INVESTIGATING THE METABOLIC POTENTIAL OF WILD STREPTOCOCCUS THERMOPHILUS STRAINS (ACIDIFICATION, AROMATISATION) FOR POTENTIAL NEW DAIRY BIOTECHNOLOGICAL USES.

ABDELMALEK MERIBAI*[1] & ABDELOUAHAB DIAFAT[2]

1*.Faculty of Nature and Life Sciences, El Bachir El Ibrahimi University, (34000) Bordj Bou Arreridj, Algeria. Laboratory of Characterization and Valorization of Natural Products.
2. Pr. Abdelouahab DIAFAT: Department of Biological Sciences, Faculty of Nature and Life Sciences, El Bachir El Ibrahimi University, (34000) Bordj Bou Arreridj, Algeria. Laboratory of Characterization and Valorization of Natural Products.

Corresponding author*[1] E- mail: abdelmalek.meribai@univ-bba.dz.

Received: 25- 04- 2024 **Published:** 28- 11- 2024

Abstract:

Background*: Streptococcus thermophilus generates the aromatic chemicals acetaldehyde and diacetyl from lactose, pyruvate, and citrate. These molecules are found in yoghurts, cheeses, and other fermented beverages. However, there is a lack of clarity regarding the regulation of the metabolic pathways involved in biosynthesis. In developing nations, there are issues with the methods used to choose strains and dose these scents.* ***Objective****: The study aims the selection of wild S. thermophilus strains, of plant origin, isolated from Algerian black fermented table olives, under the constraint of long cryopreservation, to explore their potential for aromatization (Acetaldehyde), acidification (decrease in pH and lactate production) in an assimilated industrial environment.* ***Material & Methods****: Following strains reactivation on M17 broth and Reconstituted Skim Milk (RSM), flavouring strains are chosen using polarography (Figure: 1), and the acetaldehyde dosage is determined using a photometric technique. Acidification kinetics (pH drop, lactate generation) are tracked on RSM at 4H, 8H, 16H, and 24H. Probiotic capabilities of chosen strains were investigated by tracking their growth and survival on M17 medium supplemented at 1% and 0.5% with several prebiotic chemicals, including: Gome Arabic (GA), Gluco-Oligo-Saccharid (GOS), Fructo-Oligo-Saccharid (FOS), and Psylium.* ***Results:*** *study allowed the selection of three S. thermophilus strains (MTS1, MTS2, MTS3), having acidifying profile: (pH: 4.91 (STM1), 4.88 (STM2) and 4.61(STM3). Lactate (D): 83ºD (STM1), 94ºD (STM2) and 103ºD (STM3), production of acetaldehyde on polarograph and by photometric method: Acetaldehyde (in ppm): 0.03 (STM1), 0.066 (STM2) and 0.095 (STM3) after 24H.* ***Conclusion:*** *This is the first report, conducted in Algeria, contributing to the exploration/evaluation of the behavior of wild Streptococcus thermophilus strains, of plant origin, in an assimilated industrial environment. They also provide a theoretical basis for understanding/mastering the strains metabolism/a new experimental plan for the selection of indigenous S. thermophilus strains with acidification and aromatization potential.* **Keywords:** Acetaldehyde, Acidification, Dairy technology, Polarography, *Streptococcus thermophiles*

RÉSUMÉ:

Contexte: *Streptococcus thermophilus* génère des composés aromatiques acétaldéhyde et diacétyle à partir du lactose, du pyruvate et du citrate. Ces molécules sont présentes dans les yaourts, les fromages et autres boissons lactées fermentées. Cependant, la régulation des voies métaboliques impliquées dans la biosynthèse de ces aromes n'est pas clairement établie. Le choix des methods analytiques pour dosages des aromes, techniques pour selection des souches lactiques performantes, pose probleme dans les pays sous développés. **Objectifs**: L'étude vise la sélection des souches autochtones S. thermophilus, d'origine végétale, isolées d'olives noires fermentées d'Algérie, sous la contrainte d'une longue cryoconservation, afin d'explorer leur potentiel d'aromatisation (acétaldéhyde), d'acidification (diminution du pH et de la production de lactate) en milieu industriel assimilé. **Matériel et méthodes**: Après réactivation des souches sur bouillon M17, lait écrémé reconstitué (RSM), les souches aromatisantes sont selectionnées par polarographie et le dosage en acétaldéhyde est déterminé par une technique photométrique. Les cinétiques d'acidification (chute de pH, génération de lactate) sont suivies sur RSM à 4H, 8H, 16H et 24H. Aptitudes probiotiques/survie des souches selectionnées, ont été étudiées suivant leur croissance/ survie sur milieu M17 supplémenté à 1% et 0,5% par plusieurs produits chimiques prébiotiques, dont: Gome Arabic (GA), Gluco-Oligo-Saccharide (GOS), Fructo-Oligo-Saccharide (FOS) et Psylium. **Résultats**: L'étude a permis la selection de trois souches de *S. thermophilus* (MTS1, MTS2, MTS3), ayant un profil acidifiant: pH: 4.91 (STM1), 4.88(STM2) and 4.61(STM3), Lactate (In D): 83ºD (STM1), 94ºD(STM2) and 103ºD(STM3). Production d'acétaldéhyde au polarographe et par méthode photométrique: (Acetaldehyde (in ppm): 0.03 (STM1), 0.066 (STM2) and 0.095 (STM3) apres 24H d'incubation.**Conclusion:** Il s'agit du premier rapport, réalisé en, contribuant à l'exploration/évaluation du comportement de souches autochtones *S. thermophilus,* d'origine végétale, dans un environnement industriel assimilé, fournisse également une base théorique pour comprendre/maîtriser le métabolisme de l'espèce/un nouveau plan expérimental pour la sélection de souches lactiques indigènes de *S. thermophilus* avec un potentiel d'acidification et d'aromatisation.

Mots clés: Acetaldehyde, Acidification, Technologie laitiere, Polarographie, *Streptococcus thermophiles*

Abréviation

CFU: Colony Forming Unit
FOS: Fructo-Oligo-Saccharid
GA: Gum Arabic
G-C: Gas chromatography
GLC: Gas Liquid Chromatography.
GOS: Gluco-Oligo-Saccharid
GRAS: Generally Recognized As Safe.
HDME: Hanging Dropping Mercury Electrode.
HMF: Homofermentative.
HTF: Heterofermentative.
LAB: Lactic Acid Bacteria.
M17media: Selective Media used for Lactococci
mg/kg. milligram/kilogram
MPN/ml: Most Probable Numbers (MPN) per milliliter.
OD: optical density
PPM: Parts Per Million
Psych: Psyllium
RSM: Reconstituted Skimmed Milk 10% V/W
S. thermophilus: Streptococcus thermophilus
SHMT: Serine Hydroxymethyltransferase
V: Volume
W:Weight
WHO: World Health Organization

1. Introduction

Lactic acid bacteria constitute a heterogeneous group, are generally thermophilic, mesophilic, some are psychrotolerant or thermotolerant. According to Frank and Nino, (2002), thermophilic lactic species: genres: *Streptococcus thermophilus and Lactobacillus ssp*, thrive at temperatures between 37ºC and 60°C. In milk technologies that need thermophilic processes, such as acidified fermented milks/ beverages, yoghurts, and cooked cheeses, this bacterial group is more frequently utilised. Thermophilic, homofermentative lactic species are commonly used in dairy technology, particularly in operations that need high temperatures (about 45°C) such making yogurt, acidified fermented milks, and various types of cheese. Frank and Nino, (2002); the species *S. thermophilus* is distinguished by its non-pathogenic nature (saprophytes), its primary habitat of milk and dairy products, the fact that some strains have been isolated from plants, decaying vegetables and fruits (Michaylova *et al*., 2002; 2007), and the fact that it is a GRAS species (Hols *et al*., 2005; Delorme, 2008). *S. thermophilus* differs from the majority of other *streptococci* due to its resistance to temperature, capacity to grow at 52°C, urease, and the comparatively high rate of acidification of milk. El Sharoud et al., *(*2013) state that *Streptococcus thermophilus* is one of the most commonly used lactic, homofermentative, thermophilic species in dairy technology, ranking second only to *Lactococcus lactis* (Hols et al., 2005). It is the only lactic species with urease enzyme (Mora *et al*., 2004; 2005; Zotta *et al*., 2008; El Sharoud et al.,2013; Yu *et al*., 2020; Arioli *et al*., 2017; 2022), ensuring rapid acidification kinetics, by conversion of lactose into lactate, secretion of exopolysaccharides (Wu et al., 2014; Mostefaoui et al., 2015; Taj et al., 2022; Wa et al., 2022) and milk proteolysis (Boulay et al., 2020; 2021; Yu et al., 2020). The synthesis of vitamins, including folic acid (Iyer et al., 2010; 2010; Chaves et al., 2003) and the production of aromatic compounds, including acetaldehyde (Chavez et al., 2003; Benaama et al., 2011; 2012; Tong et al., 2012; Zhao et al., 2024). The species has historically been used in yoghurts, in co-culture with *Lactobacillus bulgaricus* (Courtin and Rul, 2004; Angelov et al., 2009; Herve-Jimenez et al., 2009; Dan et al., 2017), and in mixed cultures with thermophilic lactobacilli and certain actinomycetes species like *Bifidobacterium spp*. (Oliveira et al., 2009; Almeida et al., 2009). In some cheeses in co-culture with the species *Lactobacillus helviticus* (Bernardeau et al., 2008; Zhao et al., 2024) and cooked cheeses such as Emmental, Gruyère and Grana (McSweeney & Souza, 2000). The species *S. thermophilus* having the presumption of safety (GRAS), status proposed by the European Food Safety Authority (Delorme, 2008; Taj *et al.*, 2022). Acetaldehyde is a simple chemical molecule that smells strongly of fruit and is frequently compared to almonds. It can help give some fermented foods—like yoghurt, cheese, sauerkraut, and other fermented beverages/products- their distinctive flavour and scent. Many fruits and alcoholic beverages naturally contain acetaldehyde, which is also frequently added to food for its flavouring qualities. Acetaldehyde is the primary taste of yogurts, some cheeses (Ott *et al*., 1997; Rau et al., 2022), and several acidified fermented milks (Ott et al., 2000; Valero et al., 2001), according to Gezginc et al. (2015). Due to this odour, yoghurts are loved by consumers for their delicious fruity flavour (Bongers et al., 2005; Ott et al., 1997; 2000). Lactic acid bacteria's metabolic processes for acetaldehyde synthesis and control seem to be strain-specific (Chavez et al., 2002; Rau et al., 2022). According to some studies (Xanthopoulous et al., 1994; Georgala et al., 1995; Beshkova et al., 1998) *Lactobacillus delbrueckii subsp. bulgaricus* produces more acetaldehyde in mixed culture than *Streptococcus thermophilus*. However, other studies have found the opposite (Schirch et al., 1985; Ott et al., 1997; Chavez et al., 2002; 2003). According to Chavez et al. (2003), Bongers et al. (2005), and Gezgincet al. (2015), lactic acid bacteria can produce acetaldehyde either directly during milk fermentation by decarboxylating pyruvate through the action of pyruvate decarboxylase or indirectly from acetyl Co-enzyme-A (Acetyl- CoA) through pyruvate dehydrogenase and aldehyde dehydrogenase. Acetaldehyde is also produced from threonine in the lactic species *Streptococcus thermophilus* by cleaving threonine and producing acetaldehyde and glycine through the enzymatic action

of serine hydroxymethyl transferase (SHMT) (Chavez *et al*., 2003; Benaama et al., 2011; 2012; Tong *et al*., 2012; Rau *et al*., 2022). Furthermore, the functional and technological characteristics of lactic strains, such as their ability to produce exopolysaccharides, flavour (by producing diacetyl and acetaldehyde), acidify, and proteolyze, determine their usage in technological applications. One significant industrial problem is the identification of novel thermophilic lactic ferments from milk and dairy products. New strains with particular characteristics have been chosen and developed as a result of the advancement of knowledge in this area. These strains need to be more widely recognised, and their metabolic processes need to be well studied and understood. Furthermore, the generation of aromatic compounds during lactic fermentation is a highly intricate biochemical process that is connected to the physiological and biochemical traits of the flavouring lactic strain as well as other exogenous factors, including the culture medium's oxidation-reduction potential (Martin et al., 2011), the culture conditions and fermentation medium composition (Escamilla-Hurtado et al., 2005; Baranowska, 2006), the fermentation's duration and temperature (Guo et al., 2021), the conservation process (Valero et al., 2001), the conservation technique, and the final product's packaging (Saint-Eve et al., 2008). Acetaldehyde can be generated via the metabolism of sugar, amino acids, nucleotides, and pyruvate in the lactic species *S. thermophilus* (Chavez et al., 2002; Zha et al., 2015). Preheating the milk (Lorenzen et al., 2003), the temperature and duration of fermentation, and heat shock to thermophilic starter cultures (Ozer and Ataso, 2002) are other variables that could affect the synthesis of acetaldehyde. Acetaldehyde production levels in *S. thermophilus* species seem to be caused by metabolic pathways of peptide and amino acid degradation; however, it’s unclear how these biochemical pathways are regulated and what part important enzymes-particularly urease-play in acetaldehyde production. In this context, the study intends to investigate the industrial behaviour of native wild lactic strains *Streptococcus thermophilus*, isolated from fermented black table olives from Algeria, in pure culture, and evaluate their metabolic activity on a restricted fermentation medium: skimmed milk reconstituted at 10%W/V. The kinetics of acidification (production of lactate) and aromatisation (production of acetaldehyde) will be monitored over the course of 24 fermentations. Research on their development on selective medium M17 (Tarzaghi & Sandine, 1975) supplemented at 1% and 0.5% with several peribotic chemicals, including Gome arabic (GA), Fructo-Oligo-Saccharid (FOS), Gluco-Oligo-Saccharid (GOS) and Psylium.

2.Material and methods

2.1. Origin of *S. thermophilus* strains

The lactic strains *S. thermophilus* are part of the collection of *Laboratory of Characterization and Valorization of Natural Products- El Bachir El Ibrahimi University, (34000)- Bordj Bou Arreridj, Algeria*. Initially isolated from the varieties of Algerian black table olives- were identified, characterized, preserved at (-80 °C) on M17 Broth medium supplemented with 30% glycerol as cryoprotecteur.

1.2.Revivification/reactivation of *S. thermophilus* strains

The reactivation of lactic strains was carried out by double inoculation with a 24-hour interval, on M17 broth (Tarzaghi and Sandine, 1975) at 43ºC then by incubation for 24 hours, on the fermentation medium (Reconstituted Skimmed Milk: 10% W/V).

1.3.Characterization of lactic strains

The lactic strains *S. thermophilus* are part of the collection of *Laboratory of Characterization and Valorization of Natural Products- El Bachir El Ibrahimi University, (34000)- Bordj Bou Arreridj, Algeria*.

Initially isolated from the varieties of Algerian black table olives- were identified, characterized, preserved at (-80°C) on M17 Broth medium supplemented with 30% glycerol as cryoprotecteur.

1.4.Selection of lactic acid strains by polarography

The idea is to apply a decreasing potential to the mercury drop, which enables the measurement of the current intensity caused by the chemicals in the medium oxidising or reducing in accordance with the applied potential. When the potential approaches a threshold value that can reduce or oxidise the substance, the presence of an electroactive component in the solution will cause the current to increase quickly. All traces of oxygen are eliminated by bubbling nitrogen or an inert gas because oxygen can also change the quantitative response of an element being analysed (Bilinski et al., 1976; Gilbert, 1997).

2.5. Selection of flavoring lactic strains by polarography

2.5.1. Principle of polarography

The selection of strains *S. thermophilus*, and detection of aroma (acetaldehyde), in trace form, on polarograph (*Pol 150 Polarographic Analyzer Radiometer analytical-Trace Lab- France*) consisted of a passage of the standard: Acetaldehyde: (Table- 1) then injecting a volume of culture media for the *S. thermophilus* strains. The selection of strains was done by eliminating strains that did not give acetaldehyde, after 24 hours of fermentation, on the restricted medium: skimmed milk reconstituted at 10% (W/V).

2.6. Monitoring of the acetaldehyde kinetics production

2.7. Photometric method

The kinetics of acetaldehyde formation were monitored using a photometric approach in accordance with the methodology suggested by Yüksekdağ et al.,(2004).

The inoculation of thermophilic lactic strains (*S. thermophilus*) was carried out on restricted fermentation medium (reconstituted skimmed milk 10%) and concerned only the strains (flavoring) which gave positive results on polarograph. The inoculation was done in a ratio of 2% (V/V) (2ml of the reactivated/revified bacterial suspension, in 100ml of the fermentation medium (RSM: Reconstituted Skimmed Milk 10% V/W) then by incubation at 43ºC. The reading of the results (after 4H, 8, 16H, and 24H) was carried out on a spectrophotometer (Shimadzu UV-Spectrophotometr- China) against a standard solution (Raw Acetaldehyde Etalon Table 1) of pure acetaldehyde (Conversion factor on packaging: 1.8 mg/m3=1 ppm at 25°C) at different incubation time intervals: 04H, 08H, 16H and 24H. Results are expressed in parts per million (ppm).

2.8.Acidification kinetics (titration of lactic acid production)

Only the strains that had been previously chosen on the polarograph were involved in the acidification kinetics monitoring. As per the method outlined by Demirci and Gunduz, (1994) and utilised by Chamba and Prost, (1989); Thomas and Chamba, (2000), it was carried out by titrating lactate in Dornic degree (°D) on fermentation medium (skim milk reconstituted at 10%) and then incubating for 24 hours at various intervals: 04H, 08H, 16H, and 24hours.

Table 1: Characteristics of Acetaldehyde Standard

Packaging: 5ML
Molecular weight: (g/mol) 44.05
Storage temperature: 2- 8°C
Density: 0.785g/ml at 25°C
Melting point (°C): -125 C(lit.)
Boiling point (°C): at 1013 hPa

Molecular formula: C_2H_4O
Purity ACS reagent. >=99.5%

2.9. Comparaison/exploitation of résults

The results obtained were compared with those cited in the scientific literature

3. Results

Table 2: Characterization of *S. thermophilic* strains after their reactivation, selection on polarograph			
Aspects of colonies on M17 medium	Whitish, creamy rounded		
Selected *S. thermophilus* strains	**MTS1**	**MTS2**	**MTS3**
Appearance of cells in the fresh state	Coccies, Diplococci, short chains		
Gram stain	+	+	+
Mobility	–	–	–
Catalase test	–	–	–
Oxidase test	–	–	–
Nitrate reductase test	–	–	–
Growth at temperature: 10°C	–	–	–
Growth at temperature: 45°C	+	+	+
Growth at temperature: 55°C	+	+	+
Thermo resistance for 30 min at 60ºC	+	+	+
Growth in the presence of 02% Na Cl	+	+	+
Growth in the presence of 04.5% Na Cl	+	+	+
Growth in the presence of 06.5% Na Cl	+	+	***D**
Growth on alkaline medium (pH: 9.6)	–	–	–
Growth on Sherman blue milk	–	–	–
Action on litmus milk	+	+	+
Homofermentation test	***HMF**	***HMF**	***HMF**
Citratase search	+	+	–
Acetaldehyde	+	+	+
Acetoin production (Test)	+	+	+
*MTS: S. thermophilus, strains (+) positif test, *HMF: homofermetative, *HTF: Heterofermentative, *D: variable			

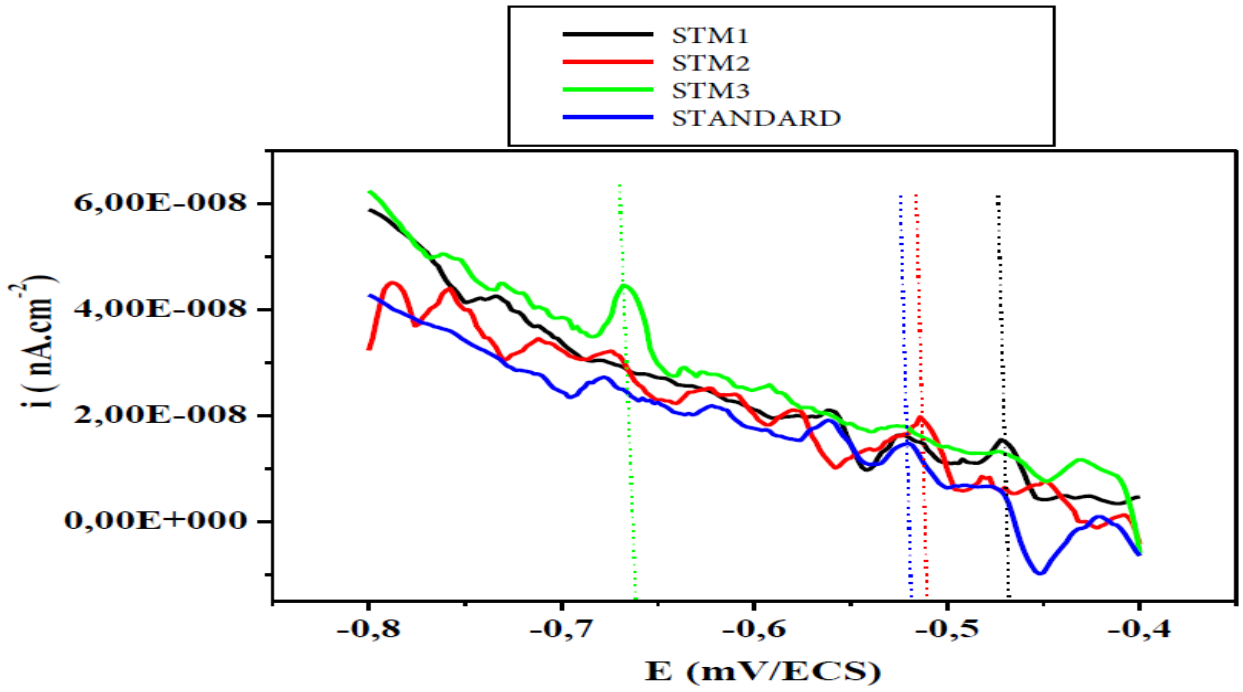

Figure 1: Selection of acetaldehyde-producing strains by polarography

Tableaux: 3, 4 and 5- Growth and survival of strains on M17 medium pH Values (Table: 3), acidification kenitics (Tabe: 4) and Acetaldehyde production kinetics (in ppm) in Table 5:

Table 3: Monitoring of pH values for 24 Hours

	Table 3: Monitoring of pH values for 24 Hours				
Strains/Time	**4H**	**6H**	**8H**	**16H**	**24H**
STM1	6.22		5.61	5.11	4.91
STM2	6.12		5.48	5.29	4.88
STM3	6.17		5.21	4.87	4.61

Table 4: Acidification kinetics (Lactate in °D)

	Table 4: Acidification kinetics (Lactate in °D)				
Strains/Time	**4H**	**6H**	**8H**	**16H**	**24H**
STM1	38	43	54	68	83
STM2	42	49	59	72	94
STM3	44	51	63	79	103

Tableaux: 6, 7, 8 abd 9- Growth and survival of strains on M17 medium supplemented with 1% and 0.5% prebiotics :

Table 6: Growth kinetics on M17 media supplemented with *G.A

Medium	M17 added with 1% G.A			M17 added with 0.5% G.A		
Time (H)	24h	48h	72h	24h	48h	72h
MTS1	0.095	0.097	0.184	0.083	0.083	0.083
MTS2	0.312	0.493	0.632	0.391	0.417	0.499
MTS3	0.215	0.233	0.261	0.273	0.293	0.300

*GA: Gum Arabic

Table 7: Growth kinetics on M17 media supplemented with GOS

Medium	M17 added with 1% GOS			M17 added with 0.5 % *GOS		
Time(H)	24h	48h	72h	24h	48h	72h
MTS1	0.191	0.211	0.225	0.075	0.115	0.135
MTS2	0.189	0.207	0.215	0.231	0.249	0.256
MTS3	0.195	0.213	0.332	0.272	0.324	0.341

*GOS: Gluco-Oligo-Saccharid

Table 8: Growth kinetics on M17 media supplemented with *FOS

Medium	M17 added with 1% FOS			M17 added with 0.5 % *FOS		
Time(H)	24h	48h	72h	24h	48h	72h
MTS1	0.098	0.118	0.123	0.112	0.123	0.136
MTS2	0.289	0.305	0.315	0.231	0.249	0.256
MTS3	0.295	0.311	0.342	0.162	0.172	0.194

*FOS: Fructo-Oligo-Saccharid

Table 9: Growth kinetics on M17 media supplemented with Psylium

Medium	M17 added with 1% Psylium			M17 added with 0.5 % Psylium		
Time(H)	24h	48h	72h	24h	48h	72h
MTS1	0.217	0.226	0.237	0.337	0.334	0.367
MTS2	0.278	0.324	0.360	0.321	0.332	0.348
MTS3	0.232	0.253	0.278	0.347	0.366	0.406

4. Discussion

As the ideal growth temperature for the species under study in pure culture, 43°C appears to be a good choice for the incubation temperature. For the isolation, incubation, and/or development of *S. thermophilus*, multiple authors have employed the same T°C (Degeest et al., 1999; 2002; Lorenzen et al., 2003; Grade et al., 2004). Zisu and Shah (2003) investigated the impact of temperature, pH, and protein supplementation on the species *S. thermophilus1275's* ability to produce exopolysaccharides. The results of various combinations of these parameters indicated that the highest amounts of E.P.S. were generated at 37°C and 40°C at pH: 4.08; E.P.S. production of was reduced at the high temperature of 45°C. Accordingly,

several authors (Degeest et al., 2002; Tabasco et al., 2007). The polarography selection procedure (Figure:1), which was followed by the colorimetric dosage used for the main aroma (acetaldehyde), revealed that all strains achieve their maximum production at 43ºC, the ideal temperature in relation to the thermophilic lactic strains under study, after about 16 hours of incubation (Chavez et al., 2003). Singh et al., (1980) had previously observed that *S. thermophilus* species produces higher acetaldehyde and lactate when grown in pure culture as well as when co-cultured with *Lactobacillus bulgaricus*. Acidification and the generation of acetaldehyde at 37ºC and 42ºC are entirely the species fault. Additionally, Benaama et al., (2011; 2012) reported that after just 10 hours of incubation at 42ºC in pure culture, on a culture medium (skimmed milk reconstituted at 10% (W/V)), (native) strains of *S. thermophilus* isolated from raw cow's milk produced the highest amount of acetaldehyde. Monitoring the kinetics of aroma production for all of our *S. thermophilic* strains over the of a 24-Hour incubation period revealed two successive stages: an ascending stage following 16 hours of fermentation, during which acetaldehyde is used to synthesise aromas, and a second, declining stage (descending), during which the medium's aromatic compounds are depleted. This second phase of decline, common to all our studied strains, MTS1, MTS2 and MTS3 (Table: 2), can be explained by a probable exhaustion of aroma precursors in the culture medium which is nutritionally poor. Added to this are the experimental constraints, linked to the losses of a part of the aromatic compounds, due to their volatile nature. According to Imhof et al., (1994), the production of aromatic compounds in the culture medium occurred during the first twenty hours of fermentation and during the coagulation of milk. Furthermore, Beshkova et al., (1998) observed that thermophilic lactic strains in both pure and mixed cultures exhibited the highest aromatising activity (production of aromas) as early as 07 hours of milk coagulation, while the highest acetaldehyde concentrations were obtained between 22 and 23 hours of fermentation. These findings are completely consistent with our own. The production of acetaldehyde seems to be lactic strain dependent (Chavez et al., 2002; 2003). Xanthopoulous et al.,(1994) and Yüksekdağ et al.,(2004) developed the colorimetric method for determining acetaldehyde, which Lindsay and Day,(1965) used. This method is more sensitive, allowing for the determination of aromas even at low concentrations or even at trace levels in PPM (Xanthopoulous et al., 1994). Additionally, the colorimetric methods are more precise and reproducible, enabling the dosage of aromas at low traces: acetoin at 57 µM and diacetyl at 12 µM, according to the comparative study of aromatic compounds and for the same samples (Xanthopoulous et al., 1994). Furthermore, the most popular fast analytical technique for measuring several aromatic chemicals, including acetaldehyde, ethanol, and diacetyl, in concentration ranges that match those of fermented milks and other dairy products is gas-liquid chromatography. The high repeatability limitations of this method, which range from 65 µM for ethanol to 250 µM for acetaldehyde, are a drawback (Xanthopoulous et al., 1994). Georgala et al., (1995) investigated the production of the primary aromatic compounds (acetaldehyde, diacetyl, and acetoin) by gas chromatography (G-C) in a study on raw sheep's milk and yoghurt made from the same milk. The fermentations were carried out by five strains of *Streptococcus thermophilus*, four strains of *Lactobacillus bulgaricus*, and twenty combinations of these two species. Among the volatile chemicals examined, the study found that the generation of aromas appears to be strain dependant. Acetaldehyde, the main product of the strains in both pure and mixed cultures, reaches maximum values of 13 to 14 mg/kg. However, very little acetoin and diacetyl were generated. In the same, the authors found that *Lactobacillus bulgaricus* strains generate significantly more acetaldehyde than *Streptococcus thermophilus* strains. Acetaldehyde formation is reciprocally stimulated by the strains in a mixed culture. However, Beshkova et al.,(1998) found that the lactic starter strains of yoghurt in mixed cultures had the highest aromatic activity when comparing the generation of aromatic compounds in pure and mixed cultures. The strain *Lactobacillus bulgaricus*, on the other hand, had, a greater capacity for flavouring, producing organic acids between 2 and 10 degrees Celsius. Imhof et al. (1994) claim that

alcohols and aldehydes are produced straight from milk and do not come from fermentation. Only five ingredients actually affect the finished product's scent. Only three of the 32 aromatic molecules that were measured by gas- chromatography had an aromatic effect on the fermentation product, according to Imhof et al. (1995). Acetaldehyde production appears to be strain-dependent, with thermophilic starter strains producing 03 to 04 times more butanedione in mixed cultures. In this regard, Baranowska, (2006), in a study on the effect of milk composition as a culture medium, enriched with different components: (lactose (10g/L), glucose (0.70g/L), sodium proteinate (25g/L), sodium citrate (03 g/L), citrate (01g/L) and threonine (01 and 03 g/L) on the formation of aromas of different fresh yogurts, after 07 and 14 days of storage, showed that threonine positively affects the rate of acetaldehyde formation and reaches values of 22, 24 and 56 mg/L, in several types of yogurts studied. Furthermore, strains of *Streptococcus thermophilus* were used to study the growth (biomass) and acetaldehyde production on skimmed milk that had been reconstituted at 10% and supplemented with 05 Mmol and 10 Mmol of threonine. The results showed that after 10 hours of fermentation, the amino acid had no effect on growth or biomass production, but acetaldehyde production increased significantly (Benaama et al., 2011). According to Benaama et al., (2012), after the addition of 0.5% and 03% lactose and sucrose to the M17 medium (Tarzaghi and Sandine, 1975) containing three thermophilic (indigenous) lactic strains *Streptococcus thermophilus* BN1, BN2 and BN3, isolated from cow's milk, a significant increase in acetaldehyde production was recorded.Despite the constraints of long-term freezing (- 80ºC), our strains STM1, STM2 and STM3 exhibited acidifying profiles at 43ºC, technologically interesting with: pH: 4.91 (STM1), 4.88(STM2) and 4.61(STM3). Lactate (In D) : 83ºD (STM1), 94ºD(STM2) and 103ºD(STM3). Acetaldehyde (in ppm): 0.03 (STM1), 0.066 (STM2) and 0.095 (STM3).According to Lucas and Reyrolle (1989), the acidity in Dornic degree (ºD), is the expression of the acidity developed in the fermentation medium (skimmed milk reconstituted at 10% W/V), by fermentation of lactose and production of lactic acid. It should be noted that our lactic strains, previously isolated, preserved in M17 broth supplemented with 30% glycerol, at (- 80°C), revived, having failed to coagulate the reconstituted skimmed milk, after 24H, at 43ºC, were eliminated from this study Table: 2. This process allowed us to select strains that were technologically interesting. According to Chamba and Prosts, (1989) rule, any lactic strain of *Streptococcus thermophilus* that exerts a variation of ΔpH of 0.50 over a period of 04 hours, is considered technologically interesting, especially when we take into account the limitations of freezing, pure culture, and the buffering power exerted by the fermentation medium Tables 3, 4 and 5. According to a comparative analysis of the effects of freezing and lyophilization as two methods of lactic strain preservation over a six-month period, the first method (freezing) produced lactic strains with the highest survival rates, intracellular enzymatic activity, and the lowest rate of autolysis (Kendyl and Al Soda, 2015). The growth kinetics of *S. thermophilus* strains were monitored for three days: 24H, 48H and 72H. Using a scanning spectrophotometer (JENWAY7315- Japan) to measure the optical density (OD) at 600 nm. Hanoune et al., (2015) used the same wavelength for the absorbance of lactic acid bacteria isolated from plants Table 6. Overall, the prebiotic extracts accelerated the exponential growth of the strains, especially at a rate of 0.5%. Tables: 6, 7, 8 and 9. Research has observed that prebiotic extracts have activating/accelerating effects on the growth of LAB, specifically on *S. thermophilus* strains: G.A. or exudates from acacia Senegal (*Senegalia senegal*) trees (Calame et al., 2008, Elawad et al., 2021). Furthermore, Tandon et al., (2019); M'hir et al.,(2021) acknowledged the beneficial impacts of FOS extracts on LAB development in general. Elli et al.,(2008); Lotfipour et al., (2012); Shree et al.,(2017) have also reported the beneficial effects of Psylium extract or *Plantago Ovata growth* on LAB.

5. Conclusion

Despite the artisanal experimental conduct, the wild strains of *S. thermophilus:* (MTS1, MT2, and MT3) of plant origin, reactivated after a lengthy cryopreservation period and displayed aromatising (acetaldehyde) and acidifying (pH decrease, lactate production) profiles of technological interest. They were cultured in poor (simple) SRM medium without any additives, taking into account the buffer effect and constraints of the culture medium. All things considered, the strains' development was exponentially accelerated by the prebiotic extracts employed with M17 medium, particularly when the rate was 0.5%. Exploring their potential in mixed culture, with thermophilic and mesophilic lactic species present, on media supplemented with prebiotics and amino acids would be interesting in perspective.

P.S. To our knowledge, this is the first report, conducted in Algeria, contributing to the exploration/evaluation of the behavior of wild *S. thermophilus* strains, of plant origin, in an assimilated industrial environment. They also provide a theoretical basis for understanding/mastering the metabolism of the species/an experimental plan for the selection of indigenous *S. thermophilus* lactic strains with aidification and aromatization potential.

NB: The study's findings add to our knowledge the molecular compounds (acetaldehyde) and aroma characteristics of milks fermented by wild *Streptococcus thermophilus* strains.

They also offer an experimental foundation for the selection of autochthonous thermophilic lactic strains that have the ability to acidify and aromatise milk.

During fermentation and storage, it helps to enhance the flavour and smell of dairy products.

Conflict of interest: The authors hereby declare that they have no known conflict of interests could have appeared to influence the work reported in this paper.

Acknowledgments:This work was supported by the Algerian Ministry of Higher Education and Scientific Research (DGRSDT, MESRS), Algeria.

6. Références

Almeida KE, Tamime AY, Oliveira MN (2009). Influence of total solids contents of milk whey on the acidifying profile and viability of various lactic acid bacteria. LWT- Food Science and Technology 42 : 672– 678 Aresearch note.

Angelov M, Kostov G, Simova E, Beshkova D, Koprinkova- Hristova P. (2009). Protocoopération factors in yoghurt starter cultures. Revue de Génie Industriel 3: 05- 12.

Arioli, S., Della Scala, G., Martinović, A., Scaglioni, L., Mazzini, S., Volonté, F., ... & Mora, D. (2022). In *Streptococcus thermophilus*, ammonia from urea hydrolysis paradoxically boosts acidification and reveals a new regulatory mechanism of glycolysis. *Microbiology Spectrum*,*10*(3), e02760- 21.

Arioli, S., Della Scala, G., Remagni, M. C., Stuknyte, M., Colombo, S., Guglielmetti, S., ... & Mora, D. (2017). *Streptococcus thermophilus* urease activity boosts *Lactobacillus delbrueckii subsp. bulgaricus* homolactic fermentation. *International journal of food microbiology*, *247*, 55- 64.

Baranowska M (2006).Intensification of the synthesis of flavour compounds in yogurt by milk enrichment with their precursors. Pol J Food Nutr Sci 15(56): 5- 11.

Bennama R, Ladero V, Alvarez MA, Fernández M, Bensoltane A (2012). Influence of lactose and sucrose on growth and acetaldehyde production by three strains of *Streptococcus thermophiles*. International Conference on Applied Life Sciences (ICALS 2012) Turkey September 10- 12 2012.

Bennama R, Rechidi-Sidhoum N, Bensoltane A (2011). Effect of Threonine on Growth and Acetaldehyde Production by Streptococcus thermophilus. World Applied Sciences Journal 15(2): 160- 163.

Bernardeau M, Vernoux J.P, Henri-Dubernet S, Guéguen M (2008). Safety assessment of dairy microorganisms: The Lactobacillus genus. *International Journal of Food Microbiology* 126: 278- 285.

Beshkova D, Simova E, Frengova G, Simov Z (1998). Production of flavor compounds by yogurt starter cultures. Journal of Industrial Microbiology & Biotechnology 20: 180- 186.

Bilinski, H., Huston, R., & Stumm, W. (1976). Determination of the stability constants of some hydroxo and carbonato complexes of Pb (II), Cu (II), Cd (II) and Zn (II) in dilute solutions by anodic stripping voltammetry and differential pulse polarography. *Analytica Chimica Acta*, *84*(1), 157-164.

Bongers SR, Hoefnagel MHN, Kleerebezem M (2005). High-level acetaldehyde production in Lactococcus lactis by metabolic engineering. Applied and Environ Microbiol 71(2): 1109- 1113.

Boulay, M., Al Haddad, M., & Rul, F. (2020). *Streptococcus thermophilus* growth in soya milk: Sucrose consumption, nitrogen metabolism, soya protein hydrolysis and role of the cell-wall protease PrtS. *International Journal of Food Microbiology*, 335, 108903.

Boulay, M., Metton, C., Mézange, C., Oliveira Correia, L., Meylheuc, T., Monnet, V., ... & Juillard, V. (2021). Three distinct proteases are responsible for overall cell surface proteolysis in Streptococcus thermophilus. *Applied and Environmental Microbiology*, *87*(23), e01292- 21.

Calame, W., Weseler, A. R., Viebke, C., Flynn, C., & Siemensma, A. D. (2008). Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. *British journal of nutrition*, *100*(6), 1269-1275.

Chamba J F, Prost F (1989). Mesure de l'activité acidifiante des bactéries lactiques, thermophiles pour la fabrication de fromage à pâte cuite. Lait 69 : 417- 431.

Chaves AC, Fernandez M, Lerayer AL, Mierau I, Kleerebezem M, Hugenholtz J (2002). Metabolic engineering of acetaldehyde production by *Streptococcus thermophiles*. Applied and Environmental Microbiology, 68(11): 5656- 5662.

Chaves AC, Ruas- Madiedo P, Starrenburg M, Hugenholtz J, Lerayer AL (2003). Impact of engineered *Streptococcus thermophilus* strains over-expressing glyA gene on folic acid and acetaldehyde production in fermented milk. Brazilian Journal of Microbiology 34(suppl.1): 114- 117.

Courtin P, Rul F (2004). Interactions between microorganisms in a simple ecosystem: yogurt bacteria as a study model. Lait 84:125- 134. Delorme C (2008). Safety assessment of dairy microorganisms: *Streptococcus thermophilus*. *International Journal of Food Microbiology* 126: 274- 277.

Dan, T., Wang, D., Wu, S., Jin, R., Ren, W., & Sun, T. (2017). Profiles of volatile flavor compounds in milk fermented with different proportional combinations of *Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus*. *Molecules*, *22*(10), 1633.

Degeest, B., Mozzi, F., De Vuyst,L.,(2002). Effect of medium composition and temperature and pH changes on exopolysaccharide yields and stability during Streptococcus thermophilus LY03 fermentations. International Journal of Food Microbiology.79, 161- 174.

Degeest, B. and De Vuyst, L. (1999). Indication that the Nitrogen source influences both amount and size of exopolysaccharide produced by Streptococcus thermophilus LY03 and modelling of the bacterial growth and exopolysaccharide production in a complex medium. Appl. Environ. Microbiol. 65:2863- 2870.

Demirci M & Gunduz H (1994). Dairy technology handbook Turkey. Hasad Press. Pp. 184.

El Sharoud WM, Delorme C, Darwish MS, Renault P (2013). Genotyping of *Streptococcus thermophilus* strains isolated from traditional Egyptian dairy products by sequence analysis of the phosphoserine phosphatase (serB) gene with phenotypic characterizations of the strains. Journal of Applied Microbiology 112: 329- 337.

Elawad, F. A. E., Sulieman, M. A., & Elkhalil, E. (2021). Effect Of Gum Arabic As Prebiotic On Probiotics And Quality Of Yoghurt During Cold Storage. *Bacterial Empire*. Vol. 4, NO. 2, 236 1.

Elli, M., Cattivelli, D., Soldi, S., Bonatti, M., & Morelli, L. (2008). Evaluation of prebiotic potential of refined psyllium (Plantago ovata) fiber in healthy women. *Journal of clinical gastroenterology*, *42*, S174-S176.

Escamilla-Hurtado ML, Valdés-Martínez SE, Soriano-Santos J, Gómez- Pliego R, Verde-Calvo JR, ReyesDorantes A, Tomasini- Campocosio A (2005). Effect of culture conditions on production of butter flavor compounds by *Pediococcus pentosaceus and Lactobacillus acidophilus* in semisolid maize-based cultures. International Journal of Food Microbiology 105(3): 305- 316.

Frank J. C. and Nino M (2002). The lactic acid bacteria: A literature survery. Critical. Rev.in Microbiology. 28: Pp: 282.

Georgala A, Tsakalidou E, Kandarakis I, Kalantzpoules G (1995). Flavour production in ewe's milk and ewe's milk yoghourt, by single strains and combinations of *Streptococcus thermophilus and Lactobacillus debruekii subsp bulgaricus* isolated from traditional Greek yoghourt. Lait 75: 271-283.

Gezginc, Y., Topcal, F., Comertpay, S., & Akyol, I. (2015). Quantitative analysis of the lactic acid and acetaldehyde produced by *Streptococcus thermophilus and Lactobacillus bulgaricus* strains isolated from traditional Turkish yogurts using HPLC. *Journal of dairy science*, *98*(3), 1426-1434.

Gilbert, B., (1997). Méthodes électrochimiques d'analyse, Faculté des Sciences, Université de Liège. Belgique.

Grade, S., Avila, M., Medina, M.and Nunuz, M. (2004). Fast induction of nisin resistance in Streptococcus thermophilus INIA463 during growth in milk. International Journal of Food Microbiology, 96:165- 172.

Guo, S., Wu, T., Peng, C., Wang, J., Sun, T., & Zhang, H. (2021). Metabolic footprint analysis of volatile metabolites by gas chromatography-ion mobility spectrometry to discriminate between different fermentation temperatures during *Streptococcus thermophilus* milk fermentation. *Journal of Dairy Science*, 104(8), 8541- 8553.

Gyosheva B, Petrova I, Mutafchieva M (1995). Preservation of Streptococcus thermophilus strains after long term storage in lyophilized state. Journal of Culture Collection1: 34- 37.

Hanoune S, Djeghri-Hocine B, Kassas Z, Derradji Z, Boudour A & Boukhemis M (2015). Optimization of *Lactobacillus fermentum DSM 20049* growth on whey and lupin based medium using response surface methodology. *Advance Journal of Food Science and Technology*.(9): 679- 685.

Herve-Jimenez, L., Guillouard, I., Guedon, E., Boudebbouze, S., Hols, P., Monnet, V., ... & Rul, F. (2009). Postgenomic analysis of *Streptococcus thermophilus* cocultivated in milk with Lactobacillus delbrueckii subsp. bulgaricus: involvement of nitrogen, purine, and iron metabolism.*Applied and Environmental Microbiology*, *75*(7), 2062- 2073.

Hols P, Hancy F, Fontaine L, Grossiord B, Prozzi D, Leblond- Bourget N, Decaris B, Bolotin A, Delorme C, Ehrlich D, Guedon E, Monnet V, Renault P, Kleerebezem M (2005). New insights in the molecular biology and physiology of *Streptococcus thermophilus* revealed by comparative. FEMS Microbiology Reviews 29: 435- 463.

Imhof R, Glattli H, Bosset JO (1994).Volatile organic aroma compounds produced by thermophilic and mesophilic mixed strain dairy starter cultures Lebensm Wiss U Technol 27: 442- 449.

Imhof R, Glattli H, Bosset JO (1995). Volatile organic compounds produced by thermophilic and mesophilic single strain dairy starter cultures. Lebensm Wiss U Technol 28: 78- 86.

Iyer R, Tomar SK, Kapila S, Mani J, Singh R (2010). Probiotic properties of folate producing *Streptococcus thermophilus* strains. Food Research International 43 : 103- 110.

Iyer R, Tomar SK, Maheswari TU, Singh R (2010). *Streptococcus thermophilus* strains: Multifunctional lactic acid bacteria. International Dairy Journal 20: 133- 141.

Kendyl S, El Soda M (2015). Influence of Freezing and Freeze Drying on Intracellular Enzymatic Activity and Autolytic Properties of Some Lactic Acid Bacterial Strains. Advances in Microbiology 5: 371- 382. Volume 32(4). Published August, 01, 2016 www.jnsciences.org E-ISSN 2286- 5314

Lindsay RC, Day EA (1965). Rapid quantitative method for determination of acetaldehyde in lactic starter culture. Journal of Dairy Science 48 : 665- 669.

Lorenzen PC, Ebert E, Clawin I, Schlimme E (2003). Influence of heat impact in reconstitute skim milk on the properties of yogurt fermented by roppy or non ropy starters cultures. Nahrung Food 47(5): 349- 353.

Lotfipour, F., Mirzaeei, S., & Maghsoodi, M. (2012). Preparation and characterization of alginate and psyllium beads containing Lactobacillus acidophilus. *The Scientific World Journal*,*2012*(1), 680108.

Lucas, S., & Reyrolle, J. (1989). Etude d'un lot de ferments lactiques mésophiles. Equilibre des flores au cours de la première étape de la fabrication du levain. *Le lait*, *69*(2), 121-130.

M'hir, S., Filannino, P., Mejri, A., Tlais, A. Z. A., Di Cagno, R., & Ayed, L. (2021). Functional exploitation of carob, oat flour, and whey permeate as substrates for a novel kefir-like fermented beverage: An optimized formulation. *Foods*, *10*(2), 294.

Martin F, Cachon R, Pernin K, De Coninck J, Gervais P, Guichard E, Cayot N (2011). Effect of oxidoreduction potential on aroma biosynthesis by lactic acid bacteria in nonfat yogurt. J Dairy Sci 94 : 614– 622 doi: 10.3168/jds.2010- 3372.

McSweeney, P. L., & Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. *Le Lait*, *80*(3), 293-324.

Michaylova M., Isawa K. and Vlachkova. L. (2002). Study on yogurt bacteria isolated from plants in Bulgaria. In: Book of abstracts of the 7th Symposium on *Lactic Acid Bacteria*. Egmondaan Zee. The Netherlands, A 40.

Michaylova M., Minkova S., Kimura K., Sasaki T. and Isawa. K. (2007). Isolation and characterization of Lactobacillus delbrueckii ssp.bulgaricus and Streptococcus thermophilus from plants in Bulgaria. *FEMS Microbiol Lett*, 269, 160- 169.

Mora D, Majuin E, Masiero M, Parini C, Ricci G, Manacini PL, Daffonchio D (2004). Characterization of urease genes cluster of Streptococcus thermophilus. Journal of Applied Microbiology 96: 209- 219.

Mora D, Monnet C, Parini C, Guglielmetti S, Mariani A, Pintus P, Molinari F, Daffonchio D, Manachini PL (2005). Urease biogenesis in *Streptococcus thermophilus*. Int Research Microbiology 156: 897- 903.

Mostefaoui A, Hakem A, Yabrir B, Boutaiba S, Badis A (2015). Evaluation of biofilm formation by exopolysaccharide producer strains of thermophilic lactic acid bacteria isolated from Algerian camel milk. Emirates Journal of Food and Agriculture 27(6): 513- 521.

Oliveira DRP, Perego P, Converti A, De Oliveira MN (2009). Effect of inulin on growth and acidification performance of different probiotic bacteria in co-cultures and mixed culture with *Streptococcus thermophilus*. Journal of Food Engineering 91: 133- 139.

Oliveira DRP, Perego P, Converti A, De Oliveira MN (2009). Growth and acidification performance of probiotics in pure culture and co-culture with *Streptococcus thermophiles* : The effect of inulin LWT. Food Science and Technology 42: 1015- 1021.

Ott A, Fay LB, Chaintreau A (1997). Determination and origin of the aroma impact compounds of yogurt flavor. Jo Agric Food Chem 45: 850- 858.

Ott A, Germond JE, Chaintreau A (2000).Vicinal diketone formation in yogurt13 (C) precursors and effect of branched chain amino acids. J Agri Foo Chem 48(3): 724- 731.

Özer B, Andataso Y (2002). Effect of addition of aminoacids, treatment with galactosidase and use of heatshocked cultures on the acetaldehyde level in yoghurt. International Journal of Dairy Technology 55: No 4.

Rau, M. H., Gaspar, P., Jensen, M. L., Geppel, A., Neves, A. R., & Zeidan, A. A. (2022). Genome-scale metabolic modeling combined with transcriptome profiling provides mechanistic understanding of *Streptococcus thermophilus* CH8 metabolism. *Applied and Environmental Microbiology*, *88*(16), e00780- 22.

Saint-Eve A, Levy C, Le Moigne M, Ducruet V, Souchon I (2008). Quality changes in yogurt during storage in different packaging materials. Food Chemistry 110 : 285- 293.

Schirch V, Hopkins S., Villar E, Angelaccio S (1985). Serine hydroxy-methyltransferase from Escherichia coli : Purification and properties. J Bacteriol 163: 1– 7.

Shree, K. D., Deshpande, H. W., & Bhate, M. A. (2017). Studies on exploration of psyllium husk as prebiotic for the preparation of traditional fermented food "Buttermilk". *Int. J. Curr. Microbiol. App. Sci*, *6*(7), 3850-3863.

Singh, J., Khanna, A., & Chander, H. (1980). Effect of incubation temperature and heat treatments of milk from cow and buffalo on acid and flavor production by *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. *Journal of food protection*,*43*(5), 399- 400.

Tabasco,R.,Paarup,T.,Janer,C.,Pelaez,C.and Requena,T.(2007). Selective enumeration and identification of mixed cultures of Streptococcus thermophilus Lactobacillus delbrueckii sub sp bulgaricus Lb acidophilus Lb paracasei sub sp paracasei and Bifidobacterium lactis in fermented milk.International Dairy.Journal. 17: 1107- 1114.

Taj, R., Masud, T., Sohail, A., Sammi, S., Naz, R., Sharma Khanal, B. K., & Nawaz, M. A. (2022). In vitro screening of EPS-producing *Streptococcus thermophilus* strains for their probiotic potential from Dahi. *Food Science & Nutrition*, *10*(7), 2347- 2359.

Tandon, D, Monzoorul, Haque M, Gote, M, Jain, M, Bhaduri, A, Kumar, Dubey A, & Mande, S. (2019). A prospective randomized, doubleblind, placebo-controlled, doseresponse relationship study to investigate efcacy of fructooligosaccharides (FOS) on human gut microfora. *Scientific Reports* 9: 54.

Tarzaghi BE, Sandine WE (1975). Improved medium for lactic *Streptococcus* and their bacteriophages, Appl Microbiol 29: 807- 813.

Thomas A, Chamba JF (2000). Mise en évidence de l'évolution des aptitudes technologiques des bactéries lactiques thermophiles utilisées dans les fromages à pâte pressée cuite. Sciences des aliments 20: 159- 167.

Tong, D., Xia, C., Hua, B. Q., Jun, L. W., Chao, Z. J., & Ping, Z. H. (2012). Effect of L-Threonine concentrations on acetaldehyde production and glyA gene expression in fermented milk by Streptococcus thermophilus. *Food Biotechnology*, *26*(3), 280- 292.

Valero E, Villamiel M, Miralles B, Sanz J, Martinez-Castro I (2001). Changes in flavor and volatile components during storage of whole and skimmed UHT milk. Food Chemistry 72(1): 51- 58.

Wa, Y., Zhang, C., Sun, G., Qu, H., Chen, D., Huang, Y., & Gu, R. (2022). Effect of amino acids on free exopolysaccharide biosynthesis by *Streptococcus thermophilus* 937 in chemically defined medium. *Journal of Dairy Science*, *105*(8), 6460- 6468.

Wu Q, Tun HM, Chi-Ching LF, Shah NP (2014). Genomic insights into high exopolysaccharide-producing dairy starter bacterium *Streptococcus thermophilus* ASCC 1275. Scientific Reports 4: 4974 | DOI: 10.1038/srep 04974.

Xanthopoulous V, Picque D, Bassit N, Boquien CY, Corrieug G (1994). Methods for the determination of aroma compounds in dairy products: a comparative study. Journal of Dairy Research 61: 289- 297.

Yu, P., Li, N., Geng, M., Liu, Z., Liu, X., Zhang, H., ... & Chen, W. (2020). Lactose utilization of *Streptococcus thermophilus* and correlations with β-galactosidase and urease. *Journal of Dairy Science*,*103*(1), 166- 171.

Yüksekdağ, Z. N., Beyatli, Y., & Aslim, B. E. L. M. A. (2004). Determination of some characteristics coccoid forms of lactic acid bacteria isolated from Turkish kefirs with natural probiotic. *LWT-Food Science and Technology*, *37*(6), 663-667.

Zha, M., Yu, J., Zhang, Y., Wang, H., Bai, N., Qin, Y., ... & Bilige, M. (2015). Study on *Streptococcus thermophilus* isolated from Qula and associated characteristic of acetaldehyde and diacetyl in their fermented milk. *The Journal of General and Applied Microbiology*, *61*(2), 50- 56.

Zhao, X., Ge, Y., Yu, X., Liu, C., Li, H., Wang, X., & Yao, S. (2024). Fermentation Characteristics of Fermented Milk with *Streptococcus thermophilus* CICC 6063 and *Lactobacillus helveticus* CICC 6064 and Volatile Compound Dynamic Profiles during Fermentation and Storage. *Molecules*, *29*(6), 1257.

Zisu B, Shah NP (2003). Effect of pH, temperature, supplement with whey protein concentrate and adjunct cultures on the production of exopolysaccharides by *Streptococcus thermophilus* 1275. Journal of Dairy Science 86(11): 3405- 3415.

Zisu, B., Shah, N.P., (2003). Effect of pH, temperature, supplement with whey protein concentrate and adjuncte cultures on the production of exopolysaccharides by Streptococcus thermophilus1275. Journal of Dairy Science.86,(11): 3405- 3415.

Zotta T, Ricciardi A .M, Rossano R, Parente E (2008). Urease production by *Streptococcus thermophilus*. Food Microbiology 25 : 113- 119.