.

Tolerance for Acids in Fermented Cider.—Freshly fermented cider was sterilized in 4-ounce bottles and was then inoculated with the cultures the organisms under investigation. The inoculated bottles

were stored at room temperature for a period of two months. The cider after sterilization contained 4 per cent alcohol and 0.58 per cent acid as malic.

Of the twenty-one organisms under investigation, only Nos. 1, 2, 3, 5, 6, 8, 11, 12, 13, 14, 15, and 16 grew in the above medium. Organisms 4, 7, 9, 10, 17, 18, 19, 20, and 21 failed to grow in the cider. This would indicate a marked variation in the ability of different *Mycoderma* to utilize natural media for growth. The former group of organisms did not grow luxuriantly in the fermented cider. The addition of 0.5 per cent acetic acid to the fermented cider medium inhibited the growth of only organism 13, but the addition of 1 per cent acetic acid prevented the growth of all organisms. When 2 per cent malic acid was added, the growth of all organisms but Nos. 11, 12, 13, 14 was inhibited, when 3 per cent malic acid was added the growth of all but No. 11 was inhibited, and the addition of 4 per cent malic acid prevented all growth. A lower concentration of acetic acid than of malic acid was necessary to inhibit growth. That the addition of acetic acid will prevent the development of *Mycoderma* in fermented cider has been known previously. [7, 10, 11]

EFFECT OF SODIUM CHLORID

Very little research has been reported in the literature upon the salt tolerance of *Mycodermas* and molds. In studying the conservation of meat and fish by salt, Pettersson[12] has found that certain wild yeasts can develop in fish in 25 per cent of salt. Bitting[13] found that 5 grams of salt per 100 cc had no effect on molds in meat bouillon while 30 grams per 100 cc prevented the mold. The yeasts studied by him did not develop in a solution containing 15 grams per 100 cc. Thom[14] found that 15 per cent salt inhibited the growth of certain *Penicillium* molds. Karaffa-Korbutt[15] found salt to possess a weak bactericidal power and found that many yeasts were capable of growing in a 25 per cent solution. Mitra[16] found the concentration of salts that inhibit growth of *Saccharomyces ellipsoideus* cells to be 2.2 M potassium chlorid, 1.2 M magnesium chlorid, 0.7 M calcium chlorid and 0.2 M sodium chlorid in a synthetic medium.

Cruess[9] found that the addition of salt reduced the rate of destruction of added lactic acid by the *Mycodermas* and mold studied by him. Several cultures, including a mold, grew at 16 per cent salt and caused considerable decrease in acidity. One was inhibited at 16 per cent salt but grew slightly at 14 per cent salt. None of the

organisms grew at 18 per cent salt. However, a mixed culture of *Mycoderma* and mold from cucumber brine grew to some extent at 18 per cent salt.

Recently Spearman, Gee, and Luck[17] investigated the influence of sodium chlorid on the growth and metabolism of *Saccharomyces cerevisiae* in a wort medium. They found that *S. cerevisiae* will ferment wort containing up to 10 per cent salt, but the weight of the yeast crop obtained decreased as the salt concentration increased and the lag phase of the fermentation period was progressively lengthened by increasing concentrations of salt.

In our experiments the effect of salt on the growth and metabolism of cultures 1 to 21 was studied. In a preliminary test flasks of sterile cucumber juice to which various amounts of salt had been added were inoculated with 1 cc portions of cultures 1 to 21. Before inoculation the medium used contained 9 grams lactic acid per 100 cc and was of 3.6 pH value. It was found later the pH value of the medium has a marked influence on the salt tolerance of these organisms.

The addition of 4.6 per cent salt had little influence on the rate of oxidation of the lactic acid except by organisms 7 and 20, whose activity was much retarded. The addition of 8.2 per cent salt only slightly retarded the action of organisms 1, 2, 3, 4, 5, and 8, but considerably retarded that of the other organisms. Only organisms 7, 9, and 10 showed growth at the end of 35 days in the 12.5 per cent salt solution. During the 35-day incubation period acid titrations were made at regular intervals. Curves showing the rates of destruction for several organisms are given in figure 3. The retarding effect of the salt is very evident.

The pellicle formed at about equal rates in the natural medium and in that containing 4.6 per cent salt but much more slowly in that containing 8.2 per cent salt and still more slowly in that of 12.5 per cent salt. In this experiment salt exerted a greater retarding effect on the rate of growth of the molds than of the *Mycodermas*.

The activity of the *Mycodermas* as measured by the reduction in total acidity was found to be proportional, within the experimental error, to the amount of growth as determined by centrifuging a known volume of the solution and noting the volume of sediment. It would appear that the amount of acid oxidized by a given volume of the *Mycoderma* in a given time is fairly constant and reduction brought about in the rate of oxidation of lactic acid by the addition of salt is caused by decrease in the size of the *Mycoderma* crop and not in its activity.

It was noted that the addition of moderate amounts of salt, e.g., 2 to 4 per cent, increased the *Mycoderma* crop of some cultures and the amount of lactic acid oxidized in a period of 30 days. However, this evidence of growth stimulation was not consistent and not very conclusive.

Salt Concentration Required to Inhibit Growth.—In one experiment a cucumber-juice medium was brought to pH 3.6 by the addition of lactic acid and the following percentages of salt were added

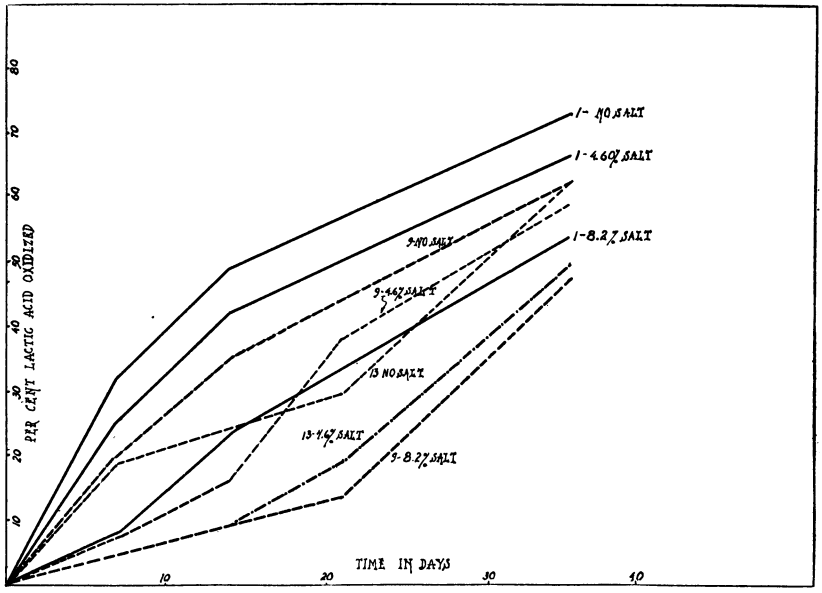

Fig. 3. Effect of salt on the rate of oxidation of lactic acid.

and checked by titration with silver nitrate: 0, 4.6, 8.2, 12.5, and 15 per cent. After sterilization, inoculation, and 30 days' incubation it was found that all cultures had grown in the media of 0, and 4.6 per cent salt; all except No. 13 had grown in 8.2 per cent salt; only Nos. 7, 9, 10, 12, and 21 had grown in 12.5 per cent salt and none had grown in 15 per cent salt.

In dill brine the tolerance of the organisms for salt was somewhat less than in cucumber juice, probably because the dill brine was much poorer in nutrient substances and was of somewhat lower pH value, namely 3.2. All grew in brine of 6° Baumé; all except No. 13 grew in brine of 9.5° Baumé; Nos. 1, 2, 4, 5, and 8 grew in brine of 12° Baumé; Nos. 1, 2, 4 and 8 grew in brine of 14° Baumé; only No. 4 grew in brine of 16° Baumé and none grew at 18° Baumé. The percentage of salt was somewhat less than the Baumé degree.

The tolerance for salt in a cucumber-juice medium of pH 5.10 was found to be greater than the tolerance in dill brine or in acidified cucumber juice. Organisms 1 to 21 previously cultured in plain cucumber juice were transferred to tubes of sterile cucumber juice containing 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 per cent added salt. After six weeks' incubation it was found that the minimum amount of salt necessary to inhibit growth under these conditions was as follows: The addition of 8 per cent salt inhibited the growth of only organism No. 13; 10 per cent salt inhibited growth of Nos. 11, 12, and 16; 12 per cent salt inhibited growth of Nos. 3, 6, 14, and 15; 14 per cent salt was necessary to inhibit growth of Nos. 1, 2, 4, 5, 8, 17, and 20; 20 per cent salt for 7, 9, 10, and 19; and growth of organism 21 occurred in the 20 per cent salt solution.

It was thought the tolerance of the film yeasts for salt might be affected by the salt content of the medium in which they had previously been cultured. Accordingly several of the film yeasts were cultured in cucumber juice containing no added salt and in that containing 10 per cent added salt. Transfers were made to tubes of sterile juice containing 2, 4, 6, 8, 10, 12, 14, 16, and 18 per cent added salt.

After six weeks incubation it was found that most of the cultures previously grown in a medium containing 10 per cent salt possessed a tolerance for 2 per cent more salt than those previously grown on a medium containing no added salt. For examples, organisms, 1, 2, 5, and 8, previously grown in the absence of salt tolerated 12 per cent salt; those previously grown in 10 per cent salt tolerated 14 per cent salt; organism 3 previously grown in the absence of salt tolerated only 10 per cent salt; that grown in 10 per cent salt tolerated 16 per cent salt. Organisms 12, 14, and 15 tolerated 2 per cent more salt when previously grown in 10 per cent brine. Cultures from dill brine (which contains 4 to 5 per cent salt) possessed greater tolerance for salt than those from plain cucumber juice.

The effect of the pH value of the medium was emphasized by the foregoing experiment. The pH value of the medium in that experiment was 5.6, while another similar experiment the pH value was 3.6. At pH 5.6 the tolerance of the organisms for salt was much greater than at 3.6. Thus at pH 3.6 organisms 7, 9, 10, and 19 tolerated 12.5 per cent salt but not 15 per cent, whereas in a medium of pH 5.6 they tolerated 18 per cent salt. Organism No. 21 tolerated 12.5 per cent salt at pH 3.6 and 20 per cent at pH 5.6. In another experiment organisms 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 17, 18, 19, and 20 grew in cucumber

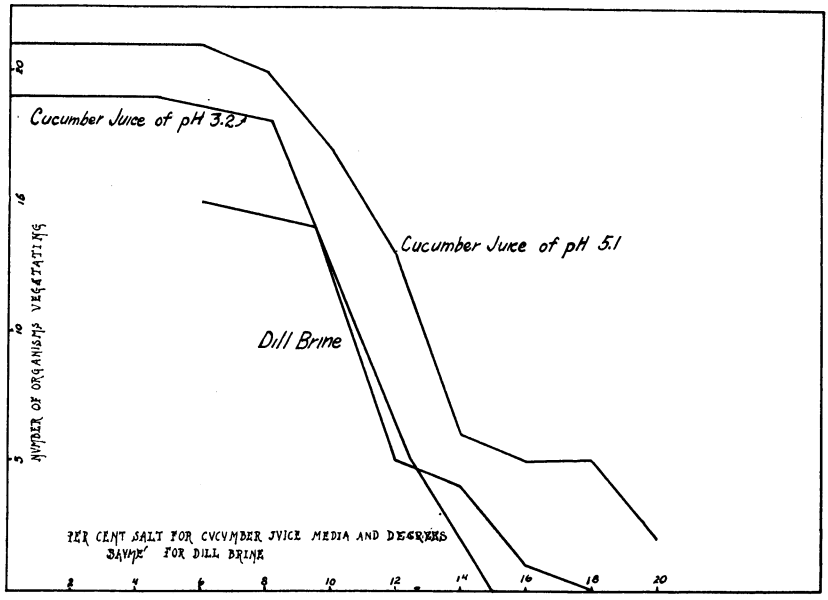

Fig. 4. Salt tolerance in cucumber juice and dill brine.

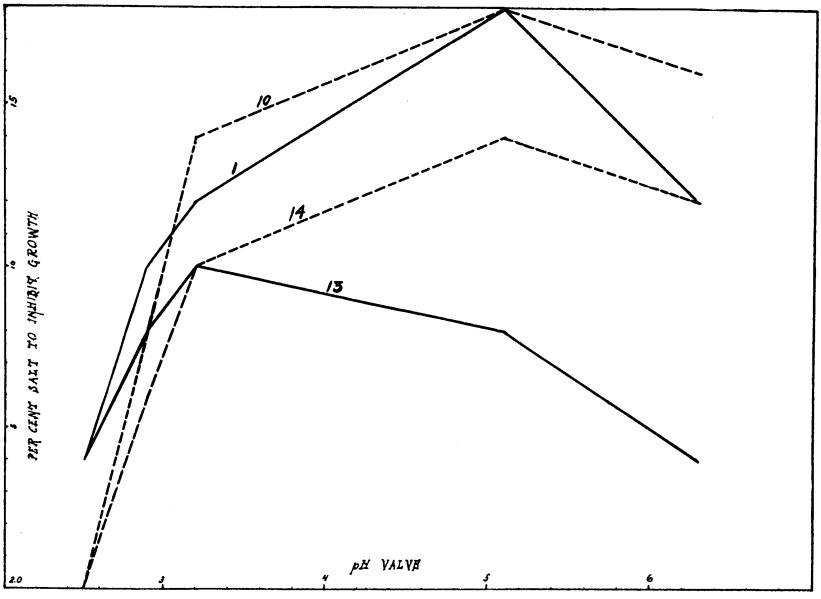

Fig. 5. Effect of pH on salt tolerance.

juice containing 10 per cent salt while only No. 20 grew in the same medium acidified with 1.5 per cent lactic acid. In order to test the effect of pH value on salt tolerance more accurately seven of the organisms were cultured in cucumber-juice media of pH 2.5, 2.9, 3.2, 5.1, and 6.3 to which various concentrations of salt were added. The results of this series of experiments are summarized in table 10.

TABLE 10

EFFECT OF pH VALUE ON SALT TOLERANCE

Organism	pH				
	2.5	2.9	3.2	5.1	6.3
	Per cent salt required to inhibit growth				
1	4	10	12	18	12
6	6	10	12	14	12
10	0	8	14	18	16
13	4	8	10	8	4
14	4	10	12	14	12
17	0	6	10	14	12
21	0	4	10	18	16

The data clearly show that the lower the pH, the less the amount of salt necessary to inhibit growth. However, the maximum amount of salt necessary to inhibit growth naturally varied with the organism.

The salt tolerance of a few of the organisms studied was found to be less in apple cider than in cucumber juice. However, this difference probably was caused not only by the difference in pH value but also by differences in the general composition of the two media.

As previously noted for other experiments, it was noted that the volume of visible growth decreased with increase in salt concentration. This effect was not marked in solutions of low salt content but became very noticeable in solutions of higher salt concentration. In all these tests distinct pellicle formation was taken as the criterion for growth. The results of the tests are summarized in figures 4 and 5. In figure 4, the number of organisms showing growth out of the total of twenty-one organisms studied is plotted against the percentage of salt for cucumber juices of pH 3.2 and pH 5.1 and against the Baumé degree for dill brine. In figure 5 the percentage of salt required to prevent the growth of five organisms is plotted against pH value.

EFFECT OF DISTURBING THE FILM ON GROWTH AND RATE OF ACID DESTRUCTION

In determining the rate of oxidation of lactic acid in a previous test, it was necessary to thoroughly mix the contents of the culture flask in order to secure a representative sample. It was suspected then that this periodic disturbance of the film might influence the true rate of oxidation of lactic acid. To test this assumption the following experiment was conducted:

Eight portions of 70 cc each of sterile dill brine in 4-ounce bottles were inoculated with loopfuls of organism 10. One set of bottles was left undisturbed at room temperature. Another set was shaken two or three times daily. After a period of three weeks the remaining acid

TABLE 11

EFFECT OF DISTURBING FILM ON OXIDATION OF ACID

(Per cent of acid destroyed)

Organism No.	2	10
Undisturbed	97.0	94.0
Shaken several times a day	26.2	2.5
Aerated	97.5	65.5

was determined by titration. In the undisturbed set, 99 per cent of the acid had been destroyed, while in the shaken set only 18.2 per cent of the total acid had been destroyed.

Growth was much heavier in the undisturbed bottles. The periodic shaking did not altogether prevent the appearance of film growth, although it prevented its normal development.

However, in these experiments the factor of aeration was not controlled. That aeration markedly increases the multiplication of yeasts, probably by removing carbon dioxide, has been known for many years. Recently Devereux and Tanner[18] have reviewed the previous work and have found that the growth of the yeasts investigated by them in their synthetic nutrient medium was markedly increased by aeration. They found the effect of aeration to be most marked with *Pichia farinosus*. The maximum unaerated and aerated counts for this organism were respectively 24.0 and 320.0 millions per cubic centimeter. As this yeast was the only one which formed a pellicle, they thought that there might be some correlation between pellicle-forming yeasts and those which respond positively to aeration. This point was not investigated further by them.

In a second experiment the ability of a *Mycoderma* to destroy lactic acid was studied in the absence of film formation when supplied with air by passage of a continuous stream of air through the culture. Air was filtered through five inches of cotton and bubbled through 70 cc portions of dill brine in 125 cc Erlenmeyer flasks, inoculated previously with 1 cc of cultures of organisms 2 and 10. The inoculated medium, however, frothed badly and some growth occurred along the sides of the flask, above the liquid. However, the experiment showed that *Mycodermas* were capable of growing in the bottom of a flask of liquid medium when sufficient air was supplied.

For comparison the effect of disturbing the film by intermittent shaking at infrequent intervals was again determined, and the loss in acid determined at the end of 10 days. The liquids in the aerated flasks were brought to volume before titration to compensate for loss from evaporation. The results obtained are shown in table 11.

Active oxidation of the acid occurred in the continuously aerated liquids, although film formation was prevented.

EFFECT OF RATIO OF SURFACE OF MEDIUM TO VOLUME ON RATE OF DESTRUCTION OF ACID

The changes in the nutrient medium caused by the growth of film-forming organisms are normally, in the absence of aeration, most profound at the surface of the medium where growth takes place. Increasing the surface exposed per unit volume should increase the amount of acid destroyed in a given time. If this were strictly so, then the ratio of the percentage of acid oxidized to the surface exposed per unit volume of the nutrient solution would be constant. To test this hypothesis the following experiment was conducted:

To synthetic nutrient medium previously described was added lactic acid at the rate of 25 cc to 500 cc of solution. Ten, 20, 30, 40,

TABLE 12

EFFECT OF RATIO OF SURFACE TO VOLUME ON AMOUNT OF ACID OXIDIZED

Volume added *cc*	Height *cm*	Surface/volume	Acid oxidized *per cent*	K*
10	4.6	0.217	36.5	168
20	9.1	0.110	19.2	171
30	13.4	0.075	12.8	170
40	17.7	0.057	9.7	171
50	22.3	0.045	7.7	171
60	26.5	0.038	6.5	.....

* Ratio of percentage acid oxidized to surface/volume.

and 50 cc of the acidified medium were placed in flat-bottomed Nessler tubes of approximately uniform bore, 1.7 cm in diameter and sterilized. The tubes were inoculated with organism 10 and the decrease in acid content determined after incubating for ten days at room temperature. The results are shown in table 12.

It is evident that there is very good agreement for the values for K (ratio of percentage of acid oxidized to the surface exposed per unit volume) in this case. This proves that the oxidation of lactic acid occurs mainly at the surface. In preliminary tests in which oil-sample bottles were used and the surface per unit volume varied from 0.08 to 0.80, less close agreement for the value of K was observed, probably because of the greater experimental error involved. But even in these tests, the agreement was such as to show definitely that the rate of oxidation of lactic acid varies in proportion to the surface exposed per unit volume.

EFFECT OF A LAYER OF NEUTRAL OIL ON GROWTH

In some vinegar and pickle factories the vinegar stock or pickle brine in the storage vats is covered with a layer of neutral mineral oil in order to prevent the growth of *Mycodermas.* The oil layer retards or, if thick enough, prevents growth of *Mycoderma* by retarding diffusion of oxygen into the liquid beneath. It was thought desirable to determine the thickness of such an oil layer necessary to prevent growth. Accordingly the following experiments were conducted:

TABLE 13

EFFECT OF THICKNESS OF OIL LAYER ON PERCENTAGE OF ACID OXIDIZED

Thickness of oil film	Organism No.	
	10	21
	Acid oxidized	
mm	*per cent*	*per cent*
0	Basic	Basic
0.5	Basic	91.5
1.0	100.0	81.0
1.5	55.5	62.2
2.0	24.0	44.6
3.0	3.8	15.5
6.0	0	0
8.0	0	0
11.0	0	0
17.0	0	0
26.0	0	0

To 50 cc portions of dill brine in 4-ounce bottles enough neutral mineral oil was added to give oil films of the following thicknesses: 0.5, 1, 1.5, 2, 3, 6, 8, 11, 17, and 26 mm. One set of these bottles in duplicate was inoculated with 1 cc portions of a culture of organism 10 and another set with 1 cc portions of a culture of organism 21. The percentages of acid oxidized in 10 days are shown in table 13.

Although the presence of 6 mm of oil prevented decrease in acidity, slight growth of the mold and the yeast took place just beneath the oil film. A very faint growth also occurred where films thicker than 6 mm were used. This slight growth may have been made possible by the small amount of air which gained entrance during inoculation.

EFFECT OF pH VALUE UPON GROWTH

It has been shown earlier in this paper that the pH value of the medium has a marked influence on the salt tolerance of these organisms and that dill brine will not support the growth of all of the organisms studied. This latter condition was thought to be due partly to the relatively low pH value of the medium. In order to obtain further data on the effect of pH value on the growth of film organisms, dill brine was brought to pH values of 2.35, 2.6, 2.85, 2.90, 3.2, 5.6, 6.7, and 9.8, by the addition of lactic acid or of sodium hydroxid. The flasks of the sterilized brine were then inoculated with loopfuls of organisms 1 to 21 and examined for growth after a period of thirty days. That the pH value of the medium is of importance can be seen from the following summary.

None of the organisms grew at pH 2.35 and 9.8; Nos. 1, 2, 4, 5, 6, 8, and 11 to 15 grew at pH 2.6; these, and also Nos. 16 and 19 grew at pH 2.85 and 2.90; all except Nos. 17, 18, 20, and 21 grew at pH 3.2; all grew at pH 5.6; and only Nos. 7, 9, 10, 17, and 21 grew at pH 6.7.

NITROGEN FIXATION

Growth of *Mycoderma* occurs on the surface of 'salt stock' tanks for a period of two years or more even when the film is skimmed at frequent intervals. The fact that this recurring growth can take place in a medium naturally so poor in nitrogen might indicate that nitrogen fixation occurs. Analysis showed the scum yeast to be but little poorer in nitrogen content than brewer's yeast grown in a medium rich in nitrogen.

Lipman[19] has shown that *Mycoderma vini* is capable of fixing nitrogen in media very low in nitrogen.

Using the technique devised by Lipman[19] for his experiments, cultures of the twenty-one organisms were grown for two months in cucumber juice containing only 0.01 per cent nitrogen. Total nitrogen was then determined by analyzing the entire individual cultures. It was found that under the conditions of this test, no nitrogen or at most only a negligible amount was fixed. Evidently these organisms can thrive in a medium low in nitrogen.

EFFECT OF OXYGEN SUPPLY

As stated earlier in this paper the organisms studied were found to be strongly aerobic. Growth did not occur in agar plates stored in an evacuated desiccator, and in slab cultures in agar and gelatin, growth was confined to the surface. As shown elsewhere growth and activity were retarded by thin layers of mineral oil and in some cases entirely inhibited by thick-layers of this soil.

To further determine the effect of size of headspace on growth and activity in a sealed container 8-ounce bottles were completely filled with dill brine lightly inoculated with a mixed *Mycoderma* culture, and then 5, 10, 20, 50, and 100 cc respectively were withdrawn and the bottles sealed with crown caps. After incubating for one month at room temperature the bottles were examined for growth and the loss in acid was determined. It was found that only in the cotton-plugged controls was growth heavy and the decrease in acidity marked. In the sealed bottles a slight pellicle formation was observed in the bottle with 100 cc head space. Surface growth did not occur in any of the other bottles. No significant differences in volume of sediment were obtained by centrifuging 20 cc portions of the cultures. The difference in the acid content was also very slight.

This test was repeated using non-acidified cucumber juice, a more favorable medium, in 4-ounce bottles. Headspaces of 0, 5, 10, 20, 25, 50, 75, and 100 cc were provided as previously described. These bottles were heavily inoculated with a mixed *Mycoderma* culture, sealed with crown caps and incubated. After 24 hours a slight film growth occurred in all except that with 0.0 cc headspace. The pellicle in the bottle with 5 cc headspace was very slight and when disturbed did not re-occur. After seven days' incubation the relative amount of film growth was in proportion to the oxygen supply, being very heavy in the bottles plugged with cotton; somewhat less but still heavy at 100 cc headspace; moderate at 50 and 75 cc headspace; light at 25, 20, and 15 cc; very light at 10 cc and negative at 5 cc headspace.

When the bottles were shaken, the turbidity of the liquid was much greater in the bottles plugged with cotton than in any of the sealed bottles. The amount of growth as determined roughly by volume of sediment and by counting by microscope was in proportion to the volume of headspace.

These observations corroborate the recent investigations of Ayers, Barnby, and Voight.[20]

EFFECT OF CERTAIN ANTISEPTICS AND GERMICIDES

Owing to the fact that the growth of *Mycoderma* on food storage brines causes deterioration and loss, it is desirable to ascertain whether growth can be prevented by the use of permissible antiseptics and in what manner the efficacy of such antisepetics is affected by the composition of the medium. For this reason the antiseptic power of certain chemicals toward the mixed *Mycodermas* normally occurring in salt stock brines was studied.

Comparison of Various Antiseptics.—The experiments with antiseptics were conducted first to compare the toxicity of a number of antiseptics to *Mycodermas* and secondly to determine the effect of salt concentration and pH value of the medium on the toxicity of sodium benzoate.

In the first experiment, salt stock brine procured from the California Conserving Company was used. This brine was 17° Baumé and showed a total acid content expressed as lactic of 0.46 grams per 100 cc.

The following antiseptics were added to this brine: acetic acid, citric acid, hydrochloric acid, lactic acid, oxalic acid, sulfurous acid, sodium sulfid, sodium thiosulfate, sodium sulfite, sodium metabisulfite, sodium cyanid, potassium permanganate, potassium chromate, mercuric chlorid, barium chlorid, calcium chlorid, cupric acetate, cupric sulfate, ferric sulfate, ferric chlorid, ferrous sulfate, potassium alum, zinc sulfate, formaldehyde, hydrogen peroxid, analine oil, carbon bisulfid, toluène, xylene, benzine, and phenol. Acetic and lactic acids were used in concentrations of .1–1.5 per cent hydrochloric, citric, and oxalic acids in concentrations of 0.1–1.0 per cent; the other acids and salts in concentrations of 0.01–0.10 per cent and mercuric chlorid in concentrations of 0.001–0.100 per cent. The oils such as carbon bisulfide, xylene, and toluene, were added as drops, 1–10 drops being used per 100 grams of brine.

As the brine used in the first series was poor in bacterial food and high in salt content, it was diluted to 10° Baumé and to each 1,000 cc was added 10 grams of dextrose, 1 gram peptone and 0.5 gram dipotassium phosphate. The resulting brine was 11° Baumé and contained 0.24 grams acid per 100 cc expressed as lactic. It was much more favorable to the growth of the *Mycodermas* present.

TABLE 14

CONCENTRATION OF VARIOUS ANTISEPTICS REQUIRED TO PREVENT GROWTH OF MIXED CULTURE OF MYCODERMA ON 17° BAUMÉ BRINE AND ON 11° BAUMÉ FORTIFIED BRINE

Antiseptic	Concentration necessary to prevent growth	
	On 17° Baumé *per cent*	On 11° Baumé *per cent*
Acetic acid	0.40	1.0
Citric acid	0.70	.....
Hydrochloric acid	0.20	0.20
Hydrofluoric acid	0.06	.....
Lactic acid	1.50	.....
Oxalic acid	0.30	0.80
Sulfurous acid	0.01	0.06
Sodium benzoate	0.01	.....
Sodium sulfite	0.01	.....
Sodium metabisulfite	0.01	.....
Mercuric chlorid	0.002	0.001
Formaldehyde	0.02	0.04
Hydrogen peroxid	0.06	.....
Aniline oil	6 drops	6 drops
Benzine	30 drops	.....
Toluene	8 drops	8 drops
Xylene	8 drops	6 drops
Phenol	.....	0.06

The treated brines and untreated check samples were stored for a period of four months at room temperature. At the end of that period luxuriant growth had occurred in the untreated samples of brine. Growth was inhibited by the antiseptics listed in table 14 when used in the concentrations shown. Other antiseptics tested did not prevent growth in the concentrations used. Heavy growth consisting of the usual white *Mycoderma* film occurred in most samples in which insufficient antiseptic was used. In some samples the surface was not completely overgrown, although a white ring formed on the sides of the container and was taken as evidence of growth.

The following additional experiments were made: Salt itself was used as an antiseptic in one experiment and it was found that growth occurred at 21° Baumé after 1½ months incubation and at 22° Baumé after 4 months; 23° Baumé prevented growth.

Although growth was prevented by 0.06 per cent hydrofluoric acid it was not prevented by 0.10 per cent ammonium fluorid, the maximum concentration used.

Owing to its observed strong toxic effect further tests were made with sulfur dioxid solutions. Various amounts were added to 50 gram portions of salt stock brine of 18.3° Baumé and 0.08 per cent acidity. Growth occurred in all concentrations at the end of two weeks except at 0.035 per cent. In this same brine the addition of as much as 0.08 per cent sodium benzoate did not prevent growth. The addition of 0.02 per cent hydrofluoric acid prevented all growth in this brine.

The use of alkali as an antiseptic to prevent the growth of normally occurring organisms in salt stock brine was roughly determined. Salt stock brine was brought to the following pH values by the addition of sodium hydroxid; pH 4.5, 5.5, 7, 8.2, 9.0 and 9.8. After incubation at room temperature for four months, growth occurred at all pH values except the most alkaline, namely, pH 9.8.

The results obtained, especially those with sulfurous acid and sodium benzoate, indicate that the concentration of the antiseptics required to prevent *Mycoderma* growth varies considerably with the medium.

Of the antiseptics investigated, sulfurous acid, hydrofluoric acid, and sodium benzoate were the best both from standpoint of efficiency and of their probable effect on the pickles.

Of these three, sulfurous acid was most efficient, 0.04 per cent preventing all growth. When the brine was sufficiently acid, only 0.01 per cent sodium benzoate was required to inhibit growth. This is also true for the sulfites and sulfurous acid. However, when the acidity was low, even 0.08 per cent sodium benzoate did not prevent growth. Hydrofluoric acid was the most efficient of the mineral acids with the exception of sulfurous acid. Although 0.06 per cent hydrofluoric acid was required in one experiment, growth in solution of higher concentrations than 0.01 per cent was restricted to very slight ring formation. In concentrations of 0.02 per cent it practically inhibited growth.

Effect of Salt Concentration on the Toxicity of Sodium Benzoate.— Preliminary experiments showed that in cucumber juice of pH 5.1 and in the presence of 15 per cent salt, less than 0.1 per cent of sodium benzoate was required to inhibit growth. In solutions of lower salt content much larger amounts were necessary.

These observations led to the following experiments with cucumber-juice media of various salt and benzoate concentrations and inoculated with the following cultures:

A—Mixed *Mycodermas* freshly isolated from dill brine.

B—Mixed *Mycodermas* and mold spores from olive brine.

C—Organism No. 6 preserved in a 10 per cent NaCl cucumber-juice brine at 32° F.

D—Organism No. 3 preserved as above.

E—Organism No. 8 preserved as above.

F—Organism No. 21 from cider culture.

TABLE 15

EFFECT OF SALT CONCENTRATION ON THE CONCENTRATION OF SODIUM BENZOATE REQUIRED TO PREVENT GROWTH

Salt	Culture tested					
	A	*B*	*C*	*D*	*E*	*F*
	Concentration of sodium benzoate to prevent growth					
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0	0.40	0.20	0.20	0.10	0.20	0.20
5	0.15	0.15	0.05	0.10	0.10	0.10
10	0.05	0.05	0.05	0.05	0.05	0.05
15	0.05	0.05	0.05	0.05	0.05	0.05

Cucumber juice was brought to pH 5.10 and 15 per cent salt content and to the plain cucumber juice as well as that containing added salt the following percentages of sodium benzoate were added: 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50 respectively. The prepared media were then tubed, sterilized, and inoculated with the above cultures, and incubated for 10 days. At the end of this period members of this series showing no growth were re-inoculated. Final observations were made after an incubation period of one month. The amount of sodium benzoate necessary to inhibit growth is shown in table 15. Growth of all the organisms studied occurred in the absence of sodium benzoate at all salt concentrations.

The mixed cultures *A* exhibited somewhat greater resistance than the others to benzoate at 0 per cent and 5 per cent salt but at 10 per cent and 15 per cent salt all were of about the same resistance. Much less benzoate was required to prevent growth at 10 and 15 per cent salt than at 0 and 5 per cent.

Effect of pH Value on the Toxicity of Sodium Benzoate to Mycodermas.—To tubes of apple juice adjusted to various pH values by the addition of citric acid or of sodium hydroxid and containing various concentrations of sodium benzoate, was added a mixed culture of *Mycoderma* from dill brine and salt-stock brine. The tubes were incubated for 6 months and the presence or absence of growth noted with the results shown in table 16.

TABLE 16

EFFECT OF pH VALUE ON THE TOXICITY OF SODIUM BENZOATE TO MYCODERMAS

pH value	Benzoate required to prevent growth *grams in 100 cc*
2.7	0.05
3.8	0.06
4.7	0.50
5.4	1.00
7.3	4.00
7.8	More than 1.0
10.0	0.70

It will be seen that the pH value of the medium exerted a strong effect on the concentration of sodium benzoate required to prevent growth and that maximum tolerance lay at some pH value between 7.3 and 10.0.

EFFECT OF LIGHT

Although no extensive investigations on this point were conducted, it was observed that the growth of the organisms investigated took place in both diffused light and in the dark, but not in direct sunlight. This was found to be true both for liquid and solid media. This condition was also found true in practice since growth of *Mycoderma* and mold does not occur on salt stock tanks exposed to the sun.

DEATH-TEMPERATURE STUDIES

Although the death temperatures and the factors influencing them have been extensively studied for various bacteria, references to the death temperatures of yeasts and molds in the literature are not so numerous, and the available literature on the death temperature of *Mycodermas* is very scanty. In 1905, Takahashi[21] isolated several varieties of *Mycodermas* from sake, kaji, and sake mash and studied their properties. He determined killing temperatures for his cul-

tures to range from 54.4° C to 60° C. Later the same author[22] isolated another *Mycoderma* which was killed in a moment at 70° C, or on exposure to 64° C for 5 minutes.

Ayers and associates[20] determined the death point of a culture of *Mycoderma* isolated from cloudy olive brine by heating in olive infusions containing various percentages of salt. They state that "In a 4 per cent salt brine the organism was not destroyed by heating the brine to 130° F (54.4° C) and maintaining this temperature for five minutes. However, in an 8 per cent salt brine it was destroyed at this temperature when maintained for 5 minutes." They found that in a 14 per cent salt brine the death temperature was lowered to 125° F provided this temperature was maintained for 10 minutes. Growth occurred in the 4 and 10 per cent salt brine after the same treatment.

TABLE 17

DEATH POINT OF MYCODERMAS IN CUCUMBER JUICE

Organism	Death point
A	60° C for 10 min.
B	60° C for 30 min.
6	50° C for 30 min.
3	60° C for 10 min.
8	60° C for 30 min.
21	65° C for 30 min.

In our investigations the death temperatures of five cultures of *Mycodermas* and one culture of *Penicillium* were determined. The cultures were designated *A, B,* 6, 3, 8, and 21 to conform with previous designations. *A* was mixed culture of *Mycoderma* yeast from 1928 dill brine and *B* was a mixed culture of mold spores and *Mycoderma* yeast from 1928 olive brine. 5 cc of sterile cucumber juice in small thin glass test tubes (1 cm in diameter and 12 cm long) were inoculated with small loopfuls of the organisms and heated in water baths held at 50, 55, 60, 65, 70, and 75° C respectively for 5, 10, and 30 minutes. Upon removal from the heating bath the tubes were immediately cooled in cold water and incubated at room temperature and were examined periodically until no further growth occurred. The results of the final observation are shown in table 17.

The death-temperature tests were then extended to include the effect of amount of inoculum, pH value of the juice and its salt content. Cucumber juice of pH 5.05 was brought to pH 2.75 by the addition of lactic acid and to pH 7.5 by the addition of sodium hydroxid. It was also brought to 2, 4, 6, 8, 10 and 12 per cent salt content. Five cubic centimeter portions of the prepared medium were

placed in test tubes (these tubes were larger than those used previously, measuring 1.6 cm in diameter, 15 cm long and 1.5 mm thick) and sterilized. The media were then inoculated with 5 drops of suspension of each of the cultures studied. This inoculum produced a turbid solution. The tubes were then heated for 5 minutes in water baths held at 50, 55, 60, 65, 70, and 75° C, respectively, and incubated. Distinct pellicle formation was taken as evidence of growth. The results are shown in table 18.

TABLE 18

EFFECT OF SALT AND pH ON DEATH TEMPERATURE

pH	Salt	Death temperature with 5 minutes' exposure					
		Organism					
		A	*B*	3	6	8	21
	per cent	*per cent*	°C	°C	°C	°C	°C
2.75	0	55	55	55	50	50	50
5.05	0	70	70	65	65	60	75
7.5	0	70	70	65	65	65	75
5.05	0	70	70	65	60	60	75
5.05	2	70	70	65	60	60	75
5.00	4	70	65	65	60	60	75
5.05	6	70	65	65	60	60	75
5.05	8	70	65	65	55	60	75
5.05	10	65	65	65	55	60	55
5.05	12	60	60	60	55	60	55

The death points of the organisms studied were higher than those reported by Takahashi[21] and Ayers[20] for their organisms. At a low pH the death temperature was considerably lowered. Moderate concentrations of salt did not affect the death temperature but high concentration lowered it considerably for most of the organisms studied.

CLASSIFICATION OF THE STRAINS OF MYCODERMA STUDIED

The various strains of *Mycoderma* resembled each other fairly closely in macroscopical appearance but differed in their microscopical appearance and in their tolerance for salt and for acid. In their tolerance for salt, especially, the organisms studied have retained the characteristics exhibited in their original environment.

On the basis of their tolerance for salt, the sixteen strains of *Mycoderma* studied may be classified into the following three groups:

1. Growth inhibited in cucumber juice by the addition of 10 per cent salt. Organisms 11, 12, 13, and 16 fall in this group.

2. Growth inhibited in cucumber juice by the addition of 15 per cent salt. Organisms 1, 2, 3, 4, 5, 6, 8, 14, and 15 fall in this group.

3. Growth inhibited in cucumber juice by the addition of 20 per cent salt. Organisms 7, 9, and 10 fall in this group.

In group 1, organism 13 differs from the other three in its lower salt tolerance, both in cucumber juice and in dill brine. In fact it had the lowest salt tolerance of all the organisms studied. Organisms 11, 12, and 16 are very similar in their salt tolerance. Organism 13 exhibited a maximum salt tolerance in cucumber juice of pH 3.2 and lower salt tolerance at other pH values. In cucumber-juice medium, organisms 16 oxidized lactic acid more rapidly than 13. In dill brine, organisms 11 and 12 oxidized the acids present at about the same rate. The initial rate was rapid but decreased with decrease in concentration of acid. In the case of organism 16, however, the rate of oxidation of acid was slow at first but increased as the concentration of acid decreased. Organism 13 oxidized lactic acid least rapidly of all the organisms in this group. Organism 11 was more tolerant for acetic acid than Nos. 12, 13, and 16, but less tolerant for citric acid. Organism 11, 13, and 16 were less tolerant for malic acid, oxalic acid, and tartaric acid than No. 12.

On cucumber juice, the films formed by organisms 11 and 13 are thin and only lightly wrinkled, particularly that of No. 13. They both show a turbid medium, much fine sediment, and a considerable growth on the walls of the tube. Organism 12 forms a thick, wrinkled film under which numerous granules form and settle to the bottom as a loose, granular sediment. Organism 16 forms a smooth, thin film, with heavy compact sediment. Unlike the others it produces notable quantities of gas. In microscopical appearance No. 13 differed in the predominance of short sausage-shaped cells, No. 16 in the predominance of branched chains of spherical to sausage-shaped cells. Organisms 12 and 13 were very similar to each other in size, No. 11 was smaller than the others and No. 16 differed from Nos. 11, 12 and 13 in the predominance of large spherical cells.

In group 2, organisms 3, 6, 14, and 15 were less salt tolerant both in cucumber juice and in dill brine than the others in this group. In dill brine, No. 5 was less salt tolerant than Nos. 1, 2, 4, and 8; No. 4 was the most salt tolerant of all organisms in dill brine. Organisms 1, 6, and 14 showed maximum salt tolerance in cucumber juice at pH

5.1 Organisms 1, 4, 8, and 14 oxidized lactic acid in cucumber juice more rapidly than Nos. 2, 5, 6, and 15, and organism 3 oxidized lactic acid in cucumber juice very slowly. In dill brine, organism 3 oxidized the acid slowly, also, but the others rather rapidly. These organisms were somewhat tolerant to acetic acid, Nos. 1, 2, and 3 being more so than the others; Nos. 6, 14, and 15 were more tolerant for citric acid than the others and No. 4 least tolerant; Nos. 2 and 6 were more tolerant for malic acid than the others and No. 8 least tolerant; No. 6 was found to be most tolerant for oxalic acid and No. 3 least tolerant; No. 14 was most tolerant for tartaric acid and Nos. 1, 4, 5, and 8 least.

On cucumber juice Nos. 1, 2, 4, 5, 6, and 8 grew similarly, No. 3 differed in that the film was thinner and only lightly wrinkled, the sediment was finer and the medium was more turbid; the film for No. 14 was thicker and only slightly wrinkled, there was but little sediment and much gas; No. 15 had only a slight film growth but a much heavier sediment and evolved gas. In microscopical appearance, Nos. 1, 4, and 6 were sausage-shaped to filamentous with very common chain formation; Nos. 2 and 8 were spherical to long rod-shaped with predominating sausage-shaped cells; No. 5 short sausage-shaped; No. 14 was sausage-shaped and the cells occurred in branched chains; Nos. 3 and 15 were spherical to ellipsoidal in shape. On the average No. 14 was considerably larger than the others.

In group 3 all the organisms were much less tolerant to salt in dill brine than in cucumber juice. The organisms of groups 1 and 2 were about equally tolerant in each, or even slightly more tolerant in dill brine. Organism No. 10 exhibited its maximum salt tolerance at pH 5.1 (natural cucumber juice) and had a lower salt tolerance in media of less and greater pH. Organism No. 9 oxidized lactic acid in cucumber juice more rapidly than did Nos. 7 and 10. In dill brine their rate of oxidation of acid was similar. They showed similar tolerance for acetic acid, citric acid, malic acid, oxalic acid, and tartaric acid. In the presence of 10 per cent salt, organism No. 10 was more tolerant for citric acid than No. 7. On cucumber juice, Nos. 9 and 10 differed from No. 7 in forming a more wrinkled, thicker film and a more compact sediment. In other media, growth was similar for all three. In microscopical appearance No. 9 differed from Nos. 7 and 10 in the predominance of short sausage-shaped cells and the cells were larger than either No. 7 or No. 10.

On the basis of this classification, the sixteen strains may be divided into varieties as follows:

Group	Variety	Organisms	Chief criteria for differentiation from other members of same group
1	*A*	13	Microscopical appearance, rate of oxidation of lactic acid, tolerance for acid and salt.
1	*B*	11, 12, 16	
2	*C*	1, 4, 6	
2	*D*	2, 3, 8, 15	Microscopical appearance.
2	*E*	5, 14	
3	*F*	9	Rate of oxidizing lactic acid and microscopical appearance.
3	*G*	7, 19	

The division of varieties *C, D,* and *E* was probably the most arbitrary as it was based chiefly on microscopical appearance. It is believed that with this exception the groups of organisms show sufficient difference in their properties to be classified into varieties and not merely designated as strains of the same species.

SUMMARY AND CONCLUSIONS

Some of the morphological and physiological properties of 16 strains of *Mycodermas,* one of *Torula* and 4 of molds from food-storage brines have been studied and the results reported in this paper.

1. The *Mycodermas* resembled each other fairly closely in macroscopical appearance but marked differences were found in their microscopical appearance. The molds were typical in appearance for the genera represented, namely, *Penicillium* and *Mucor;* the strain of *Torula* was also typical.

2. It has been found that the organisms studied retained the characteristics exhibited in their original environment, even after prolonged growth in various media.

3. The various strains of *Mycodermas* differed in their tolerance for salt and for acid.

4. All of the organisms studied were capable of growth in nutrient sucrose, dextrose, maltose, lactose, mannite, glycerin, acetic acid, citric acid, lactic acid, malic acid, oxalic acid, and tartaric acid. Growth in these media, in most cases, was not accompanied by visible gaseous fermentation of the medium.

5. Of the acids studied, citric acid was found most suitable for the development of the molds and lactic acid for the development of the *Mycodermas,* especially of strains isolated from pickle brines. Oxalic acid was most toxic. Acetic acid was less toxic than was expected. Many of the *Mycodermas* were capable of growth even in moderately high concentrations of this acid.

6. The concentration of salt necessary to inhibit growth of the various organisms was found to depend upon the composition of the

medium especially its pH value upon the manner of inoculating, and upon the previous method of growing the respective cultures. In general, the lower the pH of the medium, the lower was the concentration of salt required to inhibit growth. Although a few of the organisms were capable of growing in the presence of 20 per cent or more of salt, most of them were inhibited by the presence of 15 per cent salt.

7. The action of the organisms on the nutrient medium was apparently limited principally to the surface, for it was found that their activity was markedly influenced by disturbing the film or changing the ratio of surface to volume.

8. The activity of the organisms was retarded by the presence of a layer of neutral mineral oil. A certain minimum thickness of layer was found necessary to completely inhibit growth; this was 6 mm, under the conditions of our test.

9. The organisms were strongly aerobic and their development and activity was easily checked by limiting their oxygen supply.

10. No nitrogen fixation occurred in dilute cucumber juice.

11. Exposure to direct sunlight inhibited growth of all of the organisms studied.

12. Of the antiseptics studied, sodium benzoate, sulfurous acid, and hydrofluoric acid were most efficient and most practicable for factory use. Prevention of the growth of *Mycoderma* by the organic acids used or by salt was not found feasible, as too large a concentration of each is required when used alone. However, in the presence of moderately large amounts of salt, lower concentrations of acid are required. The toxicity of the antiseptics studied was increased by increasing the salt and acid content. This was especially true of sodium benzoate.

13. The death temperature of some of the organisms was determined. It was found to be influenced by pH, salt concentration, and amount of inoculum used.

14. The sixteen strains of *Mycodermas* studied were classified into three groups and into seven varieties.

LITERATURE CITED

[1] JORGENSEN, ALFRED.
1911. Microorganisms and fermentation. XX + 420 p. Figs. 1–40. J. B. Lippincott Company, Philadelphia.

[2] CHESTER, F. D.
1901. A manual of determinative bacteriology. VI + 401. Figs. 1–13. The Macmillan Company, New York City.

[3] CRUESS, W. V.
1918. The fermentation organisms of California grapes. Univ. California Publ. Agr. Sci. **4**:1–66.

[4] ROUND, L. A., and H. L. LANG.
1917. Preservation of vegetables by fermentation and salting. U. S. Dept. Agr. Farmers' Bul. **881**:1–15.

[5] LEFEVRE, D. J.
1924. Making fermented pickles. U. S. Dept. Agr. Farmers' Bul. **1438**:1–16.

[6] SHINKLE, C. A.
1912. American commercial methods of manufacturing preserves, pickles, canned foods, etc. IX + 160 p. Figs. 1–30. Published by author. Philadelphia.

[7] CRUESS, W. V.
1924. Commercial fruit and vegetable products. VII + 530 p. Figs. 1–100. McGraw-Hill Book Company, Inc., New York City.

[8] JOSLYN, M. A.
1927. The effect of the pickle scum on the pickle brine. Fruit Products Jour. and Amer. Vinegar Indus. **6**(1):16–18.

[9] CRUESS, W. V.
1928. Characteristics of microorganisms occurring in brines. Canning Age. **9**:375–376.

[10] CRUESS, W. V.
1921. Vinegar from waste fruits. California Agr. Exp. Sta. Bul. **287**:1–19. (Out of print.)

[11] CRAWFORD, S. L.
1921. Loss in vinegar by evaporation and fermentation. Canner **53**(25): 46.

[12] PETTERSSON, A.
1900. Experimentelle Untersuchungen über das Conservieren von Fisch and Fleisch mit Salzen. Arch. Hyg. **37**:171–175.

[13] BITTING, A. W.
1909. Experiments on the spoilage of tomato ketchup. U. S. Dept. Agr. Bur. Chem. Bul. **19**:1–16.

[14] THOM, C.
1914. The salt factor in the mold ripened cheeses. Storrs Agr. Exp. Sta. Bul. **79**:387–394.

[15] KARAFFA-KORBUTT, K.
1912. The influence of common salt on the life and growth of micro-organisms. Zeit. Hyg. **71**:162–170.

[16] MITRA, S. K.
1917. Toxic and antagonistic effects of salts on wine yeast. Univ. California Publ. Agr. Sci. **3**:63–102.

[17] SPEARMAN, H. B., A. H. GEE, and J. M. LUCK.
1928. The influence of sodium chloride on the growth and metabolism of yeast. Jour. Bact. **15**:319–325.

[18] DEVEREUX, E. D., and F. W. TANNER.
1927. Observations on the growth of yeasts in pure nutrient solutions. Jour. Bact. **14**:317–334.

[19] LIPMAN, C. B.
1911. Nitrogen fixation by yeasts and other fungi. Jour. Biol. Chem. **10**:360–365.

[20] AYERS, S. HENRY, H. A. BARNBY, and E. L. VOIGHT.
1928. Clouding of olive brine. Glass Container **7**:5, 6, 18, 34, 36, 38.

[21] TAKAHASHI, T.
1905. Some new varieties of Mycoderma yeast. Tokyo Imp. Univ. Col. Agr. Bul. **6**:387–402.

[22] TAKAHASHI, T.
1906. A new variety of Mycoderma yeast as a cause of a sake disease. Tokyo Imp. Univ. Col. Agr. Bul. **7**:101–105.

The titles of the Technical Papers of the California Agricultural Experiment Station, Nos. 1 to 20, which HILGARDIA replaces, and copies of which may be had on application to the Publication Secretary, Agricultural Experiment Station, Berkeley, are as follows:

1. The Removal of Sodium Carbonate from Soils, by Walter P. Kelley and Edward E. Thomas. January, 1923.

4. Effect of Sodium Chlorid and Calcium Chlorid upon the Growth and Composition of Young Orange Trees, by H. S. Reed and A. R. C. Haas. April, 1923.

5. Citrus Blast and Black Pit, by H. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.

6. A Study of Deciduous Fruit Tree Rootstocks with Special Reference to Their Identification, by Myer J. Heppner. June, 1923.

7. A Study of the Darkening of Apple Tissue, by E. L. Overholser and W. V. Cruess. June, 1923.

8. Effect of Salts on the Intake of Inorganic Elements and on the Buffer System of the Plant, by D. R. Hoagland and J. C. Martin. July, 1923.

9. Experiments on the Reclamation of Alkali Soils by Leaching with Water and Gypsum, by P. L. Hibbard. August, 1923.

10. The Seasonal Variation of the Soil Moisture in a Walnut Grove in Relation to Hygroscopic Coefficient, by L. D. Batchelor and H. S. Reed. September, 1923.

11. Studies on the Effects of Sodium, Potassium, and Calcium on Young Orange Trees, by H. S. Reed and A. R. C. Haas. October, 1923.

12. The Effect of the Plant on the Reaction of the Culture Solution, by D. R. Hoagland. November, 1923.

13. Some Mutual Effects on Soil and Plant Induced by Added Solutes, by John S. Burd and J. C. Martin. December, 1923.

14. The Respiration of Potato Tubers in Relation to the Occurrence of Blackheart, by J. P. Bennett and E. T. Bartholomew. January, 1924.

15. Replaceable Bases in Soils, by Walter P. Kelley and S. Melvin Brown. February, 1924.

16. The Moisture Equivalent as Influenced by the Amount of Soil Used in its Determination, by F. J. Veihmeyer, O. W. Israelsen and J. P. Conrad. September, 1924.

17. Nutrient and Toxic Effects of Certain Ions on Citrus and Walnut Trees with Especial Reference to the Concentration and Ph of the Medium, by H. S. Reed and A. R. C. Haas. October, 1924.

18. Factors Influencing the Rate of Germination of Seed of Asparagus Officinalis, by H. A. Borthwick. March, 1925.

19. The Relation of the Subcutaneous Administration of Living Bacterium abortum to the Immunity and Carrier Problem of Bovine Infectious Abortion, by George H. Hart and Jacob Traum. April, 1925.

20. A Study of the Conductive Tissues in Shoots of the Bartlett Pear and the Relationship of Food Movement to Dominance of the Apical Buds, by Frank E. Gardner. April, 1925.