A STUDY OF PSYCHROPHILIC, MESOPHILIC, AND HALOPHILIC BACTERIA IN SALT AND MEAT CURING SOLUTIONS

by

HAROLD EUGENE TICKNER

B. S., Kansas State College
of Agriculture and Applied Science, 1949

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1950

TABLE OF CONTENTS

INTRODUCTION

The preservation of food by man has been, and is still, an important problem with which he has to cope. Many types of foods have been treated with salt to hold them in an edible condition for extended periods.

Since salt is one of the most important componants of a meat curing solution, there has been from time to time considerable discussion as to whether salt harbors meat spoilage organisms. In order to obtain information on this subject, this work dealt with the examination of commercial brands of sodium chloride to determine if they contained organisms harmful to foods, meat in particular.

The initial interest of this work revolved around organisms which could be grown at a low temperature. The first phase of the work dealt with isolation of "psychrophilic" bacteria from salt samples. The term "psychrophilic" in this work refers to those organisms which generally exhibit growth at a temperature of 5°C. This is the temperature most frequently employed in curing meats. "Mesophilic" bacteria will be considered as those which showed growth at a temperature of 37°C.

The definition of "halophilic" bacteria will be reviewed, since no definition has been agreed upon as to the correct usage of the word. This definition will be discussed in the Review of Literature.

REVIEW OF LITERATURE

Salt has been used for centuries in a variety of ways. It was once used as an unit of exchange and could be traded for other materials necessary to the comfort and well-being of the people. Jensen (1945) stated that the use of salt in the preservation and curing of meats was practiced and considered a well-known method before the time of Homer; i.e., 1000 B.C. Jensen also stated that the three main economic necessities of the ancient world were salt, spices, and incense, and that these substances were uniformly associated with microbial action.

It is not known exactly when it was discovered that salt could be used for preserving meats; however, it is believed that "salting" was first practiced in the saline deserts of Asia and along sea coasts where salt was easily obtainable. The advent of modern refrigeration during the past half century has brought about a decrease in the use of heavily salted foods to a great extent. This factor has presented a new and interesting problem to the bacteriologist, because concomitant with greater use of refrigeration, the lowering of the salt content of foods has brought about changes in the microbic flora.

The literature has numerous references reporting that there are various bacteria in salt used for human consumption. Many of the bacteria present have found their way into the salt by way of exposure to air, man, and by contamination from the tools used in processing salt into an usable form.

The definition for "halophilic" bacteria is not clear,

because many workers present different salt requirements for the growth of such organisms. Clayton and Gibbs (1927) stated that halophilic cultures cannot be obtained on ordinary bacteriologic nutrient media unless 15 percent sodium chloride is present. ZoBell (1946a) reported that a concentration of more than 5 percent sodium chloride is necessary before an organism can be classified as a halophilic organism. It is interesting to note that ZoBell states that few typical marine organisms are considered as halophilic organisms since they do not develop, or grow, in a concentration greater than 5 percent sodium chloride.

In looking over the requirements of halophilic organisms, it is difficult to find agreement as to what kind of an organism comes under the designation of a halophilic organism. Sturges and Heideman (1924) give the following requirements for halophilic organisms, and also group the growth requirements of the organisms in relation to their requirements of sodium chloride in the following manner:

HALOPHILIC ORGANISMS
Organisms which grow on media containing 12 per cent NaCl.

GROUP I. SALT OBLIGATE
Organisms requiring for growth salt in concentrations greater than one per cent.

GROUP II. SALT PREFERENTIAL
Organisms which grow more luxuriantly in media containing more than one per cent salt than on standard media and usually exhibiting extreme irregularities on the latter.

GROUP III. SALT FACULTATIVE
Organisms which grow with equal luxuriance on standard and on 12 per cent NaCl media.

GROUP IV. SALT TOLERANT
Organisms which grow on 12 per cent media (often exhibiting irregular forms) but grow more luxuriantly on standard media.

In the present study of salt and meat curing solutions, halophilic organisms will be considered as those organisms which require a concentration of at least 5 percent sodium chloride. The reason for this arbitrary definition is that the organisms which will grow on a concentration of 15 percent salt will show one set of morphologic characteristics at that concentration; whereas, at a concentration of 5 percent salt, they will present another entirely different morphologic picture. This feature will be brought out more clearly in another section of this thesis.

Edwin LeFevre (1918) isolated 24 cultures from the contents of pickle vats. The brines had a concentration of approximately 10 percent sodium chloride. On pickle juice agar, the colonies had reddish centers when viewed from the bottom of the plates. Four of the cultures were micrococci, and the rest were rod forms which fell into four groups. One outstanding feature of the organisms was their preference for sodium chloride. Only one organism of the group would grow in a concentration of less than 5 percent sodium chloride, while the three other groups were found to grow in a concentration of 25 percent sodium chloride in broth. In this same work (LeFevre, 1918) stated that both micrococci and rod forms were involved in the spoilage of salted products.

The flora of meat curing solutions was studied to a certain degree by Sturges (1923). He stated that the brines used at that time had a salt concentration of 15 percent, a slight amount of

nitrate, and a small percentage of cane sugar. After this type of curing solution was used in the curing of meat, he isolated many organisms from the cured hams. He stated that it would be possible to isolate hundreds of species of bacteria from old brines since he had isolated 100 cultures which he studied in detail. The following are a few of the more interesting organisms which he investigated: 1. A yeast (Torula); 2. a motile, Gram-negative bacillus, resembling Escherichia coli* in morphology; 3. a very pleomorphic form; 4. a bacillus (?) which persistently curved around to form V shapes and perfect rings; 5. a bacillus which invariably developed long filaments; 6. a vibrio resembling the cholera vibrio in morphology; and 7. an extremely motile, Gram-negative, spherical form. His No. 3 organism showed extremely varied morphology. In young cultures on salt agar, the cells had the form of spheres of one-half micron in diameter. In about a week, the cells became enlarged from 4 to 6 times the original size, and filaments began to develop. Also, old cultures on ordinary media without salt showed very grotesque forms.

The typical organism in Sturges' (1923) cultures was a Gram-negative, nitrate-reducing, motile, non-sporulating form which developed readily at low temperatures and in the presence of 15 percent sodium chloride. It had weak fermentative powers, and did not liquify gelatin. As for classification, Sturges urged that a group of halophilic organisms should be recognized, since

---

* Names of all bacteria cited conform to Bergey's Manual of Determinative Bacteriology, 6th Edition, 1948.

he had difficulty in fitting many of these forms into the accepted scheme of classification at that time.

At about the same time Heideman (1924) studied abnormalities of meat curing solutions. Ropiness, or sliminess, of meat curing solutions had been known for a long time. His work was mainly the study of 5 organisms in standard and pickle media at 30°C.

Heideman stated:

> All of the organisms, with one exception produced ropiness throughout the pickle media (above pH 6.0). Growth was best at a pH of 8.0 and very slight or absent at a pH below 6.0. In only one instance did the ropiness persist after a pH of 6.0 had been reached. None of the organisms tested seemed to produce ropiness without the meat extractives which are present in an old pickle, although in some cases growth was noted in new pickle containing no protein material. The presence of carbohydrates and the reaction of the medium appears to be of vital importance in ropy pickle production.

A "pickle," or meat curing solution, is usually made up of 15 to 20 percent salt, a slight amount of nitrate, and cane sugar. As curing proceeds, the salt is taken up by the meat and the meat protein diffuses into the pickle, making it an excellent medium for the growth of microorganisms.

Yesair (1930) examined a series of 125 samples of salt. The total counts on these salt samples ranged from 0 to 1,470. He found that the largest counts were on solar salt samples. In the identification of these organisms, no organisms of the coliform, "flat sour," or "sulfide spoilage" groups were present. He stated that in the process of refining high grade salt, the majority of bacteria are destroyed, but that there are putrefactive anaerobes present in mined salt. Therefore, mined (or rock) salt should not be used in curing solutions. He also

isolated organisms which were morphologically and physiologically similar to Pseudomonas salinaria. This organism has been associated with reddening and discoloration of hides and salt fish.

In relation to the work that Yesair (1930) did on the bacterial content of salt, it is interesting to note that Weinzirl (1924) had isolated bacteria from samples of spoiled meat by means of aerobic agar plates. These organisms he identified and reinoculated into sterile meat which was incubated at 37°C. The cultures never gave an indication of putrefaction or strong hydrogen sulfide production, although it was noted that souring took place with certain of the species. The putrid meat samples were also tested for anaerobic organisms, and they were always found to be present. These anaerobic organisms were, in turn, inoculated into meat, and, in many cases, they produced typical putrefaction. Weinzirl then concluded that the aerobic count could serve as an indication of putrefaction, since both aerobic and anaerobic organisms develop at the same time. He also suggested a standard of 10,000,000 aerobic bacteria per gram for adjudging the degree of contamination.

Rockwell and Eberty (1924) grew Micrococcus pyogenes var. aureus in a standard nutrient broth solution to which various stimulating and inhibiting agents were added. The next step was measuring the inhibition of proteolytic effects. This was done by measuring the liquefaction produced in coagulated blood serum by Proteus vulgaris. From these results, they found that the preservation of proteins had other factors involved besides the dehydration effect of salt. Some of these factors are dehydration,

direct effect of chloride ion, removal of oxygen, sensitization against carbon dioxide, and interference with the rapid action of proteolytic enzymes.

Organisms found in sea water are too numerous to mention; however, 28 genera are reported in Bergey's Manual of Determinative Bacteriology, 6th Edition. Although the genera of organisms listed in Table 1 have been reported in Bergey's Manual to have been isolated from salt and salted materials, they are not necessarily to be considered indigenous to environments of high salt concentration.

Sturges and Heideman (1924) isolated 101 cultures from "pickles" used in curing hams. These organisms consisted of every morphologic type known at that time. There were also forms which they were unable to fit into the traditional bacillus-coccus-spirillum catagory--the usual recognized forms of bacteria.

LeFevre and Round (1919) reported on five closely related groups of organisms which were isolated from "pickle" scum. The differentiation of the organisms studied was based upon differences in Gram stain, morphology, presence or absence of spores, and colony formation. The concentrations of sodium chloride used were from 5 to 25 percent, and growth at these concentrations was characteristic of these halophilic organisms.

There have been many types of media used in the cultivation of halophilic organisms. In many cases the media used were composed of materials similar to the type of material in which the organisms were found growing. For example, Stuart, Frey and James (1933) used a medium which was prepared from hides, rice,

Table 1. Organisms reported in salt and salted materials taken from Bergey's manual of determinative bacteriology, sixth edition.

Source	Organism
Salt and salted materials	Chromobacterium Clostridium Pseudomonas
Brines	Bacillus Desulfovibrio Pseudomonas Sarcina Vibrio
Salted codfish, reddened	Bacillus Flavobacterium Micrococcus Pseudomonas
Salted fish	Bacillus
Salted hides	Pseudomonas
Salted intestines (wiener skins)	Tetracoccus
Salted sardines, anchovies, etc.	Vibrio
Salt ponds, red	Pseudomonas
Salt waters	Spirillum
Solar salt	Pseudomonas Ristella Sarcina
Salt seas and lakes	Flavobacterium Halobacterium Pseudomonas Urobacterium Thiobacillus

and salt in culturing organisms associated with the "reddening" of salted hides. They also subcultured mixed species of red chromogenic organisms on media which contained a high concentration of sodium chloride in dextrose. After employing a large number of dilutions and plating, they were able to isolate a red, chromogenic micrococcus, apparently in pure culture.

LeFevre and Round (1919) suggested the following medium for the cultivation and isolation of halophilic bacteria:

Cucumber juice	1000 ml
Agar-agar	Amount not stated
NaCl	100 g

The method of sterilization was not given. They did find that the organism grew equally well on meat extract with 5 percent salt.

For isolation and enumeration of bacteria from sea materials, ZoBell (1946b) used different media which had been suggested by various workers, and finally developed a medium which he called Medium 2216. It has the following composition:

Sea water (aged)	1000.0 ml
Bacto-peptone	5.0 g
Ferric phosphate	0.1 g
Bacto-agar	15.0 g

This medium has a pH of 7.5 to 7.6 after sterilization at 120°C. for 20 minutes. The sea water used in this medium should be aged by storing raw sea water in glass bottles in the dark for a few weeks or longer. During this storage, the organic content in the water may be reduced from an initial 4 or 5 milligrams per liter to one-tenth this amount. If germ-proof filters are used in filtering the sea water, the filtrate should be inoculated

with some raw sea water to be sure that organisms are present which decompose the organic matter present in sea water. ZoBell stated that this medium was the best for isolation and reproducible plate counts on marine materials and for the cultivation of most aerobic heterotrophs in the sea. He did not report as to whether this medium had ever been used for the cultivation of halophilic organisms, but it seems that it would be a good medium to use, since the only variation would be the concentration of sodium chloride that would have to be added to it for growing and isolating halophilic organisms.

ZoBell (1926b) compared his Medium 2216 with that of another worker, Reuszer (1933). Reuszer's medium differed from ZoBell's in that it contained glucose, a lower concentration of protein, and no ferric phosphate. In comparative tests ZoBell found that only 53 to 78 percent as many colonies were elicited on Reuszer's medium as on Medium 2216, and furthermore that with the addition of ferric phosphate to Reuszer's medium, counts were nearly as high as those obtained on Medium 2216.

Another medium that has proven useful is one developed by Moore (1940). She used silica gel in the medium which gave several advantages over other previous media used in the cultivation of halophilic organisms. Because highly acid and alkaline solutions are used in the medium, it was found that sterilization was unnecessary, since control plates did not show growth. Another factor here is the high concentration of sodium chloride (3 molar) which inhibits growth of any contaminating organisms from the air. The medium also can be poured at a lower temperature

then ordinary media containing agar-agar. Moore carried out a comparison with another similar medium in which she replaced the silica gel with agar-agar. The growth rate was found to be greater when using the silica gel incorporated in the medium; also, growth was more profuse. The more abundant growth, along with the increased speed of growth, makes Moore's medium a very desirable one for bacteriologic examination of salted products. In the examination of salted products, it has been found by many workers that halophilic organisms are very slow in showing up on cultivation plates.

In 1939 Chambard and Gastellu suggested the use of egg-albumin which contained 20 percent sodium chloride. This medium was used in the examination of organisms associated with salted hides.

Clayton and Gibbs (1927) reported that they obtained excellent growth of chromogenic strains of organisms on a medium made up of rice and fish broth. After the medium was prepared, it had a clean, white surface free of water. The product being examined could be placed directly upon the surface of the medium. Later, after it was found that rice favored rapid development of halophilic cultures, a filtrate of rice was incorporated into an agar medium along with fish water--one pound of minced cod to 1 liter water.

In the case of "reddening" of salted fish, Browne (1921-22) found that this discoloration was caused by two distinct micro-organisms--one a spirochete which produced an opaque, pink coloration, and the other a bacillus which produced a transparent,

red coloration. He observed that these two organisms grew in close harmony, and the coloration was likely to vary from a pale pink to a dark red. It was also noted that the two organisms were difficult to separate and grow in pure culture. These organisms showed typical, halophilic characteristics, because they grew well in products of commercial value such as heavily salted fish, brines, sea salt, and fish-agar medium saturated with sea salt. It was also noted that growth was not found on media containing a concentration of less than 16 percent sea salt by weight. Here again the morphology of the organisms depended upon the concentration of the salt in the medium. Brown found the organisms to vary from 14 microns in length in a saturate of medium to small spherical forms (2 microns in diameter) in concentrations of 18 percent. There were also intermediate forms present. He stated that the amount and character of colonial growth did not seem to be affected by differences of salt concentration, the changes being cellular only. The optimum growth temperature for these organisms was 50° to 55°C.; however, they grew at lower temperatures, but their morphology was changed, and there was a loss of pigment production. When these organisms were recultured at their optimum growth conditions, their original characteristics reappeared. Browne believed the source of these organisms to be the sea salt which is used in treatment of the fish, and that any attempt to eliminate these organisms would have to be based on destroying the organisms in the salt.

It was pointed out by Pierce (1914) that red, chromogenic

bacteria were the principal cause of pink to red coloration of San Francisco Bay salterns, but he did not identify the bacteria. He reported that the organisms were obligate halophilic bacteria which would grow only in concentrated brines, and when grown on salted codfish would produce a red discoloration. In contrast to solar salt, it was pointed out by Rahn (1934) that rock salt and crystallized salt manufactured from rock salt were practically free from bacteria, with the exception of a few bacteria which are contaminants. Clayton (1931) also stated that marine, or sea salt, and solar salterns often carry halophilic bacteria. Also, Clayton and Gibbs (1927) found that a pink halophilic organism was responsible for the discoloration of salted hides, and also produced pink blotches on salt fish. Bacterium halophilicum* and Spirochaeta halophilicum* were found to be associated with the discoloration of salted fish, and were also isolated from sea salt by Browne (1922). Petrowa (1936) stated that the red, brownish, and other spoilage organisms were traced to saline lakes from which salt was obtained. In work done by Harrison and Kennedy (1922), it was shown that the discoloration of salted codfish was caused by Pseudomonas salinaria which had been introduced with the solar salt used in the curing of the codfish. In 1936, Stuart found proteolytic and chitinoclastic bacteria in nearly all of the 27 samples examined, of solar salt from different parts of the world.

---

* Not listed in Bergey's Manual of Determinative Bacteriology, 6th Edition. 1948.

Very little work has been done in connection with the effect of different concentrations of protein on halophilic organisms. A study was made by Stuart (1940) in which he found that by increasing the concentration of protein in the substrate, the unfavorable influence of a low pH appears to be offset when the NaCl concentration is not greater than 3.8 molar.

Winslow and Hotchkiss (1921-22) used salts in the form of chlorides and added them to a basic medium in varying concentrations. The basic medium was a 1 percent peptone solution and a pH of 6.8 to 7.0. They determined the rate of growth by comparing the turbidity produced with a suspension of dead bacterial cells. It was found that a broad, general relationship existed between the toxicity and solution tension. It is important to note that they found that a definite, stimulating action was exerted by concentrations of salt below the inhibition level, resulting in more rapid growth than that which occurred in the plain peptone medium. Since the inhibition level varies for different species of halophilic organisms, one might wonder if this would be a definite factor in selective spoilage of salted products. For example, a spoilage organism present in a salted product containing a concentration of salt that is just below the inhibition level, or in the level that would definitely stimulate the organism to abnormal growth, would cause spoilage.

ZoBell et al. (1937) found an average of 167 organisms per ml in water from the Great Salt Lake, northern Utah. Most of these organisms proved to belong to the obligate halophilic group, whose growth required 6 to 15 percent sodium chloride. As for

activity of the organisms, they stated that organic matter in the lake undergoes microbial decomposition as a result of their activity.

EXPERIMENT I
PREPARATION OF MEDIA FOR PLATE COUNTS

The medium used for the determination of the plate count of bacteria in the salt samples under test was nutrient agar plus 0.1 percent glucose and various percentages of sodium chloride, adjusted to pH 7.6. Since the bacteria to be considered were psychrophilic organisms, it was desirable to have a medium which would have a solidification point below 45°C. However, when 15 grams of agar-agar per liter were used, it was found that the nutrient agar media containing 25 percent and 30 percent sodium chloride had a solidification point higher than 45°C. Therefore, it was necessary to determine the maximum amount of agar-agar needed to obtain a medium which could be poured at 45°C. In this determination, bottles containing 100 ml of a 25 percent sodium chloride solution, with varying amounts of agar-agar added in gradations of 0.1 gram, starting at 0.5 gram through 1.5 grams, were used. The bottles were then heated for 20 minutes in an Arnold steamer, removed, cooled at room temperature for 5 minutes, and placed in a 45°C. water bath. The results of this determination are shown in Table 2.

Table 2 shows that 1.0 g of agar-agar per 100 ml of 25 percent sodium chloride solution is in a liquid condition when cooled to 45°C. Thus, the nutrient agar media containing 25 percent and 30 percent sodium chloride for the enumeration of bacteria by plate counts would have to contain not more than 1 g of agar-agar per 100 ml of medium rather than the usual 1.5 g. It was found that similar results were obtained using 1 g of

agar-agar per 100 ml of 30 percent sodium chloride medium.

Table 2. Determination of grams of agar-agar in 100 ml of a 25 percent sodium chloride solution to give a solidification point below 45°C.

Grams Agar-Agar	State of medium melted at 100°C. and cooled to 45°C.
0.5	Liquid
0.6	Liquid
0.7	Liquid
0.8	Liquid
0.9	Liquid
1.0	Liquid
1.2	Solid
1.3	Solid
1.4	Solid
1.5	Solid

EXPERIMENT II
PLATE COUNTS OF HALOPHILIC ORGANISMS IN COMMERCIAL SALT SAMPLES

Thirty-eight samples of sodium chloride, one sample of sodium nitrate, and one sample of sodium nitrite were examined for total numbers of bacteria per 10 grams of sample, employing the plate count technic.

Materials

In this experiment for the enumeration of halophilic bacteria, the basic medium used was nutrient agar plus 0.1 percent glucose with sodium chloride added to obtain desired salt concentrations. The salt concentrations used were 0.85 percent, 5 percent, 10 percent, 15 percent, 20 percent, 25 percent, and 30 percent.

Procedure

Ten grams of the salt to be examined were weighed into sterile dilution bottles. Ninety grams of sterile distilled water were added to obtain a dilution of 1 to 10. Quantities of 1 ml and 0.1 ml of the 1 to 10 dilution were plated using the basic medium containing 0.85 percent, 5 percent, 10 percent, 15 percent, 20 percent, 25 percent, and 30 percent sodium chloride to enumerate the bacteria in the samples of salt. Violet red bile plates without sodium chloride were also used to check the samples for coliform organisms. Counting of the plates were made 1, 2, 3, 5, 7, 10, and 14 days following plating.

Results at 5°C. Incubation

In all cases there were less than 10 organisms per gram of sample examined.

The plates were then placed in a 37°C. incubator and held for 14 days, or until the media dried out.

Results at 37°C. Incubation

Out of 40 samples, 39 showed a count of less than 10 organisms per gram of sample examined. One sample showed 10 organisms per gram of sample, and this organism was isolated on nutrient agar medium containing 5 percent sodium chloride. Violet Red Bile Agar plates without sodium chloride were also used to check the samples, and coliform counts were less than 10 organisms per gram of sample examined.

EXPERIMENT III
ISOLATION OF PSYCHROPHILIC- AND MESOPHILIC-HALOPHILIC BACTERIA

The counts were less than 10 organisms per gram of sample in all but one of the salt samples, and to obtain representative bacteria from the samples, broth tubes were employed using the same concentrations of sodium chloride as were used in the plate counts.

Materials and Procedure

Nutrient broth pH 7.6 was prepared and 25 ml of the medium was placed in tubes and sterilized at 121°C. for 20 minutes. To obtain the desired sodium chloride concentrations, the salt samples were aseptically weighed out and added directly to the medium to obtain concentrations of 0.85 percent, 5 percent, 10 percent, 15 percent, 20 percent, 25 percent, and 30 percent. Only representatives of the bacteria in the salt sample were desired, and not total numbers. Consequently it was possible to use the sodium chloride to be examined in order to obtain the varied percentages of sodium chloride.

The tubes were then placed in the 5°C. incubator and held for a period of from 11 to 16 days. At the end of that time, smears of the tubes were placed upon slides, stained with Hucker's Gentian Violet for 1 minute, and checked for the presence of bacterial cells. If cells were present, indicating possible bacterial growth, the culture was streaked out on poured plates containing nutrient agar plus 0.1 percent dextrose with a concentration of sodium chloride the same as that of the broth tube

under consideration. Tubes were then placed in a 37°C. incubator and mesophilic bacteria were isolated using the same procedure as used for isolation of psychrophilic bacteria.

Results of this experiment are shown on Tables 3 and 4.

Table 3. Isolation of pure cultures of bacteria from salt samples in glucose sodium chloride nutrient broth tubes incubated at 5°C. and 37°C. for 11 to 16 days.

Number of broth tubes showing growth as evidenced by turbidity and simple stain	Percent of sodium chloride in basic medium	Numbers of cultures actually growing on streaked plates
	Results at 5°C. Incubation	
8	0.85	3
1	5.0	0
3	10.0	0
2	15.0	0
3	20.0	0
1	25.0	0
2	30.0	0
	Results at 37°C. Incubation	
15	0.85	19
12	5.0	13
10	10.0	10
4	15.0	4
3	20.0	1 scant & lost
2	25.0	0
1	30.0	0

Table 4. Results of identification of cultures isolated from commercial grades of sodium chloride.

Culture number	Name of organism isolated	Percent of NaCl in glucose nutrient broth and agar used for original isolation
9	Bacillus brevis	0.85
14	Bacillus cereus	0.85
10	Bacillus lentus	0.85
4	Bacillus pumilus	0.85
13	Bacillus pumilus	0.85
18	Bacillus pumilus	0.85
5	Bacillus subtilis	0.85
11	Bacillus subtilis	0.85
12	Bacillus subtilis	0.85
16	Bacillus subtilis	0.85
17	Bacillus subtilis	0.85
19	Bacillus subtilis	0.85
22	Bacillus subtilis	0.85
2	Flavobacterium solare	0.85
3	Micrococcus candidus	0.85
6	Micrococcus flavus	0.85
7	Micrococcus freudenreichii	0.85
8	Micrococcus freudenreichii	0.85
1	Micrococcus roseus	0.85
36	Bacillus laterosporus*	0.85
14	Micrococcus epidermidis*	0.85

Table 4. (Cont.)

Culture number	Name of organism isolated	Percent of NaCl in glucose nutrient broth and agar used for original isolation
21	Bacillus brevis	5.0
29	Bacillus cereus	5.0
31	Bacillus cereus	5.0
28	Bacillus firmus	5.0
33	Bacillus lentus	5.0
20	Bacillus lentus	5.0
23	Bacillus pumilus	5.0
25	Bacillus pumilus	5.0
32	Bacillus subtilis	5.0
26	Micrococcus flavus	5.0
27	Micrococcus luteus	5.0
41	Alcaligenes faecalis	10.0
42	Bacterium mutabile	10.0
44	Flavobacterium solare	10.0
45	Micrococcus flavus	10.0
35	Undetermined	10.0
36	Undetermined	10.0
37	Undetermined**	10.0
38	Undetermined**	10.0
40	Undetermined	10.0
43	Undetermined	10.0
48	Bacillus firmus	15.0

Table 4. (concl.)

Culture number	Name of organism isolated	Percent of NaCl in glucose nutrient broth and agar used for original isolation
47	Bacillus lentus	15.0
46	Undetermined**	15.0
50	Undetermined**	15.0

* Isolated at 5°C.
** See Experiment XIII.

EXPERIMENT IV
PLATE COUNTS OF CURING SOLUTIONS

Since sodium chloride is used in the preparation of meat curing solutions and has long been a subject of discussion as a possible source of meat spoilage, it was deemed desirable to obtain information of psychrophilic and psychrophilic-halophilic bacteria in meat curing solutions.

Materials and Procedure

Meat curing solutions were obtained from the Meats Laboratory, Kansas State College, and from a local meat packing plant. The media used in making plate counts of the curing solution were 0.85 percent, 5 percent, 10 percent, 15 percent, 20 percent, 25 percent, and 30 percent sodium chloride in nutrient agar plus 0.1 percent glucose. The media were adjusted to a pH of 7.6 and sterilized at 121°C. It was first expected that the counts would be low, similar to the low counts that were obtained in platings of the sodium chloride samples. However, dilutions of 1-100, 1-1000, 1-10,000, and 1-100,000 had to be employed because the counts ran very high. After aliquot portions of the dilutions were plated, the plates were placed in a 5°C. incubator until good growth of the colonies was observed and counts could be obtained. Violet Red Bile plates were also used for checking the samples for coliform bacteria, and these plates were incubated at 37°C.

Results

The results of plate counts of psychrophilic and psychrophilic-halophilic bacteria in the curing solutions are given as the original count in Tables 5, 6, 7, and 8, following Experiment V. Cultures of psychrophilic organisms were isolated from the plates, and Table 9, given in Experiment VI, shows the genus and species of the organisms.

EXPERIMENT V
DETERMINATION OF NUMBERS OF PSYCHROPHILIC ORGANISMS IN THE CURING SOLUTIONS

Microorganisms are responsible for many types of spoilages in meats which have been treated or cured with "pickles." The "pickles" which were used in the original plate counts in Experiment IV were held in refrigeration in 12-ounce screw-cap bottles at 5°C., which is the temperature that is used in the refrigeration rooms where meats are being treated with "pickles."

Four curing solutions were collected from barrels which held the meat and "pickle." The meat had been "put down," or placed in the "pickle," and held for 30 to 45 days before the curing solution samples were taken.

Plate counts were made four times on 2 of the 4 samples over a period of 91 days, and the other two samples were plated out 3 times over a period of 45 days to determine changes in the bacterial counts. Trends in the numbers of bacteria in the "pickles" are shown on Tables 5, 6, 7, and 8.

Table 5. Curing solution number 8 which was 30 days old at the time the first plate count was made.

% of NaCl in glucose nutrient agar	Total bacteria per ml on plates incubated at 5°C.			
	Original count	17 days	73 days	91 days
0.85	TNTC (1-10)	59,000	3,960,000	540,000
5.0	TNTC (1-10)	6,300	170,000	520,000
10.0	TNTC (1-10)	Less than 1,000	97,000	200,000
15.0	TNTC (1-10)	Less than 1,000	Less than 1,000	Less than 1,000
20.0	Less than 10	Less than 1,000	Less than 1,000	Less than 1,000
25.0	Less than 10	Less than 1,000	Less than 1,000	Less than 1,000
30.0	Less than 10	Less than 1,000	Less than 1,000	Less than 1,000
Violet Red Bile	Negative in 0.1 ml	Negative in 0.1 ml	Negative in 0.1 ml	Negative in 0.1 ml

Table 6. Curing solution number 9 which was 45 days old at the time the first plate count was made.

% of NaCl in glucose nutrient agar	Total bacteria per ml on plate incubated at 5°C.		
	Original count	27 days	45 days
0.85	6,600	58,700,000	87,040,000
5.0	2,700	117,000,000	24,960,000
10.0	Less than 1,000	70,400,000	30,720,000
15.0	Less than 1,000	13,000	Less than 1,000
20.0	Less than 1,000	Less than 1,000	Less than 1,000
25.0	Less than 1,000	Less than 1,000	Less than 1,000
30.0	Less than 1,000	Less than 1,000	Less than 1,000
Violet Red Bile	Negative in 0.1 ml	Negative in 0.1 ml	Negative in 0.1 ml

Table 7. Curing solution number 10 which was 45 days old at the time the first plate count was made.

% of NaCl in glucose nutrient agar	Total bacteria per ml on plate incubated at 5°C.		
	Original count	27 days	45 days
0.85	51,000	700,000	770,000
5.0	750,000	510,000	580,000
10.0	Less than 1,000	12,000	7,000
15.0	Less than 1,000	1,000	3,000
20.0	Less than 1,000	Less than 1,000	Less than 1,000
25.0	Less than 1,000	Less than 1,000	Less than 1,000
30.0	Less than 1,000	Less than 1,000	Less than 1,000
Violet red bile	Negative in 0.1 ml	Negative in 0.1 ml	Negative in 0.1 ml

Table 8. Curing solution number 7 which was 30 days old at the time the first plate count was made.

% of NaCl in glucose nutrient agar	Total bacteria per ml on plates incubated at 5°C.			
	Original count	17 days	73 days	91 days
0.85	TNTC (1-10)	TNTC (1-10T)	503,000	23,000
5.0	TNTC (1-10)	TNTC (1-T)	2,100,000	30,000
10.0	TNTC (1-10)	4,600	113,000	6,000
15.0	TNTC (1-10)	300	Less than 100	3,000
20.0	Less than 10	Less than 100	Less than 100	Less than 100
25.0	Less than 10	Less than 100	Less than 100	Less than 100
30.0	Less than 10	Less than 100	Less than 100	Less than 100
Violet Red Bile	Negative in 0.1 ml	Negative in 0.1 ml	Negative in 0.1 ml	Negative in 0.1 ml

EXPERIMENT VI
TESTING PURE CULTURES OF PSYCHROPHILIC ORGANISMS IN OLD STERILE CURING SOLUTIONS

To determine if the isolated cultures of psychrophilic bacteria could grow in pure culture and produce a "Ropy" condition in curing solution, all of the organisms isolated in Experiment III at 5°C. and 37°C. from the salt samples and curing solutions were inoculated into sterile curing solution.

Three samples of curing solution were obtained from the Kansas State College Meats Laboratory. The three samples were taken from different barrels containing meat and the "pickle" which had been "put down" for a period of 30 days.

The samples were thoroughly mixed, filtered through coarse filter paper, and centrifuged to remove fat particles and other extraneous materials from the solutions. They were then passed through Seitz filters to sterilize the solutions. The curing solutions could not be autoclaved since, upon heating the solutions, the protein present would be coagulated, and the solution would thereby be altered and of no value in checking for the production of "rope."

One-tenth ml, 1.0 ml, and 5.0 ml portions of the sterilized curing solution were checked for sterility in tubes containing 10 ml of nutrient broth. All tubes were sterile after incubation at 5°C. for 48 hours, and at 37°C. for an additional 48 hours.

Materials and Procedure

When it was established that the curing solution was sterile, it was transfered aseptically, in approximately 7 to 8 ml amounts,

to sterile test tubes and inoculated with the pure cultures of organisms that were isolated at 5°C. from the salt and curing solution samples. All of the pure cultures of psychrophilic organisms would grow at a temperature of 21°C. Since it was necessary to conserve time, the cultures were incubated in the curing solution medium at a temperature of 21°C. for a period of 30 days.

Results

The results of this experiment as to growth, name of organism and condition of the medium are shown on Table 9.

Table 9. Results of inoculating pure cultures of psychrophilic bacteria isolated from salt and curing solutions into a sterilized curing solution which was 30 days old. Incubated at 21°C.

Culture number	Genus and species	Percent of NaCl in isolation medium	Abnormalities produced in the media by the growth of the pure culture		
			Sub-surface growth	Surface growth	Sediment
24	Achromobacter surydice	10.0	Turbid	None	None
4	Achromobacter delicatulum	5.0	Flocculent	None	Flocculent, moderate
5	Alcaligenes faecalis	0.85	Flocculent	None	Flocculent, abundant
7	Achromobacter liquefaciens	10.0	Flocculent	None	Moderate, flocculent
11	Achromobacter liquefaciens	0.85	Turbid	None	None
37	Alcaligenes metalcaligenes	10.0	Turbid	None	Scant, compact
2	Achromobacter stenohalis	0.85	Flocculent	None	Flocculent, moderate
36	Bacillus laterosporus*	0.85	Turbid	None	Scant, compact
15	Bacillus lentus	5.0	Turbid	None	Compact, scant

Table 9. (Cont.)

Culture number	Genus and species	Percent of NaCl in isolation medium	Abnormalities produced in the media by the growth of the pure culture		
			Sub-surface growth	Surface growth	Sediment
23	Bacillus pumilus	0.85	Turbid	None	Compact, scant
3	Bacterium linens	5.0	No growth	No growth	No growth
33	Flavobacterium solare	5.0	Turbid	None	Scant, compact
8	Micrococcus aurantiacus	10.0	Heavy turbidity	None	Abundant, flocculent
12	Micrococcus aurantiacus	0.85	Turbid	None	Moderate, flaky
21	Micrococcus aurantiacus	0.85	Turbid	None	Abundant, flocculent
27	Micrococcus aurantiacus	0.85	Turbid	Pellicle	Abundant, compact
31	Micrococcus aurantiacus	5.0	Turbid	None	Scant, compact
35	Micrococcus aurantiacus	0.85	Turbid	None	Moderate, flaky
19	Micrococcus candidus	10.0	Turbid	None	Compact, scant

Table 9. (Cont.)

Culture number	Genus and species	Percent of NaCl in isolation medium	Abnormalities produced in the media by the growth of the pure culture		
			Sub-surface growth	Surface growth	Sediment
41	Micrococcus candidus	15.0	Flaky	None	Flocculent, Abundant
14	Micrococcus epidermidis*	0.85	Flocculent	None	Moderate, flocculent
34	Micrococcus epidermidis	0.85	Abundant, turbidity	None	Scant, compact
22	Micrococcus flavus	5.0	Turbid	None	Compact, scant
10	Micrococcus freudenreichii	0.85	Scant turbidity	None	Abundant, flocculent
28	Micrococcus freudenreichii	0.85	Turbid	None	Abundant, compact
29	Micrococcus freudenreichii	0.85	Turbid	None	Slightly ROPY
16	Micrococcus pyogenes var. albus	10.0	Turbid	None	Compact, scant
42	Micrococcus pyogenes var. albus	5.0	Heavy turbidity	None	Moderate, compact

Table 9. (Cont.)

Culture number	Genus and species	Percent of NaCl in isolation medium	Abnormalities produced in the media by the growth of the pure culture		
			Sub-surface growth	Surface growth	Sediment
17	Micrococcus varians	15.0	Turbid	None	Compact, scant
6	Pseudomonas fragi	0.85	Turbid	None	Compact, scant
30	Pseudomonas solopium*	0.85	Turbid	None	Scant, compact
9	Yeast	5.0	Heavy turbidity	None	Moderate, compact
13	Yeast	0.85	Scant turbidity	Pellicle	ROPY, abundant
18	Yeast	10.0	Turbid	Pellicle	ROPY, abundant, compact
26	Yeast	0.85	Scant	Pellicle	ROPY, abundant
38	Yeast	10.0	Turbid	Pellicle	ROPY, abundant
39	Yeast	10.0	Turbid	Pellicle	ROPY, abundant

Table 9. (Concl.)

Culture number	Genus and species	Percent of NaCl in isolation medium	Abnormalities produced in the media by the growth of the pure culture		
			Sub-surface growth	Surface growth	Sediment
25	Unidentified	5.0	Turbid	None	Moderate, compact
	Control		Clear	None	None

* Cultures originally isolated from salt.

EXPERIMENT VII
PREPARATION OF CURING SOLUTION TO CHECK GROWTH OF PSYCHROPHILIC- AND MESOPHILIC-HALOPHILIC BACTERIA IN A NEW CURING SOLUTION

The composition of the curing solutions used in curing meats is generally consistent, in that it is composed of sodium chloride, sucrose, potassium nitrate, and water. In order to check the growth of pure cultures of bacteria isolated from the salt samples and "pickles," a curing solution was prepared containing the following ingredients:

Distilled Water	1000	ml
NaCl	200.0	g
Sucrose	60.0	g
KNO_3	3.75	g

Ten ml portions of the prepared curing solution were placed in test tubes and sterilized. After sterilization by autoclaving at 121°C., 0.1 ml portion of sterile goat serum was added as a source of protein. The tubes were held for a period of 48 hours at 5°C., and also for 24 hours at 37°C. to check for sterility.

Inoculations were then made using the pure cultures of psychrophilic and psychrophilic-halophilic bacteria previously isolated. These tubes were incubated for a period of 5 weeks at 5°C. and at 37°C. for 1 week.

Results

No growth was observed in any of the tubes at the end of each incubation period.

It is definitely known that bacteria do develop in the curing solutions in the presence of meat, as shown in Experiments V and VI. Therefore, it was desirable to analyze a curing solution

to find out what changes have occurred after the "pickle" has come in contact with the meats. It was necessary to determine the changes in the amount of sodium chloride and protein in the solution so a sterile curing solution could be prepared to determine if the psychrophilic organisms that had been isolated could grow in a meat curing solution in the absence of meat.

EXPERIMENT VIII
DETERMINATION OF NUMBERS OF MESOPHILIC AND MESOPHILIC-HALOPHILIC BACTERIA IN MEAT CURING SOLUTIONS

The numbers of psychrophilic bacteria were determined in Experiment IV, and in order to obtain a better conception of the numbers of bacteria present in the curing solutions, a number of plate counts of "pickles" were made and incubated at a temperature of 37°C.

Materials and Procedure

The same procedure was used in this determination as in Experiment IV with the exception that the plates were incubated at a temperature of 37°C. over a period of 10 days.

Results

The total number of bacteria per ml of the curing solution is recorded in Table 10.

Table 10. Results of plate counts at 37°C. incubation of curing solutions. Solutions had been in contact with meats for a period of 30 days.

Percent of sodium chloride in basic medium	Total numbers of bacteria per ml on glucose sodium chloride nutrient agar indubated at 37°C. for 10 days					
	Solutions					
	7	8	9	10	10-B	11
0.85	1,000	30,000	1,000	4,000	820,000	150,000
5.0	<1,000	10,000	1,000	19,000	61,000	90,000
10.0	1,000	7,000	<1,000	6,000	5,000	27,000
15.0	<1,000	5,000	<1,000	6,000	2,000	9,000
20.0	<1,000	700	<1,000	200	<1,000	<1,000

EXPERIMENT IX
DETERMINATION OF CHLORIDE CONTENT IN OLD USED CURING SOLUTIONS

It was determined by Experiment VII that pure cultures of psychrophilic and psychrophilic-halophilic bacteria would not grow in a curing solution that had not been in contact with meat. Experiment VI showed that the pure cultures could grow in an old curing solution which had been sterilized by filtration. Thus, it was upon the basis of these two experiments that an analysis of the amount of sodium chloride present in old curing solutions was made in order to determine if there was a decrease in the amount of sodium chloride, and if so how much.

Materials and Procedure

Four samples of curing solutions were obtained that had been in contact with meat for a period of 30 days at a temperature of 5°C. Since the protein in the samples would interfere in the determination of chloride, a protein-free filtrate of the curing solution was prepared according to the method of Folin and Wu, as outlined in the War Department Technical Manual, TM 8-227, 1941. After this filtrate was prepared, the quantities of sodium chloride were determined by the method of Whitehorn, as given in the War Department Manual 1941, on the four samples of curing solutions.

Results

The amounts of chloride expressed as sodium chloride are recorded in Table 11. Along with the experimental results, the

amounts of sodium chloride added to a new "pickle" is given as a comparison to show the relative loss of sodium chloride from the curing solution.

Table 11. Results obtained from the analysis of four "pickles" for the quantities of sodium chloride expressed as percent by volume.

Curing solution and sequence	Age of curing solution when taken for barrel	NaCl percentage by volume
Solution A	30 days	11.51
Solution B	34 days	11.33
Solution C	27 days	16.09
Solution D	30 days	13.43
Average of A, B, C, and D	30 days	14.43
New pickle		20.00

EXPERIMENT X
DETERMINATION OF AMOUNT OF PROTEINS IN OLD USED CURING SOLUTIONS

The results of Experiment VII indicated that pure cultures of psychrophilic and psychrophilic-halophilic bacteria would not grow in a curing solution which had not been in contact with meat.

Therefore, in order to determine whether the proteins in meats diffuse out into the "pickle," and if so how much, a quantitative analysis was made to obtain information about the protein content of curing solutions.

Materials and Procedure

The same curing solution samples used in Experiment IX were tested for the protein according to the Kjeldahl-Gunning method as stated by Pierce and Haenisch (1940).

Results

The amounts of nitrogen recorded in the form of protein (factor 6.25) are reported in Table 12. The average amount of protein was determined in the four curing solutions, and it is this amount of protein in the form of serum which was used to prepare a new meat curing medium, as explained in Experiment XI.

Table 12. Amount of protein in four curing solutions as determined by the Kjeldahl-Gunning method.

Curing solution	Age of curing solution when taken for barrel	Protein percentage by volume
Solution A	30 days	1.490
Solution B	34 days	1.800
Solution C	27 days	0.964
Solution D	30 days	1.181
Average of A, B, C, and D	30 days	1.358
New pickle		0.000

EXPERIMENT XI
PREPARATION OF A "SYNTHETIC AGED CURING SOLUTION" EMPLOYED AS A MEDIUM FOR CHECKING GROWTH OF PURE CULTURES OF ORGANISMS ISOLATED FROM SALT AND MEAT CURING SOLUTIONS

The results of the chloride and protein determinations, along with knowledge of present-day compositions of curing solutions, were used in preparing a "synthetic aged curing solution," or medium. This medium was prepared to use in pure culture studies of bacteria isolated from salt and meat curing solutions.

A medium such as this can be utilized to check materials used in making curing solutions in order to determine if the constituents contain organisms that cause meat spoilage or an abnormal condition of meat curing solutions.

Materials and Procedure

The average percentage of chloride in the form of sodium chloride was determined in Experiment IX, and chemically pure sodium chloride was used in preparing the medium.

The percentages of nitrogen expressed in the form of protein was shown in Experiment X. Cow's serum was the form of protein which was added to the medium.

The other constituents of present-day meat curing solutions have definite values, and the formula of the meat curing medium was:

Protein in Serum	1.358 percent
Sodium Chloride (C.P.)	14.430 percent
Sucrose	6.000 percent
Potassium Nitrate	0.375 percent

The sodium chloride, sucrose, and potassium nitrate were dissolved in distilled water. The amount of protein in serum was

then calculated, based on serum containing 6.5-8.2 percent total serum protein according to Levinson and MacFate (1943). In order to inactivate the complement in the serum, it was heated at a temperature of 57°C. for 10 minutes. The serum was then added to the medium and made up to a total volume of 1 liter. A Seitz filter was employed to sterilize the medium.

Ten-ml portions of this medium were placed in sterile test tubes and inoculated with the pure cultures of psychrophilic and mesophilic organisms which had been isolated from salt and meat curing solutions.

Results

The results of this Experiment are recorded in Table 13. This table lists the genus and species of the pure cultures inoculated into the medium, the amount of sodium chloride in the isolation medium, and the visible reactions which the organisms produced, or abnormal conditions of meat curing solutions produced by the bacteria.

It was found that 78.2 percent of the mesophilic organisms isolated from the salt samples would not grow at 5°C. in nutrient broth when incubated for 30 days. Therefore, only the characteristics listed above are given for the psychrophilic organisms.

Table 13. Results of inoculating pure cultures of psychrophilic bacteria isolated from salt and curing solutions into a "synthetic aged curing solution." Incubated at 5°C.

Culture number	Genus and species	Percent of NaCl in isolation medium	Abnormalities produced in the media by the growth of the pure culture		
			Sub-surface growth	Surface growth	Sediment
24	Achromobacter eurydice	10.0	No growth	No growth	No growth
4	Achromobacter delicatulum	5.0	Scant turbidity	None	Compact, moderate
5	Alcaligenes faecalis	0.85	Scant turbidity	None	Scant, compact
7	Achromobacter liquefaciens	10.0	Scant turbidity	None	Scant, flaky
11	Achromobacter liquefaciens	0.85	Scant turbidity	None	Scant, flaky
37	Alcaligenes metalcaligenes	10.0	None	None	Scant, compact
2	Achromobacter stenohalis	0.85	None	None	Scant, flaky
36	Bacillus laterosporus*	0.85	Scant turbidity	None	Scant, compact
15	Bacillus lentus	5.0	Scant turbidity	None	Scant, flaky

Table 13. (Cont.)

Culture number	Genus and species	Percent of NaCl in isolation medium	Abnormalities produced in the media by the growth of the pure culture		
			Sub-surface growth	Surface growth	Sediment
23	Bacillus pumilus	0.85	No turbidity	None	Moderate, compact
3	Bacterium linens	5.0	Scant turbidity	None	Moderate, flaky
33	Flavobacterium solare	5.0	Scant turbidity	None	Moderate, compact
8	Micrococcus aurantiacus	10.0	Moderate turbidity	None	Scant, compact
12	Micrococcus aurantiacus	0.85	Scant turbidity	None	Moderate, compact
21	Micrococcus aurantiacus	0.85	Scant turbidity	None	Scant, compact
27	Micrococcus aurantiacus	0.85	None	None	Moderate, compact
31	Micrococcus aurantiacus	5.0	Scant turbidity	None	Moderate, compact
35	Micrococcus aurantiacus	0.85	No turbidity	None	Scant, compact

Table 13. (Cont.)

Culture number	Genus and species	Percent of NaCl in isolation medium	Abnormalities produced in the media by the growth of the pure culture		
			Sub-surface growth	Surface growth	Sediment
19	Micrococcus candidus	10.0	No turbidity	None	Moderate, compact
41	Micrococcus candidus	15.0	Scant turbidity	None	Moderate, compact
14	Micrococcus epidermidis*	0.85	No turbidity	None	Moderate, viscid
34	Micrococcus epidermidis	0.85	Scant turbidity	None	Scant, compact
22	Micrococcus flavus	5.0	Scant turbidity	None	Moderate, compact
10	Micrococcus freudenreichii	0.85	No growth	No growth	No growth
28	Micrococcus freudenreichii	0.85	No turbidity	None	Scant, compact
29	Micrococcus freudenreichii	0.85	Scant turbidity	None	Scant, compact
16	Micrococcus pyogenes var. albus	10.0	Scant turbidity	None	Moderate, compact

Table 13. (Cont.)

Culture number	Genus and species	Percent of NaCl in isolation medium	Abnormalities produced in the media by the growth of the pure culture		
			Sub-surface growth	Surface growth	Sediment
42	Micrococcus pyogenes var.	5.0	Scant turbidity	None	Moderate, compact
17	Micrococcus varians	15.0	No turbidity	None	Scant, compact
6	Pseudomonas fragi	0.85	Slight turbidity	None	Moderate, compact, viscid
30	Pseudomonas solopium*	0.85	No growth	No growth	No growth
9	Yeast	5.0	No turbidity	None	Scant, compact
13	Yeast	0.85	No turbidity	Moderate	Viscid
18	Yeast	10.0	No turbidity	None	Scant, compact
26	Yeast	0.85	Scant, flaky turbidity	None	Scant, flaky
38	Yeast	10.0	No turbidity	None	Moderate, flaky

Table 13. (Concl.)

Culture number	Genus and species	Percent of NaCl in isolation medium	Abnormalities produced in the media by the growth of the pure culture		
			Sub-surface growth	Surface growth	Sediment
39	Yeast	10.0	No turbidity	None	Scant, compact
25	Unidentified	5.0	Slight turbidity	None	Scant, viscid
	Control		Clear	None	None

* Cultures originally isolated from salt.

EXPERIMENT XII
A COMPARISON OF BACTERIA FOUND IN SALT AND MEAT CURING SOLUTIONS

The source of meat spoilage organisms in cured meats has long been a subject much discussed among industrial and research workers. Some workers are of the opinion that the source of the spoilage organisms might be from salt, which is an important constituent of "pickles." Others contend that the organisms gain entrance and cause spoilage of meats by contamination or mishandling of the "pickles" and/or the meat while they are being prepared to undergo the cure.

Therefore, it was desirable to make a comparison of the bacteria which were isolated and identified from the salt samples and curing solutions. In making this comparison, the genus and species of the organisms found in the salt are compared with those found in the "pickles" to see if it were possible for the bacteria present in the "pickles" to have found their way into the "pickles" via the salt.

The results of this experiment are shown in Table 14.

Table 14. Comparison of pure cultures which were isolated and identified from salt and curing solutions.

Organisms found in salt that were also present in curing solutions	Number of times isolated from salt	Number of times isolated from curing solutions
Flavobacterium solare	2	1
Micrococcus candidus	1	2
Bacillus pumilus	5	1
Micrococcus freudenreichii	2	3
Bacillus lentus	5	1
Micrococcus candidus	1	2
Micrococcus flavus	2	1
Bacillus pumilus	5	1

EXPERIMENT XIII
ORGANISMS REQUIRING SODIUM CHLORIDE FOR GROWTH

Four cultures were isolated from salt samples which would not grow on ordinary bacteriological media unless sodium chloride was incorporated in the media. In studying the morphological and cultural characteristics of these organisms, it was found that 5 percent sodium chloride added to the media was sufficient for growth. The cultures could not be identified according to the classification of Bergey's Manual of Determinative Bacteriology, Sixth Edition, 1948.

The four cultures described in Table 15, as well as many other cultures which were salt tolerant, exhibited pleomorphic characteristics when grown in a high concentration of sodium chloride.

Table 15. Morphological and cultural characteristics of four cultures isolated from salt which could not be identified according to the present classification in Bergey's Manual of Determinative Bacteriology, Sixth Edition, 1948.

Culture number	Gram stain	Dextrose	Lactose	Form	Nitrate reduced	Skim milk without salt	Starch hydrolysis
50	-	Acid	Acid	Irreg. rod forms	-	-	-
46	+	-	-	Rods in chains	+	-	-
38	+	-	-	Rods in chains	+	Peptonization, acid curd.	+
37	+	-	-	Rods club shaped	+	Peptonization, acid curd.	+

DISCUSSION

This work, dealing with the possibility of meat spoilage organisms in salt, was carried out under a number of different approaches.

Various grades of sodium chloride, which are an important constituent in making a "pickle," were examined in the first phase of this problem. Since the plate counts of bacteria in the salt samples were low, it would appear that the salt manufacturers are producing grades of salt of good quality. However, the organisms isolated from these salt samples fell into many of the genera that have caused meat spoilage. Many of the organisms can develop at a temperature of 5°C. in a "pickle," indicating that they may be a source of trouble to meat packers.

Numerous studies have been made of the different operations necessary to prepare a meat product in meat packing plants. During these operations, the necessary handling and exposure to air are possible sources of contamination which cannot be overlooked.

Excluding the possibility of external contamination of the meats and curing solutions, this work indicated that many of the salts examined contained organisms which were harmful. These bacteria are especially significant where packers allow meats to undergo the curing process over a period of 30 to 40 days and do not control the temperature or concentration of sodium chloride in the "pickle."

The examination of several curing solutions indicated that they were of a very low quality from the standpoint of numbers

of bacteria per ml, as compared with six counts obtained by Jensen (1945b) which fell in a range from 200,000 to 1,500,000 bacteria per ml.

Landerkin (1940) reported that bacteria develop largely at the immediate surface of the side of meat, and within the membranes of the side itself. However, in Experiment VI of this thesis, it was found that bacteria do develop in used curing solution without meat being present. This shows that if salt containing spoilage organisms is used in preparing a "pickle," these organisms would contribute to a low quality "pickle" and meat product.

On the bases of these facts, it is important that the constituents of a "pickle" be carefully examined before they are combined and employed in processing meats.

It was found that the composition of a "pickle" changes considerably, because the sodium chloride content decreases and the protein content in the "pickle" increases. This change is brought about by sodium chloride passing into the meat and proteins passing out of the meat into the curing solution. This change makes the "pickle" an excellent medium for growth of many bacteria which are halophilic or salt tolerant.

Another aspect of the work dealt with the preparation of a medium which could be used in examining for spoilage organisms in materials to be used in making a "pickle." This medium could be used for control work in a meat packing plant.

It is interesting to note that 54.5 percent of the organisms isolated from the salt samples at 37°C. were of the genus

Bacillus, and 15.6 percent were Micrococcus species. However, of the organisms isolated from the curing solutions, only 7.6 percent were members of the genus Bacillus, and 44.7 percent were of the genus Micrococcus. This indicates that there is an inverse ratio between these particular types of organisms in the salt samples and those in the curing solutions.

The majority of the organisms isolated from the salt samples were members of the genus Bacillus, and this is to be expected because they are more resistant than the genus Micrococcus. However, the greatest percentage of organisms isolated from the curing solutions were of the genus Micrococcus, and this seems to indicate that they are more salt tolerant. The members of both of these genera contribute largely to meat spoilage.

Table 16 shows the types of salts examined, and lists the salt samples that contained organisms which have been known to contribute to meat spoilage.

The results of the experiments in which the sterile old curing solution and the sterile "synthetic aged curing solution" were used as media indicate that either medium could be employed in examining individual components of a meat curing solution for spoilage organisms.

Table 16. Different types of salt samples examined.

Types of salt samples	Number of cultures isolated
Number 7 rock salt	0
Flake salt	2
K.D. granulated salt	3
Canners' flake salt	1
K.D. medium	0
Cheese flake	2
Granulated salt	2
Granulated salt	2
Fine flake salt	2
Rock salt	0
Sodium nitrate salt	0
Sodium nitrite salt	0
Pickling, cooking and canning salt	2
Cracker topping salt	0
Butter salt	0
Hi-grade bakers' salt	2
Granulated salt	1
Flake salt	1
Bakers' salt	1
Free running salt	1
Granulated butter salt	1
Granulated flour salt	1

Table 16. (Concl.)

Types of salt samples	Number of cultures isolated
Iodized salt	1
Flake flour salt	3
Meat salt	3
Crude stack salt	3
Crude wet salt	2
K.D. mild cure salt	0
K.D. ½ granulated	2
Vac. refined butter salt	1
Vac. refined treated salt	0
Vac. refined iodized shaker salt	2
Prepared curing powder salt	0
Purified salt	1
Rock salt	0
Rock salt	1
Bakers' salt	1
Number 1 salt	0
Rock salt (mined)	1

CONCLUSIONS

In meat packing plants which employ a procedure whereby the meats are held in the "pickle" for an extended period, or if the new "quick curing process" is not used, caution should be exercised in handling the meats before they are placed in the curing solution. The curing solutions should be prepared as aseptically as possible by using materials which are free from spoilage organisms. Temperature and concentration of the sodium chloride content of the "pickle" should be controlled and checked periodically during the curing process.

It was found that salt does harbor meat spoilage organisms, but that the number of these organisms in the salt is so low they are insignificant. The reason for this insignificance is that the normal contaminating organisms picked up by meats during processing are so numerous that, in comparison, the contaminating organisms derived from salt could not be considered harmful.

In preparing solid media which contain high concentrations of sodium chloride, the amount of agar-agar had to be lowered to obtain a liquid media at a temperature of 45°C. which would become solid at a lower temperature.

The number of aerobic organisms in the salt samples tested was less than 10 organisms per gram of sample when using a standard plate count technique.

The types of organisms isolated were salt-tolerant in many cases; however, only 4 cultures were isolated that could be

classified as halophilic.

The number of psychrophilic organisms was also found to be low, as only 3 cultures were isolated at a temperature of 5°C. from the salt samples.

As the "pickles" aged, the numbers of organisms were found to increase enormously. Thus, the "pickles" examined in this work were of a low quality. High counts were obtained at temperatures of 5°C. and 37°C., indicating that there were large numbers of psychrophilic organisms as well as mesophilic organisms developing in the "pickle."

The "sterile old curing solution" and the "synthetic aged curing solution" media can be used in examining for spoilage organisms in materials to be used in making a "pickle." These media could also be used for control work in meat packing plants.

It was found that there is an inverse ratio between the salt concentration and protein content in a "pickle." The longer the curing process, the lower the salt concentration and consequent raising of the protein content in the curing solution.

The majority of cultures isolated from the salt samples was found to belong to the genus Bacillus. The greater number of organisms isolated from the curing solution were members of the genus Micrococcus. This indicates that members of the genus Micrococcus are more salt tolerant than the genus Bacillus.

ACKNOWLEDGMENTS

The author wishes to thank Dr. T. H. Lord, his major instructor, for his help and encouragement in work on this problem. He wishes to thank Dr. P. L. Gainey, Head of the Department of Bacteriology for many helpful suggestions. He also wishes to thank Dr. J. O. Harris, and Professor V. D. Foltz for materials and suggestions.

LITERATURE CITED

Bergey, D., R. S. Breed, E. G. D. Murray, and P. A. Hitchens.
Bergey's Manual of Determinative Bacteriology, 6th Ed.
Baltimore. Williams and Wilkin. 1948.

Browne, W. W.
Halophilic bacteria. Proc. Soc. Exper. Biol. Med.
19:321-322. 1921-22. Cited in Abst. Bact. 9:198. 1925.

Chambard, P. and C. Gastellu.
Curing damages in salted skins. Jour. Intern. Soc.
Leather Trades Chem. 23:151-64. 1939. (in French)
Cited in Chem. Abst. 33:5222. 1939.

Clayton, W.
The bacteriology of common salt. Food Manufacture.
6:133. 1931.

Clayton, W. and W. E. Gibbs.
Examination for halophilic micro-organisms. Analyst.
52:395-397. 1927.

Harrison, F. C. and M. E. Kennedy.
The red discoloration of cured codfish. Trans. Roy. Soc.
Canada. Ser. 3, Sec. 5, 16:101-152. 1922.

Heidemen, A. G.
Abnormalities of meat curing solutions. Viscid brine:
so-called "ROPY PICKLE." Preliminary Report. Abst.
Bact. 8:14. 1924.

Jensen, L. B.
Microbiology of meats. Garrard Press. Champaign, Ill.
2nd Ed. p. 1. 1945.

Jensen, L. B.
Microbiology of meats. Garrard Press. Champaign, Ill.
2nd Ed. p. 113. 1945b.

Landerkin, G. B.
Studies on the bacteriology of Wiltshire bacon. I. Methods
for quantitative analysis of curing pickle. Food Research.
5:205-214. 1940.

LeFevre, E.
A preliminary study of salt organisms. Abst. Bact.
2:19. 1918.

LeFevre, E. and L. A. Round.
A preliminary report upon some halophilic bacteria. Jour
Bact. 4:177-182. 1919.

Levinson, S. A. and R. P. MacFate.
Clinical Laboratory Diagnosis. Lea and Febiger. Philadelphia. 2nd Ed. 5:191. 1943.

Moore, H. N.
The use of silica gels for the cultivation of halophilic organisms. Jour. Bact. 40:409-413. 1940

Petrowa, E. K.
Salt water from saline lakes, as a source of sowing micro-organisms on the salt products. Arkh. Biol. Nauk. 43 (ser. 2-3) 201-207. Russian with English summary. 1936. Cited in Marine Microbiology by C. E. ZoBell. Chronica Botanica Co. p. 197. 1946.

Pierce, G. J.
The behavior of certain microorganisms in brine. The Salton Sea. Carnegie Inst. Wash. Publ. No. 193:49-69. 1914. Cited in Marine Microbiology by C. E. ZoBell. Chronica Botanica Co. p. 196. 1946

Pierce, W. C. and E. L. Haenisch.
Quantitative Analysis, John Wiley & Sons, Inc. New York. 2nd Ed. pp. 123-127. 1940.

Rahn, O.
Salt. A study of its bacterial content. Nat. Provisioner. 2 p. 1934.

Reuszer, H. W.
Marine bacteria and their role in the cycle of life in the sea, III. The distribution of bacteria in the ocean waters and muds about Cape Cod. Biol. Bull. 65: 480-497. 1933. Cited in Marine Microbiology by C. E. ZoBell. Chronica Botanica Co. p. 42. 1946.

Rockwell, G. E. and E. G. Eberty.
How salt preserves. Jour. Infect. Dis. 35:573-575. 1924

Stuart, L. S.
A note of halophilic chitinovorous bacteria. Jour. of Amer. Leather Chem. Assoc. 31:119-120. 1936. Cited in Chem. Abst. 30:4532. 1936.

Stuart, L. S.
Effect of protein concentrative and cysteine on growth of halophilic bacteria. Jour. Agr. Research. 61:267-275. 1940.

Stuart, L. S., R. W. Frey, and L. H. James.
Microbiological studies of salt in relation to the reddening of salted hides. U. S. Dept. Agr. Tech. Bul. 383. p. 25. 1933.

Sturges, W. S.
Studies on halophilic microorganisms. The flora of meat curing solutions. Abst. Bact. 7:11. 1923.

Sturges, W. S. and A. G. Heideman.
Studies of halophilic microorganisms. II. The flora of meat curing solutions. Abst. Bact. 8:14-15. 1924.

Weinzirl, J.
Concerning the relation of the bacteria count to the putrefaction of meat. Abst. Bact. 8:14. 1924.

Winslow, C. and M. Hotchkiss.
Studies on salt action. The influence of various salts upon bacterial growth. Proc. Soc. Exp. Biol. and Med., N. Y. 19:314-315. 1921-22. Cited in Abst. Bact. 9:253. 1925.

Yesair, J.
Bacterial content of salt. Canning Trade. 52:112-115. 1930.

ZoBell, C. E.
Marine Microbiology. Chronica Botanica Co. p. 196. 1946a.

ZoBell, C. E.
Marine Microbiology. Chronica Botanica Co. pp. 41-42. 1946b.

ZoBell, C. E., D. Q. Anderson, and W. W. Smith.
The bacteriostatic and bactericidal action of Great Salt Lake water. Jour. Bact. 33:253-262. 1937.