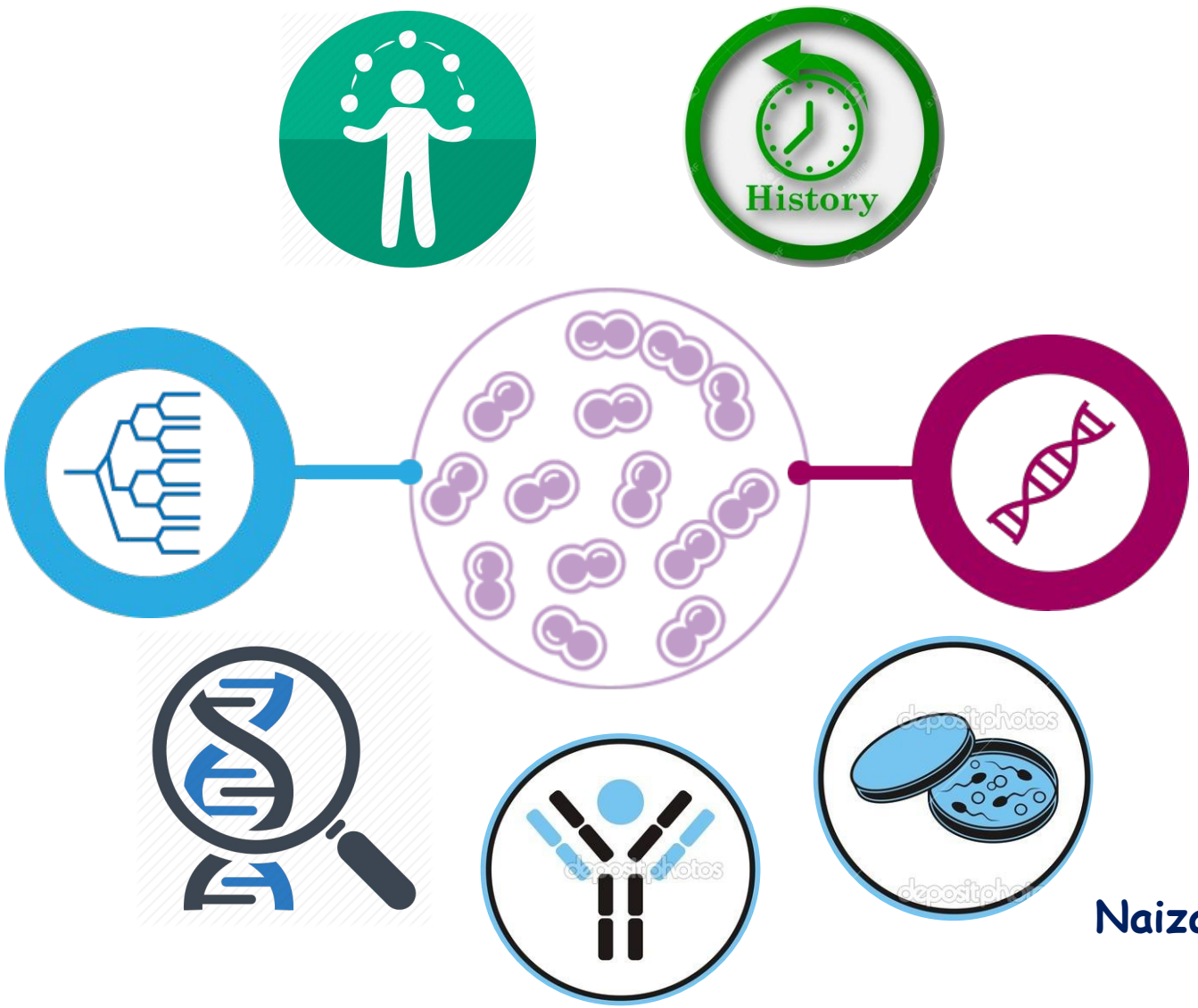
Lactococcus sp.



Done by:
Naizabayeva D.
BT 16-02

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Introduction

Lactic acid bacteria are among the most important groups of microorganisms used in food fermentations. They contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid. As agents of fermentation LAB are involved in making yogurt, cheese, cultured butter, sour cream, sausage, cucumber pickles, olives and sauerkraut, but some species may spoil beer, wine and processed meats.

Meanwhile, they has a great application in sphere of pharmacy, due to their antimicrobial ability and safety for the human, taken a probiotics, produce practically important bactericines , and recently practiced as a delivery agent for therapeutics and vaccines.

2.History and discovery

The common organism associated with the souring of milk was first described by Lister (1878) as 'Bacterium lactis'. In the next thirty years a variety of names and descriptions, including Str. lacticus of Kruse (1903), were added to the literature, and it was left to Lohnis (1909) to clarify the situation. He agreed that it should be placed in the genus Streptococcus and suggested that it should be named 'Streptococcus lactis', thus keeping Lister's original species name. The various synonyms encountered are very numerous and were summarized by Breed (1928). Bergey (1939) has adopted this list in its entirety. Orla Jensen's (1919) description of Str. lactis is as follows. The optimum temperature for growth is 30°C., and when freshly isolated it will coagulate sterile milk at this temperature in less than 24 hr. At the optimum temperature it occurs as a diplococcus or as short chains. Growth below 10 °C. and above 40°C is in general poor. Str. lactis is characterized by failure to ferment sucrose.

2.History and discovery

This group of bacteria, previously designated the lactic streptococci (*Streptococcus lactis* subsp. *lactis* or *S. lactis* subsp. *cremoris*) was placed in this new taxon in 1985 by Schleifer. This discovery were investigated according to results of nucleic acid hybridization studies and immunological relationships of superoxide dismutase, which in its turn demonstrated that *Streptococcus lactis* (and its subspecies), *Lactobacillus xylosus, Lactobacillus hordniae, S. garvieae, S. plantarum* and *S. raffinolactis* are closely related to each other but not to other streptococci. Therefore it was proposed that these taxa be transferred to a new genus *Lactococcus* gen.nov. as *Lactococcus lactis* subsp. *lactis* (including former S. *lactis* subsp. *diacetilactis* and *Lactobacillus xylosus)* comb.nov., L.*lactis* subsp. *cremoris* comb.nov., L.*actis* subsp. *hordniae* comb.nov., L.*garvieae* comb.nov., L.*plantarum* comb.nov. and L.*raffinolactis* comb.nov. The relatedness of these organisms has also been demonstrated by the similarity of their lipoteichoic acid structures, lipid pattern, fatty acid and menaquinone compositions.

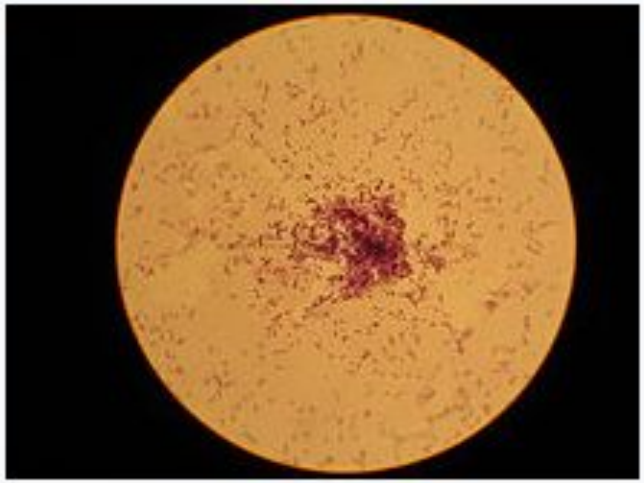
2.History and discovery

Table 1 Comparison of suggested groupings for streptococci and enterococci

Sherman 1937	Jones 1978	Bridge and Sneath 1983	Bergey 1986	Schleifer and Kilpper-Bälz 1987
Pyogenic	Pyogenic	Pyogenic Parapyogenic	Pyogenic	Pyogenic
	Pneumococci	Pneumococcal		*Strep. oralis* group (including '*Strep. milleri*')
Viridans	Oral	Viridans Paraviridans	Oral	*Strep. mutans* group
	Other streptococci Anaerobic streptococci	Thermophilic	Other streptococci Anaerobic	Other streptococci Not streptococci
Enterococcus	Faecal	Enterococcal	Enterococci	*Enterococcus* (separate genus)
Lactic	Lactic	Lactic	Lactic acid cocci	*Lactococcus* (separate genus)

3. Taxonomy

Lactococcus



Lactococcus lactis

Scientific classification

Kingdom:	Bacteria
Division:	Firmicutes
Class:	Bacilli
Order:	Lactobacillales
Family:	Streptococcaceae
Genus:	***Lactococcus*** Schleifer *et al.* 1986

Species

- *L. chungangensis*
- *L. formosensis*
- *L. fujiensis*
- *L. garvieae*
- *L. lactis*
  - *L. lactis* subsp. *cremoris*
  - *L. lactis* subsp. *hordniae*
  - *L. lactis* subsp. *lactis*
  - *L. lactis* subsp. *tructae*
- *L. piscium*
- *L. plantarum*
- *L. raffinolactis*
- *L. taiwanensis*

https://en.wikipedia.org/wiki/Lactococcus

4. Biological features: morphological

Lactococci are homofermentative, microaerophilic Gram-positive bacteria characterized by ovoid cells 0,5-1,2x0,5-1,5 mkrm in size, which appear individually, in pairs, or in chains. Non-motile, do not form spores or capsules.

4, Biological features: physiological

Genus	*Lacto-bacillus*	*Entero-coccus*	*Lacto-coccus*	*Leuconostoc*	*Pedio-coccus*	*Strepto-coccus*
Characteristic						
Morphology	rods	cocci	cocci	cocci	cocci in tetrads	cocci
CO_2 from glucose*	±	–	–	+	–	–
Growth						
at 10°C	±	+	+	+	±	–
at 45°C	±	+	–	–	±	±
in 6.5% NaCl	±	+	–	±	±	–
at pH 4.4	±	+	±	±	+	–
at pH 9.6	–	+	–	–	–	–
Lactic acid configuration	D, L, DL	L	L	D	L, DL	L

+ positive; - negative; ± varies between species

***test for homo- or heterofermentation of glucose: - homofermentation**

+ heterofermentation

http://textbookofbacteriology.net/lactics.html

4. Biological features: physiological

Protein metabolism

Nitrogen source->1. free amino acids in the composition of milk
2. Casein, which composes 80% of all proteins present in milk

1. Essential amino acid for most Lactococci are isoleucine, leucine, valine, histidine, methionine. The concentration of these amino acids in milk is less than 2 mg/l. Amount of nitrogen in this case provides only 2% of the final cell density.

2. Casein, becomes the primary nitrogen source after nonprotein nitrogen is depleted.

The enzymes that form proteolytic system - a cell wall-associated proteinase, an extracellular peptidase (s), amino acid transport system, peptide transport system and intracellular peptidases.

The key enzyme - is a cell-wall associated proteinase (PI- or PIII- type proteinase [PrtP])

Transport systems- di- and tripeptide and an oligopeptide transport system.

4, Biological features: physiological

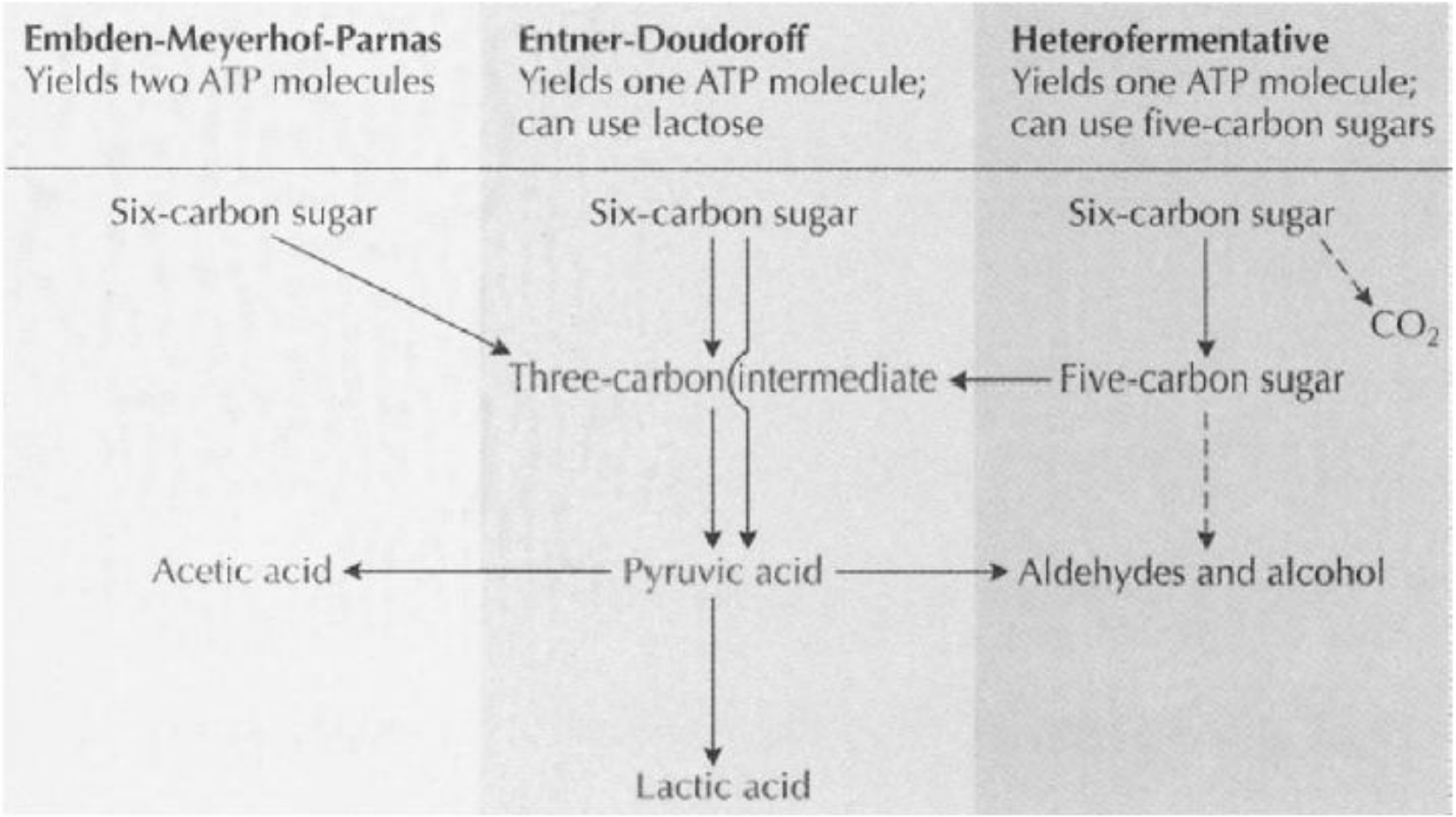
Characteristic	1	2	3	4	5	6	7	8
Growth at:								
4 °C	+	−	+	−	−	−	−	−
40 °C	−	+	−	−	−	−	−	+
Growth in 4% NaCl	−	+	−	+	−	−	−	+
Ammonia from arginine	−	+	−	−	−	−	+	+
Acid from:								
Amygdalin	+	+	+	+	−	−	−	+
Galactose	−	+	+	+	w	+	−	+
Lactose	−	−	+	−	−	+	−	+
Maltose	+	+	+	+	+	+	−	+
Melibiose	−	−	+	−	+	+	−	−
Melezitose	−	−	+	+	−	−	−	−
Methyl α-D-glucoside	−	−	+	+	−	−	−	w
Methyl α-D-mannoside	−	−	+	−	−	−	−	−
Raffinose	−	−	+	−	+	+	−	−
Sucrose	+	−	+	+	+	+	+	−
Trehalose	w	+	+	+	+	+	+	+
Turanose	w	−	+	+	−	−	−	−
D-Xylose	−	−	+	−	+	+	−	+
Hydrolysis of aesculin	+	+	+	+	−	−	−	−

Strains: 1, CAU 28T; 2, L. garvieae KCTC 3772T; 3, L. piscium DSM 6634T; 4, L. plantarum DSM 20686T; 5, L. raffinolactis DSM 20443T; 6,L. lactis subsp. cremoris KCCM 40699T; 7, L. lactis subsp. hordniae KCTC 3768T; 8, L. lactis subsp. lactis KCTC 3769T.

(+) Positive,(-) negative, w- weakly positive

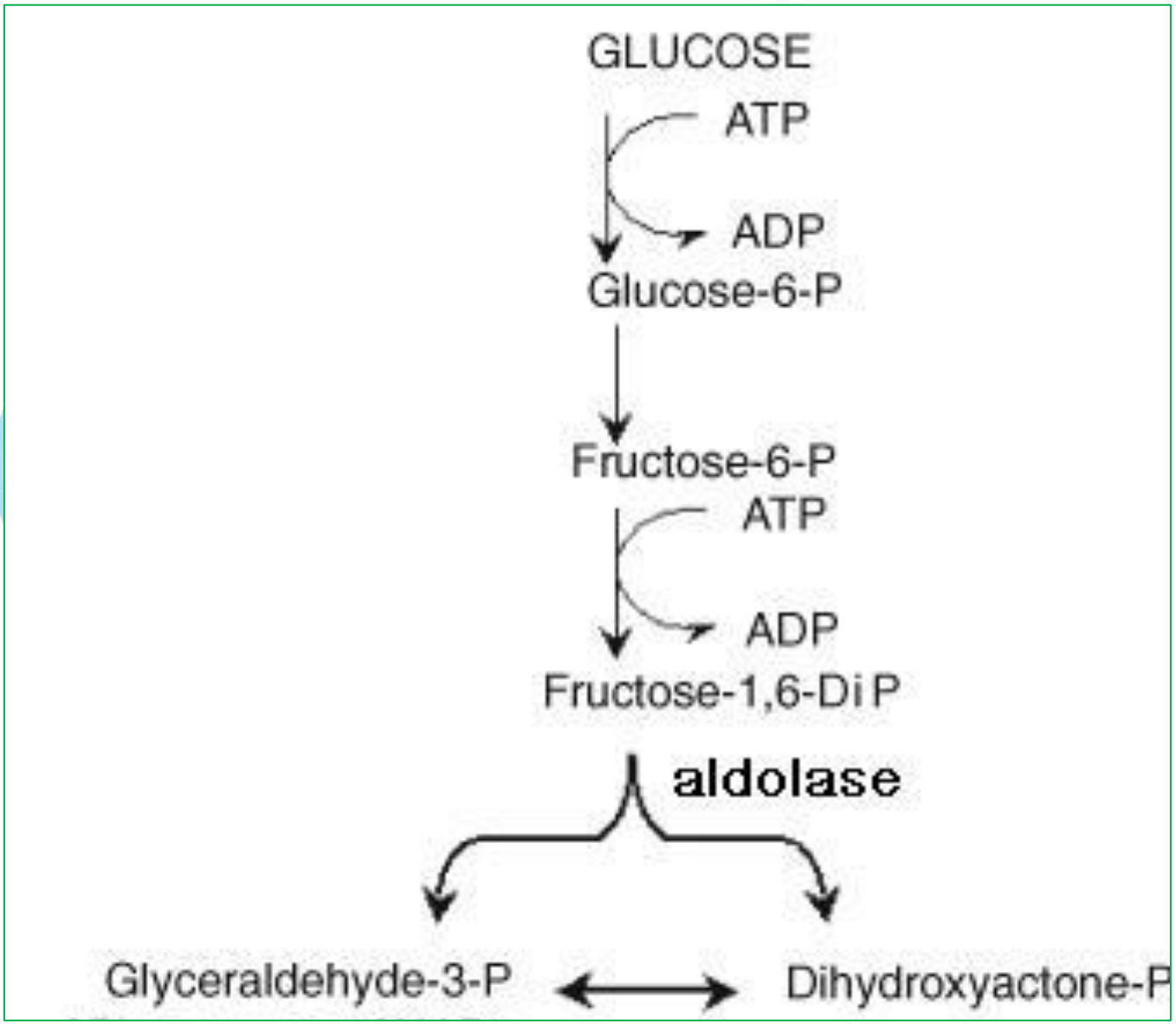
4. Biological features: biochemical

Figure 18.2 Simplified catabolic pathways used in fermented foods.



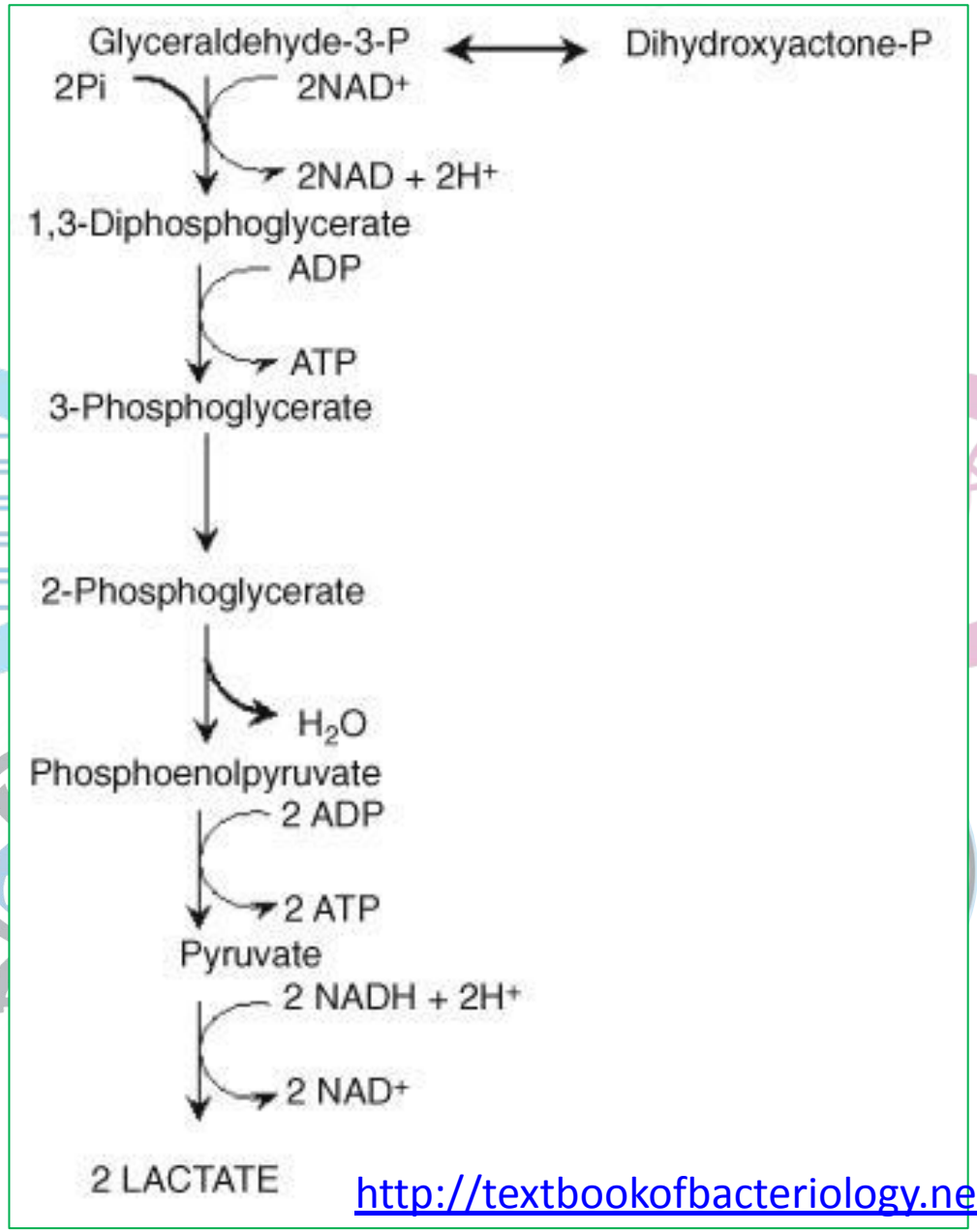
Lactococcus sp. classified as homofermentative or homolactic , cause the end product is only lactic acid and correspond to Embden-Meyerhof-Parnas catabolic pathway. [Montville et al. FOOD MICROBIOLOGY/Chapter 18]

4, Biological features: biochemical



http://textbookofbacteriology.net/lactics_2.html

4, Biological features: biochemical

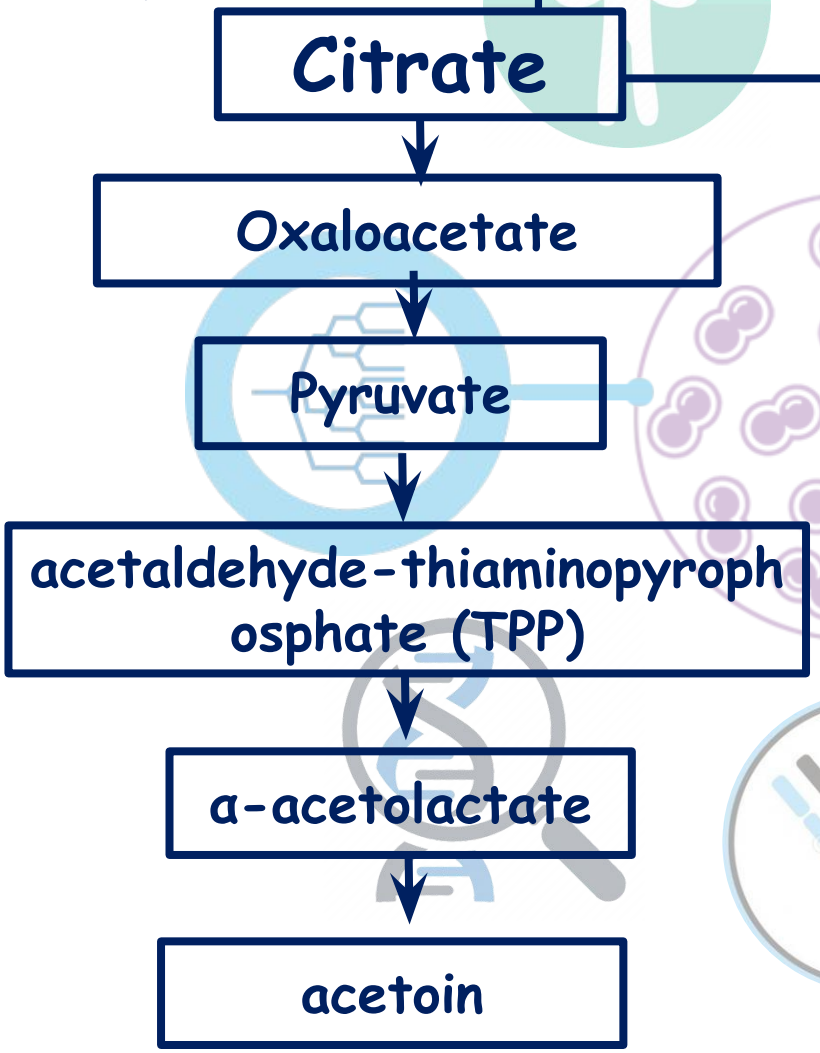


http://textbookofbacteriology.net/lactics_2.html

4, Biological features: biochemical

Capability to metsbolize citrate

Only *Lc. lactis* subsp. *lactis* biovar *diacetylactis* has the ability to metabolize the citrate present in milk.

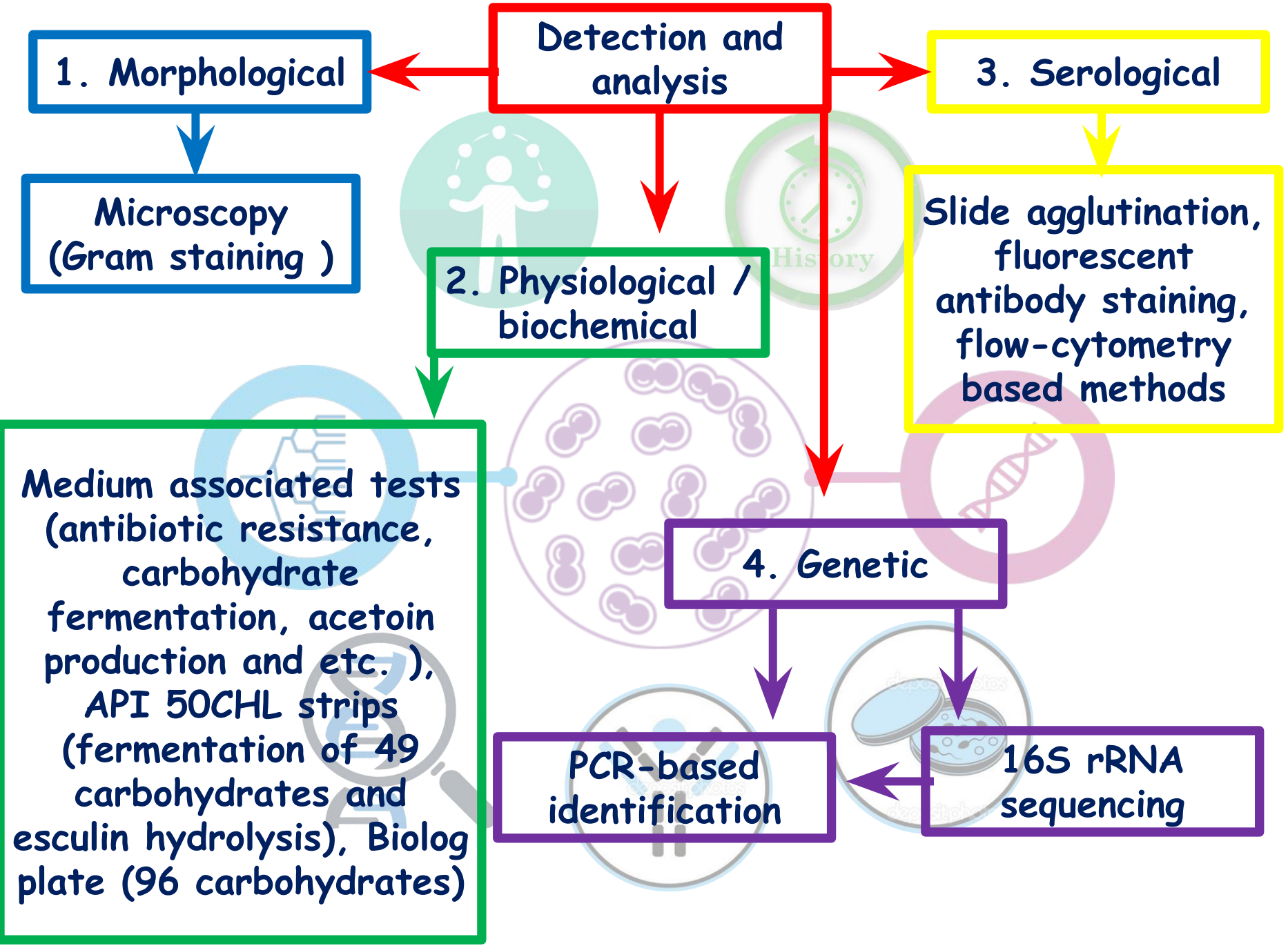


Catalysed by the citrat permease (CitP) encoded in Plasmid-citP, operon-citQRP

Decarboxylation reactions

Total endproducts:

Acetic acid, diacetyl, Acetoin, 2,3 butanediol, CO_2, which contributes flavor development of fermented food


Detection and analysis
1. Morphological
Microscopy (Gram staining)
2. Physiological / biochemical
Medium associated tests (antibiotic resistance, carbohydrate fermentation, acetoin production and etc.), API 50CHL strips (fermentation of 49 carbohydrates and esculin hydrolysis), Biolog plate (96 carbohydrates)
3. Serological
Slide agglutination, fluorescent antibody staining, flow-cytometry based methods
4. Genetic
PCR-based identification
16S rRNA sequencing

5. Phenotypic analysis: morphological

1. Cultural characteristics (color, margine, transparence of colonies and etc.)
2. Microscopic observation (Gram straining)

BUT, It often happens that cells of lactococci themselves extend into a chain, which makes them difficult to differentiate from lactobacilli. The group consisting of *Streptococcus*, *Enterococcus* and *Leuconostoc* also forms cocci that occur as chains or pairs, so it is difficult to distinguish these genera from *Lactococcus* genera on a morphological basis.

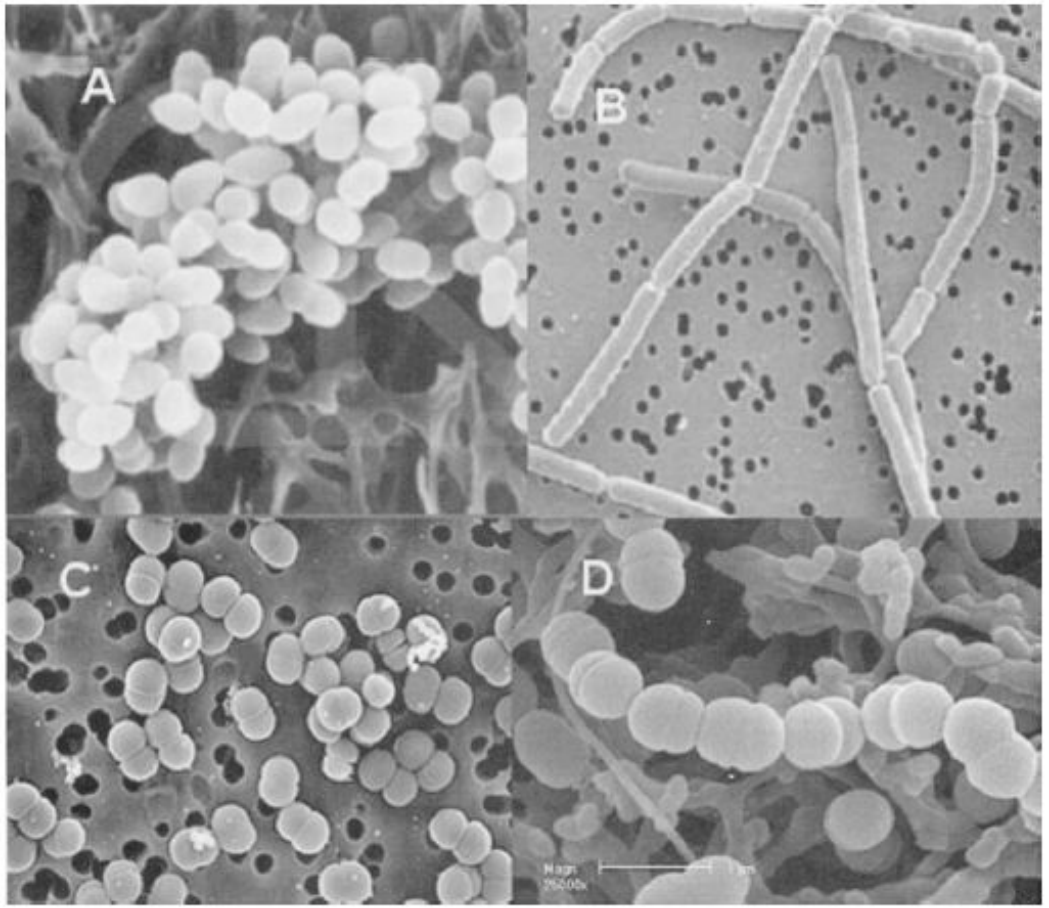


Figure 18.3 LAB associated with food fermentations. **(A)** *Lactococcus;* **(B)** *Lactobacillus;* **(C)** *Pediococcus;* **(D)** *Leuconostoc.* (B and C) Courtesy of the U.S. Department of Energy Joint Genome Institute. (D) Reprinted from G. Kaletunç, J. Lee, H. Alpas, and F. Bozoglu, *Appl. Environ. Microbiol.* **70**:1116–1122, 2004, with permission from the American Society for Microbiology.

5. Phenotypic analysis: physiological

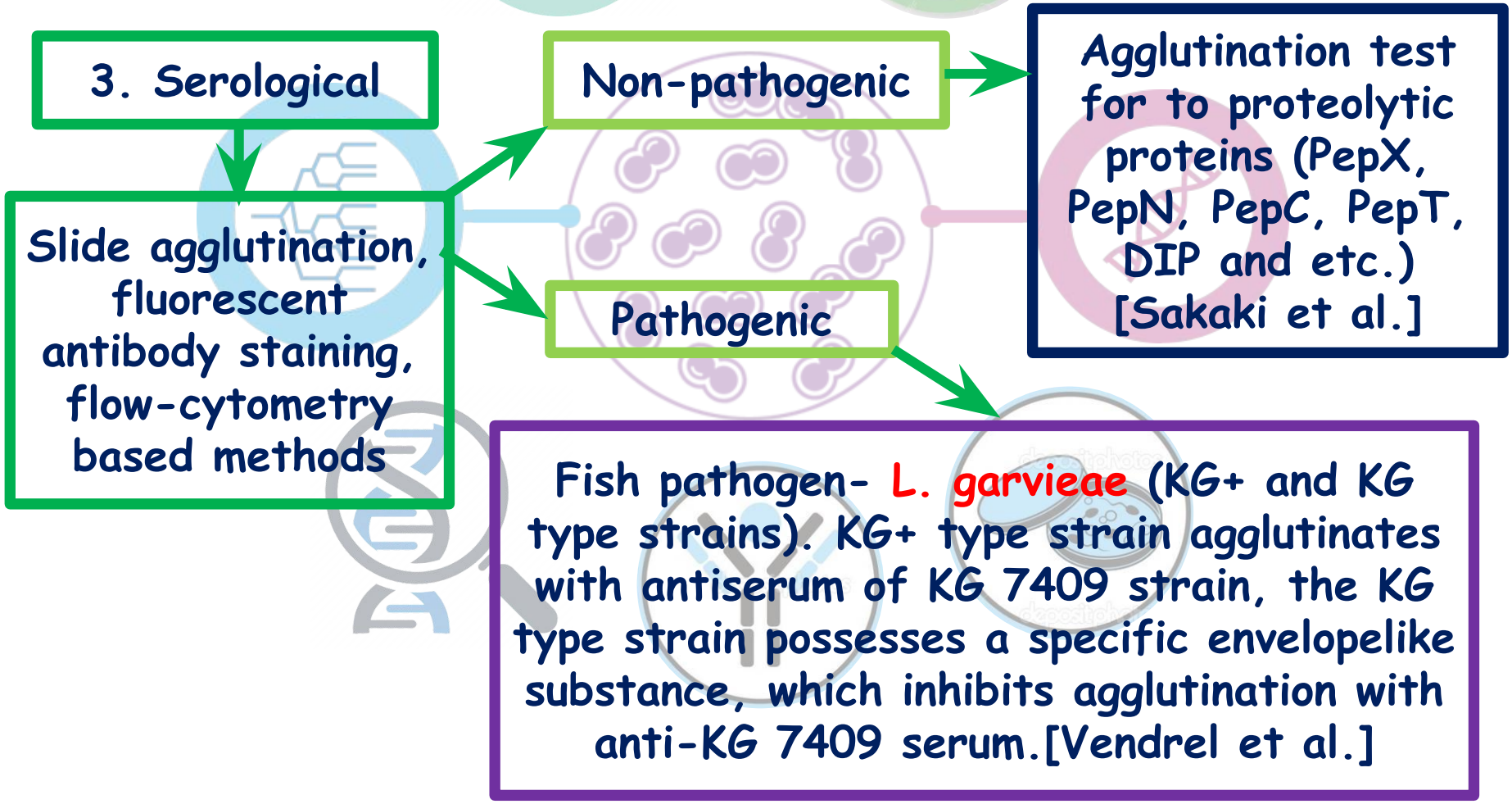
Differentiation of *Lactococcus* species

Species	PYR	VP	Arg	Lac	Man	Mel	Raf	Clind
L. lactis subsp. lactis	v	+	+	+	v	-	-	S
L. lactis subsp. cremoris	-	-	+	+	-	-	-	S
L. lactis subsp. hordiae	-	-	+	-	-	-	-	S
L. garvieae	+	+	+	+	+	-	v	R
L. plantarum	-	-	-	-	+	+	-	
L. raffinolactis	-	-	-	-	v	v	+	
L. xyloses	-	-	+	-	+	-	-	

Acid formation in: Lac=lactose, Man=mannitol, Raf=raffinose, Mel-melibiose Arg=deamination of arginine, PYR=pyrrolidonylarylaminadase, and VP=Voges-Proskauer* (acetoin detection), Clind- clindamycin, (+) = >90% positive, (-) = <10% positive, v = 60-90% strains positive

6. Immunological (serological) analysis

Generally discovery of lactococcus as a separate genera was investigated according to results of nucleic acid hybridization studies and immunological relationships of superoxide dismutase. According Lancefield et al. representatives of Lactoccus sp. were classified into serological N group.



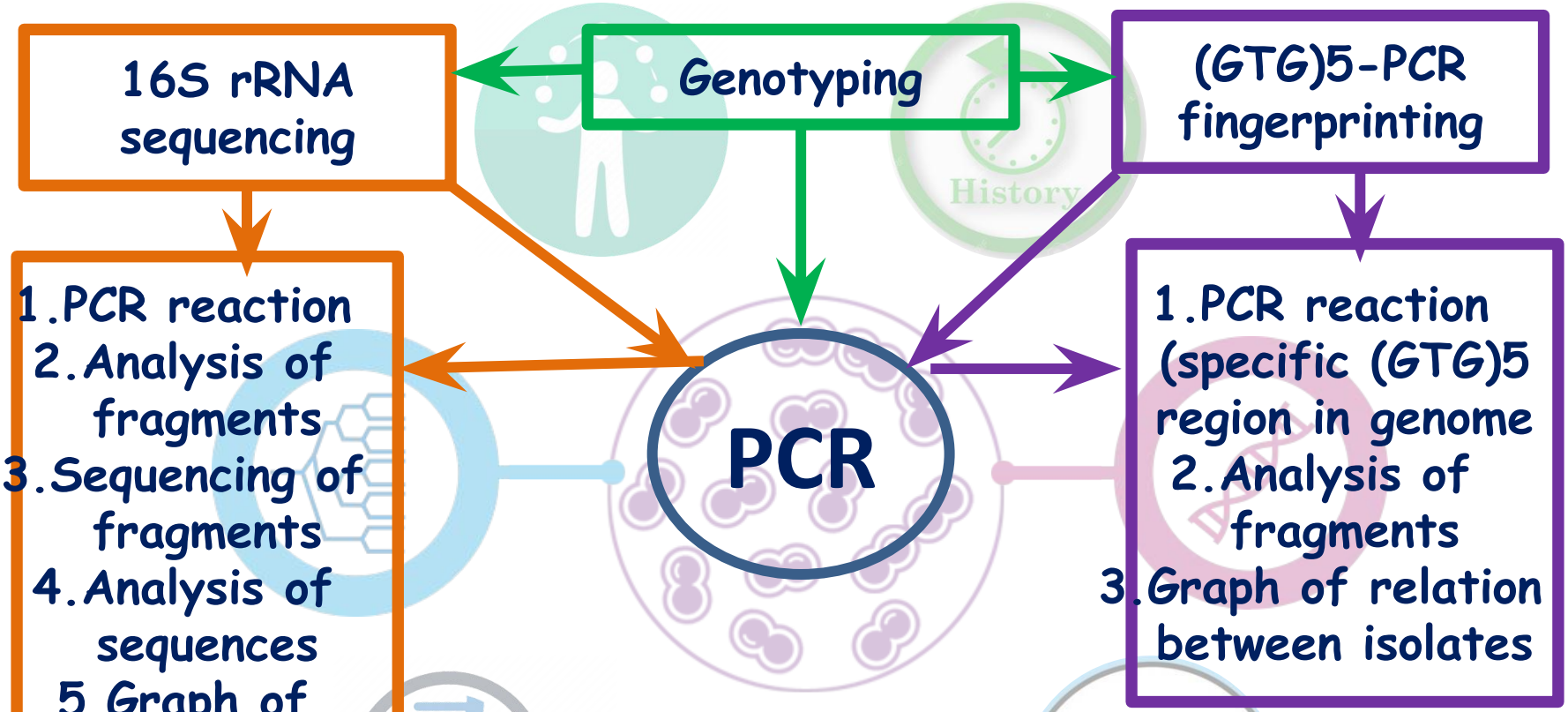
7. Genotypic techniques

Currently, there are two *L.lactis ssp. cremoris* and *L. lactis ssp. lactis*. that have been sequenced for public release. This investigations has a crucial role in understanding and manipulation of fermentation process to obtain desired product , also for fundamental researches of phylogenic diversity and epidemiology of pathogenic strains (*L. garvieae*).

Methods

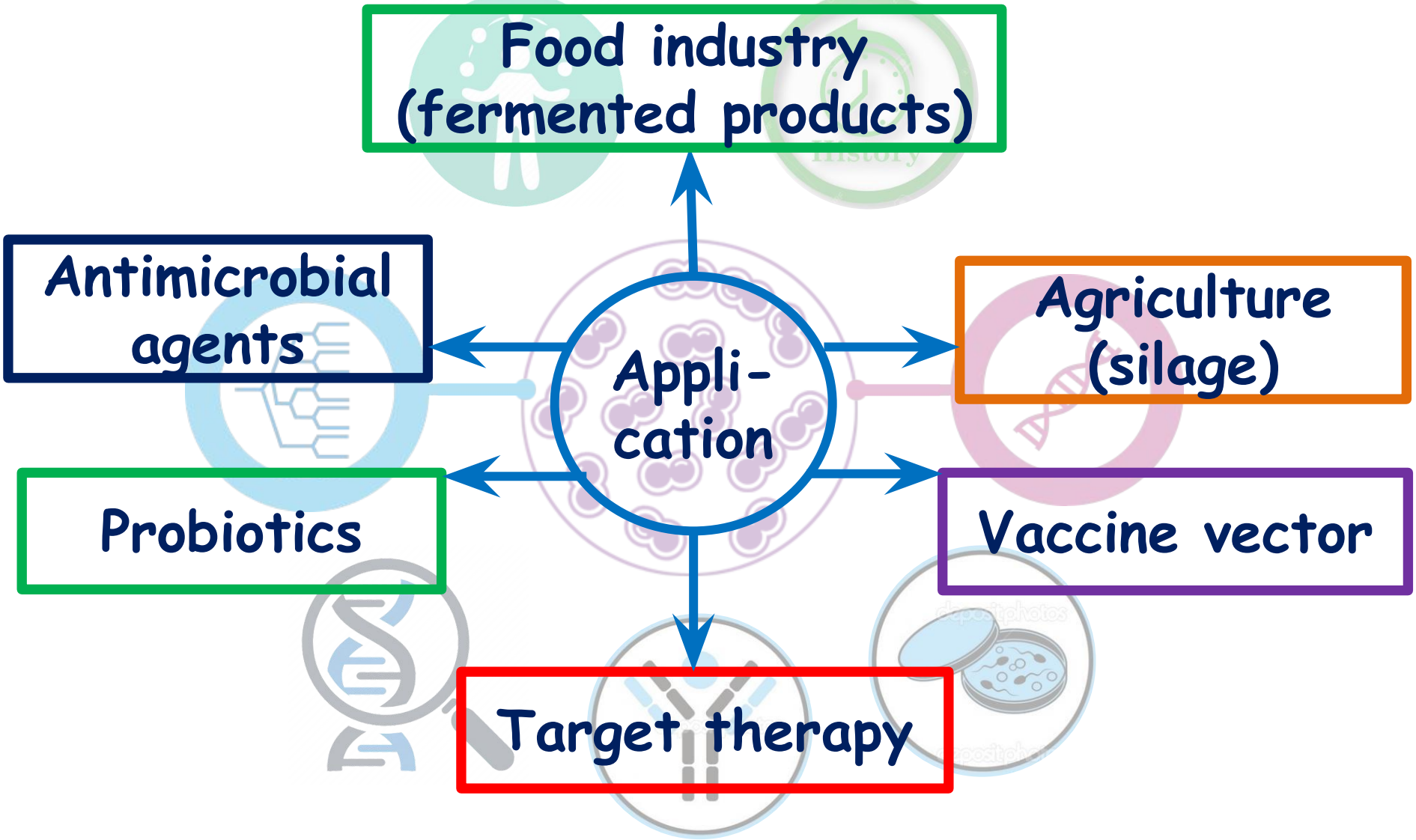
1. 16S rRNA sequencing
2. (GTG)5-PCR fingerprinting
3. Genotyping IS elements (insertion elements)
4. Randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR)
5. Multilocus sequence typing (for example 7 loci *atpA, rpoA, pheS, pepN, bcaT, pepX,* and 16S rRNA gene)

7. Genotypic techniques: Genral scheme



* So general mechanism is the same, the difference is in studying (genotyping) marker, the length and composition of which require designing specific primers and settling the reaction parameter. One more aspect that should be mentioned is the choose of marker for genotyping, cause the discriminatory index has a crucial role in diversification of isolates by strains.

8. Manipulation (genetic or non-genetic)



8. Manipulation (genetic or non-genetic)

1. Food industry: cheese, butter, buttermilk, sour cream and etc (non-genetic/genetic)
2. Agriculture, e.g. silage (non-genetic)
3. Source of antimicrobial agents and preservatives called bacteriacines. For example "Nisin". (non-genetic/ may include genetic manipulation for commercial overproduction)
4. Medicine- probiotics (non-genetic)
5. Medicine delivery factor (genetic [gene therapy])
6. New type of recombinant vaccine vector (genetic).

8. Manipulation (genetic or non-genetic)

The food-grade bacterium *Lactococcus lactis* has been extensively investigated during the last two decades as a delivery vector for therapeutic proteins, DNA and vaccine antigens. The bacterium represents a safe, genetically tractable vector capable of producing heterologous therapeutic proteins at mucosal sites. Contributing this, recombinant *L. lactis* strains have been exploited as agents to treat inflammatory bowel disease, allergy and cancer.

Examples of vaccine delivery in practice – tetanus toxin C, pneumococcal diseases, staphylococcal enterotoxin B, *H. pylory* (HspA gene), and etc.

8. Manipulation (non-genetic)

Fermented dairy products wherein *Lactococcus lactis* is the primary organism involved in manufacture.

Product	Principal acid producers	Secondary microflora
Cheese		
Colby, Cheddar, cottage, cream	*Lactococcus lactis* ssp. *cremoris*	None
	Lactococcus lactis ssp. *lactis*	
Blue	*Lactococcus lactis* ssp. *cremoris*	Citrate$^+$ *Lactococcus lactis* ssp. *lactis* *Penicillium roqueforti*
	Lactococcus lactis ssp. *lactis*	
Fermented milk		
Buttermilk	*Lactococcus lactis* ssp. *cremoris*	*Leuconostoc* spp. Citrate$^+$*Lactococcus lactis* ssp. *lactis*
	Lactococcus lactis ssp. *lactis*	
Sour cream	*Lactococcus lactis* ssp. *cremoris*	None
	Lactococcus lactis ssp. *lactis*	

8. Manipulation (genetic or non-genetic)

In sphere of Food industry, Agriculture and probiotic production general scheme of manipulation is following: Isolation -> cultivation-> biomass -> (purification, capsulation in case of probiotics or nisin production)-> treatment.

In case of genetic manipulations, the scheme provided in next order:

1. Isolation (donor of gene)-> cultivation-> DNA extraction-> identifying sequence of desired gene-> construction of vector-> transformation of recipient microorganism.
2. Isolation (recipient of gene)-> cultivation -> transformation->selection-> cultivation-> downstream processes (purification, concentration, capsulation, package)-> treatment.

9. Facts

. *Lactoococcus sp.* strains considered to be safe (GRAS) for human and used in dairy product fermentation.
. The genus contains strains known to grow at or below 7°C.
. Today they are used extensively in food fermentations, which represent about 20% of the total economic value of fermented foods produced throughout the world.
. The *Lactococcus lactis ssp. lactis* genome has 2 365 589 units (bp) of DNA, which contain 2 310 predicted genes. About 64% of the genes have assigned roles in the cell, while 20% match other hypothetical genes with unknown function. Almost 16% of the genes bear no resemblance to genes from other species and are considered to be unique to this bacterium.
. Before 1985, representative of this genera were classified as *Streptococcus* and *Enterococcus sp.*

CONCLUSION

Description of the genus *Lactococcus gen.nov.* *Lactococcus* (lac.to.coc'cus, *L.n.lac,* lactis milk., *Gr.n.coccus,* a grain or berry, M.L.masc.n. *Lactococcus* milk coccus).

Spheres or ovoid cells occur singly, in pairs or in chains, and are often elongated in the direction of the chain. Gram-positive. Endospores are not formed. Non-motile. Not β-haemolytic. Facultatively anaerobic, catalase negative. Growth at 10°C but not at 45°C . Usually grows in 4 % (w/v) NaCL with the exception of L. *lactis subsp. cremoris* which only tolerates 2 % (w/v) NaCL. Chemoorganotrophs. Metabolism- fermentative. The predominant end product of glucose fermentation is L-lactic acid. Most strains react with group N antisera. Some strains possess low levels of menaquinones.

CONCLUSION

The major glycolipid of all strains is Glc(a1-2)Glc(a1-3)acyI2- Gro, a constant minor component is Glc(a1-2), acyl- 6Glc(a1-3)acyI2Gro. All strains contain phosphatidylglycerol and cardiolipin. Lipoteichoic acid structure and occurrence of aminophospholipids are species rather than genus-specific. Non-hydroxylated long-chain fatty acids are primarily of the straight-chain saturated and monounsaturated types; some strains produce cyclopropane-ring acids. The major fatty acids are hexadecanoic and cis- 11,12-octadecenoic acids; cis-11,12-methylenoctadecanoic acid is also present in major amounts in most strains with the exception of L. *lactis* subsp. *hordniae* and L. *raffinolactis*. The G+C content of the DNA ranges from 34 to 43 mol %.

CONCLUSION

Nucleic acid hybridization and comparative immunological studies demonstrate that members of the genus *Lactococcus* are closely related to each other but not to members of the genus *Streptococcus* or *Enterococcus*. Lactococci can be distinguished from streptococci and enterococci by their ability to grow at 10°C but not at 45°C. They are not β-haemolytic; some strains show a weak α-haemolytic reaction. They are non-motile.

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Links:

1. https://en.wikipedia.org/wiki/Lactococcus
2. http://genome.jgi.doe.gov/laccr/laccr.home.html
3. http://textbookofbacteriology.net/lactics.html