

Jan 26

- ⇒ is the bacteria found in different yogurts more beneficial than others for gut health? Is there anything harmful in yogurts?
- ⇒ what are the different bacteria in these yogurts?
- ⇒ contamination?? what kinds of bacteria grow in yogurt if contaminated (eg; hands, mouth, different spoons) and are these bacteria harmful?
- ⇒ relate fermentation?

*ethics form

* Division:

• Life + Health Sciences

⇒ entries@cysf.org

* Topics

• Food science

• Gastroenterology

• Genetics/
Molecular
Microbial
biology

"Probiotic Pathways: Dairy Cultures and the Saliva Effect"

Jan. 27

Purpose: to determine which of 3 dairy products contains more beneficial bacteria (Bifidobacteria) and probiotics (Lactobacillus) for gastrointestinal health and examine the effects of (harmful and non) bacteria growth in these products as a result of contamination

How to conduct experiment? (in progress): Using cotton swabs, take samples of 3 dairy products and grow bacteria samples in agar plates (a type of petri dish). Let sit for 6 days, then take record of growth. Classify the organisms grown based off of books and bacteria guides. Do the same, but mix a contaminated spoon into the samples before swabbing them. (Lick spoon, place it on various surfaces).

(credentials)

⇒ write organized RESEARCH, hypothesis, procedure, controlled/manipulated/responding variables, materials, and finally results + conclusion and applications for improving project

Cont. of Sources

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- * Knibbs, Jessica. "What is step B, what are the symptoms of the bacterial infection and how does it differ from step A?" The Standard, 6 December 2022, standard.co.uk
- * DC. Shanson. Microbiology in Clinical Practice, 2nd Edition, Chapter 16, 1989. ScienceDirect, sciencedirect.com

Feb. 9th

● Testable Question(s): Which of 3 dairy products contains more bacteria and probiotics that are beneficial to gastro-intestinal health? Which products are "healthiest," and in what order? (i) Which?
AND... What type of bacterial growth occurs in these products once contaminated with my saliva? (ii) At what rate, and how much of the bacteria from saliva will grow? (iii)

Words highlighted in this color are ^{important} vocab/terminology
→ (make a vocab list)

Why this topic?

Gut health is something that really interests me; I've been growing an interest in nutrition and the relationship between food and your body. I want to do medicine, so why not do something about biology and food? I believe health begins with what you eat, so I want to do this experiment in order to find out how a certain class of foods affects your stomach's health.

Here is a list of the sources that guided my research:

- *Tay, Andy. "The Science of Fermentation". Lab Manager, 18 July, 2019, labmanager.com
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- * WebMD, "What are Kefir and Its Health Benefits?" webmd.com

Gram-Positive Bacteria

- Fun fact: "daege" (female servant) became "dairy"
- * thick peptidoglycan layer in cell walls so retain violet dye better (turn purple when stained) → holds onto stain strongly
 - * Simpler cell wall
 - * less resistant to antibiotics, but can still be problematic (can cause necrotizing fasciitis, sepsis, toxic shock syndrome, etc.) (easier to treat)

Gram-Negative Bacteria

- * thinner peptidoglycan layer
- * outer membrane makes cell wall more complex
 - ↳ this membrane doesn't allow violet dye to bind, and thin peptidoglycan layer is not thick enough to trap dye. Appears PINK when stained.
- * outer membrane makes these bacteria more resistant to antibiotics. (more dangerous, harder to treat)

Feb. 9 {Research}

To match a hypothesis, I researched the differences between these three dairy products and related evidence.

Terminology: polyphenols: ^{organic compounds coming from plants that have} more than one hydroxyl group that are antioxidants

fermentation: carbohydrates/glucose are broken down by the enzymes of microorganisms into gasses, acids, and alcohol. ^(ethanol) (Anaerobic respiration)

anaerobically: without oxygen. Fermentation occurs when oxygen is not present.

enzymes: proteins that speed up metabolism and break down food (catalysts)

lactose: the sugar found in milk products bacterial phyla: major taxonomic groups of bacteria, distinct genetic features

Kefir grains: symbiotic colonies of yeast + bacteria that convert milk to kefir

* Kefir is classified as the milk product with LARGEST amount of probiotics due to several factors

⇒ it is fermented with a larger variety of bacteria $\frac{1}{3}$, yeast

(more species)

⇒ LONGER fermentation time allows for more growth of probiotics

⇒ yeasts in kefir protect bacteria, so more survive

* types of ^{microorgs.} ~~bacteria~~ proven to be beneficial to gut health are...

• Bacteroidetes: found in the gastrointestinal tract, metabolize + increase nutrition. Digest fiber and polyphenols (natural substances found in plants) → gram negative (extract more energy from food)

• Firmicutes: similar functions. However they make more efficient use of calories (less) (more)
so having an imbalance between bacteroidetes/firmicutes may lead to metabolic disorders, obesity, diabetes, bowel disease, and inflammation → gram positive

• Lactobacillus acidophilus: gram positive, digestive system + mouth (a firmicute)

• Bifidobacteria: gram-positive, GI tract

Most common in kefir. ^{- dairy product.}

• Actinomyces: gram-positive, found in respiratory system, digestive, skin, and mouth
some species can be

• Pseudomonadota: can be beneficial, ^(infectious) GI tract, gram-negative

- **Saccharomyces**: a genus of yeast (not bacteria). Gram-positive. Found in fermented foods (fruits, kefir, sourdough, etc.).
- * **related conditions** due to imbalances of gut bacteria

Found an imbalance between types of organisms

• **dysbiosis**: dietary changes, intake of antibiotics and stress can result in dysbiosis. It can lead to IBD, cancer, obesity, etc.

• bacterial gastroenteritis → bacteria infection results in inflammation

• Inflammatory bowel disease (IBD) → over-activated healthy gut microbes/cells

• colorectal cancer → tumour in colon grows, inactivity, etc. irritable bowel

Yeasts are not bacteria bc.

↓ eukaryotic	↓ prokaryotic
• complex	• simple
• reproduce sexually	• cannot
• larger	• smaller
• Fungi kingdom	• Bacteria kingdom

Feb. 13

Before I continue with my research want to find an answer to:

- What risks are humans posed by probiotics in the body? (Why are they safe?)
- How much/what type of foods will benefit us the most?
- testable question again (which of the 3, and why?)
- ↳ to form hypothesis
- We find a winner of the 3 dairy products, so why are the other 2 still important? Do they have their own features that are good for our health?

Continuation of Research...

* **F/B ratio** → as mentioned, they are most dominant bacteria in gut

- provide energy - fermentation of carbs/fiber creates SFCAs (energy)
- however, little ATP. Energy is from SFCAs, used by cells lining GI tract
- high F/B ratio ~~may~~ results in several conditions eg: obesity

while low F/B ratio can lead to IBD.

→ this ratio is based on testosterone + estrogen, diet, activity, medication

→ cause ABOVE mentioned conditions, as well as diabetes, CVD, mental health decay etc.

↳ Cardiovascular disease

SFCAs: group of fatty acids produced when bacteria in GI tract ferment.

They are absorbed by **colonocytes**, which line the large intestine.

↓

cells that make up the **epithelium** → thin layer of cells that line hollow organs.

Then, enter bloodstream. →

Protein building, energy, immune system.

The way to prevent these conditions is to EAT HEALTHY, focusing on 3 dairy products.

Kefir ⊕ promotes bowel movement + digestion, nutrients, LOTS of probiotics, bone

health, etc. Vitamins A, B, C, K, D, proteins, calcium

⊖ bloating, gas, cramps, diarrhea, constipation but goes away eventually
- Contains small amounts of alcohol bc of long fermentation period

⊕ has been linked to controlling blood sugar levels, cholesterol, weight loss, lactose

intolerance

*if LPL is overly active it could lead to excessive fat storage

Milk ⊕ Calcium, Vitamin D, B12, protein, zinc, etc.

⊖ contains fat that may be harmful if consumed in large amounts and cholesterol (waxy substance found in blood to build cells, too much develops fatty deposits in blood vessels) → Clog arteries, and lipoprotein lipase enzyme breaks down

⊖ no probiotics in moderate amounts, very beneficial to GI health

triglycerides (lipids/fats) which are stored as fat into energy (absorbed by intestines)

Weight Loss Study From European Journal of Nutrition

*Fathi, Yasmeen. "Kefir drink leads to a similar weight loss, compared with milk, in a dairy-rich, non-energy restricted diet in overweight or obese premenopausal women: a randomized controlled trial." European Journal of Nutrition, 5 Feb. 2015, link.springer.com

⇒ In this trial, women were divided into 3 groups. Control group received 2 servings a day of low-fat dairy products, women in milk group received 4 servings of milk a day, and Kefir group received 4 servings of kefir/day

The result: Milk and kefir groups had lost a significantly greater amount of weight. (After 8 weeks.) No differences b/w milk and kefir groups.

→ we can draw away that milk + kefir hold the same benefits in weight loss. Is this related to the probiotic content? Milk doesn't contain probiotics, but kefir has many. How did they both encourage weight loss?

Probiotic ⊕ immune system boost ⊕ probiotics, but less than Kefir
Yogurt ⊕ supports bloodstream, growth of red + white cells
⊕ vitamins B2, B12, zinc, D, etc. weight gain + high blood sugar

⊕ flavored yogurts contain added sugars, added calories, lipoproteins, and saturated fats. *transport cholesterol and may lead to blockage of arteries if too much

HDL: high-density lipoprotein is considered healthier bc it transports cholesterol to your liver to break it down, removing it from your body

LDL: low-density lipoprotein is considered unhealthy bc if there is large amounts of it, it will lead to blockage of arteries (carries cholesterol)

Answer to my question on correlation b/w weight loss and milk consumption. ("Weight Loss Study")

"Milk doesn't contain any probiotics, but Kefir has many. How did they both encourage weight loss?"

→ Calcium is present in milk, and calcium converts energy to heat instead of storing it as fat. The more dairy products are consumed, the body knows that there is no need to store more fat.

→ Calcitriol is a form of vitamin D that extracts calcium from dairy. (Calcium promotes weight loss through thermogenesis.) If there is not enough calcium being consumed, your body creates more calcitriol, and calcitriol produces fat cells. If you consume more calcium, your body will create less calcitriol, meaning less fat.

Summary ↑ Calcium → ↓ calcitriol → ↓ fat

*This was proved by a study lead by Micheal Zemel, PhD

Fibromyalgia and the bacteria in the gut

→ research done by McGill Uni. and Uni. of Montreal and Institute for Pain Medicine at Rambam Health Care Campus proved that chronic pain is related to the concentration of certain bacteria and bile acids in the GI system. Bile acids help metabolize / absorb fat. It was found that healthier women in the study had 5x more alpha-muricholic acid (one type of bile acid) present in their gut. The presence and absence of certain microorgs. determine the symptoms of fibro. Experienced.
(Feb. 15)

Fibromyalgia: chronic pain and fatigue syndrome. Mostly in women due to hormonal changes

Feb. 16 - Changes

* I've decided to add 2 more independent/manipulated variables

→ Buttermilk and non-probiotic yogurt. (pasturized milk control)

* Adding a new part: winning product's probiotic will be grown on same plate as samples of human flora, and compared with man-made probiotic and human flora. (skin, saliva, gut).
↳ grown in milk as medium

NEW TESTABLE QUESTIONS

1) Which of 5 dairy products contains the most microflora that is beneficial to gastrointestinal health? In other words, which is the "healthiest"?

2) When probiotic bacteria from the winning variable (the dairy product containing the most probiotic) is grown on the same medium as human flora (from saliva, skin, and gut) what is the relationship? Does probiotic kill the potential pathogens (competition) or neutralism?

3) What happens when we repeat the same process as above, but with synthetic probiotic from a capsule, mixed with sterile milk as a medium? Relationship?

Title Idea: Probiotic Pathways: The Effects of Dairy Products on Gastro-Intestinal Health and the Relationship b/w probiotics (Natural and Synthetic) and Bacterial Flora of Humans (?)

Research Continued

* Probiotics can fight against potential pathogenic bacteria, therefore strengthening the immune system. Releasing bacteriocins, which are toxins, the probiotics kill bacteria that may harm humans.

Bacteriocins kill bacteria by inhibiting growth and not allowing the cell to form.

pathogenic bacteria's
||
↓

* I want to test this with parts

2 - with natural probiotic, highest abundance of probiotics

3 - with synthetic probiotics

can mix w/ pasteurized milk

Milk doesn't contain probiotics when it is fresh and not fermented. (Such as the 2% milk I'm using). There are no bacterial cultures (eg. Lactobacillus) added. → Milk is a medium for bacterial growth (eg. to make Kefir) but milk itself does not contain probiotics.

* What will be the relationship? Nothing happen or the probiotic will kill the human flora?
Other way around?

Why milk as medium? 1) It's sterile, so no interfering extra bacteria
2) dairy products are a common medium for probiotics b/c they protect the bacteria from acidity, allowing them to survive in the gut.

Feb. 17

Now let's look into the positives and negatives of my 2 NEW manipulated variables. (Buttermilk and non-probiotic yogurt)

Hypothesis

Buttermilk

- ⊕ leftover liquid from making butter and adding probiotics
- ⊕ B12 abundant, D, phosphorus, ^{protein} calcium, etc.
- ⊕ less fat and calories
- ⊖ lactose + probiotic can cause bloating, gas, diarrhea, etc.

Non-probiotic yogurt

* yogurt has to be made w/ cultures, but it does not contain added probiotics (stronger, more effective)

⊕ will still contain vitamins, protein, and calcium, and cultured bacteria

⊖ no more probiotics, not as much benefit to gut.

Forming hypotheses

To answer questions 2 and 3, we need the answer to #1. (Which of 5 dairy products...) Even though we know pasteurized milk (control variable) won't contain probiotics, we will still swab it to prove that it doesn't.

* I made a chart to compare the products and determine, which is the 'healthiest?'

(Find chart on google doc / trifold / online section)

Hypotheses

1) Kefir is the most beneficial dairy product to gastrointestinal health because it contains the greatest number of probiotics, including different bacteria and yeasts.

2) If the winning variable (hypothesizing that it will be kefir) retains lactobacillus (hypothesizing that it will) and this probiotic is grown on the same media as human flora (extracted from skin, saliva, and gut) it will kill the human flora because lactobacillus is the strongest and most effective probiotic genus.

3) If the synthetic probiotic powder mixed with milk is grown on the same media as human flora, it will kill the human flora because it contains several species of the lactobacillus genus.

* refer to picture from bottle and mention brand name

Variables

1) For first part of experiment, the CONTROLLED variable is the pasteurized milk, the MANIPULATED variable is the 4 other dairy products, and the RESPONDING/DEPENDANT variable is the abundance of probiotics observed (once samples are extracted and grown.)

2) For the second part of the experiment, the CONTROLLED variables will be a) dairy product that has highest amount of probiotics & its bacteria, b) amount, as well as the human flora's amount smeared onto media. The MANIPULATED/INDEPENDANT variable is the types of human flora that will be tested (skin, saliva, and gut.) The RESPONDING/DEPENDANT variable is the relationship that occurs b/w probiotics and human flora. (competition or neutralism?)

3) For third part of experiment, the CONTROLLED variable is the type of probiotic capsule used. The MANIPULATED/INDEPENDANT variables are a) the probiotic is now from a capsule, mixed w/ milk, not just extracted from a product, b) the types of human flora that will be tested. (skin, saliva, and gut.) The RESPOND/DEPENDANT variable is the relationship that occurs b/w probiotics and flora. (competition or neutralism?)

Research (Cont.)

Oral / Dermal Bacteria (most common)

(cocci bacteria genus are spherical/round-shaped. bacterium, pathogens)
(bacilli are bacteria genus that are rod-shaped, like a stick)

- Lactobacillus genus: gram-positive, mouth, skin
- Streptococcus genus (S. mitis, S. salivarius, S. mutans): gram-positive, mouth, skin. May be opportunistic pathogens (normally do not harm but can cause disease if host's immune system weakens.) (Cocci bacteria.)
- Granulicatella adiacens: gram-positive, found in mouth (and gut)
- Prevotella melaninogenica: gram-negative, found in mouth (and gut)
- many more but these are main ones

Streptococcal Infections

cuts, blisters, bites

*cause skin and throat infections, GAS usually through skin injuries, transfer of oral fluids. (coughing, sneezing, sharing food) and GBS through birth

GAS (Streptococcus A)

- skin / throat
 ↓ swollen throat
- strep throat, necrotizing fasciitis, impetigo, etc.
- mostly children

flesh-eating

skin infection

GBS (Streptococcus B)

- GI tract / vagina

- mostly pregnant women, newborns, and ppl w/ chronic infections
- UTIs, sepsis, pneumonia, meningitis, etc.

inflammation of brain/spine tissue

extreme reaction to infection

Feb. 20th

Dermal Bacteria (Most common)

- Staphylococcus epidermis: gram-positive, cocci genus, opportunistic pathogen (can cause endocarditis, cellulitis, meningitis, many more)
- Staphylococcus aureus: gram-positive, pathogenic, skin + nose, can cause skin infections, sepsis, respiratory tract illnesses
- Streptococcus genus: (see info from above)
- Corynebacterium genus, opportunistic infections, can cause infection in resp. tract like diphtheria.
- Diphtheroids: gram-p, non-pathogenic, also found in mouth

Feb. 22

I've begun copying all this information onto a google doc.
I will be conducting the experiment next week!

More research

Feb. 27 - Extra research! How will I identify the bacteria I grow on the agar plates?

* Moisture, temp, $\frac{1}{3}$ medium will affect what shows up on plate or not.
light, gases (CO_2 , O_2)

* Because different micro-orgs will grow in different conditions, I will use mediums that are specifically catered to support the growth of these common bacteria. (This should be included in procedure part)
↳ "materials"

↳ Selective media uses combinations of nutrients + agar

Characteristics to use for identification

- structure. (shape, size, spores, capsules, flagella, uni/multicellular, etc.)

* Spores: cells that protect the bacteria, most commonly produced by gram-pos bacteria
↳ have thick walls that resist environmental conditions

* Capsules: outermost layer of cell, made mainly of polysaccharides

↳ a type of carb

- presence of mycolic acids → LCFA's found in some cell walls that make help them thrive, and may cause diseases.

- reaction to gram stains (pos. or neg.)

- which media does it grow on? (what does it feed off of)

Selective media: allows the growth of one specific organism

Differential media: allows the growth of many types of similar bacteria and

helps identify them based on different growth patterns. (varying species will change the medium's color in different ways.)

⇒ a medium can be both. Can grow one bacteria but differentiate it's species.

- reaction to biochemical tests (I won't be doing this)

↳ can test if bacteria has catalase enzyme by placing drops of hydrogen peroxide onto a sample of bacteria.

* If pos. reaction (it does have catalase enzyme) it bubbles bc decomp. occurred. If neg. reaction, no bubbles.

enzyme found in living orgs. that breaks down H_2O_2 into $H_2O + O_2$

* 400x - 1000x best to see the bacteria + yeasts

Identifying the microorganisms I am likely to see

* morphology of a few common ones (not all though!)

Names based off of structure

Strepto: bacteria that come in chains →

Streptococcus

chain

spherical

Staphylo: bacteria that come in clusters, like grapes

(helps survive) → Staphylobacillus

also others

cluster of rod-shaped

diplo: come in pairs → diplococci pairs of cocci

* The part about media is insig. b/c I grow them all on different types

Lactobacillus: gram-positive, long rod, non-spore forming, can occur in chains, grows on MRS agar. (De Man - Rogosa - Sharpe agar)

Bacteroides: gram-negative, rod-shaped, non-spore forming, grows on BHI agar (brain heart infusion agar)

Firmicutes: gram-positive, can be spherical and rod-shaped, can be spore-forming and not, grows on Sugars

Staphylococci: gram-positive, spherical, come in clusters, non-spore forming, grows on MSA (mannitol salt agar) chains, → come in Y or V shape

Bifidobacterium: gram-positive, rod-shaped, non-spore forming, come in clusters, pairs, or independently. Grows on Bifidobacterium Selective Agar.

Saccharomyces: gram-positive, spherical or egg shaped, grows on YPD agar (yeast extract peptone dextrose agar)

Streptococcus: gram-positive, spherical, come in pairs or chains, non-spore forming, grows on CBA (Columbia blood agar)

March 1

=> my research is done, and tomorrow I'm conducting experiment. I bought the 5 dairy products today.

Brands: President's Choice Plain Kefir, 1% milk fat

Dairyland Light Buttermilk, 1% milk fat

Valley Pride Organic Milk, 2% milk fat

Activia Plain Probiotic Yogurt, 3.2% milk fat

non-prob. -> President's Choice Plain Greek Yogurt, 0% milk fat

Jamieson Wbill. Probiotic (mixed with nutrient broth)

Media Used:

Chocolate Agar ✓

Blood Agar ✓

Tryptic Soy Agar ✓

MCA (McConkey Agar) ✓

SDA (Sabouraud Dextrose Agar) ✓

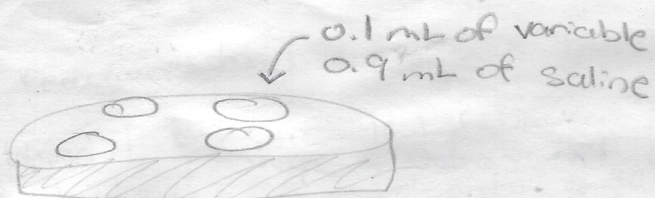
Mannitol Salt Agar ✓

Mitis-Salivarius Agar -> blue one ✓

Saliva part -> MRS (De Man - Rogosa - Sharpe Agar)

Nutrient Agar

Mueller - Hinton well diffusion (saliva)

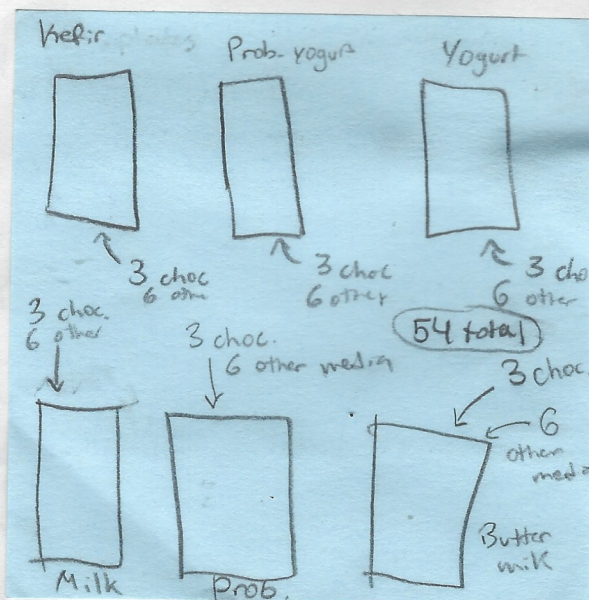


4 wells per culture → controls do not inhibit growth
(how many wells)



← 9 ml of nutrient broth

← 1 ml of product and salivary swab



Serial Dilution

x ml of initial solution : x ml of overall solution

↑
the one that sat for
1 hr

↑
diluted w/ nutrient broth

can be repeated again and again

→ for observation, ended up using the dishes that in which bacteria were diluted enough to see

10^{-7} or 1:10000000
 10^{-5} or 1:100000

March 2nd

Serial dilution: a series of dilutions where dilution factor stays the same for each step

Day 1, of experiment

* I did not brush my teeth in order to not kill bacteria for swabbing

* Poured freshly bought dairy products into 6 sterile cups. Fill about halfway. close lid IMMEDIATELY after pouring it in, to prevent contamination. {Photo}

=> MAJOR CHANGES (update on document when experiment done) → testable q's, hypotheses, and procedure changes

* added probiotic mixed w/ nutrient broth as a 6th variable

• put all 6 into test tubes, with serial dilution

• only mouth bacteria as human flora, mixed with EVERY variable, not just (in test tubes not plates)

→ once mixed w/ bacteria from one side of mouth, then other

• let sit for 1 hour

• swabbing mouth bacteria for streak plate method (on MRS agar)

to isolate colonies by diluting more and more. This was to get individual

bacteria by separation. Used an inoculating loop (sterilizing with bunsen burner)

• after letting 6 variables sit for approx. 1 hour, we diluted them

twice with nutrient broth to create 3 concentrations (original 1:10, then 1:100, and 1:1000) With 2 mechanical pipettes → but THEN even more 10^{-7} and 10^{-5}

• For 6 different media, we prepared dishes of each kind for 6 variables (36) (7th media)

• For chocolate agar, we prepared 1 dish of 1 concentrations, for 6 variables (6)

• w/ mechanical pipette, we dropped 3 concentrations of 6 products onto

chocol. and agar spread it w/ glass cell spreader (sterilized w/ ethanol and burner)

• one or two concentrations of each product onto 7 media => GREW FOR 24 HRS @ 35°C
glass cell spreader (41 plates total) or 1 GRB

- Gram stain process (ask her process of getting cells on gram stain. Maybe this won't even be included?)
- well diffusion technique → sterile borer cut wells into MRS, MSA, and Mueller-Hinton (on which we spread saliva swab) and in wells we put 6 products. The inhibition of bacteria proves strength of antimicrobial properties of dairy products. (Which bacteria did it kill? what did we identify?)
 ↪ against salivary bacteria
- identification in -phenol broth (pH indicator)
- microscope
- gram stains?

grow for 24 hrs
@ 35°C

- counted # of cells on those 41 plates

maybe choose to not track 41 plates, but only 1 concentration of each (less)
 ↳ clearest view, more diluted

→ write in new procedure

→ new hypotheses

→ test. questions (Parts to divide experiment into)

eg: instead of 10^5 use 10^7 bc less
show up

March 9th

Different dilutions in order to make counting colonies easier.

(Seeing less colonies however cells are still many)

*all photos in order of notebook

*counted colonies / did observations

*process of products w/ nutrient broth mixing with saliva (antibiotics against opportunistic pathogens) ← what is this

*glass slides for staining. ① of each agar plate. (choosing the plates based off of variety of colonies, not product.) → just to identify

*1 glass slide for MCA, 3 for MSA^(saliv.) bc of colony identification

March 9th - Going back to lab to check on what I've grown. I changed the experiment a bit, so I'm working out an organized list of observations and procedure.

CFU - colony forming units

Observations *USE THE CONCENTRATION THAT SHOWS MORE GROWTH (more diluted one) $\frac{\#CFU \times 10^6}{0.1 \text{ ml}}$

- All have dilution factor 10^2 . Too many to count

- Blood agar milk +++ growth. Small, green spots all over, and some bigger white ones
- BA Yogurt ++ growth. Small, green spots (less) and a few colonies of white
- BA kefir. + growth. Very very small green spots.
- BA Prob. Yogurt +++ growth. Green spots all over.
- BA Probiotic ++ growth. Small colonies of green and white, widespread
- BA buttermilk +++ Can see individual colonies. Very many green, widespread, not much white

• MacConkey agar milk 10^1 dilution factor. ~1200 colony count.

+++ growth. Dark red, small and big colonies. Some streaky some clumps ~12,000 individual cells

• MCA Yogurt 10^1 - 4 colony count + growth. 4 very large, pinkish colonies
- 40 individual cells

• MCA kefir 10^1 - 1 colony + growth. 1 very large, pinkish colony
- 10 indiv cells

• MCA Prob. Yogurt 10^1 - 2 colonies + growth. 2 very large *green spots are hemolysis
- 20 indiv cells

• MCA Probiotic 10^1 - Zero No growth

• MCA Buttermilk 10^1 - 3 colonies
- 30 indiv cells

• Mannitol Salt Agar milk 10^1 ~1800 colony count +++ growth.
~18,000 individual cells Many small yellow colonies

• MSA Yogurt 10^1 ~1200 colony count +++ growth. Varying sizes.
~12,000 indiv. cells Round big to small, yellow

• MSA kefir 10^1 ~84 colony count ++ growth. Big, yellow circles.
~840 indiv. cells

• MSA Prob. Yogurt 10^1 ~920 colony count +++ growth. Came in lines/streaks
like worms.
~9200 cells

• MSA Probiotic 10^1 - zero growth. Turned agar from yellow to orange from
acidity

• MSA Buttermilk 10^1 ~720 colonies +++ growth. Some colonies very
~7200 cells Small and arranged in rows, like worms.
Some circular and grouped together.

~~• Nutrient agar milk 10^5 ~680 colonies +++ growth. Very tiny circles. White.
~68,000,000 cells~~

• NA milk 10^7 ~700 colonies +++ growth. Mostly tiny circles, ~~7~~
~7,000,000,000 cells large colonies. White

• NA Yogurt 10^7 ~no growth 7.0×10^9 CFU
(colony formula units)

• NA Yogurt 10^7 ~no growth.

• NA kefir 10^5 ~360 colonies ++ growth. Some very small, some a
~36000000 cells 10^{-4} bit larger. White

• NA kefir 10^7 ~80 colonies + growth. Very little. A few larger
~80,000,000 cells 10^{-5} ones

AVERAGE:

- NA Prob. Yogurt 10^5 ~ 240 colonies ~ 24,000,000 cells ++ growth. Half worms, half round
- NA Prob. Yogurt 10^7 - 6 colonies - 60,000,000 cells + growth. 6 circles. White
- NA Probiotic 10^5 - 6 colonies - 600,000 cells + growth. 6 circles, white
- NA Buttermilk 10^5 - 47 colonies - 47,000,000 cells ++ growth. Large, singular distinct colonies. Yellow-white color
- MRS milk 10^5 - no growth
- MRS Yogurt 10^5 ~ 80 colonies ~ 80,000,000 cells ++ growth. Some individual, some stringy/worms
- MRS kefir 10^5 ~ 560 colonies ~ 56,000,000 cells +++ big, distinct colonies
- MRS Probiotic Yogurt 10^5 ~ 280 colonies ~ 280,000,000 cells +++ look like splatters, circles in a streak.
- MRS Probiotic 10^7 ~ 280 colonies ~ 280,000,000,000 cells +++ growth. Worms and streaky, also circles. Very big + distinct
↓
was very hard to count on original and 10^5 , bc so much growth. Looked like waves
- MRS Buttermilk all dilutions too hard to count. Very concentrated b/c many cells. Look like waves or spread butter.

- Mitis-Salivarius Agar milk 10^7 ~ 320 colonies +++ growth. Half worms, half circle. Blue
- ~ 3,200,000,000 cells
- MSA Yogurt 10^7 ~ 240 colonies +++ growth. Some are blue with white ring around them. Some just solid blue.
- ~ 2,400,000,000 cells
- MSA kefir 10^7 ~ 120 colonies ++ growth. White colonies (10⁵ dilution had almost black colonies)
- ~ 1,200,000,000 cells
- MSA Prob. Yogurt 10^7 - 82 colonies +++ large, distinct blue colonies
- 820,000,000 cells
- MSA Probioticz 10^5 - 9 colonies + large, blue colonies, chunky
- 900,000 cells
- MSA Buttermilk 10^7 - too many to count. Some extremely small ones and some large ones. All blue.

- Chocolate agar, saliva swab +++ growth. Too many colonies to count. Whiter with green around them.

Standard deviation: measurement that shows how far different points in a dataset are from the average of that set. If data points are further from average, there is higher deviation in the dataset

hemolysis: destruction of red blood cells

37

March 11th / 12th

*explain well procedure, process I did, and how measurements were taken

*making an excel spreadsheet + graphs for well diffusion procedure.

1 - Probiotic 2 - Kefir 3 - Butter 4 - Prob. Yogurt
5 - Yogurt 6 - Milk 7 - MRS 8 - Saline

could be *S. epidermidis* → identify

controls - no inhibition

CMSA (sal) for streptococci and Mueller-Hinton for other microorganisms

Inhibition of salival bacteria seen in well diffusion

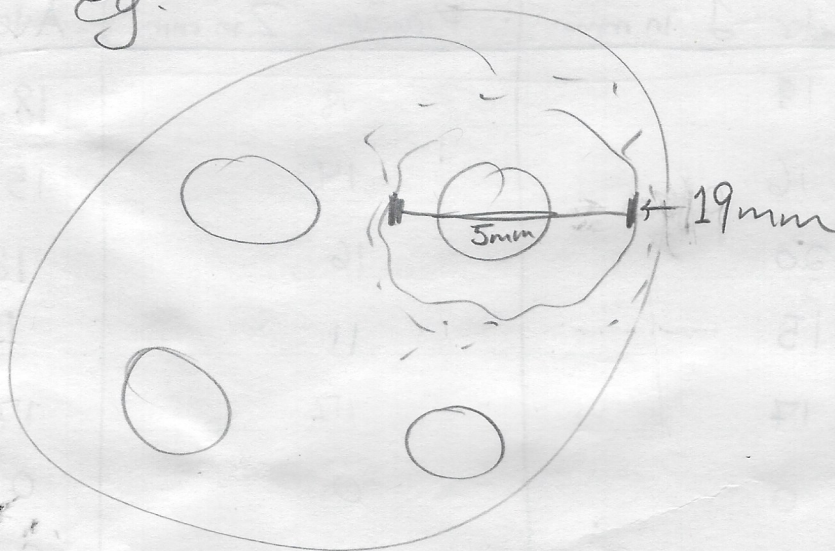
MSA 8	Diameter 1 in mm	Diameter 2 in mm	Avg.	Standard deviation
1	19	18	18.5	0.71
2	16	14	15	1.41
3	20	16	18	2.83
4	15	11	13	2.83
5	17	17	17	0.00
6	0	0	0	0.00
7	0	0	0	0.00
8	0	0	0	0.00

could be micrococci

MSA 7	d ₁	d ₂	Avg d	S.D.
1	25	30	27.5	3.54
2	24	22	23	1.41
3	22	20	21	1.41
4	16	18	17	1.41
5	21	24	22.5	2.12
6	0	0	0	0.00
7	0	0	0	0.00
8	0	0	0	0.00

note to self: diameters are total Cuell included)

eg:



Wells all 5mm in diameter

✓ could be E. coli or coliform

MCA 5	d ₁	d ₂	Avg.	S.D.
1	14	13	13.5	0.71
2	11	10	10.5	0.71
3	11	11	11	0.00
4	8	8	8	0.00
5	10	8	9	1.41
6	0	0	0	0.00
7	0	0	0	0.00
8	0	0	0	0.00

↙ could be S. aureus

MSA 6	d ₁	d ₂	Avg.	S.D.
1	16	15	15.5	0.71
2	12	12	12	0.00
3	12	13	12.5	0.71
4	8	11	9.5	2.12
5	12	12	12	0.00
6	0	0	0	0.00
7	0	0	0	0.00
8	0	0	0	0.00

March 14th

- completing online section

Dilution factors of products on agar

MSA, 10^{-1} and 10^{-2}

MRS, 10^{-4} and 10^{-6}

MCA, 10^{-1} and 10^{-2}

MSL, 10^{-4} and 10^{-6}

NA 10^{-4} and 10^{-6}

all for 24 h @ 35°C

* I made an excel graph to measure growth of products on agar plates in part 1.

* excel sheet for promotion / inhibition of oral bacteria
↳ and a graph

Notes: swabs w/ product in test tubes in order to prove whether or not products contained probiotics (and how strong those probiotics are). Also, to grow salival bacteria and see them show up individually on selective media.

5mm

image #1.
#6

microorg. #8
S. epider.

#17/18
saline 0.9%
Nace

S. aureus #6 #18

Well → 1 18/18 mm
2 16/14
3 20/16
4 15/11

diameter
5 14/14
6 0/0
7 0/0
8 0/0

MSA B

A. M. yel. no gas
B. G. yel. no gas
C. control → Ph.

1011614 broth

#2/#3 #7
1 25/30 Micrococcus?
2 24/22
3 22/20
4 16/18

#4/#5
5 21/24
6 0/0
7 0/0
8 0/0

mannitol salt agar

MSA 4

M - mannitol
G - glucose
#4. micrococcus #19

A. No lysis
B. No
C. No

101719

#4/#8 E. coli coliform #5
1 14/13
2 11/10
3 11/11
4 10/8

#4(15)/#16
5 10/8
6
7
8

MacConkey

MCA 5

#8 S. epiderm.
A. low +/-
B. low
C. low

101757

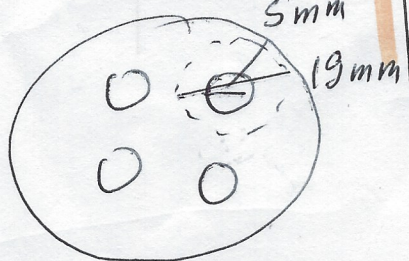
#9/#10(11) S. aureus #6
1 16/15
2 12/12
3 12/13
4 8/11

#12/#13
5 12/12
6 0/0
7 0/0
8 0/0

MSA 6

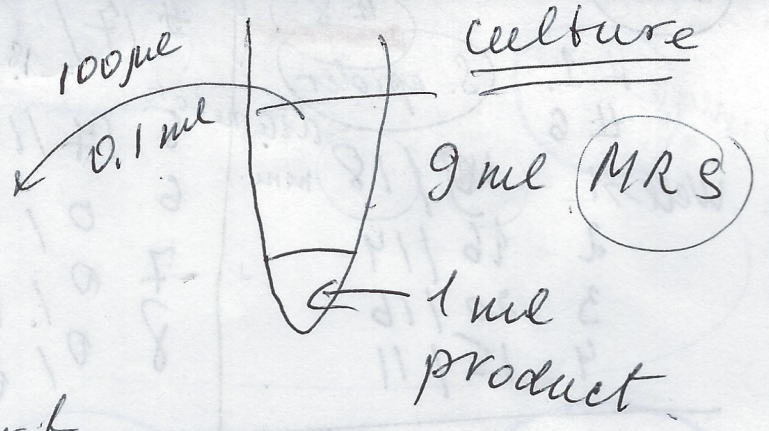
E. coli
A. M gas +++
B. G gas +
C. Lactose gas +
D. Sucrose No
E control.

102112/19



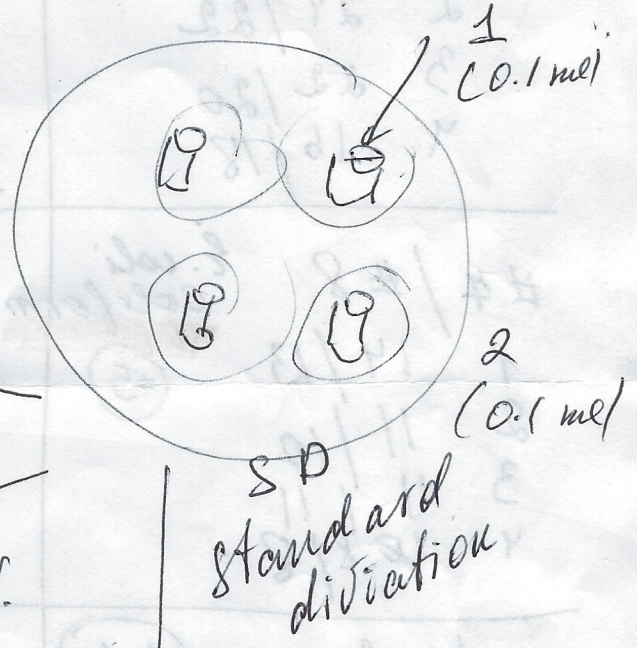
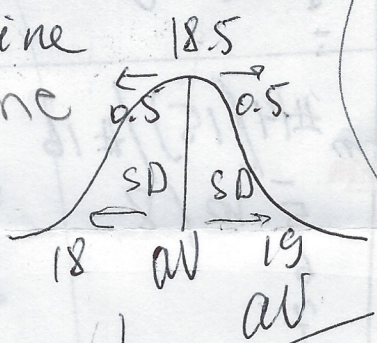
5 mm
Well x 2.

1. Probiotic
2. Kefir
3. Buttermilk
4. Propr. yogurt
5. Yogurt



24 hr at 35°C

- control {
6. milk
 7. MRS (media)
 8. Saline



culture
MSA 8

	d_1	d_2	$d_{av.}$	Standard deviation
1 Prob	19 mm	18 mm	18.5 mm	± 0.5
2 Kefir	16	14	15	± 1.0
3 Butter.	20	16	18	± 2.0
4				
5				
6				
7				
8				

