The relation of bacteria to the various industries

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THE RELATION OF BACTERIA  
TO THE  
VARIOUS INDUSTRIES.  
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Presented as a Thesis leading to the Degree of  
Master of Science.
INTRODUCTION.

Micro-organisms in recent years have come to occupy a place in the arts and industries of prime importance. Formerly all germs were looked upon as pathogenic or disease-producing for in the study of the etiology of disease, they were first discovered. Subsequent investigation has conclusively shown that they are not all harmful but are absolutely necessary for the maintenance of life.

The non-pathogenic bacteria may be divided into two classes: 1.-Beneficial, 2.-Nonbeneficial. Some of the beneficial are such as cause the souring or ripening of milk, the ripening of cheese, the fermentative, the putrefying, the nitrifying, and the nitrogen fixing. The nonbeneficial are such as cause milk and cheese to become bitter, or butter to become rancid, etc.

Since the methods of isolation have been perfected such organisms, as produce well known and desired reactions, have been used as starters instead of awaiting the natural development which is always uncertain and therefore not uniform. This certainty of action has led to their extensive use in the special industries.

The laboratory work carried on in connection with this thesis consisted in verifying the work of former investigators, also in attaining conclusions not heretofore recorded.
New facts and phenomena are very difficult to obtain on account of the former thorough investigations to determine the relation of bacteria to the different processes of decay, fermentation and nitrification.

In a number of instances it was possible to establish the specific relation of certain bacteria to forms of fruit decay.

In the thesis submitted an outline is given of the technical methods used, the characteristics of the organisms principally concerned with the arts and industries, and the general conclusions.
BACTERIOLOGICAL TECHNIQUE.

1. Culture Media.
2. Sterilization, tubes, plates, etc.
3. Fixation, staining of bacteria and spores.

1. Culture media consists of nutritive substances either carbo-hydrate or proteid, upon which bacteria will grow. Certain conditions are demanded, e.g., moisture 80%, neutral or slightly alkaline in reaction, solid or liquid. All bacteria will not grow well on the same media nor will a given bacterium produce the same kind of growth on different media, therefore a number of varieties are used. Culture media may be natural as blood serum, milk, urine, hydrocele fluid, potato, fruits, etc., or artificial as plain and glucose bouillon, plain glucose and glycerine agar-agar, plain and glucose gelatin, Dunham's solution, litmus milk, etc. These media should be clear, translucent, either liquid or solid. For the growth of nitrous and nitric organisms special media are requisite and these will be described in a subsequent chapter.

Preparation of plain bouillon:— As this is the most important and since it is the base of a number of the following it will be first considered. Formula for 1000 cc.

- Extract of beef --- 3 grams.
- Pepton siccum --- 10 "
- Sodium chloride --- 5 "
- Water --- 1000 cc.

To 1000 cc. tap water is added 3 grams of extract of beef, 10 grams of pepton siccum and 5 grams of sodium chloride and heated until all the ingredients are dissolved, stirring con-
stantly. This solution usually acid is made alkaline by use of a 10% solution of sodium hydrate added drop by drop until litmus paper shows a slight alkaline reaction. The broth is filtered while hot to free it from earthy phosphates which are precipitated. Subsequent heating should not cause further precipitation. The media is now placed in an autoclave and sterilized and is then ready for use.

Agar-agar plain,- Formula:-

Agar ------ 15 grams.
Extract of beef - 3 
Pepton siccum - 10 
Sodium chloride - 5 
Water - - - - 1000 cc.

To 1000 cc. of boiling water 15 grams of the powdered or shredded sea weed is added and this is boiled, constantly stirring until thoroughly dissolved, the extract of beef, sodium chloride, and pepton siccum are then added and dissolved. It is then made alkaline and cooled to 55° or less. To it is then added the whites of two eggs thoroughly beaten. Over a slow fire the solution, being constantly stirred, is again brought to a boil for ten minutes after which it is placed in the autoclave for fifteen minutes heated to 212° F., or a pressure of 15 pounds, following which it is filtered either through filter paper or cotton, poured into tubes, sterilized and ready for use.

Glucose agar-agar is essentially plain agar to which 10 grams of dry glucose has been added per 1000 cc. of agar.

Glycerine agar-agar is essentially plain agar-agar to which 5% or 60 cc. of glycerine C.P., has been added per
Litmus agar-agar is glucose agar-agar to which litmus has been added in sufficient amounts to color the media a deep blue. The proportion of litmus varies as to its strength.

Gelatine is a proteid substance made up of tendon, ligature, cartilage and bone. Media made therefrom is especially valuable for the differentiation of micro-organisms because some liquify it due to a proteolytic ferment. Others do not have this reaction. We may have glucose or glycerine gelatine. Formula for plain gelatine:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatine</td>
<td>100 grams</td>
</tr>
<tr>
<td>Extract of beef</td>
<td>3 &quot;</td>
</tr>
<tr>
<td>Pepton siccum</td>
<td>10 &quot;</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5 &quot;</td>
</tr>
<tr>
<td>Water</td>
<td>1000 cc.</td>
</tr>
</tbody>
</table>

The finished product of gelatine is a transparent jelly. It is essentially bouillon to which 10% of gelatine has been added. This media is quite difficult to prepare for if it is heated too long or above the boiling point the solidification will not take place on cooling. The technique is similar to the making of plain agar. Gelatine must be sterilized for 20 minutes in an Arnold steam sterilizer for three successive days.

Glucose gelatine and glycerin gelatine are essentially plain gelatine to which ten grams of glucose or 5% glycerin per 1000 cc. have been added respectively.

Litmus milk is a preparation of cow's milk with a small quantity of cream to which litmus has been added in sufficient quantities to give it a deep blue color. The litmus as a rule
is added after the milk has been sterilized to avoid loss of color but it may be added before sterilization as the color returns several hours after the media has cooled. This fact has been determined by successive experiments in the local laboratories.

Potato media:- Old potatoes are peeled and sliced with a cork borer and washed in running water for from 10 to 24 hours (to avoid turning black on being sterilized) after which they are placed in tubes and sterilized in the autoclave. Potato juice is made by grating potatoes and adding water. This makes a very good media for tuberculosis.

Dunham’s solution is a media made from pepton siccum 10 grams, sodium chloride 5 grams and water 1000 cc.

For general use culture media is usually sterilized in and kept in test tubes, which are either slanted or maintained in an upright position until solidification takes place.

2. - Sterilization.

There are three principal methods of sterilization, first superheated steam: second, flowing steam; third, hot air. The superheated steam method is used for such media as bouillon agar-agar. The vessels containing the media are placed in a chamber which can be made boiler tight, the air is expelled and steam heated to 212°F., or more for a period of fifteen minutes. This superheated steam will kill both the vegetative and spore formations of micro-organisms which might be present. This is the autoclave method. The flowing steam method of sterilization is followed for a period
of from 20 to 30 minutes on three successive days. The vegetative forms are destroyed on each application of the steam. Pasteurization is the heating of milk to 70°C., for a period of 10 minutes. Hot air method is used mostly for the sterilization of glass-ware. An oven is heated to 150°C., and the temperature maintained for several hours.

3. - Staining methods.

Fixation:-- A cover slip preparation for staining is made in the following manner for general practice. The cover slip is washed in 95% solution of alcohol to remove the grease and dirt, dried thoroughly and upon it is placed a drop of sterile water. A small sterile wire is touched upon the media or surface upon which the bacteria is growing and a very thin emulsion is made on the cover slip. This is allowed to dry in the air after which it is fixed by being passed several times through a bunsen flame, great care being exercised to avoid scorching. The slip is now ready for staining.

Various reagents will give characteristic staining reactions by which given micro-organisms can be differentiated from others similar in shape, size and growth upon culture media. The most common stains are:--


Methylene blue is used as a general stain, also as a counter stain. Gram's method does not stain all species, but some micro-organisms are stained by it while others are not, thus giving a method of identification and differentiation.
Ziehl-Nielson method is used for staining spores and such bacteria as the Bacillus Tuberculosis.

For a Methylene Blue stain apply the solution to a prepared cover slip from one to three minutes, wash in water and dry, mount in balsam. For Gram's stain cover the slip with Aniline-Gentian-Violet solution for thirty seconds, wash in water for two or three seconds, cover the slip with Gram's solution of Iodine for thirty seconds, wash in 95% alcohol until the color ceases to come out of the preparation, wash in water and mount in balsam. This stains certain bacteria dark blue or black while the nuclei are faintly or not at all stained. The use of this method has to do more with the pathogenic bacteria. Spores do not readily take up aniline dyes but when once impregnated yield up their coloring matter with great difficulty and resist decolorizing agents. This phenomena is due to the dense capsule. Abbott's method for staining spores, - Stain the cover slip preparation deeply with Methylene Blue, heating repeatedly but not continuously to the boiling point for about one minute, wash in water, wash .2% HCL alcohol, wash in water, stain 8-10 seconds in aniline-fuchsin solution, wash in water, dry and mount in balsam.

Moeller's method: - Wash cover slip preparation in chloroform for two minutes, wash in water, stain with 5% solution of chromic acid one-half to two minutes, wash in water, stain with carbol-fuchsin heating slowly until liquid boils, decolorize in 5% solution of Sulphuric acid, wash in water,
stain in aqueous solution methylene blue thirty seconds. The spores will be red, the bodies of the bacteria blue.

4. - Pure cultures and the methods of isolation.

A pure culture of a bacterium is a growth by itself upon a specially prepared medium. Since the microscopic examination may not be sufficient to identify the species its growing characteristics are noted. Isolation depends upon the fact that bacteria can be equally distributed in a liquid media and when this media solidifies in thin layers each bacterium will produce a colony. As a preliminary step it is necessary to secure a sufficient dilution of a mixed colony. This is obtained usually after three dilutions are made. From the mixed colony a loopful of material is inoculated into a tube marked "No. 1", from this tube into a tube marked "No. 2", several loopsful are placed and finally from tube marked "No. 2", several loopsful are inoculated into tube marked "No. 3." The media is in a liquid state between 42-45°C, for a temperature of 50°C, is high enough to destroy many forms of germs. When the media is impregnated with the proper dilution it is poured into sterile petri-dishes in thin layers and allowed to solidify. Agar media are placed in an incubator heated to body temperature 39°C to 98.6°F. Gelatine media are maintained at room temperature as it melts at body temperature.

Each bacterium produces a colony so the number of colonies that develops represents the number of bacteria present and where accuracy has been maintained the number of organisms per cc. is readily learned. The colonies are studied when
small, microscopically, when large, macroscopically, both with regard to, first, size; second, shape, which may be punceform, radiating, round or oval, filamentus or rhizoid, rootlike; third, surface, may be flat, elevated, concaved, or convex, moist or dry, smooth, nodular or warty, scaly, corrugated, granular, cloudy, or may show concentric rings: fourth, appearance may be transparent, translucent, opaque, dull, slimy, creamy or colored.

Isolation of species:—A platinum wire is sterilized by being heated in Bunsen flame after which it is then touched upon the culture media and cooled, then upon a colony which is inoculated into or upon a liquid or solid media. This method is called fishing.

Inoculations on solid media are of several forms. First, a stab culture is one where the bacterium is introduced into the substance of a media the growth developing along the puncture and thus showing the characteristics in growth both as to appearances and special properties, i.e., whether it is aerobic or anaerobic or facultative. In gelatine if it is a liquefying bacterium the liquified area may be saucer or nail shaped, or in the form of a sack. If not liquefying an inoculation is made in agar or gelatine producing a uniform growth along the entire line which may be beaded or arborescent.

Microscopic examinations may be made of the living organism to ascertain whether it is motile or non-motile, being carried out by means of the hanging-drop slide. Upon a clean cover-slip a drop of a freshly prepared emulsion is placed and examined. Stained preparations are made after the technique
given above to show the size, shape, and manner of grouping.

NITRIFYING BACTERIA.

The law of conservation of matter is one which as far as fertilization is concerned, is one that has begun to attract the attention of scientists. The agriculturist for centuries has been acquainted with the fact that rotation of crop and fertilizer are essential to maintain the soil in suitable condition for produce.

Nitrogen Fixing Bacteria.

Symbiosis is a living together. It is a cooperation of two associated organisms to their mutual advantage, each symbion being incapable of carrying on alone the work which the symbiotic association is to perform. It differs from parasitism for in such cases the advantage is all on one side, that of the parasite. It differs from two organisms associated for increase of virulence and function and also from two organisms living on the same media for ultimately the weaker will succumb.

The complex organic materials which are to be of service in nature's economy must be converted into simpler forms or elements and this is done by various micro-organisms more especially the saprophytic and denitrifying bacteria. Putrefactive bacteria reduce a complex organic compound into simpler constituents. Nitrifying bacteria are those which oxidize ammonia into nitrites and nitrates. Nitrification has a series of steps, the first of which reduces organic ammonia compounds to nitrites by the specific action of the
nitrous and the second of which reduces the nitrites to nitrates by the nitric organism. The oxygen supply must be adequate and the proper amount of moisture present.

Food of nitrifying bacteria may be either organic or inorganic of which phosphates are essential. Winnogradsky has experimented with solutions absolutely purified from organic matter and found that the organisms flourished. Nitrification must take place in the presence of oxygen for without it the reverse process, de-nitrification results and nitrogen gas is evolved. Another condition is that a base must be present with which the nitric acid evolved can combine. Nitrification takes place only in the feebly alkaline medium, an excess of which would retard the process. A favorable temperature about 37°C, is also necessary.

The division of nitrifying bacteria is made not biologically, but functionally into two main groups, first, nitrous and second, nitric acid producing. The preparatory preliminary process is accomplished by saprophytes which convert the compounds into ammonium compounds upon which the nitrous bacteria act forming nitrites. The nitric bacteria then act upon the nitrites and convert them into nitrates.

The nitrous organism was first isolated by Winnogradsky. They are spherical corpuscles varying in size the largest reaching a diameter of 1/1000 mm. and some are so minute as to be hardly discernible. Another form not always present has a far greater length than breadth; may be oval or larger at one end, or have truncated ends. The circular may be a
developing type. The organism differs according to the soil from which it is taken. It will grow in broth and diluted milk without producing turbidity and acting on ammonia it produces only nitrites. The elongated nitrous bacteria is about 1.8 microns and has a gelatinous capsule, a flagellum, and is motile. On silica jelly (2% dialysed silicic acid mixed with neutral salt and magnesium carbonate to solidify) it produces zoogeea and free cells. Its growth begins in about four days and reaches its greatest extent in ten days. The media required is prepared as follows: - In a sterilized flask place 100 cc. of a solution of two grams of ammonium sulphate, one gram of potassium sulphate and 1000 cc. of distilled water. To this add 1/2 gram magnesium sulphate, two grams sodium chloride, and 0.4 grams ferrous sulphate.

Examination of soil. Inoculate the above media with soil to be examined and every four or five days subculture until the process has been repeated some half dozen times. The above media is unfavorable for all but nitrous organisms, so in time a pure culture is obtained. This organism will grow in the presence of large amounts of organic matter and is able to nitrify the ammonia present.

Nitric organisms, develop freely in inorganic solutions and are able to nitrify nitrates but do not have any influence upon ammonia which in excess retards its development. This bacterium will grow upon purified agar. It prefers carbonates. The cells are elongated, rarely oval but may be pear shaped, more than one micron in length, slightly less in thickness.
and have a gelatinous membrane. It is smaller than the nitrous bacterium, otherwise differs little, except in chemical action. Conclusions reached by Adeney are:

1. In organic solutions containing ammonia, nitrous organisms thrive but nitric gradually lose their vitality.

2. Nitrous organisms cannot oxidize nitrites into nitrates in inorganic solutions.


4. The presence of peaty or humorous matter appears to preserve the vitality of nitric organisms during the fermentation of any ammonia and establishes conditions whereby it is possible for the nitric organism to thrive simultaneously in the same solution as the nitrous organism. These organisms belong to soil, river and well water, and sewage. They are found mostly in the first twelve inches, and inclay and sandy soils down from three to six feet.

**NITROGEN FIXING BACTERIA.**

If the seeds of plants are sown in a soil devoid of nitrogen, but containing all the other food stuffs of plants, they will grow and develop for a short time, then the leaves will turn yellow, dry up and the plant will die. Such a condition in the plant is called nitrogen hunger. If however, a leguminous plant, such as pea, beans or clover were sown they would flourish for a while, then the leaves would turn yellow and partly dry and remain in this condition for a few days to several weeks after which they would again begin to grow, the stalks becoming strong and juicy rapidly, turning green, producing large number of blossoms seed and haulm and containing just as much nitrogen as plants grown in more favorable conditions.
By experiment and comparison it has been shown that ordinary plants have far better yield both in dry matter and nitrogen when food stuffs contain combined nitrogen, while in the experiments and comparisons with leguminoseae absence of nitrogen found both dry matter and nitrogen in the same proportions as when the soil contained combined nitrogen.

Leguminoseae are accumulators of, while all other plants are consumers of nitrogen, however, the former, are not averse to nitrogen, manuring for this shortens the period of nitrogen hunger, while after that stage is past it has no further effect upon their growth. Green manuring is the practice of planting a leguminoseae and after its development of plowing it under rendering the soil capable of developing other plants.

There are several ways in which soil may obtain nitrogen:-

1. - From the air by the action of fungi, lichens, and algae, also brought to earth by rain, ammonia, and nitric acid.

2. - Some of the free nitrogen of the air in the soil is fixed by the alkaline and porous bodies.

3. - Some nitrogen may be absorbed by the higher chlorophyllous plants independent of bacteria.

4. - Electricity will cause nitrous and nitric acid to be formed by the air.

5. - Leguminose.

The farms of the Eastern states of this country, in the few years it has been occupied has shown a marked deterioration in the size of crops. Not so many years ago here in Iowa a crop of oats with a yield of less than 80 to 100 bushels per acre was considered poor, while now a yield of 35-40 bushels per acre is large. The soil in the United States has
been used repeatedly season after season, without receiving the proper fertilizer and a natural result has followed, depletion of plant food stuffs with decrease in yield.

Lafar holds three theories that leguminoseae receive their nitrogen:— 1. — Under the condition of symbiosis by which the plant is able to fix the free nitrogen of the air by its leaves. 2.— That the nodule organism becomes distributed within the soil and fix the free nitrogen, the resulting nitrogen compound being valuable as a source of food through the roots of the higher plants. 3.— That free nitrogen is fixed in the course of the development of the organisms within the nodule and the resulting nitrogen compound are absorbed and utilized by the host.

A leguminous nodule is a lateral swelling occurring on both the older and younger portions of the roots, the former being more abundantly supplied and can be found in all sizes from microscopic to nodules as large as a pea. A nodule consists of two portions:— a white or colorless external zone, and in internal zone pale red in young nodules, but afterwards greenish gray. The line of demarcation is marked and the outline is indented like that of the blackberry, in the cells of the inner layer the bacteria are found and this is called the bacteroidal tissue.

The bacillus can be obtained in pure culture from a node after the following manner:— Remove a nodule early and place it for a few minutes in the steam sterilizer, then wash in an antisentic solution, open with a sterilized knife. A thick creamy yellow matter exudes which upon microscopic examination
is found to be small, round-ended bacilli known as bacteriods (red radicicola). The organisms can be examined in situ by the regular process of hardening and sectioning.

M I L K.

Since milk is so largely used as food it has the opportunity of spreading infectious diseases with which it may be contaminated. Pathogenic germs such as tuberculosis are frequently found in milk of cattle suffering from that disease. Typhoid bacilli are partially excreted through the milk channels or the recepticle containing milk, having been washed with water pregnant with the germ may in this manner be inoculated into the milk. Scarlet fever, anthrax, dysentery, and cholera are also transmitted in this manner. An endemic local disease, may be made epidemic by cream separation as occurs at creameries where the milk supply of a community is separated. The returned skimmed milk is mixed and the germs thus contaminate the entire quantity.

In order to overcome the dangers lurking in milk it should be pasteurized which is accomplished by heating it to 70° C, for ten minutes in which time all the vegetative form of pathogenic bacteria such as tuberculosis, cholera, etc., will be destroyed. The anthrax spores however, require a temperature of 120° C for fifteen minutes for their destruction. However, since anthrax is not prevalent, the danger from such infection is not great.

Pasteurization does not convert any of the natural easily digested constituents of milk into unnatural compounds which are difficult of assimulation.
Condensed milk is milk which has been sterilized to such an extent that the few bacteria alive are placed in such a media as is not conducive to their reproduction. Such milk cannot be used for infant food on account of the great quantity of sugar added during its preparation.

Souring of milk is due to the bacillus lactici acidi which is normally found in the air. It acts upon the milk and converts it into two similar molecules which produce lactic and acetic acids. The bacillus does not liquify gelatine, is non-motile, 1.1-1.7 microns long, 0.3-0.4 broad and is found mostly in pairs, is aerobic and forms end spores. Besides the bacillus lactic acid some 200 other micro-organisms have been found in milk, cream, cheese and butter. Some produce very pleasant flavors, others decidedly unpleasant. The various pigmented milks are due to the action of bacilli producing blue, violet, green, and red colors.

During the World's Columbian Exposition a species of bacteria came before the notice of American Scientists who later introduced it into a number of Creameries from Maine to California and watched the results.

Bacillus No. 41, first obtained in Uruguay from milk, is a non-motile bacillus which clings together in two's but never in chains; it is 6 microns long and 1.1 wide and produces no spores. Growth takes place best at a 20-23 C, at 35 C the growth is almost stopped, and at 60 C for ten minutes the bacillus is killed. This bacterium is not an acid producer and will not cause milk to become sour.
Under the supervision of Prof. H. W. Conn the bacterium never failed to produce a higher standard of butter both as regards aroma and flavor. Later when it was introduced into the various creameries conditions such as lack of cleanliness, ignorance in maintaining pure cultures, and carelessness were factors not to be given minor consideration.

Reports were received from time to time covering a period of one year. In the vast majority of cases they were favorable in some few claim was made that it neither added or detracted, while in the remainder no action was claimed or no reports returned. Several stations where bad butter was made reported great improvement as long as Bacillus No. 41 was used, reverting to the former condition when not. In other instances the regular quantity of milk was divided into equal parts, of which one was inoculated, the other allowed to ripen naturally, and in every case the artificial starter produced the better butter.

**BUTTER.**

Butter is obtained from either sweet or sour cream. In the latter case a process of fermentation has been undergone which is called ripening which may be either natural or artificial. Natural needs no explanation as it is an occurrence with which we are all familiar.

Artificial ripening may be due to natural starters, i.e., a mixed culture taken from a favorable specimen and a small part retained each day for subsequent use and also from pure cultures. The ripening process can be controlled by cleanliness.
as regards the cows and utensils, also care in the selection of artificial starters. There are a number of varieties of bacteria which act as starters and which give good flavor and aroma to butter. There are also many forms which are the opposite. In order to have butter uniform it should be pasteurized before the starter is added. Bacteria as a rule are short lived in butter, but pathogenic may retain their vitality for quite long periods.

Cheese depends entirely upon bacteria for its process of ripening aroma and flavor. Rennet is also used to separate the casein from other products, but this may also be accomplished by acid precipitation as in sour milk. That bacteria play an important part in the ripening of cheese is proven by the fact that when they are removed or opposed, curing changes are stopped abruptly. This has been demonstrated by pasteurization and the use of antiseptics such as thymol, boric acid, etc. Four varieties of bacteria take part in the ripening process.

1. Lactic acid bacilli which produce fermentation by lactic acid.
2. The casein digesting bacteria.
3. The gas producers which cause the honeycombing.
4. Miscellaneous or extraneous bacteria present in the milk at the outset.

The process of ripening undergoes three stages: 1. The period of initial decline in the number of bacteria which occurs immediately after the removal of bacteria from the
press, this lasts only a short time and is followed by a period of two (2). Bacterial increase mostly the lactic acid group which commences about the eighth day and continues until the twentieth or thirtieth day. The casein digestion and gas producer are also present but increase only to a slight extent. 3.-The third period is one of bacterial decline and is due to the growth of the organisms which check it.

Artificial ripening is possible here as in the butter, the starter is a lactic acid producing organism which does not produce gas and has a desirable aroma free from an undesirable one. Such starters are chosen as have a vigorous development in milk. The difficulties met with in cheese making of using pasteurized milk is 1.- That such milk is not coagulable by rennet. 2.- The great difficulty in obtaining and preserving pure culture. 3.- Maintenance of a low temperature cellar will be difficult in some communities.

Poisonous cheese may be due to the ptomaine-tyrotoxican, a product of bacterial fermentation. Cheese may also be gassy due to excessive gas producing organisms. Chromogenic cheeses occur such as red, blue, etc., due to the bacteria, black due to a fungus. Bitter cheese is due to a bacillus called tyro-thrix geniculatus.

**B E E R-M A K I N G.**

The fermentation of beer is dependent upon the Saccharomyces Cervisiaeae commonly known as Baker's or Brewer's yeast. Morphologically it consists of single oval or spherical
cells separate from each other but adhering in irregular masses. Their distinctive method of reproduction is characteristic. Small buds arise from the sides of the cell increasing until as large as the original cell when they may or may not separate. The yeast grows very readily on Glucose-agar-agar.

The method in beer making herein described is the one generally used in America, the data being obtained from The Erlanger Brewing Co., Iowa City.

Barley is placed in large shallow vats and flooded with water which starts the process of germination and also floats upon its surface the lighter, poorer grains and any oats present. The temperature is fixed and every six or eight hours the grain is turned to maintain an equal or uniform advancement. When the sprout has attained a sufficient growth it is removed to a drying kiln and dried by hot air, heated to a low temperature. As the moisture lessens, the temperature is raised, the grain being in a state of agitation. This finished product is Malt.

To obtain the malt extract the malt is placed in a steamer and boiled with water which has been previously sterilized, to it is then added either Gritz (corn ground without its external coat) or ground Rice. The latter making a clearer product when completed. The addition of Gritz or Rice makes the quantity of sugar greater, and thus aids the fermentative action. After the above has been thoroughly mixed and boiled it is filtered and passed into another vat where it is heated by external steam coils. In this receptacle the hops which
There was no page 23 in the bound volume.
give color, are added and the boiling continued for two hours after which it is gradually cooled by being passed over cold water pipes, followed by ammonia pipes. Sudden cooling at this stage would destroy the light color. The Brew is now ready for fermentation.

Fermentation is begun by the use of pure cultures obtained from a previous brew, and is governed by cold ammonia pipes. The brew is passed into successive tanks at the end of every forty-eight hours as the excessive yeast settles to the bottom. The process after the first few days is continued by allowing the brew to remain in a tank from 14-16 days. In each successive tank the T. is lowered. When a given specific gravity is reached the brew is pumped into large tanks, cooled almost to the freezing point and to it then is added a 24-36 hour old ferment in definite proportions. This is maintained under a pressure of five pounds to the square inch. For several days there is a turbidity of the product but this is precipitated and the liquor becomes clear. It is then filtered and ready for market. Bottled beer is sterilized after it has been bottled by being heated in water to 48° for one hour and a half.

Beer may contain lactic acid bacilli which causes it to become bitter.

B R E A D - M A K I N G.

The normal fermentation of bread is due to the yeast plant Saccharomyces cervisicae. Great care must be exercised in the making for being so good a culture medium it is very
prone to be infected with unfavorable plants. Flour is a natural culture media and in the poorer grades germs are quite numerous and may be pathological. Baking of bread does not sterilize it and from the center of a loaf a colony may develop.

Sour bread is often due to the action of lactic or butyric acid bacilli which are found in the flour. Other acid producing germs may also be present and after the yeast has ceased to act or has been retarded they develop.

Sticky, slimy or viscous bread is due to the bacillus so often found on potatoes and therefore called the Potato Bacillus. It begins its growth in the center of the loaf. Inoculated into another loaf a similar condition is the result. Bread kept in a slightly damp place is very liable to become infected with moulds (the most common of which is mucor mucedo). Red or bloody bread is due to the chromogenic Bacillus Prodigiosus.

Putrefaction of Apples and Bananas.

Fruits are especially prone to undergo decay for they contain food which can be readily assimilated by micro-organisms. Water is one of the chief constituents and must be present in amount equal to 30%. Some bacteria even require as much as 80%. Dried fruit is such which has its water reduced to 25% or less. In such fruit bacteria may lie dormant until the proper conditions for their developments is resumed.

Apples and bananas were experimented upon and the following facts noted:
1. That there is no specific organism of decay.
2. That the cultures can be isolated and will grow upon artificial media.
3. That reinoculated in good fruit will produce decay.
4. That in some colonies the bacteria were long and broad, a typical bacillus formation.
5. That some colonies were cocci in appearance but had no general arrangement.
6. That some colonies were spore-like and would only produce such forms.
7. That some of the species of bacteria produced by the apple decay were Gram positive, others Gram negative, while still others took the Ziehl-Nielson stain.
8. That some of the bacteria isolated from banana took the Ziehl-Nielson stain, but were Gram negative.
9. That some liquified gelatin and produced gas while others did neither.
10. That none of the species isolated were motile.

Preservation of food:-

The preservation of food is an industry which came into vogue long before the discovery of micro-organisms. The canning of fruit is an example of its great utility, and is accomplished by destroying with high temperature the vegetative and spore formations of bacteria and hermetically sealing the receptacle in which the fruit is to be retained while under this temperature.

Fruits and other articles of diet may be preserved by cold, as in cold storage where the temperature is either below freezing or at a point low enough to inhibit the growth of organisms.

Food stuffs such as meat may be prepared in high altitudes and dry climates by drying in the sun's rays; in wet climates, by smoking or by coating with creosote or phenol, or by being placed in strong salt solution. The two latter methods cold and drying inhibit growth but does not destroy vitality, and
should conditions become favorable putrefactive processes will be resumed.

Food stuffs are often preserved, flavored, and colored by the use of chemicals, such as salycilic acid, boric acid, thymol, phenol, etc., which substances, however, are detrimental to health when ingested in large amounts.
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I.

Bacterioidal Tissue.

am, Starch granules.
f,-The infection threads.
k,-The nucleus with its nucleolus.
v,- Large central vacuole.
ONE

PLATE

TISSUE BACTERIODAL

ASTE BEYFRINCK
II.

Bacteriods.

u, - long rods in a high state of development.

v, - commencement of branching.

x, - a continuation of v.

y, - fully developed bacteriods.
II PLATE BACTERIOSIS

AFTER BEYERINCK
III.

Root Nodule of Legume.

mw, -Main Root.
sw, -Lateral Root.
n, - Nodule.

Cross section.

pr, -The primary integument with a few epidermal bacteria (rb)

xl, -The vascular bundles each with exylen fibre.
bact, -Strongly developed bacteriodal tissue.
PLATE

ROOT NODULE

(AFTER BEYERINCK)
IV.

Lactic Acid Bacilli.

Bacteria found in milk and cheese.

1. Origin, in sour milk.

2. Form, short thick rods, nearly as broad as long, usually in pairs.

3. Properties, immotile. Sopres large shining. Do not liquify gelatine. Breaks up sugar into lactic acid and carbonic acid gas, the casine being thereby precipitated.
Apple culture, Ziehl-Nielsen stain.

1. Origin, in decaying apple.
2. Form, various size spores.
VI,

Apple culture, Gram stain.

1. Origin, in decaying apple.
2. Form, various size spores.
APPLE CULTURE

GRAM'S STAIN.
VII.

Apple culture, methylene blue.

1. Origin, decaying apple.

2. Form, similar to cocci.

Banana culture, Methylene blue stain.

1. Origin, in decaying banana.

2. Form, either short or long rods.

IX.

Banana culture, methylene blue.

1. Origin, decaying banana.

2. Form, similar to cocci.

Banana culture, Ziehl-Nielsen stain.

1. Origin decaying banana.
2. Form, irregular size spores.
XI.

Banana culture, methylene blue stain.

1. Origin, in decaying banana.

2. Form, long narrow rods.


Ziehl-Nielsen negative.
XI PLATE

BANANA CULTURE

METHYLENE BLUE