SENIOR THESIS.

"PURITY OF YEASTS"

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Michigan Agricultural College, 1898.

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THESIS

Purity of Yeasts.

The object of the following investigation has been to demonstrate the relative purity of a few of the principal commercial yeasts and to determine the growth and action of the yeast plant and the impurities upon the different media.

In each case a portion of the yeast cake was suspended in a definite amount of sterilized water. Agar and gelatin plates were made from this suspended material; when the colonies were sufficiently developed they were carefully studied,and the microorganisms examined by means of a hanging drop. Cultures from each kind of colonies were made in tubes of gelatin,bouillon,agar,potato, milk,2% cane sugar bouillon,2% lactose bouillon,2% glucose bouillon and 1% flour starch bouillon. The cultures were grown in the ordinary temperture of the room and studied during their growth. Those of bouillon,milk,glucose bouillon,lactose bouillon,cane sugar bouillon and starch bouillon,were tested for lactic and acetic acids.

The number of colonies was counted by means of the Wolfhiigil's apparatus for the purpose of determining the ratio existing between the yeast plant and the contaminating varieties. Stained specimens,from which drawings were made,were mounted from the agar tube cultures.

Specimen No. I.

Fleischman's Condensed Yeast,24 hours old:- .01 grams of the yeast cake was suspended in 1000 cc. of sterilized water.

Gelatin and agar plates of .1cc,.4cc and .7cc were made from this suspended material. In twenty-four hours the plates had developed sufficiently to be studied. Two forms of colonies and one mould were found,which are classified as No 1,No 2 and No 3.

No. 1,-Yeast.

Colonies.- The border was regular and even,with a clear and distinct outline. The form was round,being lighter in color at the center and darker at the edges. The color of the colony was that of cream.(a,Fig 1)

Hanging-drop.- Very small round of slightly oval cells. Many were single while others were connected in chains.(b,Fig 1)

Motion.- None.

Gelatin tube cultures.- The growth was along the entire line of inoculation in distinct white dots,which were close together. The surface growth was of thin tiny white rings,the edges of which were raised above the center. Radiating lines extended outwards from the body of the colony.

Inclined agar tube cultures.- A surface growth of moist white appearance not covering the entire surface but only sprinkled over it.

Potato tube cultures.- The surface was partly covered with a dry rough growth of white connecting colonies. A few single colonies were present.

Bouillon tube cultures.- The liquid was slightly cloudy,with little colonies grouped together at the bottom and floating in the liquid.

Starch bouillon cultures.- The liquid was a little cloudy. A sediment formed later.

Cane sugar bouillon cultures.- The liquid was more cloudy than the starch or bouillon. No sediment.

Lactose bouillon cultures.- The liquid was scarcely clouded.

Glucose bouillon cultures.- The liquid was heavily clouded.

Milk tube.- Showed no change.

Growth.- Rapid.

Acids.- No acetic acid was produced in any of the cultures. Lactic acid was produced in all of the cultures, varying in intensity with milk the least, through glucose, cane sugar, bouillion, lactose and starch. None of the reactions were strong.

Behavior to gelatin.- No liquefaction.

No 2.

Colonies.- Small, with regular even borders, but a sharp outline. To the nakedeye they appeared yellow, but the microscope revealed a brown colony composed of concentric rings which became darker toward a nearly black center.(a, Fig 2)

Hanging-drop.- Small white round organisms varying in size from a tiny speck to a pin head. They were not connected.(b, Fig 2)

Motion.- A slight brownean motion.

Gelatin tube culture.- The growth extended along half the length of inoculation. The surface showed a growth of cream colored granular colonies from which a very slight white feathery growth extended toward the bottom

Inclined agar tube culture.- The surface showed tiny unconnected cream colored colonies.

Potato tube culture.- A brownish granular growth along the surface.

Bouillon tube cultures.- The liquid was cloudy with a thick cream colored ring along the surface next the tube and a slight cream colored scum on the surface.

Starch bouillon cultures.- The surface of the liquid was covered with a growth of white specks. A ring of the same color was on the surface next the tube.

Cane sugar bouillon cultures.- The liquid showed a heavy white scum on the surface.

Lactose bouillon culture.- The liquid was cloudy. A thin ring of white on the surface around the tube,and a thin scum covered the surface.

Glucose bouillon cultures.- The liquid was slightly cloudy. A very slight ring was about the tube.

Milk.- No change was visible.

Growth.- Rapid.

Acids.- Acetic acid was found in very small quantity in the starch. Lactic acid was found in small amount in glucose bouillon,increasing in the lactose bouillon,and milk.

Behavior of gelatin.- Does not liquefy.

No 3,- Mould.

Colonies.- The colonies first appeared as delicate white stars which later spread out as dry,flat whitish masses. The out-

line consisting of mycelial threads radiating from the center.(a,Fig 3)

Microscopic examination.- The spores were separated. Rectangular in shape.(b,Fig 3)

Gelatin tube cultures.- The growth occurred along the entire line of inoculation. The surface growth was a grayish white dry growth with a branching net-work of threads extending down into the gelatin.

Inclined agar tube culture.- A grayish white,thin,feathery growth, confined to the lines of inoculation and not spreading over the entire surface.

Potato cultures.- Surface covered with a heavy white growth.

Bouillon cultures.- The surface of the liquid was covered with a white feathery growth.

Starch bouillon cultures.- The surface of the liquid was about half covered with a white fan-shaped growth.

Cane sugar cultures.- The liquid showed a thick white cloud floating near the surface,but no surface growth.

Lactose bouillon cultures.- The liquid had a slight white growth throughout,with a heavy white surface growth.

Glucose bouillon cultures.- The liquid had a thick white surface growth.

Milk.- Showed no growth.

Behavior to gelatin.- No liquefaction.

In the gelatin plate made from .1 cc.,14903 colonies of No 1, 1863 colonies of No 2,and 12 colonies of No 3 were found; making a ratio of 1 colony of No 2 to 8 of No 1.

Specimen No. 2.

Sunlight Yeast(Dry,age unknown):- .1 gram of the yeast cake was suspended in 100 cc. of sterilized water. Gelatin and agar plates of .1 cc.,.5 cc.,.05 cc. and .03 cc. were made. Upon examination,when developed,two kinds of colonies and one mould were found,which are classified as No 1,No 2 and No 3.

No 1,- Yeast.

Colonies.- The colonies were star shaped with a distinct outline. The center was heavy and had lines not closely crowded together radiating from it. The color was creamy white.(a,Fig 4)

Hanging-drop.- Large white,round cells,closely massed together. (b,Fig 4)

Motion.- None.

Gelatin tube cultures.- The growth extended along the entire line of inoculation in yellowish white specks. The surface growth was white.

Inclined agar tube cultures.- The growth was of round or oval distinct colonies,varying slightly in size.

Potato tube culture.- The growth was a dry whitish mass spreading outwards from a few central cells.

Bouillon tube culture.- The liquid was very cloudy.

Starch bouillon cultures.- Somewhat cloudy.

Cane sugar bouillon cultures.- This culture showed the least growth.

Lactose bouillon cultures.- The liquid was scarcely cloudy.

Glucose bouillon cultures.- The liquid was very cloudy,but less so than the bouillon.

Milk tube.- Showed no growth.

Growth.- Not rapid.

Acids.- No acetic acid produced in any of the cultures. All gave lactic acid;bouillon gave the least,then starch bouillon, milk,lactose bouillon,cane sugar bouillon,and glucose bouillon.

Behavior to gelatin.- No liquefaction.

No 2.

Colonies.- The colonies were round with a regular and even border, and a sharp and distinct outline. The center was a light brown with a darker brown edge.(a,Fig 5)

Hanging-drop.- Small white or colorless rods about as wide as long. (b,Fig 5)

Motion.- Rapid rotary motion.

Gelatin tube cultures.- A moist yellowish white surface growth. A funnel-shaped growth extended along the entire line of inoculation.

Inclined agar tube cultures.- The surface was covered with a thin slimy yellowish white growth of connected dots.

Potato.- A slight yellowish white granular growth.

Bouillon tube cultures.- The liquid was very slightly cloudy. A white sediment formed at the bottom of the liquid.

Starch bouillon cultures.- The liquid was about as cloudy as the bouillon.

Cane sugar bouillon culture.- The liquid was cloudy and the sediment formed at the bottom.

Lactose bouillon culture.- The liquid was slightly cloudy,but not so much so as the cane sugar bouillon. A sediment formed at the bottom.

Glucose bouillon cultures.- The liquid was very cloudy. A heavy white sediment formed at the bottom.

Milk tube.- Showed no change.

Growth.- Rapid.

Acids.- None of the cultures gave lactic acid reactions. All the cultures except lactose bouillon gave the acetic acid reaction which was very slight in starch and glucose,but increased with the bouillon,milk and cane sugar.

Behavior to gelatin.- No liquefaction.

No 3.- Mould.

Colonies.- A rounded,irregular,felt-like mass at first white and then greenish or bluish gray. This was composed of many horizontally arranged straight of slightly wavy jointed threads. (Fig 6)

Microscopic examination.- The ~~first~~ Fruit organs were forked and on the ends were the intermediate spore bearers. Each of these in t turn bore a row of oval spores. The whole appeared like a

bush.(b,Fig 6)

Gelatin tube cultures.- The surface was covered with a felt-like growth,at first white but later green.

Inclined agar tube culture.- The surface was entirely covered with a felt-like growth,green at the center but white at the edges.

Potato tube cultures.- The potato was completely covered with a green felt-like growth.

Bouillon tube cultures.- The liquid had only a little of the whitish felt-like growth on the surface. Later it became green.

Starch bouillon cultures.- The surface of the liquid was only dotted with little specks of a felt-like growth. These were white at the edges and green at the center.

Cane sugar bouillon cultures.- The surface of the liquid was covered with a large folded felt-like mass,which was green at the center and white at the edge.

Lactose bouillon culture.- The surface of the liquid was half covered with a felt-like mass,green at the center and white at the edge.

Milk tube.- The milk showed a deep green felt-like growth.

Growth.- Rapid.

Behavior to gelatin.- Liquefies gelatin.

Agar plate .03 cc. of Specimen 2,contained 12022 colonies of No 1, 2769 colonies of No 2,and 1 colony of No 3; making a ratio of 3 colonies of No 2 to 14 of No 1.

Specimen 3.

Magic Yeast.(Dry,age not known):- .01 grams of the yeast cake was suspended in 100 cc. of sterilized water. Gelatin and agar plates of .1 cc., .02 cc.,and .03 cc. were made. When developed three kinds of colonies and one mould were found,which are classified as No 1,No 2,No 3 and No 4.

No 1.- Yeast.

Colonies.- Star-shaped with a distinct outline. The center was yellowish white and had lines not closely crowded together radiating from it.(a,Fig 7)

Hanging drop.- Round or oval cells. Very small and showing cell divisions. Nearly all were massed together.(b,Fig 7)

Gelatin tube cultures.- The growth occurred along the entire line of inoculation in small distinctly separate colonies. The surface growth was heavy and white.

Inclined agar tube cultures.- A whitish feathery surface growth, thicker at the center than at the edge.

Potato tube.- A yellowish white growth.

Bouillon tube cultures.- The liquid was very cloudy.

Starch bouillon culture.- The liquid was more cloudy than the bouillon.

Cane sugar bouillon culture.- The liquid was very cloudy. The most growth of any of the cultures.

Lactose bouillon culture.- The liquid was about as cloudy as the bouillon.

Glucose bouillon culture.- The liquid was slightly more cloudy than the lactose bouillon or bouillon cultures.

Milk tube.- Showed no change.

Growth.- Not rapid. Slower than yeast of Specimen 1; but about the same as that of Specimen No 2.

Acids.- None of the cultures gave the acetic acid reaction. All except bouillon gave lactic acid, starch bouillon gave the least, increasing with lactose bouillon, glucose bouillon, cane sugar bouillon and milk. The reaction was not strong in any case.

Behavior to gelatin.- No liquefaction.

No 2.

Colonies.- Border irregular, with a distinct outline. The colonies were brown and much darker in color at the edge that at the center.(a, Fig 8)

Hanging drop.- Small, white or colorless, round organisms.(b, Fig 8)

Motion.- None.

Gelatin tube cultures.- The growth extended along the line of inoculation in white specks. On the surface it was a dry white fluffy growth.

Inclined agar tube culture.- A white granular slimy growth spreading outward from a center.

Potato tube.- A yellowish white granular growth on the surface.

Bouillon tube culture.- The liquid was scarcely cloudy.

Starch bouillon cultures.- The liquid was about as cloudy as the bouillon.

Cane sugar bouillon culture.- The liquid was slightly cloudy and showed white particles floating in it.

Lactose bouillon culture.- The liquid cloudy,with a white feathery floating mass.

Glucose bouillon culture.- The liquid was not very much clouded, about the same as the bouillon.

Milk tube.- Showed no change.

Growth.- Rapid.

Acids.- Neither acetic nor lactic acid was found.

Behavior to gelatin.- No liquefaction.

No 3.

Colonies.- The surface colonies had an irregular border,but a clear and distinct outline. The deep colonies were round with an even regular border. They were dark brown at the edge and light brown at the center.(a,Fig 9)

Hanging drop.- Tiny bacilli,somewhat longer than wide. Closely connected in a mass.(b,Fig 9)

Motion.- A quick darting movement.

Gelatin tube cultures.- The growth occurred along the entire line of inoculation in white dots. The surface growth was a white spreading one.

In clined agar tube culture.- A yellowish white growth spreading over the surface. Later the agar turned green.

Potato tube cultures.- A brown unewen growth on the surface.

Bouillon tube cultures.- The liquid became green with a white scum on the surface and a white sediment at the bottom.

Starch bouillon culture.- The liquid showed very little white scum on the surface,and was less cloudy than the other cultures.

Cane sugar bouillon culture.- The liquid was cloudy with aheavy white swum on the surface,and a white sediment at the bottom.

Lactose bouillon culture.- The liquid was cloudy with a heavy white scum on the surface,and a white sediment at the bottom.

Glucose bouillon culture.- The liquid was not so cloudy as the lactose bouillon and cane sugar bouillon cultures. It showed a thin white scum on the surface and a small amount of a white sediment at the bottom.

Milk tube.- No change visible.

Growth.- Very slow.

Acids.- Acetic acid was found in very small quantity in the lactose bouillon culture. Lactic acid was found in all except the bouillon,in the following order: starch bouillon,lactose bouillon,glucose bouillon,cane sugar bouillon and milk. The amount found in starch bouillon and lactose bouillon was very small.

Behavior to gelatin.- Liquefies and turns green.

No 4.- Mould.

This was the same as that in the Sunlight Yeast:- Gelatin plate .02 cc. contained 2106 colonies of No 1, 224 colonies of No 2, 78 colonies of No 3 and 1 colony of No 4; making a ratio of 1 colony of No 2 to 9 of No 1; and 1 colony of No 3 to 27 of No 1.

Specimen 4.

Yeast Foam(Dry,age not known):- .01 grams of the yeast-cake was suspended in 100 cc. of sterilized water. Gelatin and agar plates of .02 cc.,.03 cc.,.04 cc.,.05 cc.,were made. Where sufficiently grown to be studied the plates contained two kinds of colonies and one mould,which were classified as No 1,No 2,No 3.

No 1.- Yeast.

Colonies.- Star-shaped with a clear and distinct outline. Cream color,the center being darker than the radiating lines.(a,Fig1)

Hanging drop.- Oval,white or colorless cells,often with one end slightly pointed. They were very large.(b,Fig 1)

Gelatin tube culture.- The growth was along the entire line of inoculation in small white dots. The surface growth was of a heavy white mass.

Inclined agar tube culture.- A white feathery growth,thick at the center with thinner lines radiating outwards.

Potato tube cultures.- Surface covered with a yellowish white growth.

Bouillon tube cultures.- The liquid was very cloudy.

Starch bouillon culture.- The liquid was very cloudy but not as cloudy as the bouillon.

Cane sugar bouillon culture.- The liquid was very cloudy. This gave the most growth of any of the cultures.

Lactose bouillon culture.- The liquid was more cloudy than the bouillon.

Glucose bouillon culture.- Theliquid was more cloudy than the lactose bouillon but not so much as the cane sugar bouillon.

Milk tube.- Showed no change.

Growth.- Quite rapid but less so than Specimen No 1.

Acids.- None of the cultures gave acetic acid reactions,but all except bouillon and starch gave lactic acid. Lactose bouillon gave the least,then glucose bouillon,cane sugar bouillon and milk.

Behavior to gelatin.- No liquefaction.

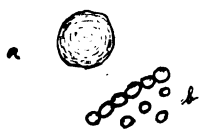
No 2.

This colony was the same as colony No 2 of Specimen 3,and gave the same growth.

No 3.

This mould was the same as in Specimens No 2 and No 3.

Gelatin plate .02 cc. contained 2773 colonies of No 1,376 colonies of No 2,and 2 colonies of No 3; making a ratio of 59 colonies of No 1 to 8 of No 2.



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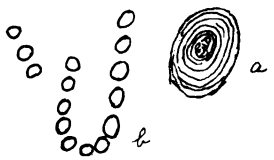

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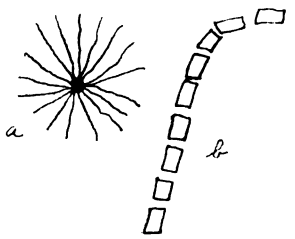

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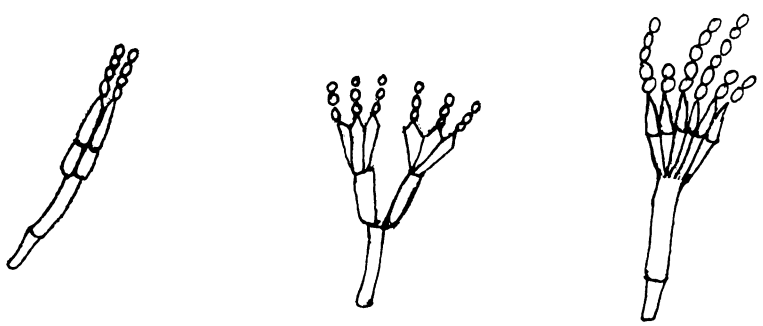

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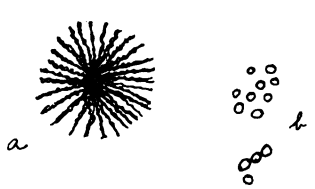

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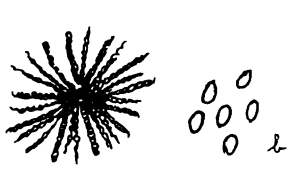

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