LONDON METROPOLITAN UNIVERSITY

Module title: Food Microbiolog y & Safety Module

LAB REPORT

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1. Introduction

In today's cheese market, consumer choices are shaped not only by flavor but also by price, fat level, and sodium content. According to Cruz Maceín, Iriondo DeHond and Miguel (2019), consumers prioritize price over fat and salt levels when making purchasing decisions, highlighting the importance of nutritional content and affordability in the cheese-buying process. Moreover, demographic characteristics such as age, education, and lifestyle exert influence on cheese consumption habits (Ouyang et al., 2021). Zhllima, Mehmeti and Imami (2021) further elaborate that educated female consumers exhibit specific purchasing behaviors, often preferring supermarkets for their cheese purchases.

Dairy consumption plays a pivotal role in global health, nutrition, and economic sustainability (Klerk and Robinson, 2022). Cheese, among dairy products, holds a prominent position as a widely cherished delicacy, featuring a diverse range of varieties including cheddar, feta, gouda, parmesan, and camembert originating from various regions worldwide. As of 2022, the global market value of cheese soared to approximately 83.4 billion U.S. dollars, with projections indicating a surpassing of the 120 billion U.S. dollar mark by 2028 (Shahbandeh, 2024). Noteworthy is the dominance of cheese consumption by the European Union, which in 2023 alone consumed around 9.2 million metric tons, significantly surpassing consumption rates in other regions. Furthermore, France emerges as a prominent leader in cheese consumption, boasting an impressive per capita consumption rate of 57.9 pounds per year (Shahbandeh, 2024).

Figure 1. Tunworth Cheese

While dairy products offer nutritional benefits, they also pose microbiological food safety hazards to consumers. Pasteurisation, which involves boiling milk to destroy harmful organisms, dramatically lowers the risk of foodborne diseases connected with dairy intake. However, unpasteurized cheese poses a significant risk, particularly to vulnerable groups such as young children, the elderly, and individuals with compromised immune systems (Klerk and Robinson, 2022).

The high risk associated with cheese consumption is multifaceted and encompasses various factors. Firstly, the presence of pathogens in cheese, particularly in high humidity and short ripening varieties, poses a significant risk to consumers (Paulina et al., 2021). elevated humidity levels and shorter ripening periods are more prone to hosting pathogens, unlike varieties with lower moisture content and longer ripening times. (Paulina et al., 2021).

Additionally, the presence of Listeria monocytogenes in traditional cheeses poses a significant risk, particularly for vulnerable populations, with the potential to cause listeriosis (Campagnollo et al., 2018). Soft cheeses have been identified as having a significantly higher risk of listeriosis compared to semi-hard cheeses (Falardeau, Trmčić and Wang, 2021; Campagnollo et al., 2018).

1.1 Risk and hazard

A risk is something that could cause potential harm due to a hazard. Risk is calculated by how likely a person might be hurt by its severity.
A hazard is something that causes harm which might lead to injury, damage, death or any other loss. It can be a substance, machine, food, work or any other process. There can be various risks in a hazard.

Three components of risk analysis:
Risk analysis typically involves several key components at identifying, assessing and mitigating potential risks. The three fundamental components of risk analysis are described in Table 1.

Table 1. Components of risk analysis.

RISK IDENTIFICATION	RISK ASSESSMENT	RISK MIGITATION
This stage involves the systematic identification of potential risks that could impact a project, operation, or organization. It's crucial to comprehensively identify all possible risks, including internal and external factors. This process often includes brainstorming, using historical data, conducting interviews, and utilizing risk checklists to ensure a comprehensive list of risks is compiled.	After identifying risks, the next step is to assess or analyse these risks to understand their potential impact and likelihood of occurrence. Risk assessment involves evaluating each risk based on its severity (impact) and probability (likelihood). This process helps prioritize risks based on their significance, allowing organizations to focus resources on managing the most critical risks first (Burgman, 2005).	Once risks are assessed, the final component involves developing and implementing strategies to mitigate or manage these risks. Risk mitigation strategies can include risk avoidance (eliminating the risk), risk reduction (implementing controls to lessen the impact or likelihood), risk transfer (shifting the risk to another party, like through insurance), or risk acceptance (acknowledging the risk and preparing to deal with its consequences if it occurs). The goal of risk mitigation is to reduce the overall impact of risks on the organization's objectives (Pérez-Rodríguez and Mercanoglu Taban, 2019).

Risk assessment involves various steps for identifying the risks involved, the key components being hazard identification, hazard characterisation, exposure assessment, risk characterisation.

- **Hazard identification:** it is an important process in risk analysis that focuses on recognising the potential sources of harm. The main aim of hazard identification is to identify the potential risks(French and Miller, 2012a).

- **Hazard characterisation:** it is a fundamental aspect that involves evaluating and describing the risk assessment through the potential adverse effects and the nature of the identified hazards.
- **Exposure assessment:** this is the crucial component of the risk assessment which involves the quantifying and the evaluation of the potential interaction between individuals, environment or communities with an agent that is hazardous.
- **Risk characterisation:** another critical component of risk assessment involving the synthesizing and the interpretation of the available information describing the risk associated by the nature and magnitude with its exposure to a situation.

1.2 Microorganisms

Hazard identification is a critical step in risk assessment processes, especially in the context of microbiological hazards such as Salmonella and enteropathogenic Escherichia coli (EPEC). This step involves recognizing and defining potential biological, chemical, or physical hazards that may pose a risk to human health through exposure.

a) **Enterobacteriaceae:** Enterobacteriaceae are a family of gram-negative bacteria that may be found in soil, water, and the gastrointestinal systems of people and animals. Within this family, enteropathogenic Escherichia coli (EPEC) and Salmonella are two important bacteria known to cause foodborne disease, offering a concern to consumers, especially when found in soft cheese.

   Enterobacteriaceae, including EPEC and Salmonella, thrive in favourable conditions with moderate temperatures (20-45°C), neutral pH values, and high moisture content (Mladenović et al., 2021). Without proper temperature control and sanitation standards during manufacture and storage, soft cheese provides an ideal environment for Enterobacteriaceae to flourish. These bacteria can persist in soft cheese for extended periods, particularly in bacterially conducive settings, and may survive even after pasteurization, especially if contamination occurs during packing or handling (Mladenović et al., 2021).

   Consuming soft cheese contaminated with Enterobacteriaceae, including EPEC and Salmonella, can lead to foodborne illness, characterized by symptoms such as diarrhoea, abdominal cramps, vomiting, and fever (D'amico, 2014). Vulnerable groups, such as children, the elderly, and individuals with weakened immune systems, are at a heightened risk of experiencing severe complications from such foodborne infections.

b) **Staphylococcus aureus:** Staphylococcus aureus, a gram-positive bacterium commonly found on human skin and mucous membranes, poses a significant risk in food processing, particularly in the manufacture of soft cheese (D'amico, 2014). Understanding the potential hazards associated with Staphylococcus aureus infection is crucial for ensuring the safety of soft cheese products.

   Renowned for its capacity to produce heat-stable toxins such as staphylococcal enterotoxins, Staphylococcus aureus increases the risk of foodborne illness upon consumption (Argudín, Mendoza and Rodicio, 2010). Even in the absence of bacterial growth, toxin synthesis can occur rapidly under favourable conditions, rendering

contaminated soft cheese potentially harmful to consumers. Thriving in conditions with moderate temperatures (10–45°C), neutral pH values, and high salt concentrations, these bacteria's finds an ideal growth environment in soft cheese, characterized by its high moisture content and favourable pH, if strict temperature control and cleanliness standards are not upheld (Myles and Datta, 2012).

Consumption of soft cheese contaminated with Staphylococcus aureus toxins can result in staphylococcal food poisoning, with similar symptoms such as nausea, vomiting, abdominal cramps, and diarrhoea. Staphylococcal enterotoxins, resistant to digestion and heat stable, present a significant health concern, particularly if infected soft cheese is ingested without sufficient heat treatment (Myles and Datta, 2012).

c) **Listeria monocytogenes:** Listeria monocytogenes is a Gram-positive bacterium capable of thriving in both aerobic and anaerobic environments, without forming spores, and displaying a remarkable adaptability to varying conditions. This pathogen has been implicated in outbreaks and recalls associated with produce, and it stands as a leading cause of severe foodborne illness, posing significant challenges to public health and food safety.

Listeria monocytogenes exhibits resilience to a variety of environmental conditions, including refrigeration temperatures (0-4°C) and high salt concentrations. Soft cheese, with its high moisture content and relatively neutral pH, provides an ideal growing environment for Listeria monocytogenes if proper temperature control and sanitation standards are not followed (Camargo et al., 2017). Additionally, Listeria monocytogenes has the ability to form biofilms on surfaces, enabling it to persist in industrial settings despite cleaning and sanitation attempts Camargo et al., 2017. Once established in a processing plant, Listeria monocytogenes becomes difficult to eliminate, posing a constant danger of contamination to soft cheese products (Rodríguez-López et al., 2018)

Consumption of soft cheese infected with Listeria monocytogenes can lead to listeriosis, a severe foodborne infection characterized by symptoms ranging from fever, muscular pains, and gastrointestinal symptoms to more serious outcomes such as meningitis, septicemia, and miscarriage in pregnant women. Vulnerable groups, including pregnant women, newborns, the elderly, and those with impaired immune systems, are more likely to develop severe consequences of listeriosis (Rodríguez-López et al., 2018)

1.3 Predictive microbiology

It is the branch of food microbiology that uses statistics and mathematical models to predict the growth and behaviour of micro-organisms in food which are exposed in various environmental conditions. Its primary aim to manage and access the microbial risks associated with the foods including the foodborne illness and its potential for spoilage.
Predictive microbiology has to do with the shelf life of the food, its model microbial growth, assess food safety risks. It models with microbial growth and its kinetics by estimating the count of microorganisms over time in the foods. By estimating the shelf life of food products by microbial population under different storage temperatures (Martínez-Martínez, Cruz and Garza, 2023).It also shows the safety margin between the hazardous microbial level by its potential and acceptability. By all the above it shows the microbial growth curves by generating

microbial growth by curves due to the population by time., it shows the critical control points by recording and monitoring the temperature, time, pH, etc to ensure food safety and finally it shows the risk assessment and the margin of safety involved.
The value of predictive microbiology in the food industry is to improve food safety by helping the food manufacturers or processors to implement preventive measures by minimising the risk of food hazards and foodborne illnesses. It also helps in enhancing the shelf life of the food.

1.4 Objective

The aim of this project is to conduct a microbial and general physicochemical evaluation of Tunworth and Petit camembert soft cheeses through isolating. The study will also evaluate phenotypic and genotypic identification methods for pasteurised and unpasteurized cheeses, with a focus on microbial composition differences.
Finally, the project seeks to discuss and interpret the results, including comparisons between raw milk and pasteurized cheese, identification of unsafe or unusual findings, and implications for risk assessment and food safety practices.

2. Methodology

2.1 Material Supply Used

List of materials and equipments used for the experiments are mentioned below.

- 1 x 10 ml pipette
- 1 x pipette filler
- x Pipette (100ul & 1000ul)
- 1 x blue pipette tips box (sterile)
- 1 x yellow pipette tips box (sterile)
- 5 x Universal bottles
- Duran bottle with distilled water (100 ml)
- 1 x Universal bottle containing Crystal violet (10ml)
- Analytical balance
- Laboratory marking pen
- Small sticker
- Small weighing boats
- 1 x Bunsen burner
- 1 x Agar plate (e.g. NA) with some individual bacterial colonies
- x 9 ml MRD (or any other diluent) in Universal bottle
- x NA plates
- 3 x Sterile plastic spreader
- 1 x metal loop

2.2 Product Introduction

Tunworth camembert cheese was used, which is a pasteurized British cheese resembling French camembert cheese made from pasteurized milk (Nunes et al., 2015).

Ingredients

- Cow's Milk (Pasteurized)
- Cheese Cultures (Penicillium candidum and Geotrichum candidum)
- Rennet
- Salt

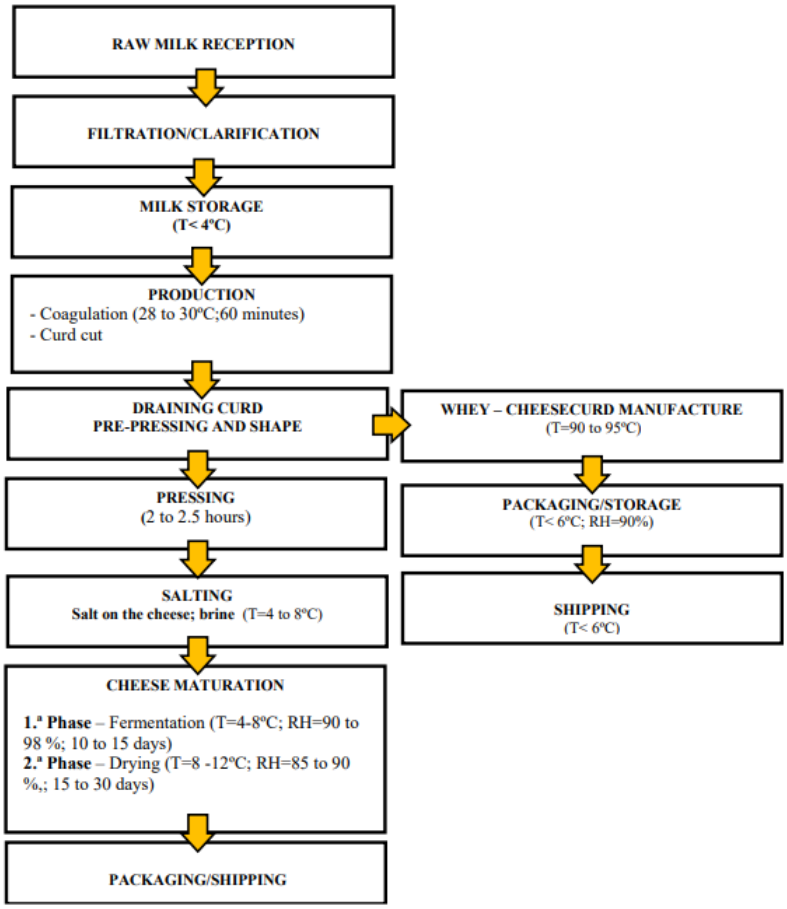


Figure 2.1 Flow diagram of cheese Production (Nunes et al., 2015)

Date of Examination
21/02/2024
Shelf Life of Cheese
About 6 to 8 weeks.

Recovery of microorganisms from cheese

The recovery of microorganisms from cheese, essential for monitoring quality and safety, involves preparing serial dilutions from 10^-1 to 10^-4 on VRBG and BP agar, and 10^-4 to 10^-7 on NA and MRS agar, as well as measuring the pH and water activity (aw) of the cheese sample for detail description refer to (Basic Microbiological Methods; Method 10).

Table 2.1 Media and dilutions used with incubation time/temperature.

Medium	Dilution used	Incubation time/temp
Nutrient Agar (NA)	10^{-2}	48h/37°C
Violet Red Bile glucose Agar (VRBG)	10^{-2}	48h/37°C
Bair-Parker Agar (BP)	10^{-3}	48h/37°C
MRS Agar	10^{-5}	48h/37°C

2.3 Phenotypic identification

This identification included several procedures to determine potential Hazards and characteristics of isolated microorganisms from VRBG and MRS Plate (Basic Microbiological Methods; Method 15).

Figure 2.2 Slope culture

•Streaking
This would help to get pure culture to do the morphological Observation which helped in microbial identification. A pure culture is essential for doing accurate tests and observation of microorganisms' characteristics (Basic Microbiological Methods; Method 1).

•Transfer to slope culture
This was done to create the stock of culture for Later testing. Slopes provide easy storage and maintenance of microorganisms for further testing (Basic Microbiological Methods; Method 1).

•Gram Staining
Isolated microorganism was classified as gram-positive or gram-negative based on their cell wall composition which helped in the selection for biochemical tests and potential antibiotic treatment (Basic Microbiological Methods; Method 6).

- **Oxidase test**

This test was performed to identify the presence of cytochrome c oxidase. This enzyme helped to categorize bacteria whether they were oxidase-positive or negative (Basic Microbiological Methods; Method 8).

- **Catalase test**

This test helped to identify the presence of catalase that breaks hydrogen peroxide into water this helps in differentiating between groups of Bacteria (Basic Microbiological Methods; Method 7).

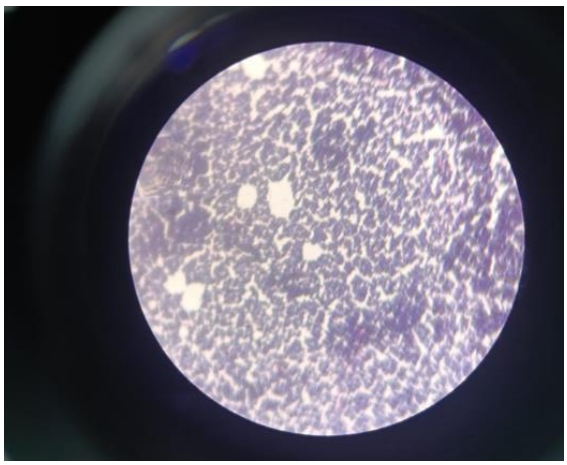


Figure 2.3 Microscopic view of Gram staining slide

- **API 20E and Software**

The API method was used to identify Enterobacteriaceae, a family of gram-positive and gram-negative bacteria e.g. salmonella, Shigella, and Yersinia, and environmental and food spoilage bacteria such as Serratia and Klebsiella. It also includes Escherichia, some members of which are pathogenic, but most are commensal (Basic Microbiological Methods; Method 15).

- **Detection of virulence factors**

To detect haemolysis on blood agar colonies were streaked from VRBG on nutrient Agar. Blood agar was used because it is a nutrient-rich medium containing sheep's blood (5%) that helps to indicate the presence of haemolytic toxins which are virulence factors (Ejiofor et al., 2018).

2.4 Use of predictive microbiology software

Combase software was used to assess the different micro-organisms like E.coli, Listeria, and Salmonella these were selected because of the wide occurrence of foodborne diseases (González et al., 2019).

- **Time Scale**

  Time scale determines the prediction of microbial load in respect to time as the microbes keep on multiplying exponentially during the log phase. This is done to predict the shelf-life and outbark investigation (González et al., 2019).

- **Initial Inoculum**

  Initial Inoculum 1 was selected based on realistic contamination levels and worst-case scenarios from minimum to maximum (González et al., 2019).

- **Temperature Examination**

  Compared different temperatures such as 5, 7, 10, 15, and 20 with various concentrations of inoculum e.g. 10 and 10^3 provide the potential risk of microbial population in various conditions (González et al., 2019).

2.5 Genotypic identification

DNA Extraction the main objective was to extract DNA from cells or its components like proteins and lipids. The process was very on the material source but mostly done through cell lysis in which the cell wall breaks and gets cellular content another technique is the removal of proteins and lipids using enzyme and the third process is DNA purification (Sönmezoğlu & Terzi, 2019).

PCR This procedure helps to create millions of copies through the amplification of specific DNA billions of times. The procedure involves DNA sequences through primers DNA stands synthesis by enzymes known as DNA polymerase and Thermal cycling where denaturalization is done at high temperatures primers bind to their sequence to a single strand called annealing and then synthesis of a new DNA strand occurs known as extension. This helps in pathogen detection (Sönmezoğlu & Terzi, 2019).

Gel Electrophoresis is used to split DNA fragments based on their size. The procedure involves loading of sample into agarose gel wells and then exciting the negatively charged DNA fragments through an electric current to move them out from the gel then Ultra violate light is used to visualize the DNA fragments after dying (Sönmezoğlu & Terzi, 2019).

Figure 2.4 Gel electrophoresis setup

Figure 2.5 Analysis of Gel from electrophoresis

3. Results

The results were obtained after the tests were conducted on culture plates of microbial load from the cheese. These results were compared with the results of **Group 4.**

3.1 Physicochemical properties and bacterial enumeration

Table 3.1 Physicochemical characteristics of cheeses

Cheese	pH	Aw
Tunworth cheese (Pasteurized)	7.79 at 22.5°C	0.972 at 22.9°C
Petit Camembert (Unpasteurized)	7.72 at 22.1°C	0.972 at 22.1°C

Table 3.2 Bacterial enumeration data.

Cheese	Medium	Dilution (30-300)	Count	CFU/g
Turnworth cheese	VRBG	10^{-2}	141	$1.41*10^{5}$
	NA	10^{-2}	42	$4.2*10^{4}$
	MRS	10^{-5}	108	$1.08*10^{8}$
	BPA	10^{-3}	279	$2.79*10^{6}$
Petit Camembert cheese	VRBG	10^{-3}	35	$3.5*10^{5}$
	NA	10^{-6}	61	$6.1*10^{7}$
	MRS	10^{-5}	55	$5.5*10^{7}$
	BPA	10^{-3}	80	$8*10^{5}$

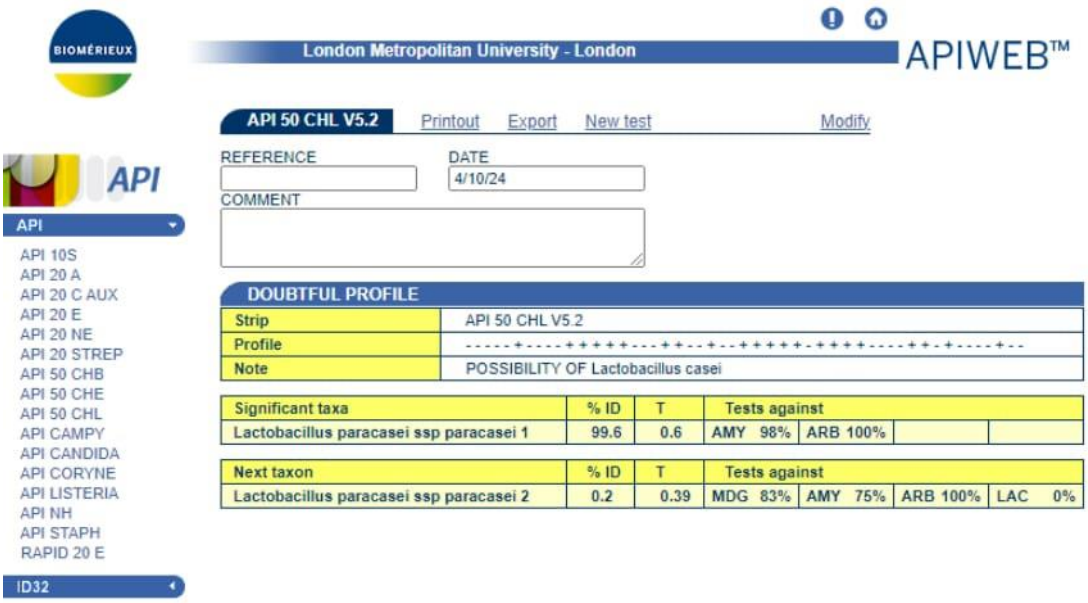
3.2 Results of Gram staining, oxidase test and catalase test

Table 3.3 Characterization of isolated bacteria.

Cheese	Plate	Gram staining	Oxidase	Catalase
Tunworth English Camembert style cheese (Pasteurized)	VRBG	-	-	+
	MRS	+	-	-
	BPA	+	-	+
Petit camembert English Camembert style cheese (Unpasteurized)	VRBG	-	-	-
	MRS	+	-	+
	BPA	+	-	+

3.3 Results of API 20E, API 50 CHL test

Figure 3.1 API 50 CHL test result after 24hrs of bacterial isolate from MRS



London Metropolitan University - London

APIWEB™

API 50 CHL V5.2 | Printout | Export | New test | Modify

REFERENCE

DATE 4/10/24

COMMENT

API

API 10S
API 20 A
API 20 C AUX
API 20 E
API 20 NE
API 20 STREP
API 50 CHB
API 50 CHE
API 50 CHL
API CAMPY
API CANDIDA
API CORYNE
API LISTERIA
API NH
API STAPH
RAPID 20 E

ID32

DOUBTFUL PROFILE

Strip	API 50 CHL V5.2
Profile	-----+----+++++---++--+--+++++-++++----++-+----+--
Note	POSSIBILITY OF Lactobacillus casei

Significant taxa	% ID	T	Tests against			
Lactobacillus paracasei ssp paracasei 1	99.6	0.6	AMY 98%	ARB 100%		

Next taxon	% ID	T	Tests against			
Lactobacillus paracasei ssp paracasei 2	0.2	0.39	MDG 83%	AMY 75%	ARB 100%	LAC 0%

Figure 3.2 API 50 CHL test result after 24hrs of bacterial isolate from MRS.

Figure 3.3 API 20E test result after 24hrs of bacterial isolate from VRBG.

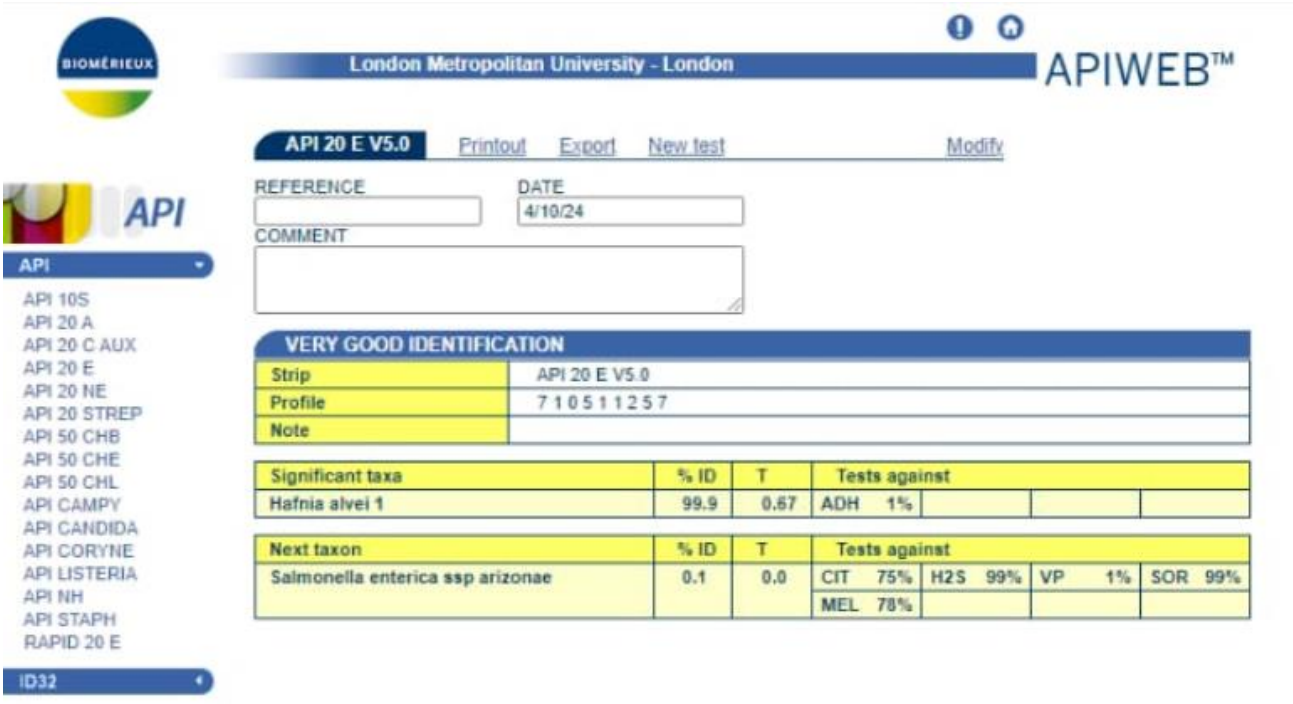


Figure 3.3 API 20E test result after 24hrs of bacterial isolate from VRBG.

3.4 Results of Virulence factor.

Blood Agar based Haemolysis test was conducted.

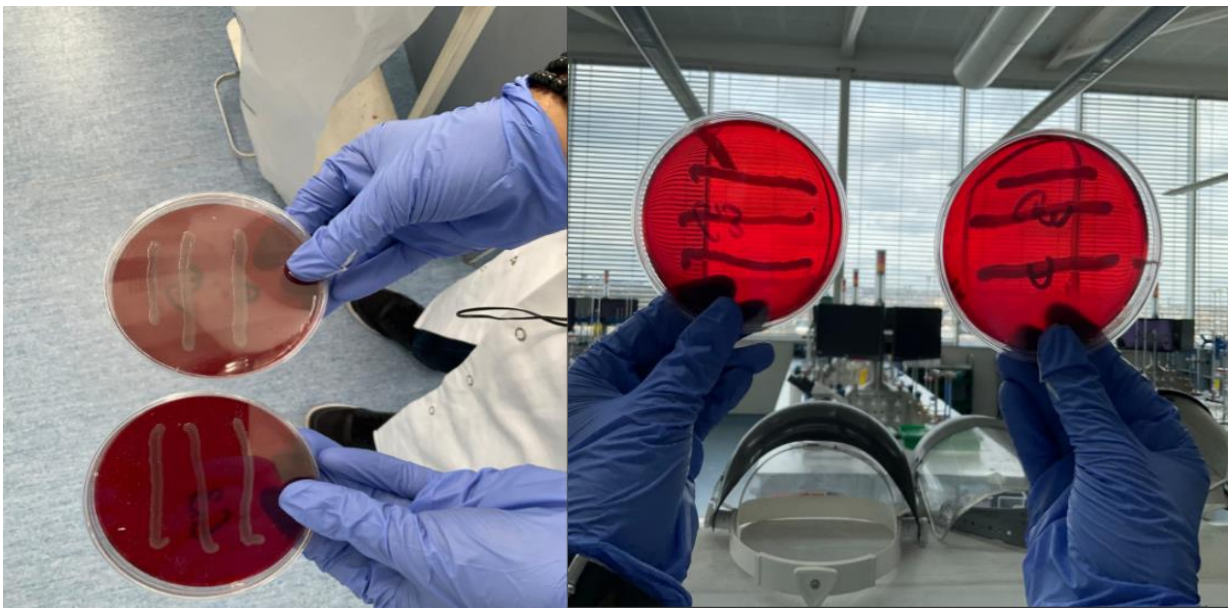


Figure 3.4 Blood Agar plates isolated from VRBG plate

Table 3.4 Hazard Characterization results on Bacteria isolated from VRBG plate.

Cheese	Haemolysis test	Staph test	Salmonella test
Tunworth	+(α)	-	-
Petit Camembert	+(α)	-	-

3.5 Predictive Microbiology

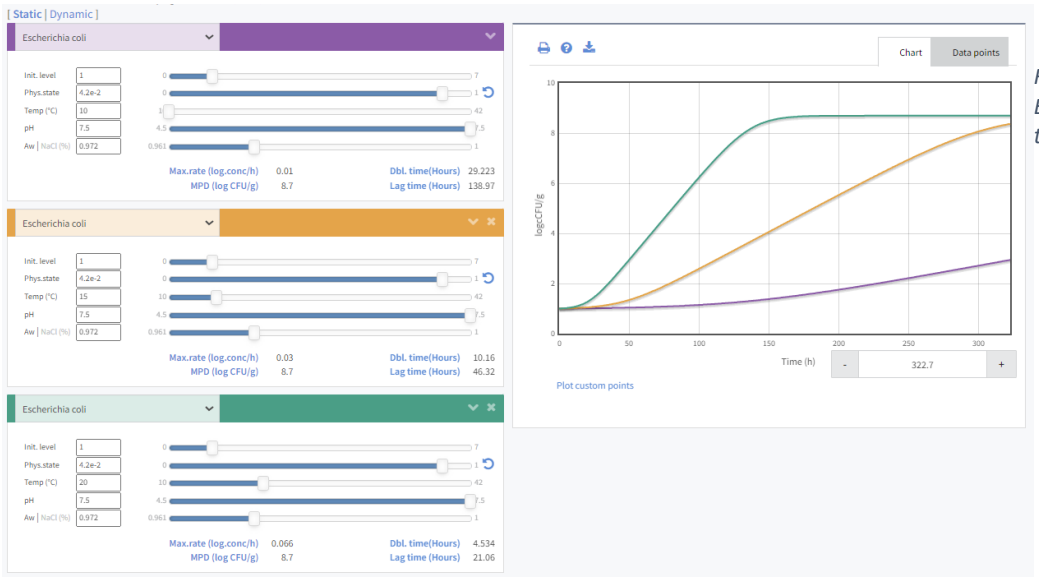


Figure 3.5 Microbial growth of Escherichia coli at different temperature.

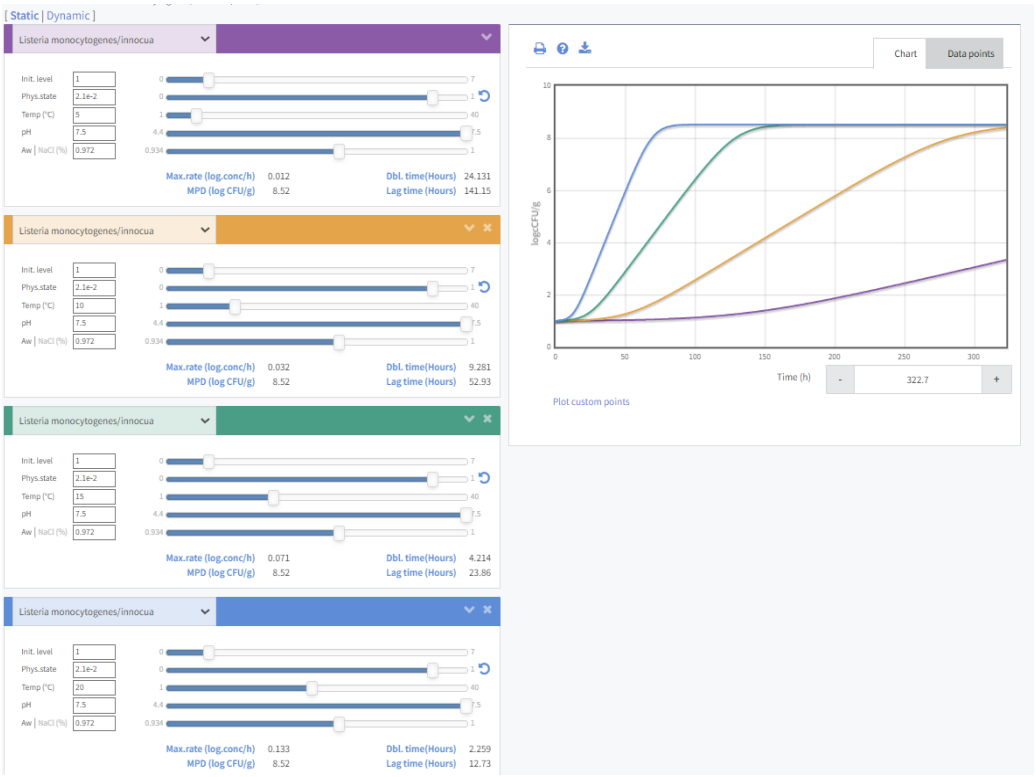


Figure3.6 Microbial growth of Listeria monocytogenes at different temperature.

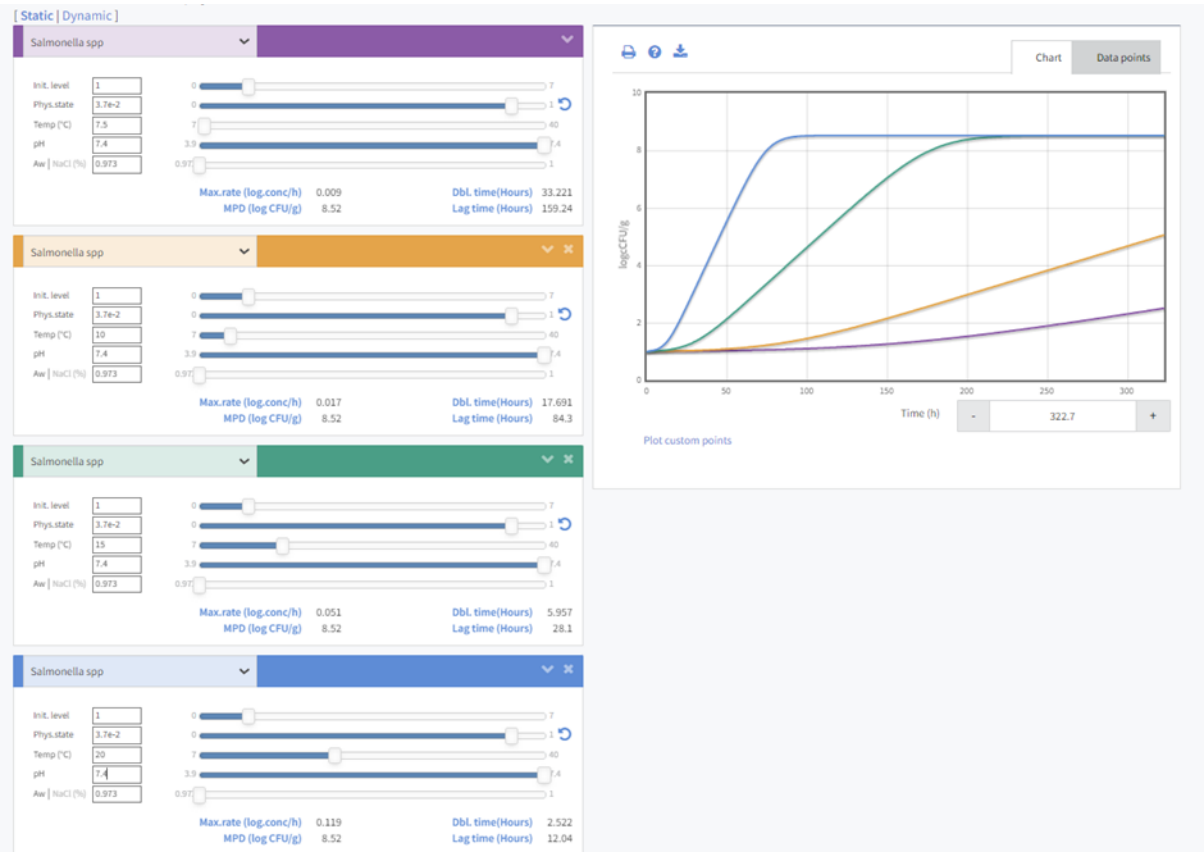


Figure 3.7 Microbial growth of salmonella spp at different temperature.

Table 3.5 Time required for Escherichia coli to reach the targeted level.

Temp (℃)	Initial level (Log 10)	Time (h) to each the target level (Log 10)
10	1	328
15	1	117.4
20	1	51

Table 3.6 Time required for Listeria monocytogenes to reach the targeted level.

Temp (℃)	Initial level (Log 10)	Time (h) to each the target level (Log 10)
5	1	295.6
10	1	113.6
15	1	51.6
20	1	27.8

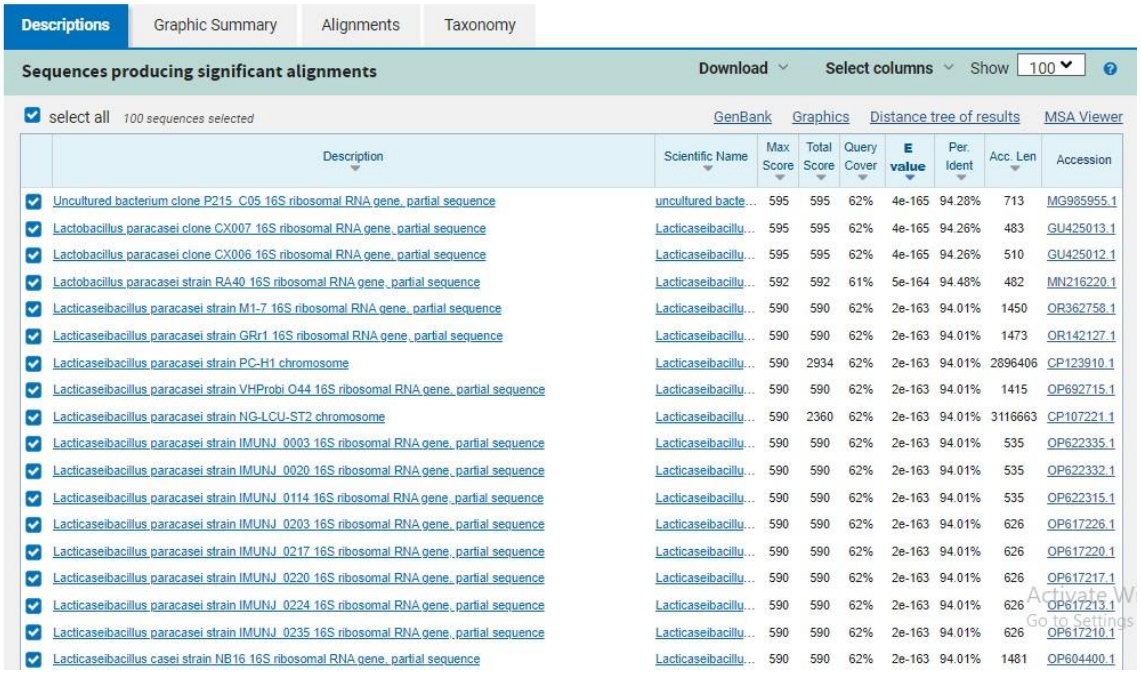
Table 3.7 Time required for salmonella spp to reach the targeted level.

Temp (℃)	Initial level (Log 10)	Time (h) to each the target level (Log 10)
7.5	1	378
10	1	201.2
15	1	67.8
20	1	28.8

Figures 3.5, 3.6, and 3.7 illustrate the growth rate of E. coli, Listeria monocytogenes, and Salmonella spp. at various temperatures. Tables 3.5, 3.6, and 3.7 present the time required for each microorganism to reach the target log10 level at different temperatures.

3.6 Genotypic Identification

The Nucleotide sequence was uploaded into blasting feature of NCBI (National Centre of Biotechnology Information) to obtain the similarity data of sequencing to pre-uploaded database.



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Uncultured bacterium clone P215_C05 16S ribosomal RNA gene, partial sequence	uncultured bacte...	595	595	62%	4e-165	94.28%	713	MG985955.1
Lactobacillus paracasei clone CX007 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	595	595	62%	4e-165	94.26%	483	GU425013.1
Lactobacillus paracasei clone CX006 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	595	595	62%	4e-165	94.26%	510	GU425012.1
Lactobacillus paracasei strain RA40 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	592	592	61%	5e-164	94.48%	482	MN216220.1
Lacticaseibacillus paracasei strain M1-7 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	1450	OR362758.1
Lacticaseibacillus paracasei strain GRr1 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	1473	OR142127.1
Lacticaseibacillus paracasei strain PC-H1 chromosome	Lacticaseibacillu...	590	2934	62%	2e-163	94.01%	2896406	CP123910.1
Lacticaseibacillus paracasei strain VHProbi O44 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	1415	OP692715.1
Lacticaseibacillus paracasei strain NG-LCU-ST2 chromosome	Lacticaseibacillu...	590	2360	62%	2e-163	94.01%	3116663	CP107221.1
Lacticaseibacillus paracasei strain IMUNJ_0003 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	535	OP622335.1
Lacticaseibacillus paracasei strain IMUNJ_0020 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	535	OP622332.1
Lacticaseibacillus paracasei strain IMUNJ_0114 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	535	OP622315.1
Lacticaseibacillus paracasei strain IMUNJ_0203 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	626	OP617226.1
Lacticaseibacillus paracasei strain IMUNJ_0217 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	626	OP617220.1
Lacticaseibacillus paracasei strain IMUNJ_0220 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	626	OP617217.1
Lacticaseibacillus paracasei strain IMUNJ_0224 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	626	OP617213.1
Lacticaseibacillus paracasei strain IMUNJ_0235 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	626	OP617210.1
Lacticaseibacillus casei strain NB16 16S ribosomal RNA gene, partial sequence	Lacticaseibacillu...	590	590	62%	2e-163	94.01%	1481	OP604400.1

Figure 3.8 Blasting description results of the sequence of isolate from MRS.

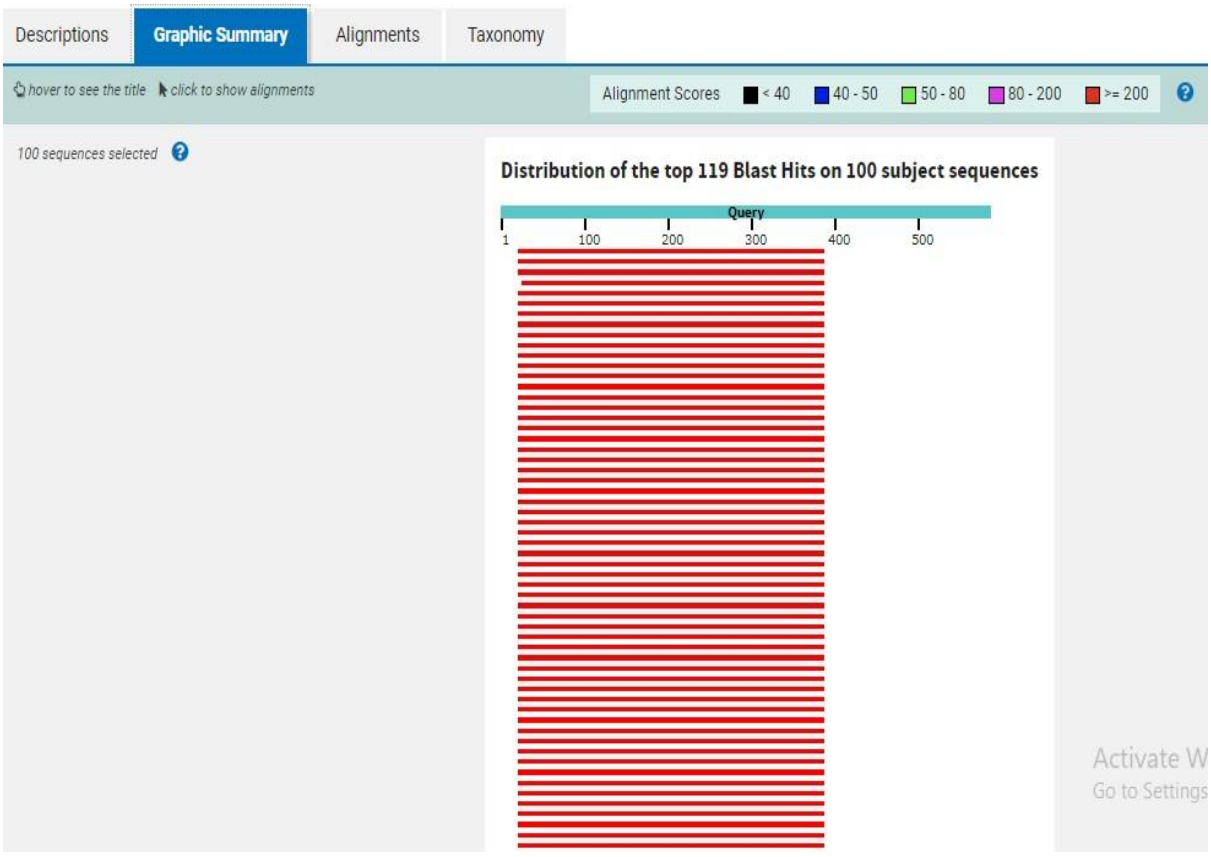


Figure 3.9 Blasting graph results of the sequence of isolate from MRS.

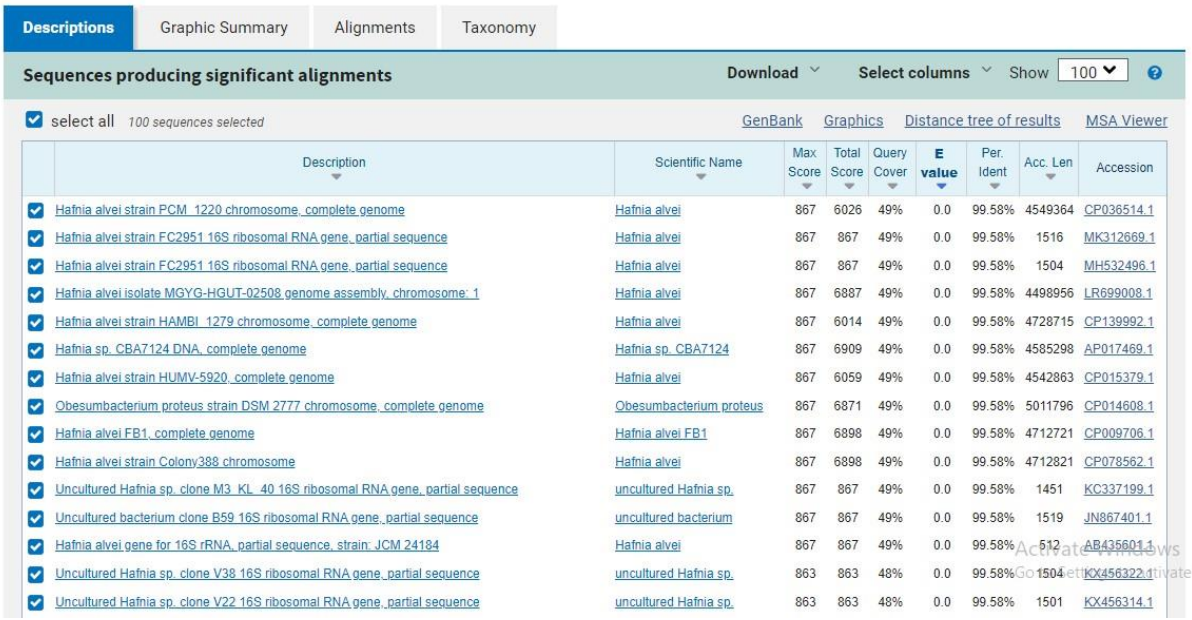
Descriptions | Graphic Summary | Alignments | **Taxonomy**

Reports | **Lineage** | Organism | Taxonomy

100 sequences selected

Organism	Blast Name	Score	Number of Hits	Description
Bacteria	bacteria		102	
. uncultured bacterium	bacteria	595	1	uncultured bacterium hits
. Lacticaseibacillus paracasei	firmicutes	595	91	Lacticaseibacillus paracasei hits
. Lacticaseibacillus casei	firmicutes	590	2	Lacticaseibacillus casei hits
. Lacticaseibacillus sp.	firmicutes	590	1	Lacticaseibacillus sp. hits
. Lacticaseibacillus paracasei subsp. tolerans	firmicutes	590	2	Lacticaseibacillus paracasei subsp. tol
. Lacticaseibacillus paracasei subsp. paracasei	firmicutes	590	4	Lacticaseibacillus paracasei subsp. pa
. bacterium Urffh22	bacteria	584	1	bacterium Urffh22 hits

Figure 3.10 Blasting taxonomy results of the sequence of isolate from MRS.



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download Select columns Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Hafnia alvei strain PCM 1220 chromosome, complete genome	Hafnia alvei	867	6026	49%	0.0	99.58%	4549364	CP036514.1
Hafnia alvei strain FC2951 16S ribosomal RNA gene, partial sequence	Hafnia alvei	867	867	49%	0.0	99.58%	1516	MK312669.1
Hafnia alvei strain FC2951 16S ribosomal RNA gene, partial sequence	Hafnia alvei	867	867	49%	0.0	99.58%	1504	MH532496.1
Hafnia alvei isolate MGYG-HGUT-02508 genome assembly, chromosome: 1	Hafnia alvei	867	6887	49%	0.0	99.58%	4498956	LR699008.1
Hafnia alvei strain HAMBI 1279 chromosome, complete genome	Hafnia alvei	867	6014	49%	0.0	99.58%	4728715	CP139992.1
Hafnia sp. CBA7124 DNA, complete genome	Hafnia sp. CBA7124	867	6909	49%	0.0	99.58%	4585298	AP017469.1
Hafnia alvei strain HUMV-5920, complete genome	Hafnia alvei	867	6059	49%	0.0	99.58%	4542863	CP015379.1
Obesumbacterium proteus strain DSM 2777 chromosome, complete genome	Obesumbacterium proteus	867	6871	49%	0.0	99.58%	5011796	CP014608.1
Hafnia alvei FB1, complete genome	Hafnia alvei FB1	867	6898	49%	0.0	99.58%	4712721	CP009706.1
Hafnia alvei strain Colony388 chromosome	Hafnia alvei	867	6898	49%	0.0	99.58%	4712821	CP078562.1
Uncultured Hafnia sp. clone M3_KL_40 16S ribosomal RNA gene, partial sequence	uncultured Hafnia sp.	867	867	49%	0.0	99.58%	1451	KC337199.1
Uncultured bacterium clone B59 16S ribosomal RNA gene, partial sequence	uncultured bacterium	867	867	49%	0.0	99.58%	1519	JN867401.1
Hafnia alvei gene for 16S rRNA, partial sequence, strain: JCM 24184	Hafnia alvei	867	867	49%	0.0	99.58%	512	AB436601.1
Uncultured Hafnia sp. clone V38 16S ribosomal RNA gene, partial sequence	uncultured Hafnia sp.	863	863	48%	0.0	99.58%	1504	KX456322.1
Uncultured Hafnia sp. clone V22 16S ribosomal RNA gene, partial sequence	uncultured Hafnia sp.	863	863	48%	0.0	99.58%	1501	KX456314.1

Figure 3.11 Blasting descriptive results of the sequence of isolate from VRBG.

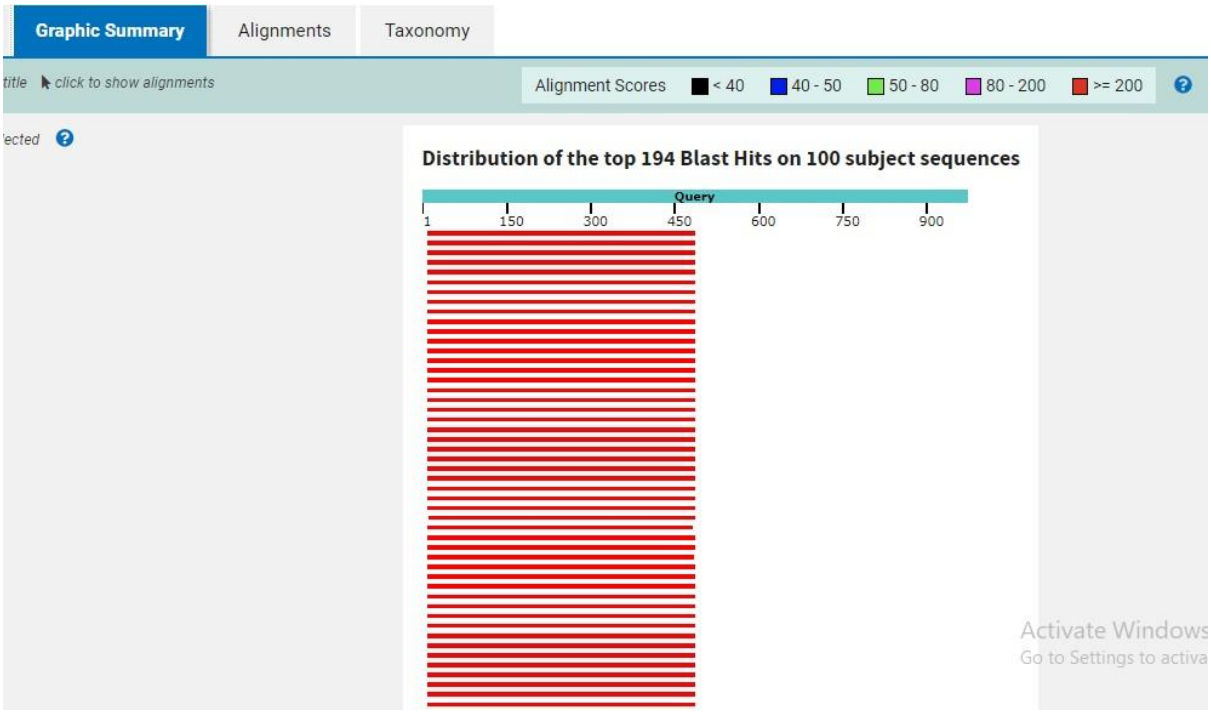
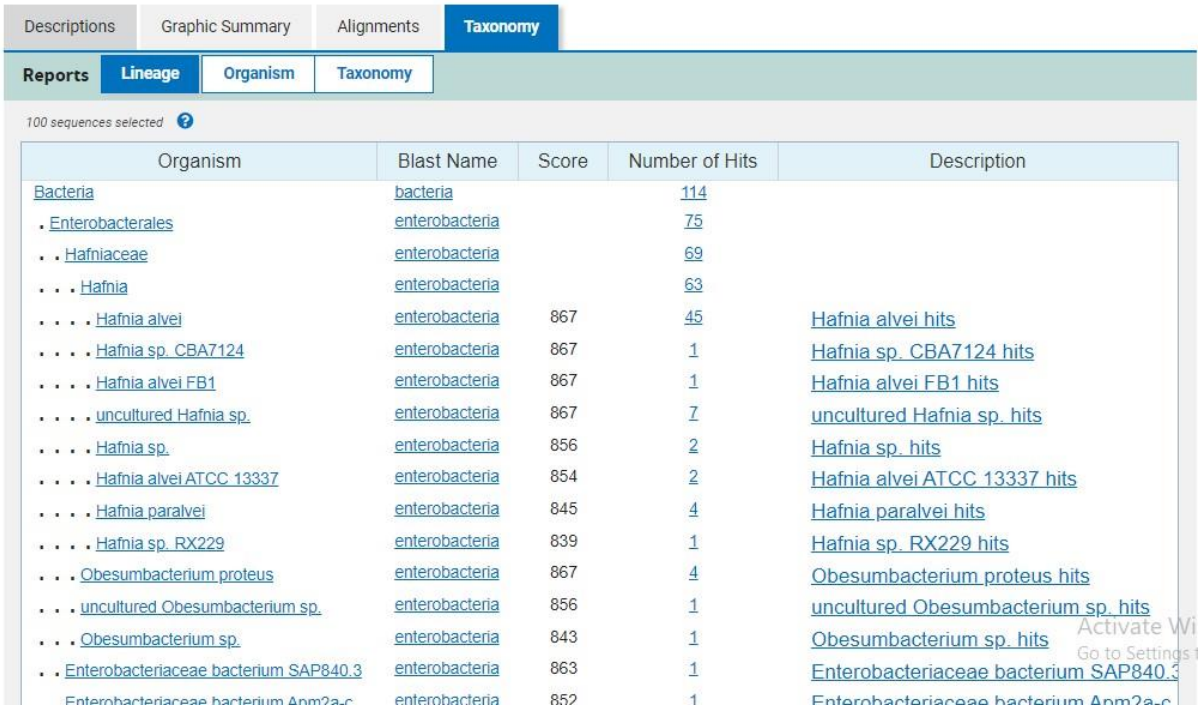


Figure 3.12 Blasting graph results of the sequence of isolate from VRBG.



Organism	Blast Name	Score	Number of Hits	Description
Bacteria	bacteria		114	
. Enterobacterales	enterobacteria		75	
. . Hafniaceae	enterobacteria		69	
. . . Hafnia	enterobacteria		63	
. . . . Hafnia alvei	enterobacteria	867	45	Hafnia alvei hits
. . . . Hafnia sp. CBA7124	enterobacteria	867	1	Hafnia sp. CBA7124 hits
. . . . Hafnia alvei FB1	enterobacteria	867	1	Hafnia alvei FB1 hits
. . . . uncultured Hafnia sp.	enterobacteria	867	7	uncultured Hafnia sp. hits
. . . . Hafnia sp.	enterobacteria	856	2	Hafnia sp. hits
. . . . Hafnia alvei ATCC 13337	enterobacteria	854	2	Hafnia alvei ATCC 13337 hits
. . . . Hafnia paralvei	enterobacteria	845	4	Hafnia paralvei hits
. . . . Hafnia sp. RX229	enterobacteria	839	1	Hafnia sp. RX229 hits
. . . Obesumbacterium proteus	enterobacteria	867	4	Obesumbacterium proteus hits
. . . uncultured Obesumbacterium sp.	enterobacteria	856	1	uncultured Obesumbacterium sp. hits
. . . Obesumbacterium sp.	enterobacteria	843	1	Obesumbacterium sp. hits
. . Enterobacteriaceae bacterium SAP840.3	enterobacteria	863	1	Enterobacteriaceae bacterium SAP840.3
. . Enterobacteriaceae bacterium Apm2a-c	enterobacteria	852	1	Enterobacteriaceae bacterium Apm2a-c

Figure 3.13 Blasting taxonomy results of the sequence of isolate from VRBG.

Table 3.8 Comparison of phenotypic and genotypic identifications of both pasteurized and unpasteurized cheese.

Cheese	Isolates	API	16s rRNA
Tunworth	Isolate from VRBG	Hafnia alvei	Hafnia alvei
	Isolate from MRS	Lactobacillus paracasei	Lactobacillus paracasei
Petit Camembert	Isolate from VRBG	Not found	Lactobacillus paracasei
	Isolate from MRS	Lactobacillus paracasei	Lactobacillus paracasei

4 Discussion

When comparing the microbial content of pasteurised and unpasteurised cheese, we observed a clear trend on VRBG, NA, and BPA plates. Cheeses made from unpasteurised milk showed significantly higher microbial counts compared to those made with pasteurised milk. However, on MRS plates, the results were unexpected. The microbial count appeared to be higher in pasteurised cheese than in unpasteurised cheese. This discrepancy could be due to an error, but the exact cause remains unclear.

Gram staining and oxidase tests yielded identical results for both cheese samples. However, catalase testing on VRBG and MRS plates produced opposite

outcomes. This suggests a high degree of similarity in the types of microbes present in the cheeses, with the primary difference being a much higher microbial count in the unpasteurised cheese. To identify the specific microorganisms, API 20 E and API 50 CHL tests were performed, and the results were analysed using APIWEB. The analysis revealed the presence of *Salmonella enterica spp.* in the unpasteurised cheese (identified by API 20 E) and Lactobacillus paracasei ssp. in both cheeses (identified by API 50 CHL)." (Fox and McSweeney, 2017).

- Clarity: It clarifies that the catalase test results were opposite, not the microbes themselves.
- Focus: It emphasizes the key findings: similar microbe types, higher count in unpasteurised cheese, and identification of specific species.
- Accuracy: It clarifies that Lactobacillus paracasei ssp. was found in both cheeses, not just the pasteurised one.
- Flow: It improves the flow by separating observations from results.

To verify the API results, DNA sequencing was performed and analysed using BLAST. While the blood agar test revealed alpha hemolytic activity, this finding may not necessarily indicate a safety concern. Hafnia alvei and Lactobacillus paracasei, both commonly found in soft cheeses, can also exhibit alpha hemolysis. The hemolysis test aimed to detect the presence of virulence factors, specifically alpha-hemolysin. Although alpha-hemolysin is a common exotoxin produced by Escherichia coli, known to enhance virulence in infections, its presence in this context doesn't automatically confirm E. coli presence. (Castro et al., 2020).

- **Clarity**: It clarifies the purpose of DNA sequencing and BLAST analysis.
- **Specificity**: It emphasizes that alpha hemolysis doesn't solely indicate a safety hazard in soft cheeses.
- **Accuracy:** It clarifies the purpose of the hemolysis test - finding virulence factors.
- **Focus:** It emphasizes that alpha-hemolysin can be produced by other bacteria besides E. coli.

5 Conclusion

Microbial examination of cheese is a very important procedure in the cheese industry for two reasons one is food safety and other one is controlling the internal microbial load to maintain the desired sensory properties of the moulded cheese.
The microbial evaluation of Tunworth cheese revealed a predominance of *Lacticaseibacillus paracasei* and *Hafnia alvei.* These species are commonly present in soft cheeses and hence are recognized as safe for human consumption.
Pasteurization has a significant role in controlling the undesired growth of microorganisms which can spoil the quality of cheese and can even turn out to be hazardous when consumed.
Raw milk cheeses have a lower shelf life and is more prone to spoilage. Proper handling and strictly following the safety protocols form farm to fork is necessary to ensure that there is no in-process and post process contamination.

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