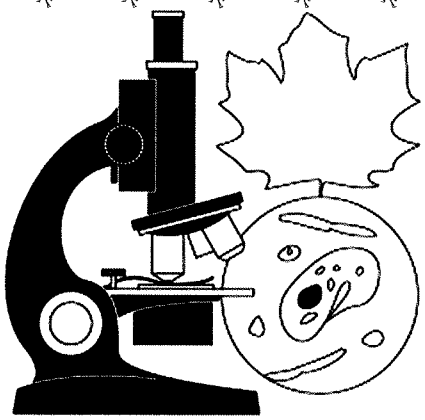


الجامعة المستنصرية

كلية العلوم

قسم علوم الحياة

المرحلة الرابعة

الغذائية العملي

للعام الدراسي

2016.2017

يطلب من

مكتبة حسنين Hassanein Library

مع التمنيات

بالنجاح والموفقية

السعر:

1250

Food microbiology: is the study of the spoilage & pathogenic microorganisms that inhabit in food, mainly Accompanied by changes in the food .

- Food is consideredas a good environment for growth of many M.Os, and these M.Os causes spoilage.
- Food also considered as a Carrier media for many pathogenic M.Os , which cause diseases .
- The impotence of food microorganisms come from prevent food contamination by these M.Os, & control or prevent reproductions of it.

Spoilage food

Is the food that unacceptable to a consumer due to its smell, taste, appearance , texture or the presence of foreign bodies .

Major reasons for food spoiled:

1-microbial growth in food.
2- chemical changes in a food.
3-physical damage.
4-changes in water content.
5- presence of foreign bodies in food.

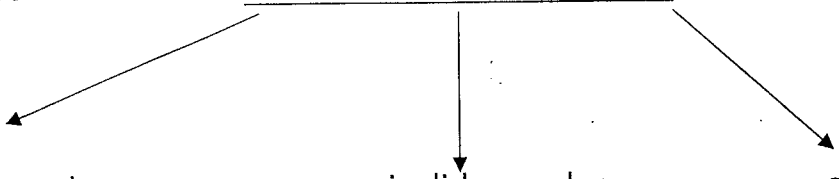
Spoilage organisms

Include bacteria , yeasts & molds that grow in food causing changes of food.

How to collect the food sample?

*Taken Under Sterile Condition To Prevent Contamination
*Randomly Taken .
*Reserved In Its Same Physical Condition (Frozen Remain Frozen , Dried Remain Dried.
*Transferred To The Lab Directly For Analysis.

Types of food samples



Liquid samples	semisolid samples	solid samples
Ex: milk, juice...ect	ex: chesses, ice-cream ..ect	fruits, grains

-In liquid sample shake before sampling for homogenization .

- In solid sample the sampling done by using sterile knife or cork borer.

- some samples done by taking thin layers from the surface.

Dealing procedures with the sample in lab

Sampling (food homogenate) :

It is about 10 gm or ml are collected from food.

The mortar

Mash or crush the solid foods and turn them into emulsion.

The container

Sterile ,wide-mouth, glass or plastic are used.

Instruments

used Probe (trial) spoon & knife to cutting & transport sample.

Sampling report

1- Date of sampling.

2-nature of food.

3-suggested tests.

4- any useful information.

Preparation & dilution of food homogenate:

Aseptically, 10 gm are transferred into sterile container, 90 ml diluted and shaken several times by mortar to obtain a 10^{-1} .the mixture is left for 3-5 min just before making dilution.

Dilutions

The food homogenate is mixed & serial ten –fold dilutions are made.

Tubes containing 9ml volume of diluents are prepared in a raw.

These are numbered in order with the ten-fold dilutions (10^{-2},10^{-3},10^{-4},ect).

1ml of the 10^{-1} dilution is transferred into the first of the 9 ml tubes .each 1 ml transferring from the previous tube into next.

Media

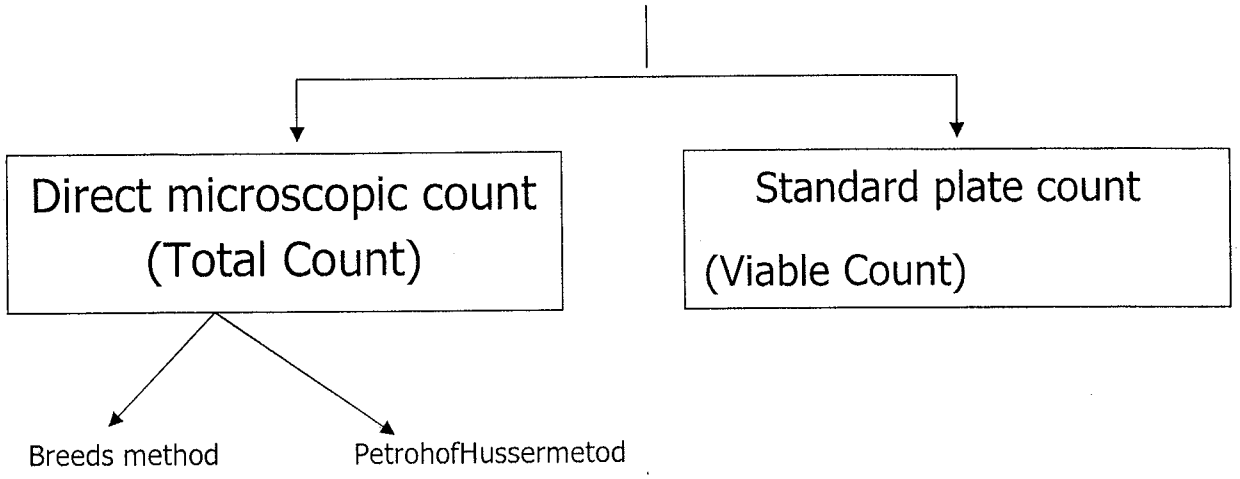
Pour plating

1 ml of each ten-fold dilution isput in Petri dishes about 15-20 ml of the molten agar & allowed to solidify.

Incubation

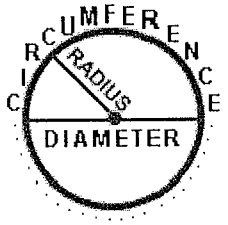
The plates are incubated in an inverted position for 24-48 hours at 37C°..

Determination of M.Os Numbers



Breed method:

This method is used to count the number of microbe cells (live and dead) is characterized as easy and the speed



Area For Circular microscopic field = π = 3.14.

Diameter microscopic field= 160 >>>> 80*80*3.14=20096

To prepare area of the bacterial film , draw a square 1 cm on slide.

Transfer 0.01 ml or drop by loop to slide and spread ,wait to dry.

By Methylene blue dye for then washed and examines

Calculate the number of microbes cells then take the rate of 10 fields .

$1cm^2$ =100 000000 Micron

$$\text{Number of microscopic fields in } 1cm^2 = \frac{\text{Area of the drawnsquare}}{\text{One Area of the microscopic field}}$$

$$= \frac{100000000}{20096} = 4976 \sim 5000 = \text{Microscopic coefficient}$$

Loopfull=100

Number of microbial cells in 1 ml = coefficient microscopic * average number of cells * invert of dilution *drop volume

Q/Calculate the number of microbial cells in half a liter of milk if you know that the loopfull from second dilution and the average number of cells 25 cells?

Standard plate count (Aerobic plate count)

Standard plate count is designed to determine viable bacterial density in food or water sample .

Standard plate count is based on mixing decimal dilutions of food sample .after incubation of plates at 37C°for 24-48 hrs ,the NO. of bacteria per ml is calculated from the NO. of colonies obtained in selected petri dishes at levels of dilutions giving significant results.

Diluent solutions → 0.1 % peptone water ⟹ protein samples.

Diluent solutions → Phosphate buffer ⟹ water &dairy products.

Dilutions:

The food homogenate is mixed or bottle should be shaken, serial decimal dilutions (ten fold) are made .for example 10^{-2}& 10^{-3} etc dilutions .

Pour plateing& incubation

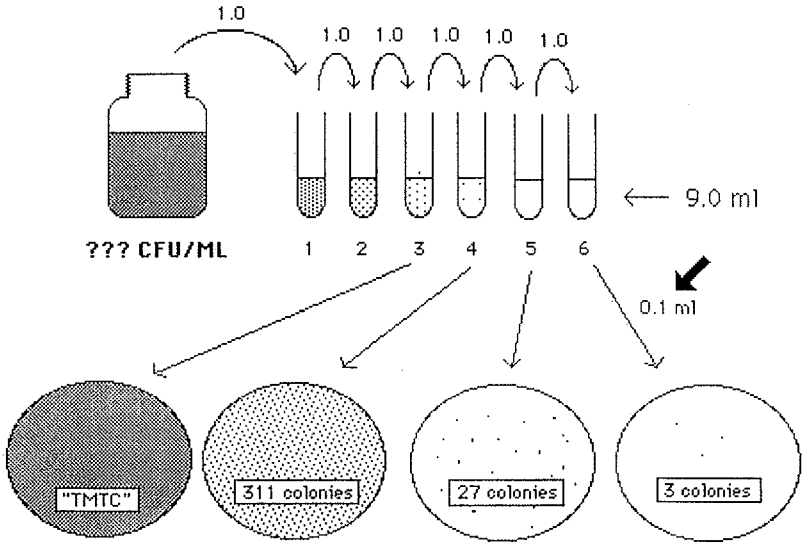
- One ml of each ten-fold dilution is added into duplicate plates .
- About 15-20 ml of the molten plate count agar (44-46C°) are added to each of the duplicate within 15 minutes & allowed to solidify .
- The plts are incubated for 24-48 hrs at 37 C°.

Counting & calculation of colony – forming units (CFU):

Only the plates containing 30-300 CFUs are counted .

When the counting the NO. of bacteria per gm or ml , the total count is calculated as follows:

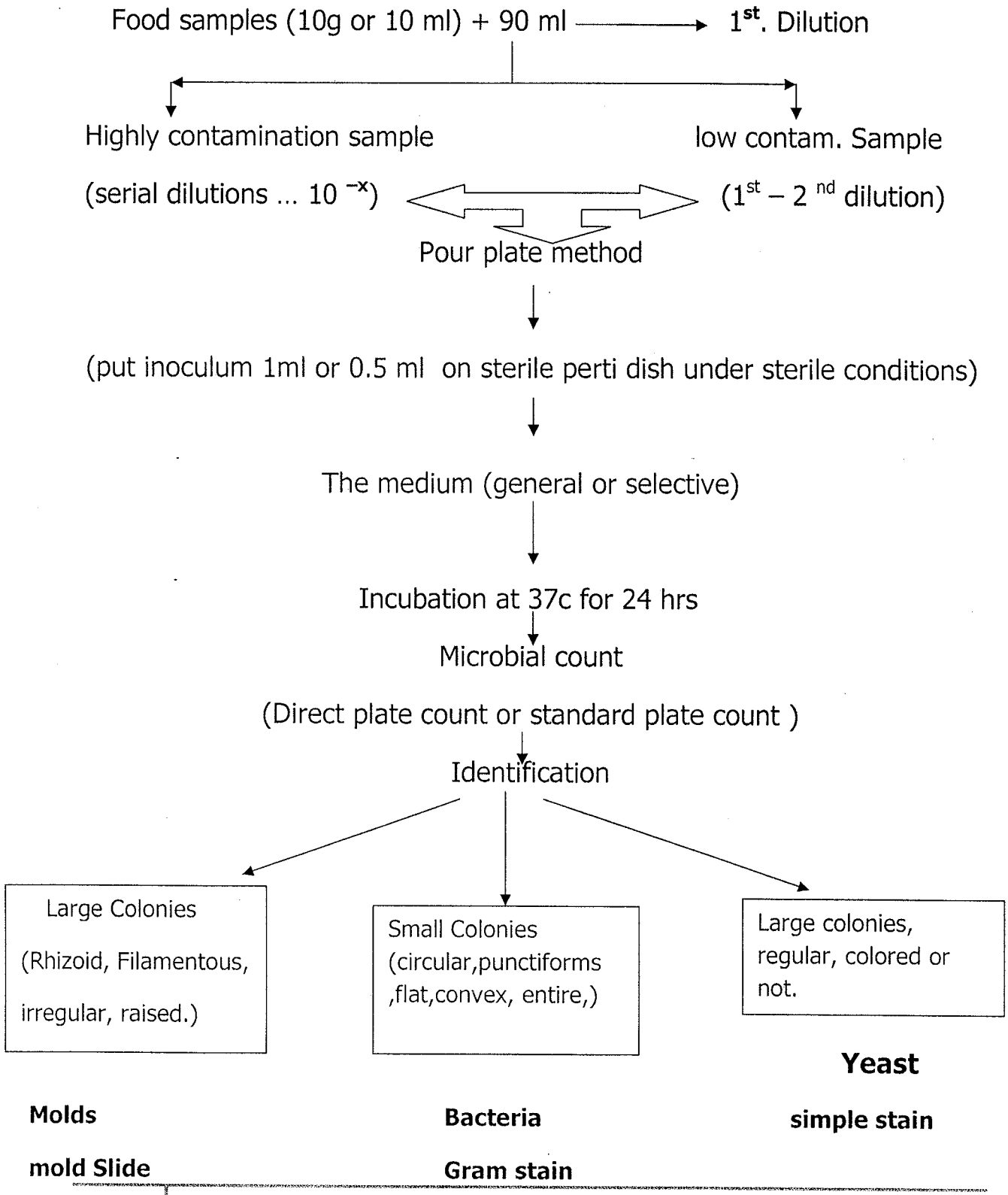
Colony forming unit (CFU) = invert of dilution factor * No. of colonies



TMTC= too many to count >>> more than 300 colonies

TFTC= to few to count >>> less than 30 colonies

LAB.Method


Food samples (10g or 10 ml) + 90 ml
1st. Dilution
Highly contamination sample
(serial dilutions ... 10 -x)
low contam. Sample
(1st – 2 nd dilution)
Pour plate method
(put inoculum 1ml or 0.5 ml on sterile perti dish under sterile conditions)
The medium (general or selective)
Incubation at 37c for 24 hrs
Microbial count
(Direct plate count or standard plate count)
Identification
Large Colonies
(Rhizoid, Filamentous,
irregular, raised.)
Small Colonies
(circular,punctiforms
,flat,convex, entire,)
Large colonies,
regular, colored or
not.
Yeast
Molds
Bacteria
simple stain
mold Slide
Gram stain

Food Microbiology

Gram Stain for Bacteria

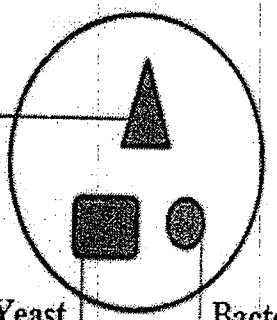
1- Put a small drop of water on the slide.
2- Take a loopfull from **one colony** from the Petri dish & mix it softly with the drop of water on the slide.
3- Fix the smear by heat (45° over the burner flame **not** through the flame) for three times.
4- Add drop from **Crystal Violet (1-1.5min)**.
5- Wash carefully with Tap water.
6- Add a drop of **Iodine** (Trapping agent) **(1min)**.
7- Add **Alcohol** (decolorizing agent) **(60sec)**.
8- Add **Safranin (1-1.5min)**.
9- Wash carefully with Tap water.
10- Dry the slide in the air at room temperature, or at the hot air of the burner flame **not** through the flame.
11- Find a clear field at 40X.
12- Move to the oil lenses (100X) after adding a **small drop** of oil on the slide.

Simple Stain for Yeasts

1- Put a small drop of water on the slide.
2- Take a loopfull from **one colony** from the Petri dish & mix it softly with the drop of water on the slide.
3- Fix the smear by heat (45° over the burner flame **not** through the flame) for three times.
4- Add drop from **Crystal Violet (1-1.5min)**.
5- Wash carefully with Tap water.
6- Dry the slide in the air at room temperature, or at the hot air of the burner flame **not** through the flame.
7- Find a clear field at 10X. & Examine at 40X.

Molds Slide Preparation

1- Place a drop of Lactophenol on a slide.
2- Dig the mold colony from the agar by loop.
3- Put it over the slide constantly **without breaking it**.
4- Put a cover slide over it.
5- Knock carefully at the left angle to spread the colony under the slide cover **without breaking it**.
6- Find a clear field under 10X. & Examine under 40X.



Molds

Yeast

Bacteria

Mold Slide

- Place a drop of Lactophenol on a slide.
- Dig the mold colony from the agar by loop.
- Put it over the slide.
- Put a cover slide over it.
- Knock at the left angle to spread the colony.
- Find a clear field under 10X.
- Examine under 40X.

Smear Preparation

- Drop of Water → loopfull of colony → Fixation by heat

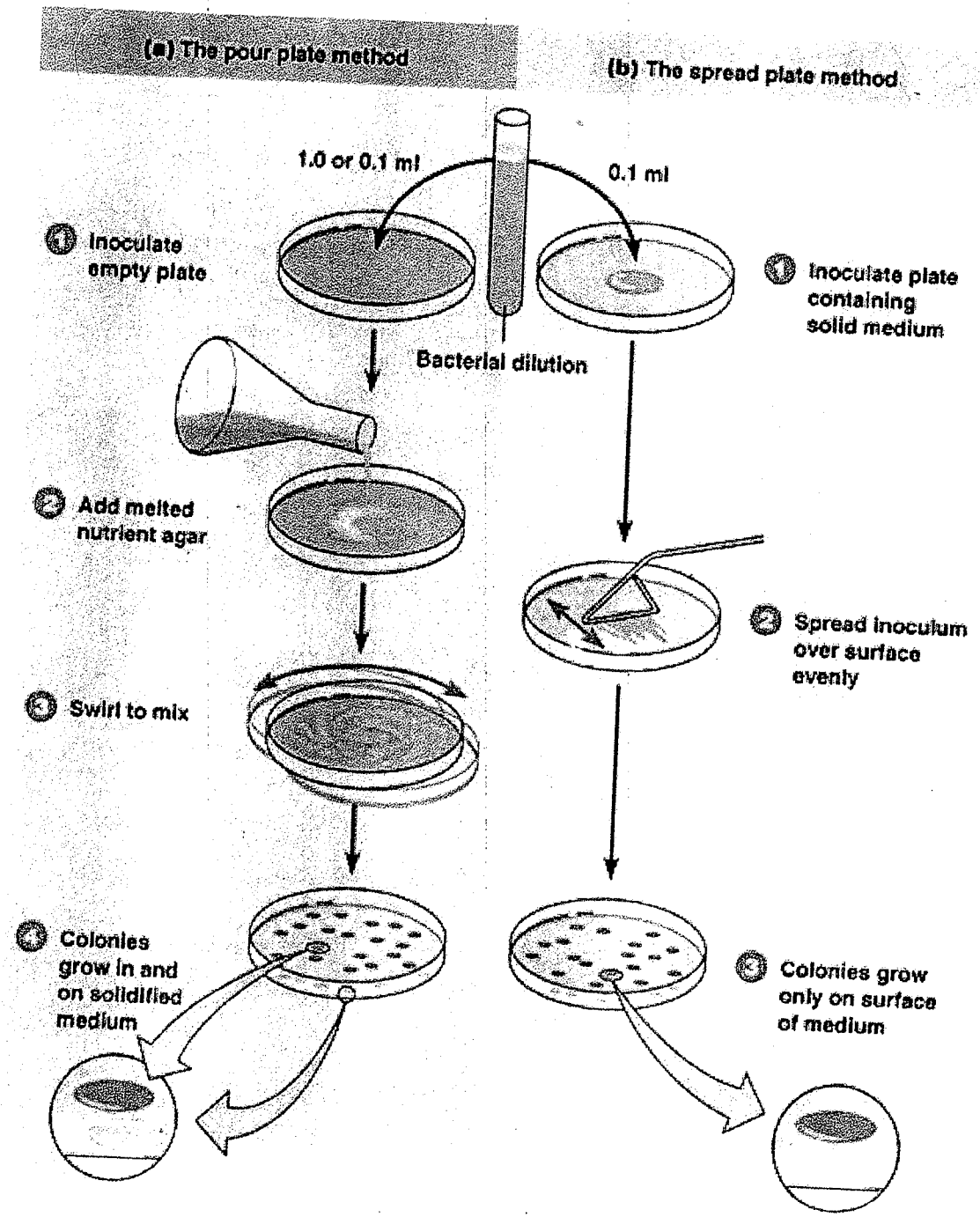
Staining

Yeast — Simple Stain

- Crystal Violet 1-1.5min
- Dry the smear in air.
- Find a clear field under 10X.
- Examine under 40X.

Bacteria — Gram Stain

- Crystal Violet 1-1.5min.
- Iodine 1min.
- Alcohol 60sec.
- Safranin 1-1.5min.
- Dry the smear in air.
- Find a clear field under 40X.
- Drop of oil.
- Examine under 100X.



Microorganisms in milk

In addition to nutritional value of the milk to human beings it is also considered as a typical media for the growth of many M.Os because it is rich in:

- Important proteins , carbohydrates , lipids , minerals & vitamins.
- Its optimal PH (6.7) & optimal moisture for microbial activity.

Sources of milk contamination:

- The milk contaminates by microbes during & after milking from (**external contaminations**) :
  Atmosphere of the farm , soil , water, air , cattle feces, insects ,files .
- From mechanical milking (milking machine , milk containers ,milk handlers . so should be all the tools , instruments & tubes clearing & sterilization .
- **Internal contaminations** :
  Several microbes can be transmitted to milk from animal itself such as *bacilli.*

Milk categories:

1- **Raw milk :** this is from healthy animal contain low number of bacteria, between 10^2-10^3 bacteria/ml
The required bacterial number that is does not cause the changes in color & taste .

2- thermized milk : this a raw milk that has been heated at 56 C° for 15 seconds.

3-pasteurized milk: the milk must be exposed to 72 C° for 15 sec or 63 C° for 30 min , to control the on spoilage , pathogenic bacteria & to prolong the storage period.

4-Boiling milk: Boil the milk to 100 C° for 5 mins.

5- sterilized milk: by using high temp. UHT treatment include very high temperatures (140-150 °C) for few seconds
Packed in a glass bottle , paper-based , or metal bottle, in this manner all microbes will be killed.

6- dried milk(powder) :

Made by the removal of water in milk with homogenization process & heat treatment to prevent spoilage.

If the examination of after treatment expose microbial growth this mean:

- The milk contamination by thermophilic bacterial spores.
- Or the milk insufficient heat treatment.
- Or the contamination happed because wrong procedure steps.

Lab work
Dye Reduction tests

The dyes most widely used for milk testing, Usually 1 ml of dye solution & 10 ml of milk are mixed in sterile-rubber stopper tubes & incubated at 37 C^o.

1-Methylene blue reduction test (MBRT)

This test is old but rapid & expensive. it indicates high or poor quality of milk .

Principle :

It depends on the reduction & decolonization of the dye by the metabolic activity of bacteria in milk & consumption of O_2 .the rate of reduction gives a measure of the degree of bacterial contamination.

Procedure:

1-carefully , pour 10 ml of the milk into test tube.

2-add 1 ml of the methylene blue solution ,the tube is closed & inverted & placed in a water bath at 37 C^o.

3- two control tubes should be done with each test tube

a- 10 ml of milk + 1 ml methylene blue solution ⟶ heated in water bath to 37 C^o for 30 min.

b-10 ml of milk + 1ml tap water.

After incubation, compare the test mixture with controls.

Reading result:

-complete de-colorization is +ve result with or without blue ring.

-colored milk with blue color is –ve result.

Note:

In untreated milk, The less time of the reduction of dye that mean increased contamination degree.

2- The Resazurin Test:

The reduction of resazurin takes place in two stages, first into blue and mauve then into the colorless.

Add 1 ml of resazorine + 10 ml of milk ⟶ examine after 10 min ⟶ Blue ⟶ pink ⟶ colorless.

3-phosphatase test :

This test is performed on pasteurized milk to determine if pasteurization has been successful or not.

Principle:

Test depends on the detection of phosphatase enzyme which is always present normally in raw milk. The enzyme is destroyed by the temperature in pasteurization .

If the phsophatase is not detected in the milk that mean the milk successfully pasteurized . if detected that mean insufficient heating or the raw milk is contamination .

In this test, add buffer substrate (nitrophenyl phosphate) which is colorless is hydrolyzed but by milk phosphatase convert to nitrophenol that is yellow .

Turbidity test

It is used to distinguish sterilized milk from untreated or sterilization process is effective or not.

Principle:

Milk that has been heated to 100 C° has had all the heat-coagulable protein denatured . if the milk not been exposed to boiled, the protein not denatured . Addition the ammonium sulphate will be detected the coagulation & turbidity of the its protein .

Procedure:

1- four gm ammonium sulphate are added to flask.
2- 20 ml of the milk are added ,shaken for 1 min & left to 5 min.
3- the mixture is filtrated by Whatman filter paper.
4- examine for turbidity.

Result:

Absence of turbidity indicates that the milk has been heated to at least 100 C°, the milk exposed to satisfactory sterilized.

Tests

1-Raw milk

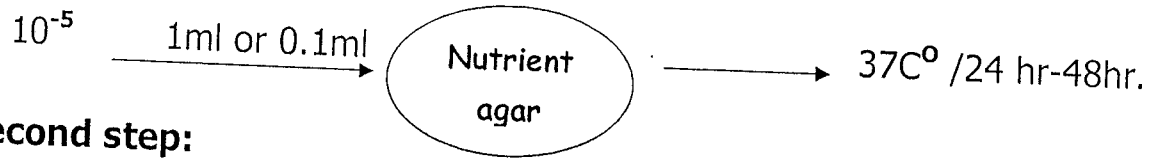
Remember} when we expect Highly contamination in the sample used No. dilution 10^{-x}.

But when expect the sample has low contamination used No. dilution 10^{-2} or 10^{-3} .

Range of dilution 10^{-1}-----→ 10^{-5}

First step:

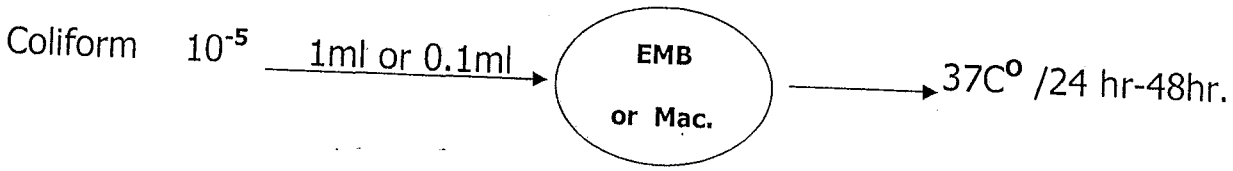
Used nutrient agar for general growth (aerobic plate count):



Second step:

used Selective media for bacteria that expected it contaminate of the sample.

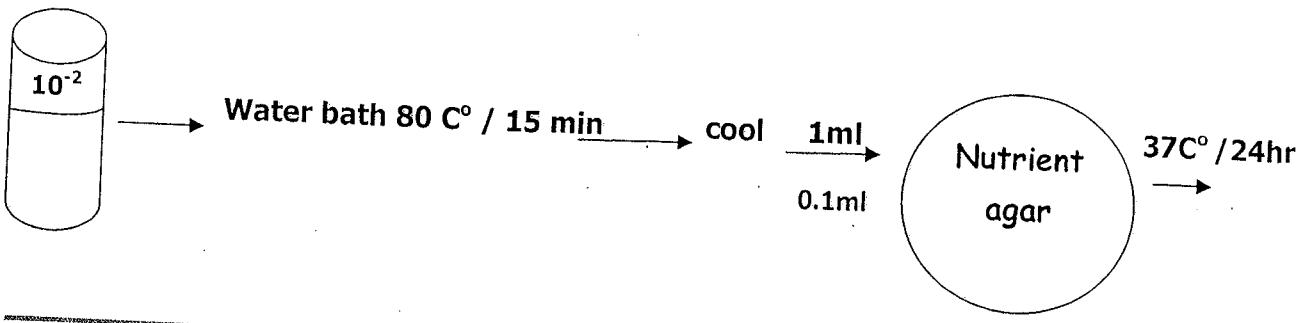
For the detection of coliform bacteria used:



*EMB= Eosin Methylene Blue

*Mac= macconkey agar

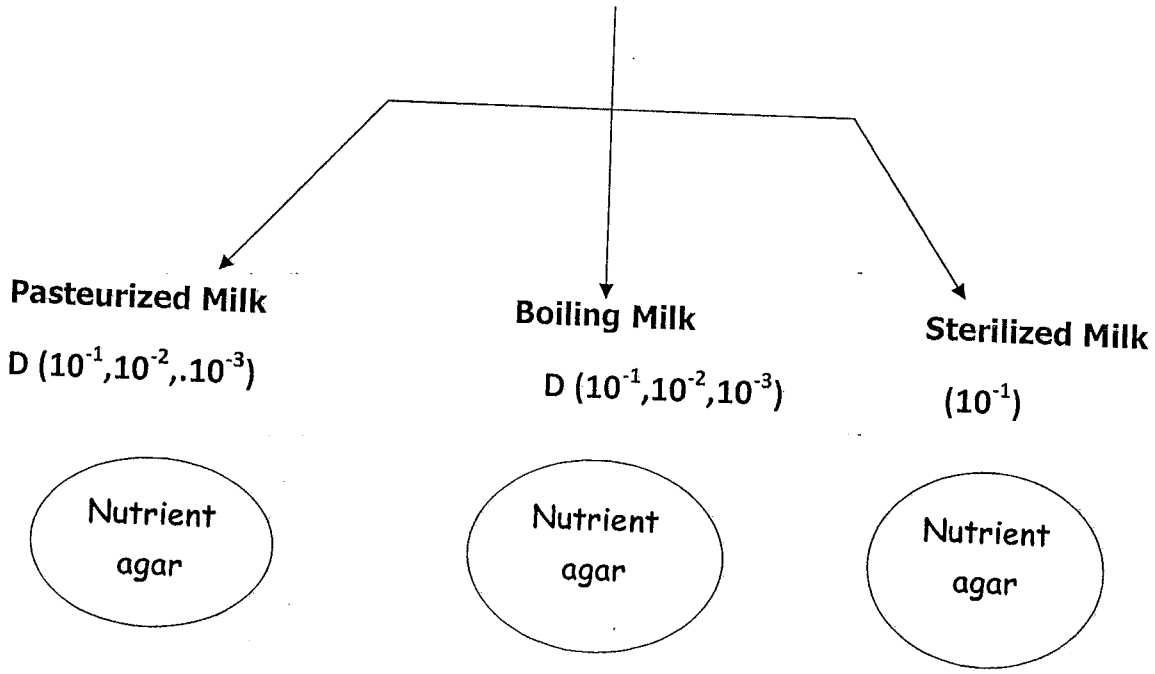
For the detection of spore former used:



By pour method(1ml) or spread method(0.1ml)

Incubated the plates at 37C°/ 24hr-48hr

In an inverted position



Third step:

Preparation slide from colonies that appeared.

Microorganisms In Dairy Products

I. **Cheeses.**
II. **Yoghurt.**
III. **Lipid dairy product.**

***Cheeses :-** is the hard product of milk. it is produced by the addition of lactic –acid bacteria as a starter or the addition enzymes or acids followed by processes to give the texture & flavor of chesses.

Classification of cheese :

Soft: moisture content 40-80%
Semi-soft :moisture content 30-40%
Hard: moisture content 30%.

Spoilage of cheese

Depend on:
1-Type or kind of chesses.
2-The moisture content.
3-Tempreture.
4-Period of storage.

The most important genera of bacteria which contaminate cheese:

-*Coliforms*: from animal or human ⟹ causes acids & gases.
-*Streptococcus Lactis* (lactic) ⟹ causes sour flavor.
-*Bacillus&Clostridium* ⟹ causes lipolyzation&proteolyzation of cheese.
-*Pseudomonas & Proteus* ⟹ causes foul odor & slime.
-Cladosporium ⟹ causes black or green color.

Procedure:

Aseptically ,taken 10 gm from various parts from chesses & put into sterile container , transfer the contents into sterile mortar.

Mix with 90 ml diluted solution and shaken several times by mortar to obtain a 10^{-1} .the mixture is left for 3-5 min just before making serial dilution.

Laboratory tests

*White cheese

1- General microbes (aerobic plate count)

10^{-3} → 1 ml (pour)/ 0.1 (spread) → Nutrient agar → 37C° /24hr

2- Detectoon Of The Coliform

10^{-1} → 1ml (pour) / 0.1 (spread) → E.M.B macConkey agar → incubation → 37C° /24hr

*E.M.B= Eosin methylene blue

3-Protelytic Bacteria :

10^{-2} → 1ml (pour) / 0.1 (spread) → Casein agar → incubation → 37C° /24hr

4-Mold & Yeast

10^{-1} → 1ml (pour) / 0.1 (spread) → Yeast extract agar → incubation → 25-28C° /48hr

***Yoghurt(fermented milks):** by lactic-acid bacteria the fresh or dried milk convert to yoghurt ,sufficient & equal number of (*Lactobacillus Bulgaricas & Streptococcus Thermophilus).*
They are added after sterilizing & cooling milk, incubate at 45-48C° thickening of the milk & it has atypical sour flavor .

The most important genera of bacteria which contaminate yoghurt:
Colifrom , mold & yeast.

Laboratory tests

*Yoghurt

1- General growth (aerobic plate count):

10^{-4} → 1 ml (pour)/ 0.1 (spread) → Nutrient agar → **37C° /24hr**

2-Mold & Yeast

10^{-2} → 1ml (pour) / 0.1 (spread) → Yeast extract agar → incubation → **25-28C° /48hr**

3- Coliform

10^{-2} → 1ml (pour) / 0.1 (spread) → E.M.B → incubation → **37C° /24hr**

*E.M.B= Eosin methylene blue

***Lipid Dairy Product**

A) Butter: Butter is less spoiled by M.Os because the butter contents of the high concentration lipid . The spoilage occur a result to moisture .

➢ M.Os that causes spoiled to butter , its capable of growing at low temperatures are called psychrophilic or its capable of lipolysis & proteolysis *(pseudomonas).*

Laboratory tests

***Butter**

1- General growth (aerobic plate count):

10^{-3} → 1 ml (pour)/ 0.1 (spread) → Nutrient agar → incubation → **37C° /24hr**

2-Mold & Yeast

10^{-1} → 1ml (pour) / 0.1 (spread) → Yeast extract agar → incubation → **25-28C° /48hr**

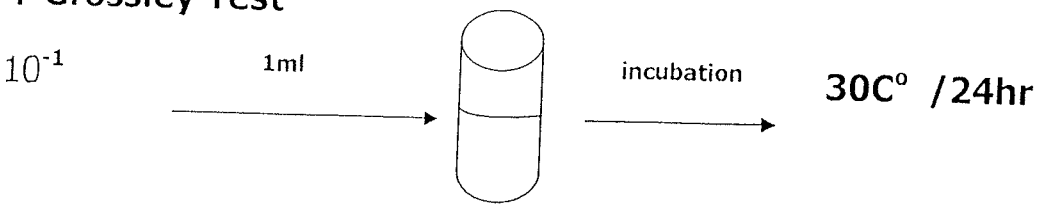
3- Detect Of the Lipolytic Bacteria

10^{-2} → 1ml (pour) / 0.1 (spread) → B.F.A → incubation → **30C° /24hr**

*B.F.A=butter fat agar

Note : After incubation, covered the plate with a solution of copper sulfate $CuSO_4$ for 5min a period. Then the lipolytic bacteria appear with a halo Bluish green surround of the colonies .

4-Grossley Test



Grossly medium

The reagent (bromocresol purple)

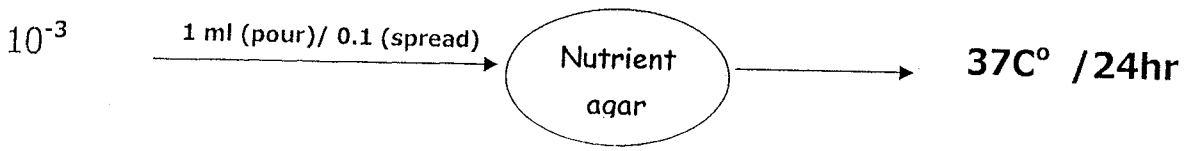
➢ **Note:** If the presence of microbial contamination in sample, after incubation of the tubes ⟶ observe the production of acetic acid which changes the medium from the violet to yellow.

B)cream: sterilize the milk &cool it, the lipid layer will appear on the surface of the milk, thickness of lipid layer depends on the lipid contents .
Spoilage may occur because presents M.Os in the original milk.

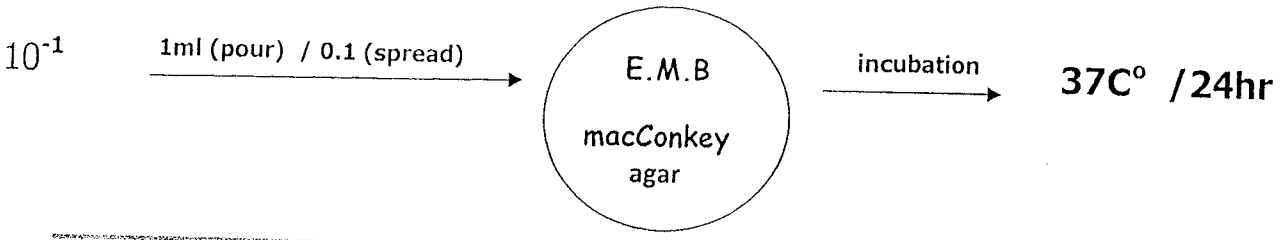
Laboratory tests

*cream

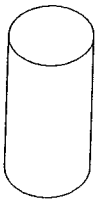
1- General growth (aerobic plate count)



2- Detection Of The Coliform



3-Grossley test :

10^{-1} → 1ml →  → incubation → **30C° /24hr**

Grossly medium

The reagent (bromocresol purple)

4-mold & yeast

10^{-1} → 1ml (pour) / 0.1 (spread) → Yeast extract agar → incubation → **25-28C° /48hr**

c) Ice cream :

1- General growth (aerobic plate count)

10^{-3} → 1 ml (pour)/ 0.1 (spread) → Nutrient agar → incubation → **37C° /24hr**

2- Detection Of The Coliform

10^{-1} → 1ml (pour) / 0.1 (spread) → E.M.B macConkey agar → incubation → **37C° /24hr**

Microorganisms in red meat ,chicken ,fish &egg

Meat is considered as an excellent growth media for a variety of M.Os due to many factors make it suitable for microbial growth & reproduction ,such as :

1-elevated moisture .

2-presence of (CHO & nitrogen) compounds.

3-minerals.

4-approprite PH for growth the M.Os.

- The meat & its products contains microbial flora on its surface.
- M.Os inside the meat come from many different sources.
- Muscles of the animal contains few of M.Os than surface but it increase after or during slaughter the animals.

Important microbes that contaminated of meat:

Bacteria ⟹ *salmonella ,staphylococci , streptococci, micrococcus, pseudomonas , lactobacilli & proteus.*

Molds ⟹ **mucor , rhizopus, cladosporium.**

Fish meat

It is spoiled faster than red meat because of:

1) high moisture.

2)high PH.

3) lipids in fish oxidize faster than lipid in red meat.

4) the tissues of fish are softer & more disassemble .

Important microbes that contaminated of fish:

Pseudomonas, Vibrio, E. Coli, Lactobacilli, Salmonella, Clostridium.

Chicken

M.Os in chickens include:

G+ staphylococci, streptococci, lactobacillus, clostridium.

G- E.coli, pseudomonas, salmonella.

Eggs

The eggs represents a perfect media for microbial growth because its contents of proteins ,lipids& vitamins .

Note:- the sample taken wiping by swab from solid shell or biopsy of the liquid (albumen).

Important microbes that contaminated of egg

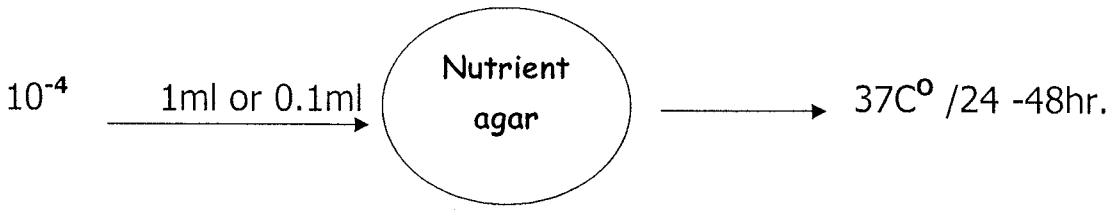
Spoilage of egg shell by colored on the shell.	*Salmonella*	*penicillium, cladosporium*
Spoilage of egg contents.	*Pseudomonas* Green putridity	*proteus* black putridity with Dislike smell .

Lab work

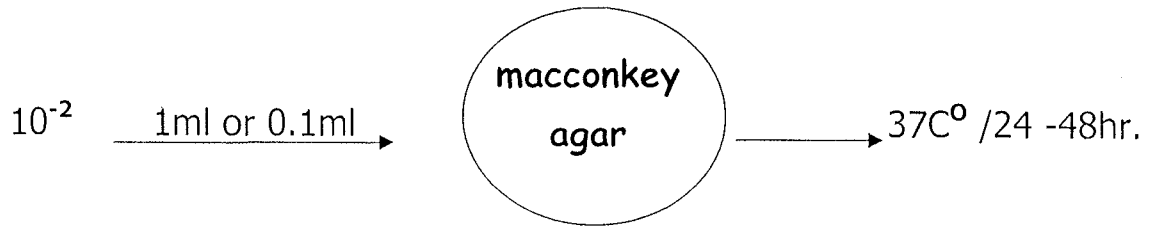
Procedure :

Samples taken from all parts of meat ⟶ mash to get a homogeneous mixture of these samples then taken 10 grams of this homogeneous mixture are transferred into sterile container, added to him dilution solution (90 ml of D.W + 1% peptone) to get the emulsion by mortar to give us a 10^{-1} .the mixture is left for 3-5 min just before making other dilution.

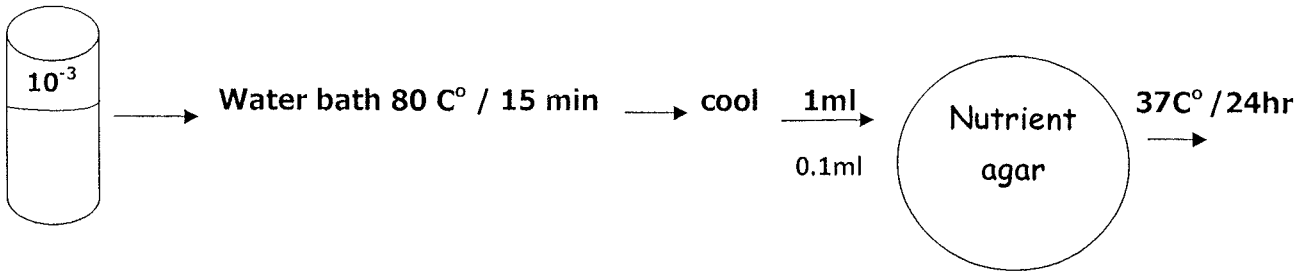
Nutrient Agar For General Growth (Aerobic Plate Count):



For the Coliform Bacteria Used:



For the spore former used:



For the Lipolytic Bacteria:

10^{-2} 1ml (pour) / 0.1 (spread) → Butter fat agar → incubation → **37C° /24hr**

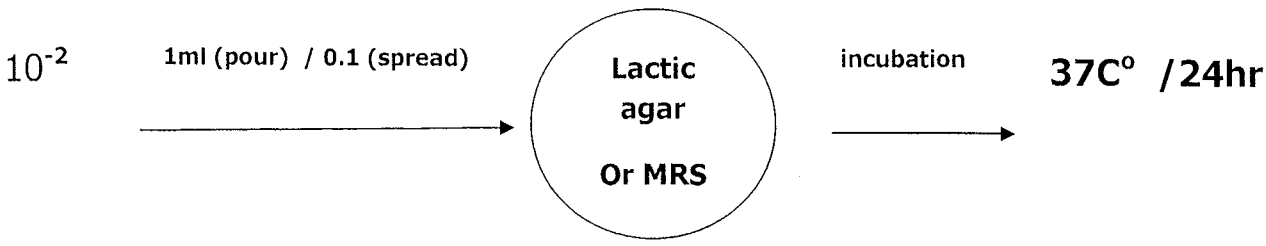
For The Proteolytic Bacteria

10^{-1} 1ml (pour) / 0.1 (spread) → Casein agar → incubation → **37C° /24hr**

For The Staphylococcus Bacteria :

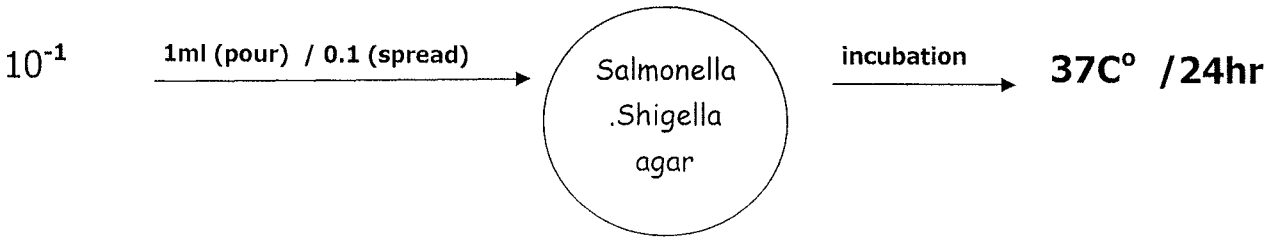
10^{-3} 1ml (pour) / 0.1 (spread) → Staph 110 agar → incubation → **37C° /24hr**

For the Lactobacilli :

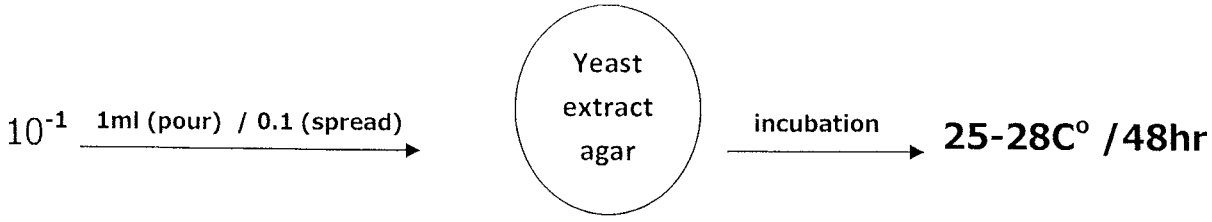


MRS= De Man, Rogosa and Sharpe

For The Salmonella Bacteria :



Mold & Yeast



Microorganisms in fruits & vegetables

Some bacteria & molds may attack of fruits & vegetables during the growth of the plant, harvesting time ,transport ,storage & cause spoilage .

- If the M.Os , can penetrate the fruit or vegetables from outer layer (natural pores on its surface)---→ can grow quickly ,reproduce & spoiled the plants.
- M.Os can enter from water,soil,air.

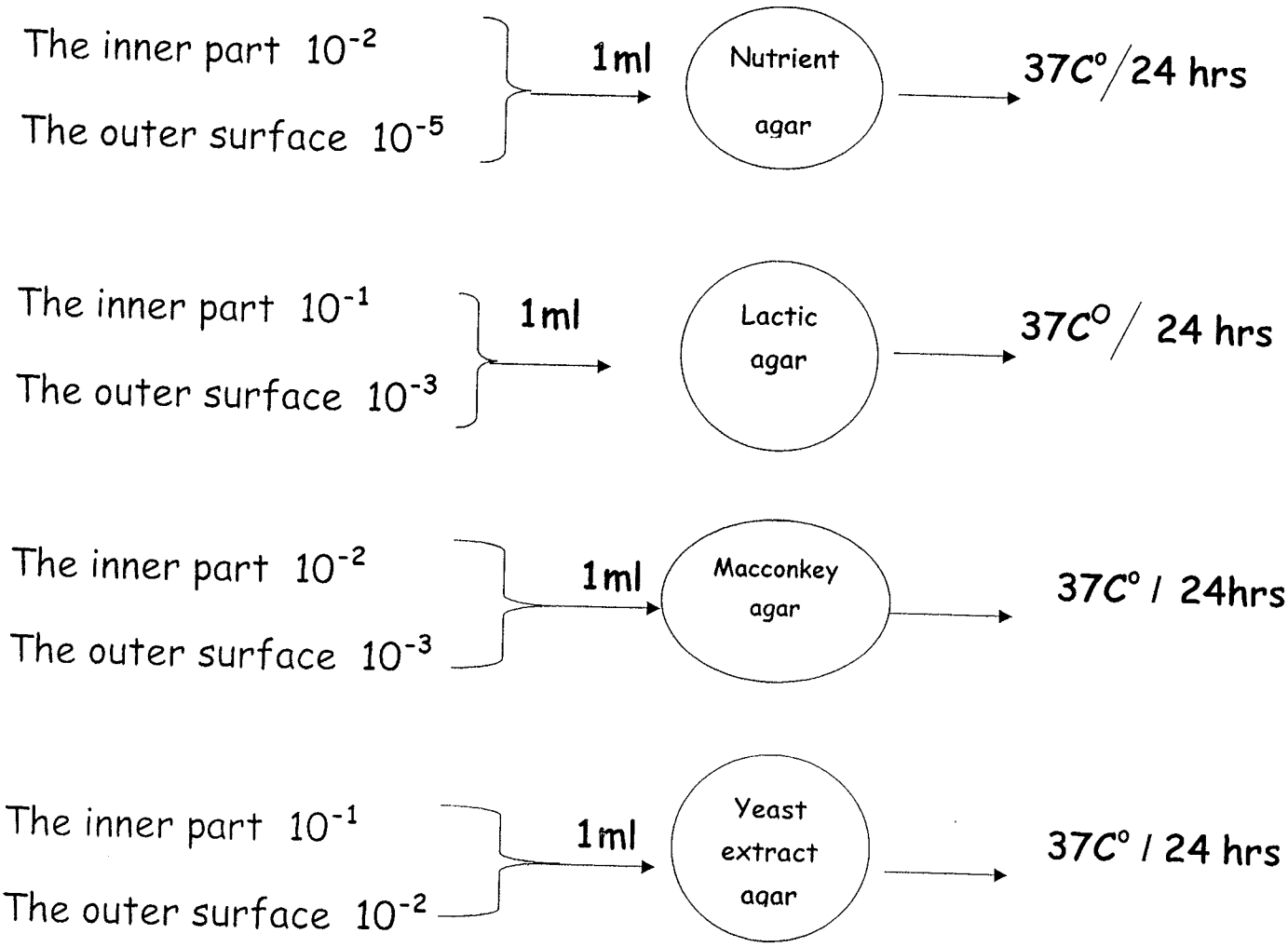
The important characters of spoilage by M.Os

Microbial Causative Agent	Nature Of Spoilage
Rhizopus	cottony growth+ black spots.
Erwinia	watery & bad odder .
Coliforms, Lactobacillus	vegetable souring.
Penicillium	Bluish-green coloration.
Aspergillus niger	Black growth

Lab work

10 gm from Internal parts or external layer are transferred into sterile container, mash the contents with 90 ml of D.W to get the emulsion by mortar to give us a 10^{-1} .the mixture is left for 3-5 min just before making other dilution.

General medium(aerobic plate count)



Microoganisms in bread & cereal

The cereal include: rice, corn, wheat &starch.

*Sources of microbial contamination:

Water ,air ,soil ,birds ,rodents &insets.

*there are 2 factors which control on microbial growth & reproduction in cereal grains:

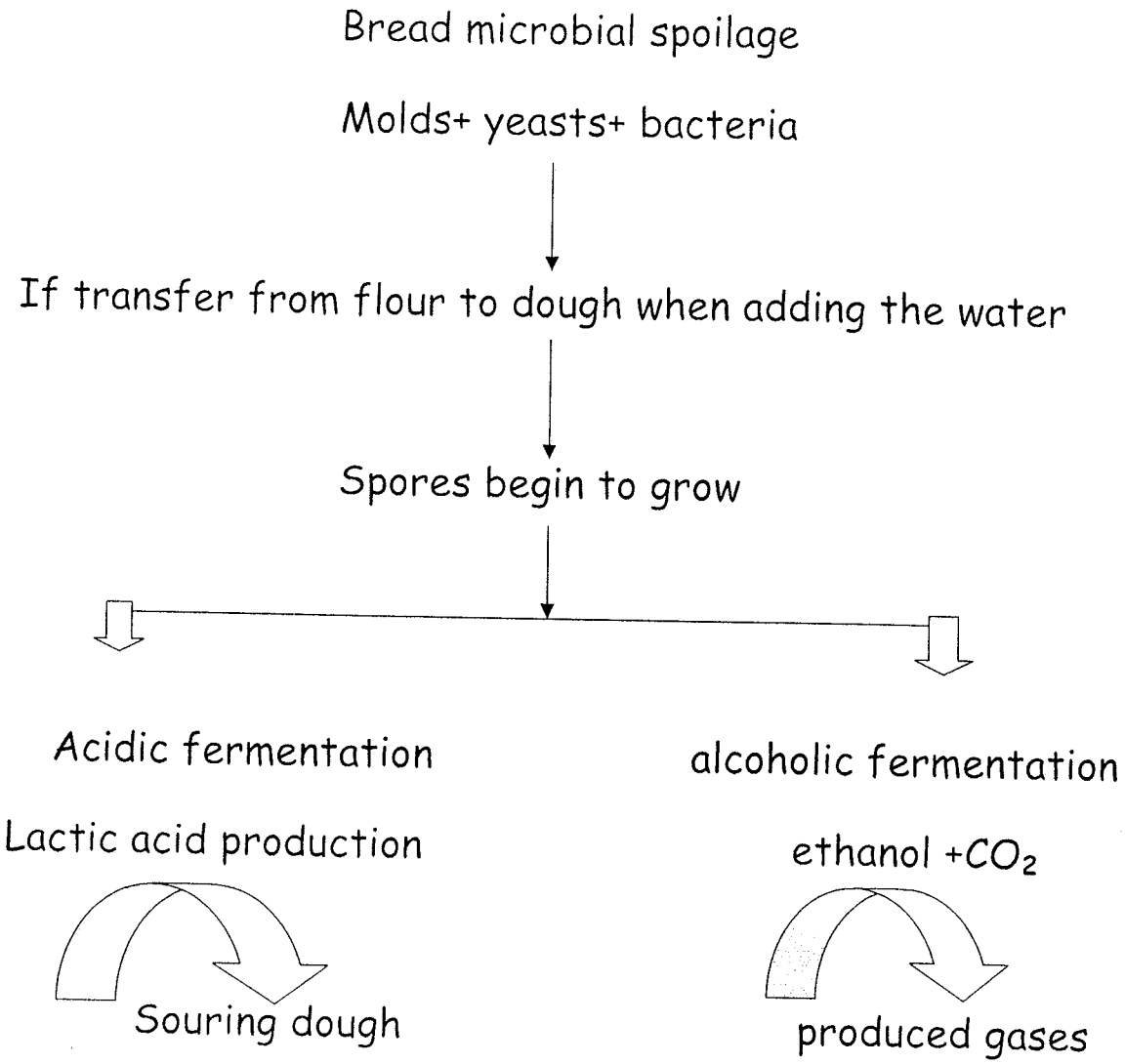
1)moisture

2)storage temp.

*the toxin producers of fungi when the cereal grains stored in a moisture place.

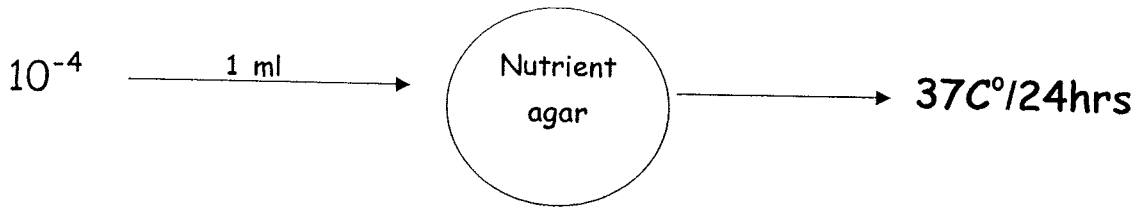
The most important microbes which spoilage :

Mold	character of spoilage
Penicillium	green growth
Mucor	white growth
Aspergillus niger	black growth
Rhizopus	white growth with black spots

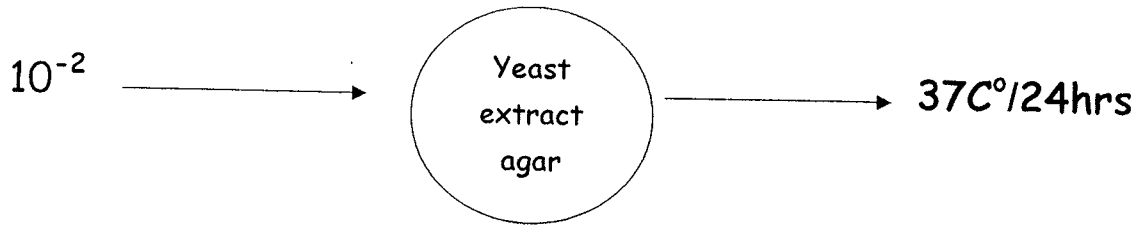

Bread microbial spoilage
Molds+ yeasts+ bacteria
If transfer from flour to dough when adding the water
Spores begin to grow
Acidic fermentation
Lactic acid production
Souring dough
alcoholic fermentation
ethanol +CO_2
produced gases

LABWork

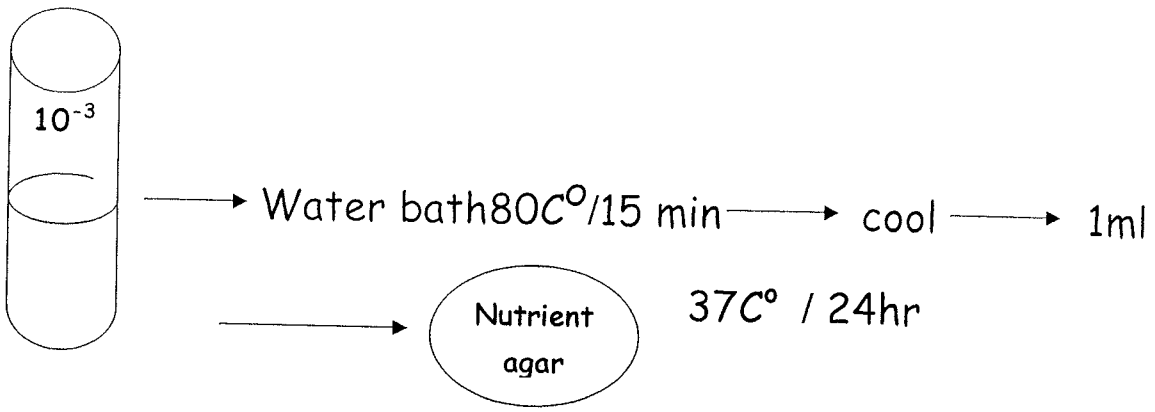
General microbes (aerobic plate count)



To detection Of the mold & yeast



To detection Of bacteria spore- forming



Microorganisms in sugary foods & pickles

1. sugary foods :

- Only Osmophilic M.Os can tolerant & growth on high sugar foods such as: Honey ,Debbis, Jams, chocolate & cake.

*Honey:

M.Os that responsible for honey spoilage are:

-Osmophilic yeasts: *Saccharomyces cerevisiae, Saccharomyces rouxii .*

-Osmophilic Molds: *Aspergillus, penecillum & mucor.*

- bacterial spores.

- Most microbes cannot spoiled the food because contain a high percentage of sugar (70-80)%.

*Debbis:

Produced from dates , the sugar concentration reach to 90%.

Example of M.O. that spoil the debbis is *Saccharomyces rouxii* ⟶ forming gases , alcohols & acids that change the taste.

*Jams:

More susceptible to contamination in sugary foods because made from different kinds of fruits that may be spoiled.

*Cake & chocolate :

Contaminated with spores of bacteria & fungal toxins from milk or nuts .

LABWORK

- To isolate osmophilic M.Os ⟶ Nutrient agar +20%sucrose.
- For sugary food used the diluents solution containing 15-20% sugar .

1- To isolate general M.Os use:

10^{-1} → 1ml(pour) or 0.1 ml(spread) → Nutrient agar+20% sucrose → incubation for → 24-48hr/ 37 C°

2- To isolate mold or yeast use:

10^{-1} → 1ml or 0.1 ml → Malt agar Or yeast extract agar → incubation for → 24-48hr/ 28 C°

2- Pickles :

- Pickles are made by lactic acid bacteria through fermentation process.
  - Vegetable chapped into small pieces with 5-15% of NaCl.
- Lactobacillus sp. (the selective media MRS agar or broth) ⟶ Responsible for all stages of fermentation.

Pickles spoilage:

Spoil by different M.Os includes the:

1. Yeasts

such as ***Candida*** ⟶ cause oxidize the lactic acid to CO2& H2O.

or ***toruiopsis*** ⟶ cause large amounts of gases.

2. Bacteria

Such as: ***Leuconostoc*** is a genus of Gram-positive bacteria,. They are generally ovoid cocci often forming chains. Cause slimy pickles.

Or ***bacillus sp.*** ⟶ Cause black pickle by H2H production.

3. molds

Such as: *penicillium & cladosporium* ⟶ soft pickles.

LABWORK

- To isolate Halophilic M.Os ⟶ nutrient agar +20%NaCl.
- For salted food used the diluents solution containing 15-20% NaCl .

1-To isolate LAB(lactic acid bacteria) ⟶ MRS agar

*MRS Agar= de Man Rogosa Sharpe agar.

2- To isolate Halophilic bacteria :

10^{-1} ⟶ Staph 110 ⟶ incubated for ⟶ 37 c° / 24-48hrs

3-To isolate molds :

10^{-1} ⟶ Malt agar or Y.E.A ⟶ incubated for ⟶ 28 c° / 24-48hrs

Soft drink (non carbonated soft drink)

Includes:

a) fruit juice
b) concentrated
c) sweeten juice
d) artificial juice

Microorganisms found in this products:

a) Lactobacillus spp. & Leuconostic
b) Acidic acid bacteria
c) Mold & yeast

carbonated soft drink :consist of

86-92 % water,7-14 nutritive sweetness CO2 as (carboic acid) & flovering, alcohol not more than (0,5%) , may contain benzoic acid 0.1%

Content & factor affecting type of microorganisms :

i. Water
ii. PH
iii. CO2
iv. Acids

Microorganisms found in carbonated soft drink

a) Yeast

Saccharomysis, pichia

b) Mesophilic acid bacteria

Lactobacillus spp. & Leuconostic

c) Mold :Aspergillus

Procedure :

diluent juice

Concentrated juice 10^{-2} ⟹ 1ml or 0.1ml ⟹ pcA ⟹ 3 days /37c

carbonated soft drink 10-3

pcA = plate count agar (N.A.+ 0.1 glucose + 0.5 % trypton)

Acidity test:

A. : diluent juice

1 ml or 0.1ml

Concentrated juice 10^{-2} ⇒ Acidic medium ⇒ 5 days/37c

carbonated soft drink 10^{-2}

Acidic medium: Tween 80 (0.02%)+ yeast glucose agar + Bromocresol purpul (0,004%)

B. Mold & yeast

C. : diluent juice

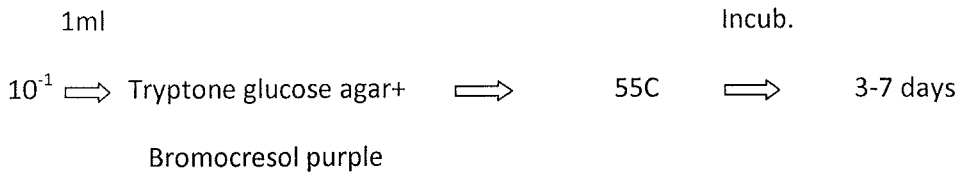
1 ml or 0.1ml

Concentrated juice 10^{-1} ⇒ YEA ⇒ 5 -7days /20-25c

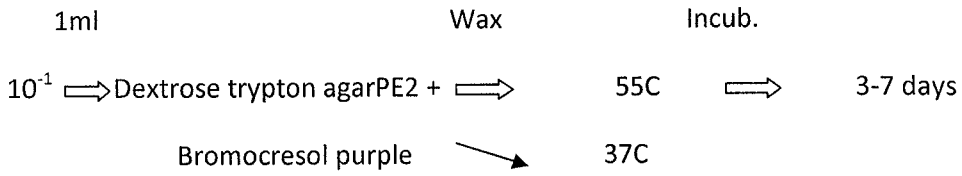
carbonated soft drink 10^{-2}

Schematic diagrame of culture procedure for low –acid canned food & high acid canned food

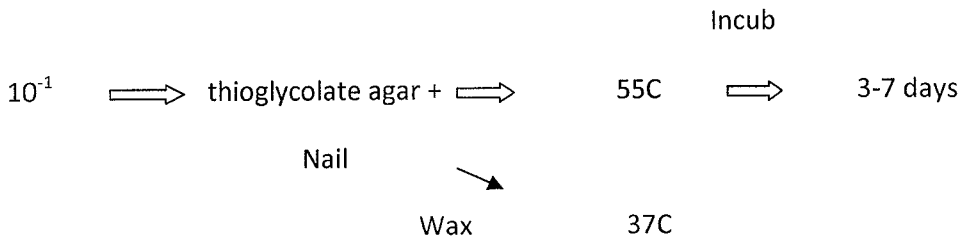
Flat sour spoilage.



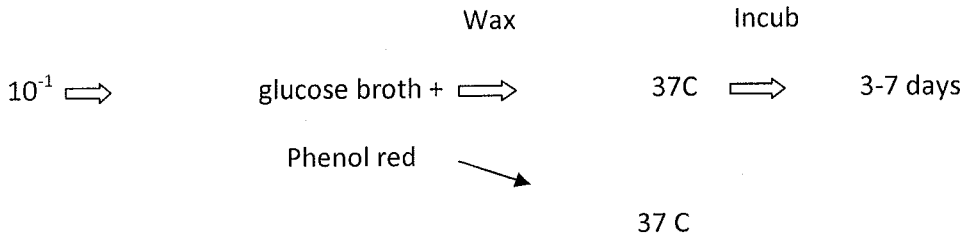
Thermophilic anaerobic spoilage.



Sulphid spoilage.



Swell spoilage.



Butyric anaerobic

10^{-1} ⟹ Dextrose trypton agarPE2 ⟹ 37C ⟹ 3-7 days

Aciduric thermophilic

10^{-1} ⟹ tryptone glucose agar ⟹ 55C ⟹ 2 days

Bromocresol purple pH=5 +

Yeast

10^{-1} ⟹YEA + Bromocresol PH =5 ⟹ 30-25C ⟹ 3-7 days

Mold

10^{-1} ⟹ water bath 80C/15min ⟹ cool ⟹ YEA

25-30/3-7days

Spoilage micro. That cause low &high acidity in various vegetable & fruit

Spoilage type	pH	example	Group of micro.	Manifestation
Thermophlic Flat sour	5.3≥	Corn,peas	Bacillus sterothermophilum	Can flat, product appearance may have slightly abnormal odor , sometimes cloudy liquor
Thermophilic anaerobic sp.	4.8 ≥	Spinach, corn	Clostridium saccharolyticum	Can swell, product appearance fermented ,sour, cheesy odor
Sulphid sp.	5.3≥	Corn,peas	Clostridium nigrificanse	Can flat , H2Sgas absorbed by product
Mesophilic **Swell sp.**	4.8≥	Corn	Clostridium botulinum	Can swell ,product may be partially digested
Butyric anaerobic	4.o≥	Tomatos ,peas	Clostridium pasturanium	Can swell ,product fermented butyric odor
Aciduric thermophilic	4.2≥	Tomato juice	Bacillus coagulans	
Yeast Mold	3.7≤ 3.7≤	fruits	Saccharomyces Byssochlamys fulva	