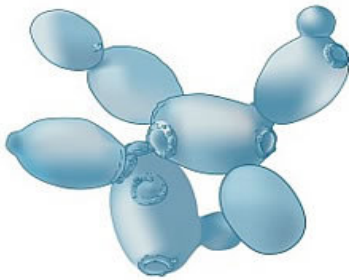
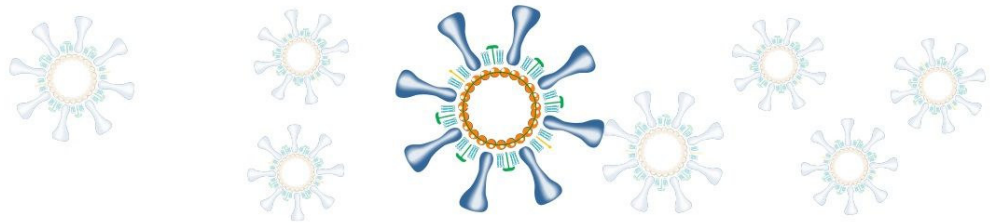
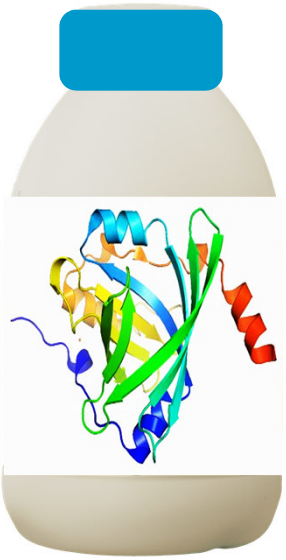




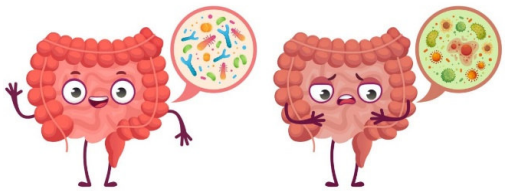
# Yeast System as Mikrobiom





Against Porcine & Respiratory Syndrom

**Dr. habil. Anna Salek, May 2024**



# Microbiome

**The microbiome refers to the entirety of all microorganisms**

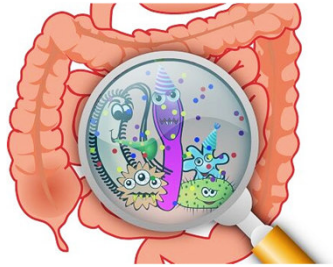
**(bacteria, fungi and protozoa) that colonize a organism (human, animal, plant).**

**Microbiomes can influence the immune system and metabolism of their host.**

**A better understanding of the roles and functionalities of these microorganisms makes**

**it possible to develop new classes of therapeutic preparations, such as live**

**biotherapeutics, probiotics, biosimilars,**

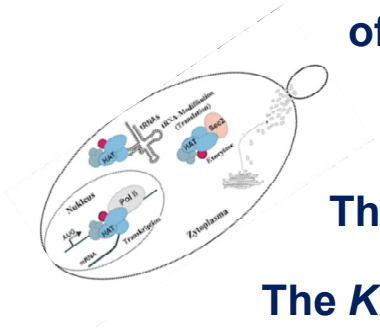


**which aim to restore the balance of the ecosystem microbiome.**

**Moreover, products based on living microorganisms generally do not act directly,**

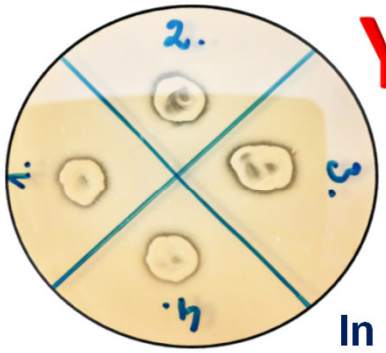
**i.e. on specific organs or tissues, but interact through a multifactorial mechanism of action.**

# Yeast System (+ killer substrains)

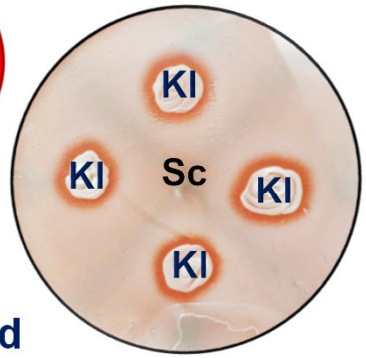



***Kluyveromyces lactis*** **is a yeast which has the ability to assimilate lactose and convert it into lactic acid.** ***Kluyveromyces lactis*** **as a representative of the so-called killer yeasts, uses this strategy to gain a selective growth advantage in the competition for nutrients via microbial competitors. These can be blocked by the effect of zymocin in cell growth and cell cycle. The** ***Kluyveromyces lactis*** **toxin is plasmid-encoded and inhibits the multiplication of the baker's yeast** ***Saccharomyces cerevisiae*** **through an irreversible block in the G1 phase of the cell cycle.**

**Sensitive target cells arrest without sprouting with a pre-replicative (1n) DNA content. Despite the heterotrimeric (αβγ) structure of the native zymocin, its toxicity is primarily caused by the γ subunit, the so-called γ-toxin.**



# Yeast System (+ killer substrains)

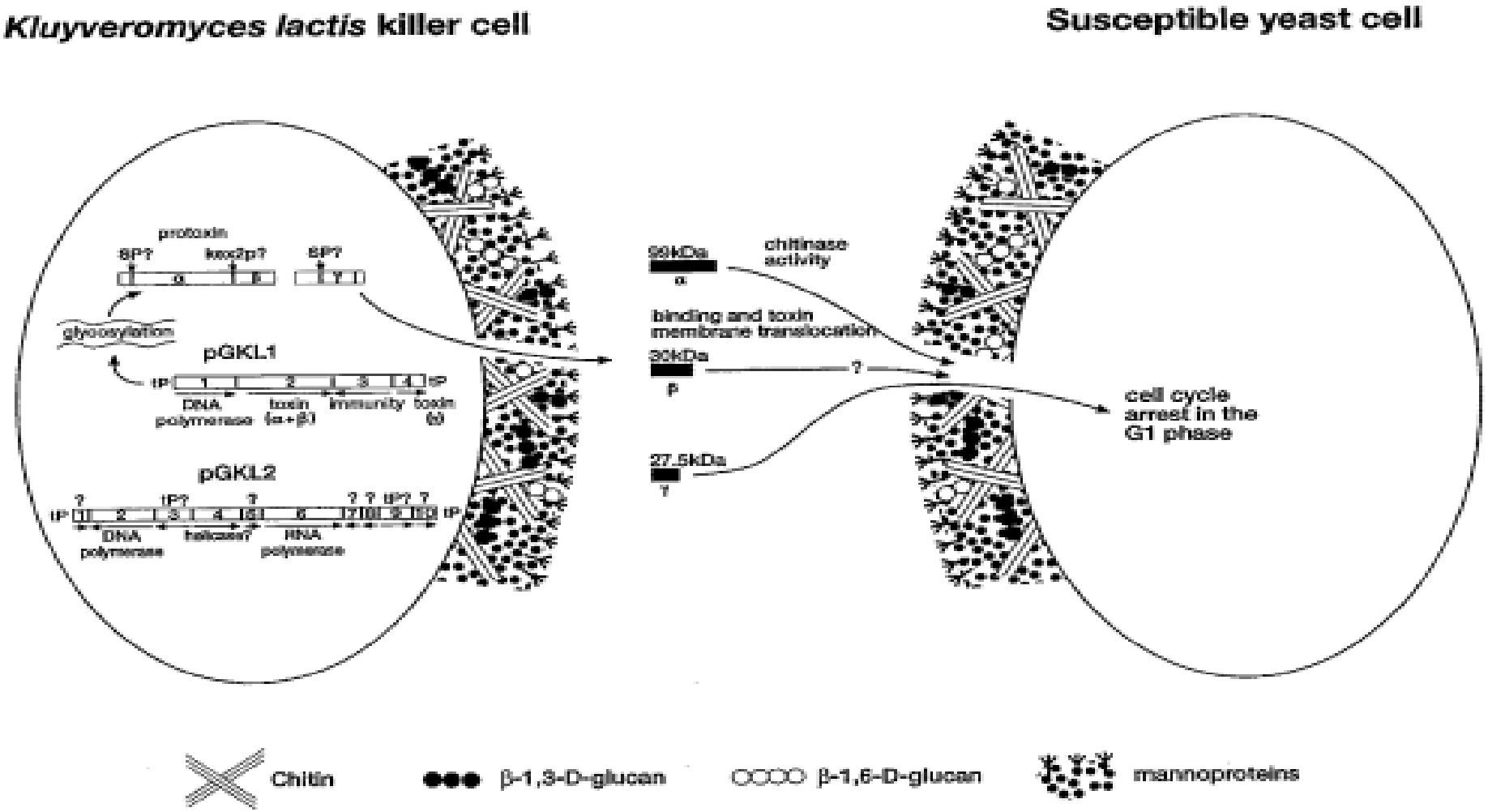



**Secretion of cytotoxic substances by microorganisms is one strategy to combat microbial competitors.**

**In yeast, numerous such killer toxin systems, either chromosomally encoded or associated with dsRNA viruses or cytoplasmic dsDNA plasmids, have evolved. Biochemically, these toxins vary by receptor specificity, by being secreted as monomers or multi-subunit complexes and by their cytotoxic effects towards target cell proliferation. The lethal interaction between *Kluyveromyces lactis* and *Saccharomyces cerevisiae* constitutes a model pathosystem that relies on zymocin, a heterotrimeric protein toxin complex secreted by the dairy yeast *Kluyveromyces lactis,* which causes an irreversible growth arrest of sensitive yeast cells.**

**This killer phenomenon is encoded by a cytoplasmic pair of killer plasmids, pGKL1 (k1) and pGKL2 (k2), and ultimately causes a G1 (growing phase) block.**

# *Kluyveromyces lactis* with linear DNA plasmids pGKI1 & pGKI2



# Yeast Killer System

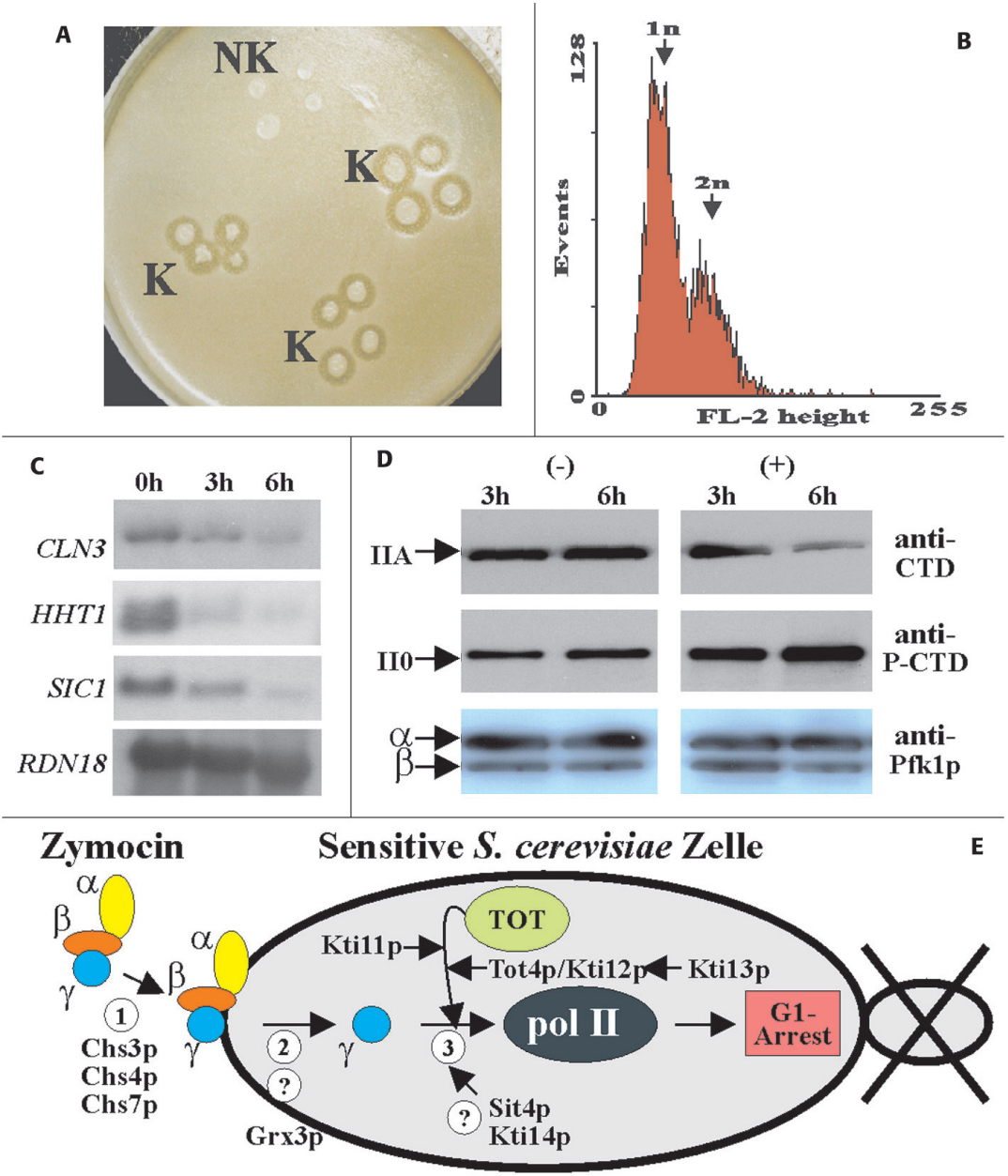


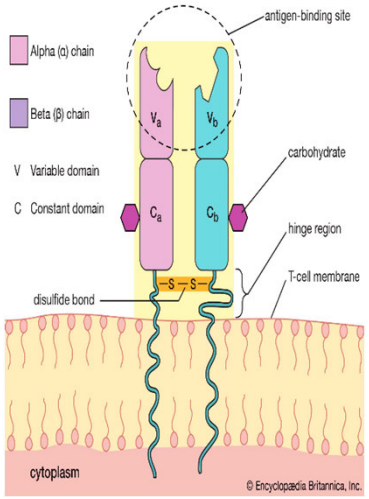

***Abb. 1: Das Killersystem von Kluyveromyces lactis. (A) Killer- (K) und Nicht-Killerstämme (NK) wurden gegen S. cerevisiae inkubiert. Hemmhöfe machen die Wachstumsinhibition sichtbar. (B) FACS-Analyse. Zymocinbehandelte S. cerevisiae-Zellen akkumulieren in der G1-Phase des Zellzyklus mit 1n DNA-Gehalt. (C) Northern-Analysen an Zymocinunbehandelten (0h) und Zymocin-arretierten Zellen (3h bzw. 6h nach Zymocingabe). Identische Gesamt-RNA wurde auf Pol I (RDN18) und Pol II (CLN3, HHT1 und SIC1) Transkription hin untersucht. (D) Phosphorylierung der C-terminalen Domäne (CTD) der Pol II-Untereinheit Rbp1p. Proteinextrakte Zymocin-unbehandelter (–) und -arretierter Zellen (+) wurden mit Antikörpern, die hypo- (anti-CTD: IIA) von hyperphosphorylierter CTD (anti-P-CTD: II0) unterscheiden, im Westernblot analysiert. Als Ladekontrolle diente anti-Pfk1p zur Detektion der α- und β-Untereinheiten der Phosphofructokinase. (E) Arbeitsmodell zum Zymocin-Signalweg, der in Bindung an die sensitive Zielzelle (1), γ-Toxinimport (2) und intrazelluläre Kommunikation des γ-Toxins mit TOT (3) einteilbar ist. Zu den Genprodukten (Chs3-4p, Chs7p, Grx3p, Kti11-14p & Sit4p) siehe Text.***

# *Kluyveromyces lactis* application

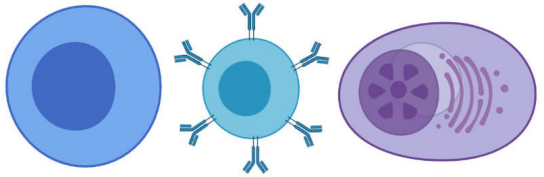
**The mucosa of respiratory and reproductive tracts is the major route of respiratory infection. It is believed that generating mucosal immunity using vaccines is the best way to prevent that infection.**

**It has been reported that some recombinant yeast *Kluyveromyces lactis* can be administered orally and in this way it deliver proteins as antigen induce mucosal immune responses.**

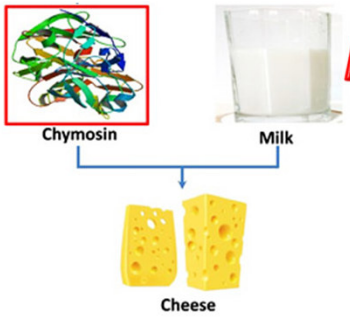
T cell




**In the our study, we isolated natural *Kluyveromyces lactis* expressing antigen to respiratory syndrome and have evaluated its ability to induce (in our organism) B cell- and T cell – mediated immune responses after oral delivery.**



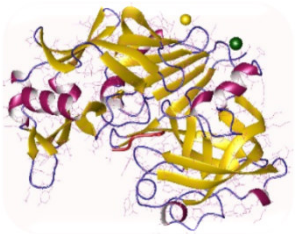
B cell, lymphocyte




# *Kluyveromyces lactis* application

**The yeast system has been shown to have advantages over conventional systems as a vaccine vehicle. For example, *Kluyveromyces lactis* is generally regarded as safe for animals and human beings. *Kluyveromyces lactis*, one of the most important non-*Saccharomyces* yeasts, has similar advantages to yeast *Williopsis mrakii*.**

**Additionally, *Kluyveromyces lactis* has a well-established track record of safe use in various food industry applications (chymosin in cheese production) and can efficiently express heterologous proteins. Moreover, components of its cell-wall such as β-1,3-glucan and mannan may have adjuvant activities. Thus, *Kuyveromyces lactis* might be a safe and ideal vaccine vehicle.**



# *Kluyveromyces lactis* application

***Kluyveromyces lactis*** **has been used as a source of lactase (β-galactosidase), an enzyme that degrades milk sugar (lactose) and is necessary for production of lactose-free dairy products.**

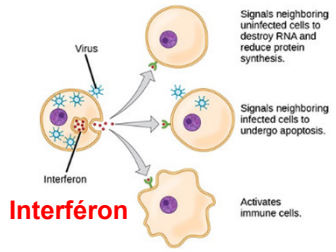
***Kluyveromyces lactis*** **is best known for its use in commercial production of the milk-coagulating enzyme bovine chymosin.**

**Today, over 40 proteins have been produced with** ***Kluyveromyces lactis*****,illustrating its utility as an alternative yeast expression system. These proteins originate from bacteria, fungi, viruses, plants, and mammals, emphasizing the ability of** ***Kluyveromyces lactis*** **To efficiently produce a diverse range of heterologous proteins.**

# *Kluyveromyces lactis* application

**The success of *Kluveromyces lactis* as a host for protein expression in the food industry suggests that this yeast could also be used for large-scale therapeutic protein production in the pharmaceutical industry.**

**In fact, numerous proteins of pharmaceutical relevance have been produced by *Kluveromyces lactis*.**



Interféron

**The mammalian proteins interleukin 1-β, interferon αA, α-lactoglobulin, lysozyme, macrophage colony stimulating factor, serum albumin and insulin precursor have been secreted from *Kluyveromyces lactis* to high titer.**

***Kluyveromyces lactis* is also proficient in the production of single-chain Fv antibodies and interesting native enzymes like inulinase, phospholipase B and chitinase.**

# *Kluyveromyces lactis* application

**In this context, *Kluyveromyces lactis* represents an interesting species.**

**While it is an attractive model for biotechnological procedures such as production of pharmaceuticals, yield of heterologous proteins.**

**This aerobic yeast is especially well-known for its ability to assimilate lactose.**

***Kluyveromyces lactis* represents one of the leading yeast contributors of dairy products.**

**In *Kluyveromyces lactis*, the lactose assimilation process (in adaptive mode) relies on a well-known pathway comprising the *LAC4* and *LAC12* genes, which encode respectively a β-galactosidase and a lactose permease, as well as the galactose-lactose regulatory genes (*LAC9* and *GAL80*) and the galactose genes (*GAL1, GAL7* and *GAL10*).**

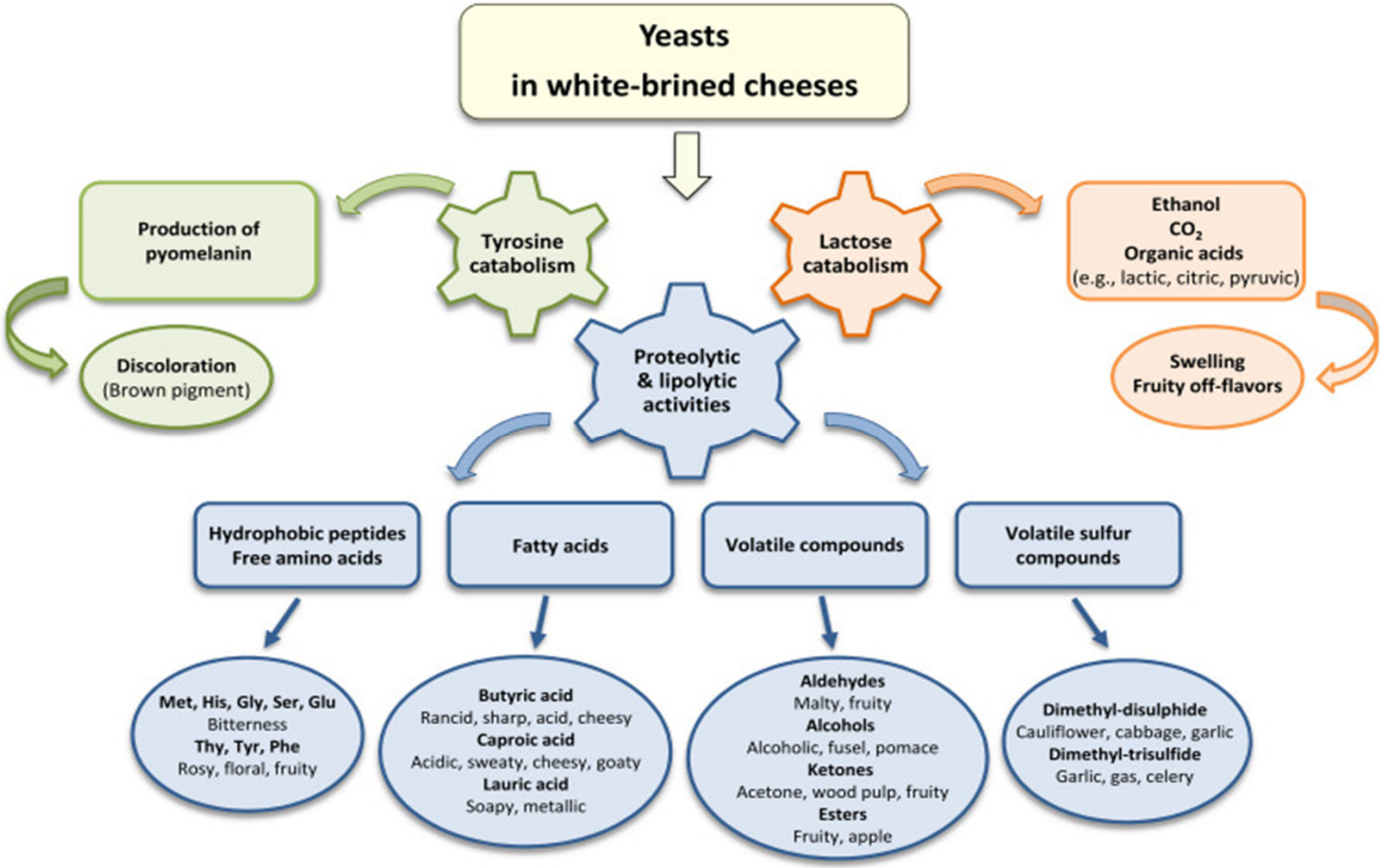
# *Kluyveromyces lactis* application

**Lactose-hydrolyzed milks also improve lactose intolerance symptoms in both children and adults. A by-product of lactose-hydrolyzed milk is increased sweetness due to the presence of free glucose.**

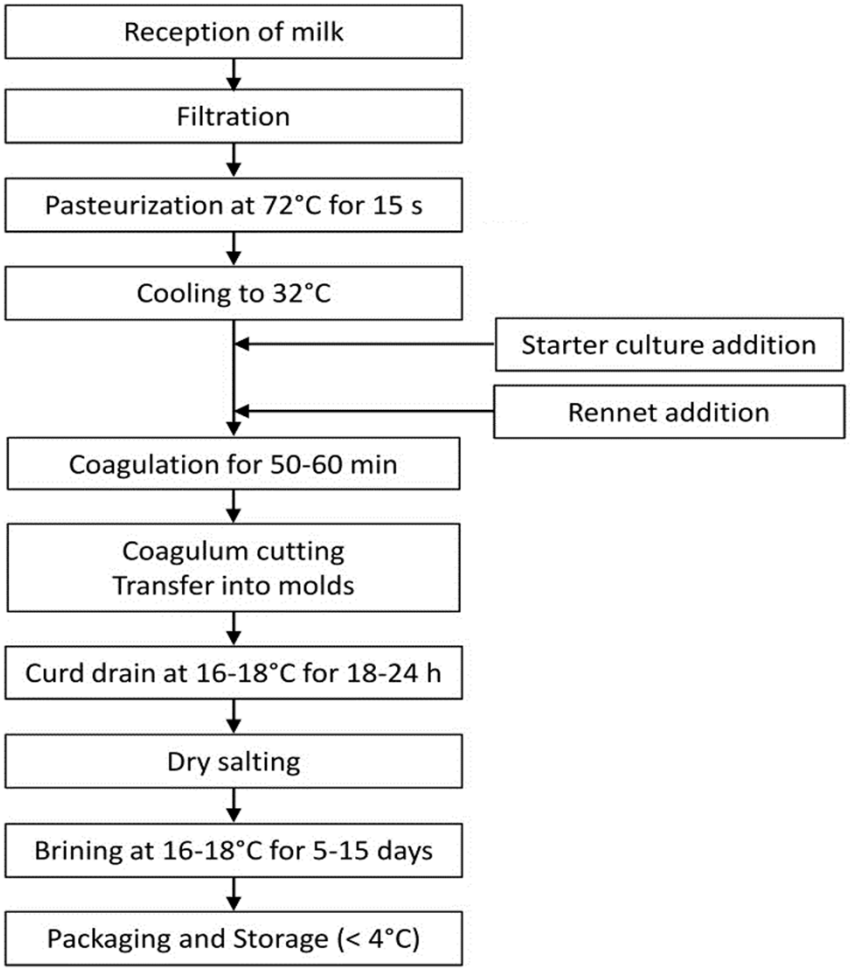
**This increased sweetness may increase its acceptability in children.**


**The ability of *Kluyveromyces lactis* to metabolize milk constituents (lactose, proteins, and fat) makes them very important in cheese ripening and fermented milk products such as kefir, as they contribute to maturation and aroma formation. *Kluyveromyces lactis* is very active for the catabolism of peptides and amino acids (proteolytic), as well as fat (lipolysis), which leads to the production of a diversity of flavours.**

# *Kluyveromyces lactis* application



# *Kluyveromyces lactis* application

# Conclusion

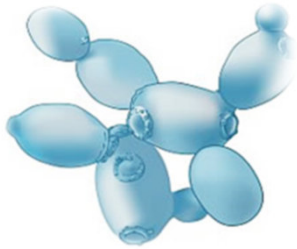
- "Kluy-lac" drink (milk with *Kluyveromyces lactis*) is: 'A protein drinks a day'.
- Protein consists of amino acids (small protein particles), known as the "building blocks of life and health".
- "Kluy-lac" drink contains bioactive peptides, amino acids and metabolites with antimicrobial properties(such as α-glycopeptides, zymocin, L(+)-lactic acid and DL-racemic lactic acid). The yeast used in "Kluy-lac" drink hydrolyze lactose to L(+) and DL(+) racemic lactic acid,thanks to the enzyme β-galactosidase, therefore this drink is lactose-free with many positive (incl. antimicrobial) effects.
- The strengthening of the immune system is due to the specific α-glycoproteins produced by metabolism of the yeast used in "Kluy-lac" Drink.
- Another valuable active ingredient in "Kluy-lac Drink is α-lactoalbumin (α-LA), which is evaluable source of important amino acids and stimulates the body's immune response.
- The refreshing effect after fermentation in "Kluy-lac" Drink is due to the resulting carbon dioxide and ethanol.

# Conclusion

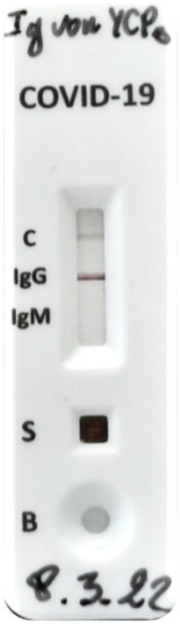
- Therapeutic uses of “Kluy-lac” Drink are mainly due to its content of lactoferrin, immunoglobulins, growth factors such as branched-chain amino acids (i.e. leucine, isoleucine and vanillin) and micronutrients (potassium, calcium, iron, phosphate and B vitamins).
- The “Kluy-lac” drink formula used improves the health of skin and hair thanks to the amino acids and presence of B-complex vitamins (especially B1 and B6).
- “Kluy-lac” can be used both as a natural food or as a dietary therapeutically supplement for patients with the following problems:
  - Chronic bacterial, viral or fungal infections;
  - Weakening or deregulation of the immune system;
  - Digestive system problems (diarrhea, constipation, dyspepsia, flatulence, etc.);
  - Loss of muscle mass or excess weight of fat mass;
  - Calcium absorption disorders, calcium deficiency, e.g. by osteoporosis.

After homogenisation:


Zum Wohl!
Cheers!
Probiotic Drinks
Kluy-lac
Lactose free
Soft cheese
Lactose free



Immunity Test
Ig = YCPs




# Thank you for your attention!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