Yeast-derived Cosmetic Ingredients

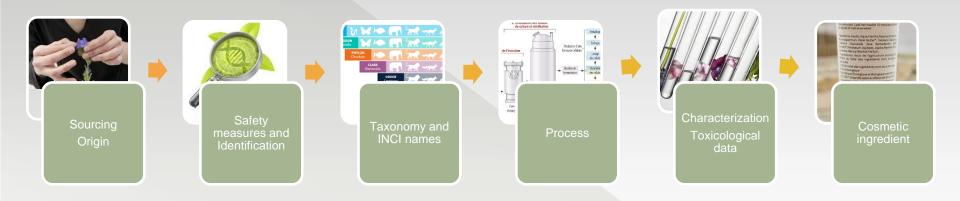
September 26 – 27th, 2022

Audrey POKRZYWA Sylvain MAZALREY (SILAB)

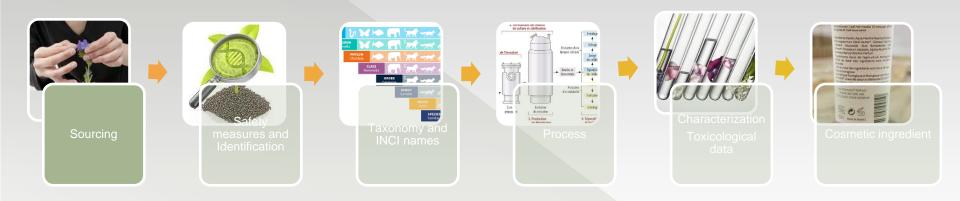


YEASTS: characteristics & identification (third edition)

Table of contents



Sourcing



Origin of the strain

- The strains can be sourced from :
 - Official collection: ATCC (American Type Culture Collection), CBS (Westerdijk Fungal Bio Diversity Institute), DSMZ (German Collection of Microorganisms and Cell Cultures), MUCL (Belgian Coordinated Collections of Microorganisms),...



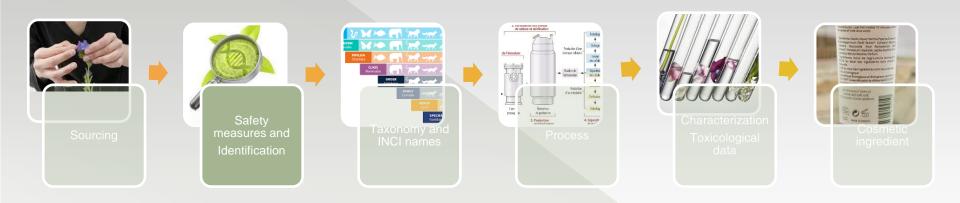






 Custom collection: partnerships with International Centers for Microbial Resources, for example: CIRM dedicated to yeasts in Montpellier (France)

Safety measures, Identification



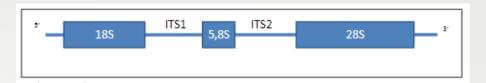
Strain identification

 To be sure to work on the right strain, it is imperative to confirm its taxonomic identification

The best way is by r-28S DNA sequencing and ITS

<u>Principle</u>: Amplification and sequencing of a portion of 28S rRNA encoding the 60S ribosomal subunit. The Internal Transcribed Space (ITS) is a region located on the genomic DNA of eukaryotes between the 28S rRNA and 18S rRNA coding genes. It is composed of three sub-regions: ITS1, ITS2 and the 5.8S gene.

The variability of the ITS seems to favour the identification of the genus and species of fungal populations.



Strain Identification

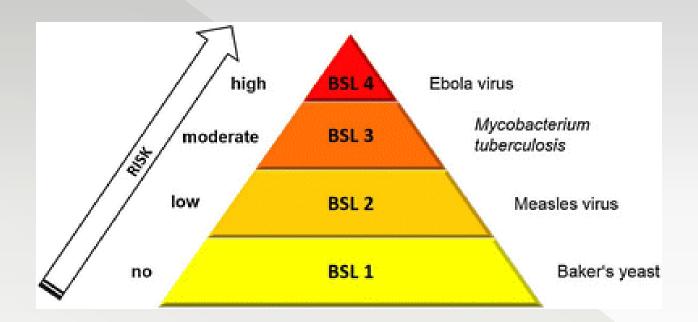


Biosafety Level	BSL-1	BSL-2	BSL-3	BSL-4
Description	No Containment Defined organisms Unlikely to cause disease	Containment Moderate Risk Disease of varying severity	High Containment Aerosol Transmission Serious/Potentially lethal disease	Max Containment "Exotic," High-Risk Agents Life-threatening disease
Sample Organisms	E.Coli	Influenza, HIV, Lyme Disease	Tuberculosis	Ebola Virus
Pathogen Type	Agents that present minimal potential hazard to personnel & the environment.	Agents associated with human disease & pose moderate hazards to personnel & the environment.	Indigenous or exotic agents, agents that present a potential for aerosol trans- mission, & agents causing serious or potentially lethal disease.	Dangerous & exotic agents that pose a high risk of aerosol- transmitted lab- oratory infections & life-threatening disease.
Autoclave Requirements	None	None	Pass-thru autoclave with Bioseal required in laboratory room.	Pass-thru autoclave with Bioseal required in laboratory room.

Strain Identification



BSL-1 (Biosafety Level One): this level is defined by the American Centers for Disease Control and Prevention (CDC)



We highly recommend only the use of BSL-1 to manufacture Cosmetic ingredients

Strain Identification

Table 1. Classification of infective microorganisms by risk group

Risk Group 1 (no or low individual and community risk)

A microorganism that is unlikely to cause human or animal disease.

Risk Group 2 (moderate individual risk, low community risk)

A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

Risk Group 3 (high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

Risk Group 4 (high individual and community risk)

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

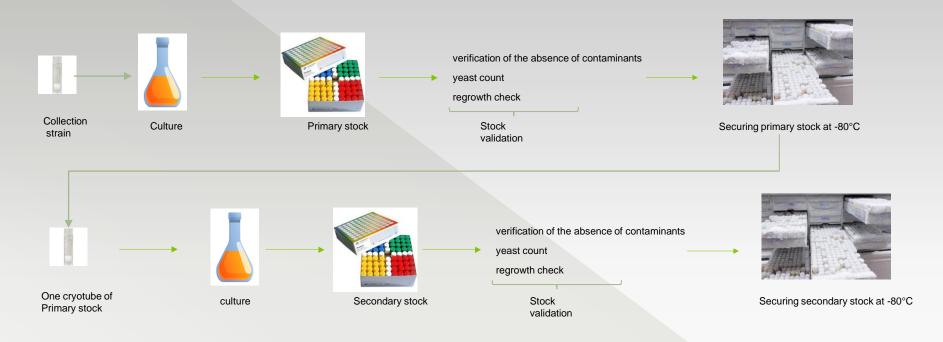
Table 2. Relation of risk groups to biosafety levels, practices and equipment

RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double- ended autoclave (through the wall), filtered air

BSC, biological safety cabinet; GMT, good microbiological techniques (see Part IV of this manual)



Safety measures



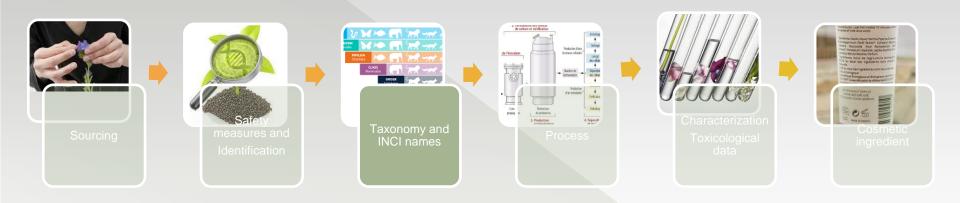
The freezers at -80°C should be under reinforced surveillance

Stocks must be placed in different freezers at -80°C

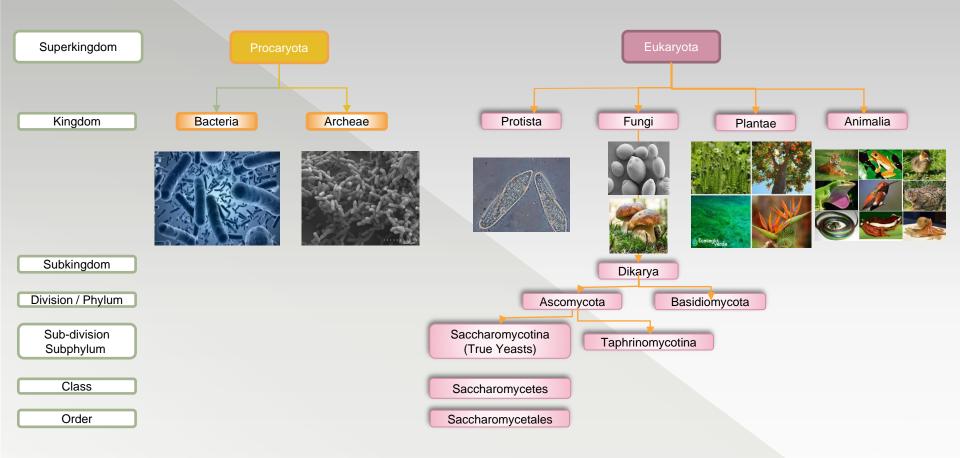


Business Continuity Plan

Taxonomy and INCI names



Taxonomy



Taxonomy - Definitions

Basionym: the originally described name, attached to the type material and species description

Homotypic synonym: names generated after the basionym (e.g. by moving it to a different genus) but sharing the same type

Heterotypic synonym: names with a different basionym and type from those mentioned above

Source: NCBI Taxonomy: a comprehensive update on curation, resources and tools

INCI names

- The purpose of this report is to be exhaustive regarding all INCI names used (at least declared) in cosmetic products
- That is why, the choice of INCI names to study is based on:
 - the review of all yeast-related INCI names in the PCPC dictionary
 - > INCI names referenced in the VCRP (Voluntary Cosmetic Registration Program) (2022)

Identification

• The objective is to check their compliance with the PCPC definition of YEAST:

Yeast is a class of microorganisms (Saccharomycetes) characterized by their lack of photosynthetic ability, existence as unicellular or simple irregular filaments, and reproduction by budding or direct division

 And to study yeasts that belong to all families of the Saccharomycetes class in order to guarantee the completeness of our study

(1/6) – July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC	
		Ascoideaceae	Ascoidea	Ascoidea rubescens	-	
			Kurtzmaniella	Candida oleophila => Undergoing modification	-	
			-	Candida saitoana => Undergoing modification	Hydrolyzed Candida Saitoana Extract	
es	selles		Debaryomyces	Debaryomyces maramus	-	
/cet	Seta		Debaryomyces	Debaryomyces nepalensis	-	
Saccharomycetes	aromyo	Debaryomycetaceae	Debaryomycetaceae	Meyerozyma	Meyerozyma caribbica Basionym: Pichia caribbica	Pichia Caribbica Ferment
Sacch	Saccharomycetales		Debaryomyces	Priceomyces carsonii Homotypic synonym : Debaryomyces carsonii Basionym: Pichia carsonii	-	
			Scheffersomyces	Scheffersomyces stipitis Basionym :Pichia stipitis Homotypic synonym : Yamadazyma stipitis	Pichia Ferment Lysate Filtrate	

Source: NCBI: National Center for Biotechnology Information

(2/6) - July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC
			Geotrichum	Geotrichum candidum Basionym: Endomyces geotrichium Heterotypic basionym: Galactomyces candidus	Galactomyces Ferment Filtrate
Saccharomycetes	Saccharomycetales	Dipodascaceae	Dipoascus	Dipodascus fermentans Basionym: Trichosporon fermentans Homotypic synonym: Galactomyces fermentans	Galactomyces Ferment Filtrate
Sacch	Saccha		Yarrowia	Yarrowia lipolytica Basionym : Endomycopsis lipolytica Heterotypic synonym : Mycotorula lipolytica	Yarrowia Lipolytica Extract Yarrowia Lipolytica Ferment Lysate Yarrowia Lipolytica Oil
		Endomycetaceae	Endomyces	Endomyces decipiens	-

(3/6) - July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC
			Metschnikowia	Metschnikowia agaves	Hydrolyzed Metschnikowia Agaves Extract Metschnikowia Agaves Polysaccharides Metschnikowia Agaves Extract
			Metschnikowia	Metschnikowia bicuspidata Basionym: Monospora bicuspidata	-
			Metschnikowia	Metschnikowia gruessii	-
	(0	a)	Metschnikowia	Metschnikowia hawaiiensis	-
etes	tales	ceae	Metschnikowia	Metschnikowia henanensis	Metschnikowia Henanensis Extract
Šc	/ce	wi wi	Metschnikowia	Metschnikowia hibisci	-
ľo	E O	įķ	Metschnikowia	Metschnikowia koreensis	-
cha	har	chn	Metschnikowia	Metschnikowia lunata	-
Sac	Saccharomycetes Saccharomycetales Metschnikowiaceae	Mets	Metschnikowia	Metschnikowia pulcherrima Heterotypic synonym: Candida pulcherrima	-
			Metschnikowia	Metschnikowia reukaufii Heterotypic synonym: Candida reukaufii	Hydrolyzed Metschnikowia Reukaufii Extract Metschnikowia Reukaufii Lysate Extract
			Metschnikowia	Metschnikowia rubicola	-
			Metschnikowia	Metschnikowia shanxiensis	Hydrolyzed Metschnikowia Shanxiensis Extract
			Metschnikowia	Metschnikowia viticola	Metschnikowia Viticola Extract

(4/6) - July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC	
			Wickerhamomyces	Wickerhamomyces alni Homotypic synonym : Pichia alni		
	Saccharomycetes Saccharomycetales Phaffomycetaceae	taceae	Barnettozyma	Barnettozyma populi Basionym : Hansenula populi Homotypic synonym : Pichia populi	Pichia Ferment Lysate Filtrate	
့		Saccharomycetales	s haffomyce	Komagataella	Komagataella pastoris Basionym : Zygosaccharomyces pastoris Homotypic synonym : Pichia pastoris	Pichia Ferment Extract Flltrate Pichia Pastoris Ferment Flltrate Pichia Ferment Lysate Filtrate
romycete			<u> </u>	Wickerhamomyces	Wickerhamomyces anomalus Basionym: Saccharomyces anomalus Homotypic synonym: Pichia anomala	Pichia Anomala Extract
Saccha				Ogataea	Ogataea minuta Basionym : Hansenula minuta Homotypic synonym : Pichia minuta	Pichia Minuta Extract
		eae	Ogataea	Ogataea naganishii Basionym : Pichia naganishii	-	
		Pichiaceae	Ogataea	Ogataea siamensis Basionym: Pichia siamensis	-	
			Pichia	Pichia heedii	Pichia Heedii Extract	
			Pichia	Pichia membranifaciens Basionym : Saccharomyces menbranifaciens	-	
			Pichia	Pichia	Pichia Extract	

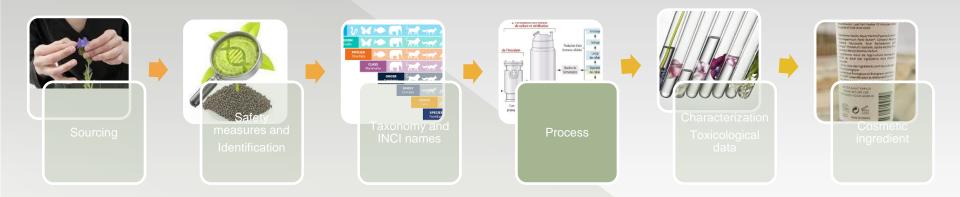
(5/6) - July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC
			Eremothecium	Eremothecium ashbyii	-
			Kluyveromyces	Kluyveromyces lactis Basionym: Torulapora lactis Homotypic synonym: Saccharomyces lactis	Kluyveromyces Extract
ycetes	cetales	etaceae	Kluyveromyces	Kluyveromyces marxianus Basionym : Saccharomyces marxianus Heterotypic synonym : Kluyveromyces fragilis Homotypic synonym : Dekkeromyces marxianus	Hydrolyzed Kluyveromyces Extract
om)	Ę	nyc	Saccharomyces	Saccharomyces cerevisiae	Saccharomyces Cerevisiae Extract
Saccharomycetes	Saccharomycetales	Saccharomycetaceae	Saccharomyces	Saccharomyces sp.	Saccharomyces Saccharomyces Extract Saccharomyces Ferment Filtrate Saccharomyces Ferment Lysate Filtrate Saccharomyces Ferment Saccharomyces Lysate Extract Filtrate Saccharomyces Lysate Extract Saccharomyces Lysate Saccharomyces Polypeptides Saccharomyces
			Torulaspora	Torulaspora delbrueckii Basionym: Saccharomyces delbrueckii	Torulaspora Delbrueckii Extract Torulaspora Delbrueckii Ferment Hydrolyzed Torulaspora Delbruekii Extract

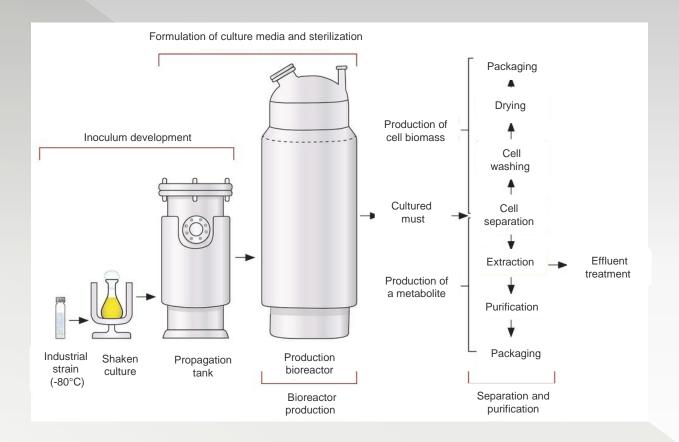
(6/6) - July 2022

Class	Order	Family	Genus	Associated Genus/Species	INCI declared to PCPC
		Saccharomycetaceae	Zygosaccharomyces	Zygosaccharomyces rouxii Basionym : Saccharomyces rouxii	-
etes	tales	Saccharomycetales incertae sedis	Starmerella	Starmerella magnoliae Basionym: Torulaspis magnoliae Homotypic synonym: Candida magnoliae	-
Saccharomycetes	Saccharomycetes	Saccharomycetales incertae sedis	Starmerella	Starmerella bombicola Heterotypic synonym : Candida bombicola	Hydrolyzed Candida Bombicola Extract
Sa	Sac	Saccharomycodaceae	Hanseniaspora	Hanseniaspora opuntiae	-
		Saccharomycopsidaceae	Saccharomycopsis	Saccharomycopsis fibuligera	-
		Trichomonascaceae	Wickerhamiella	Wickerhamiella azyma Current name : Candida azyma ; Basionym : Torulopsis azyma	-

Process

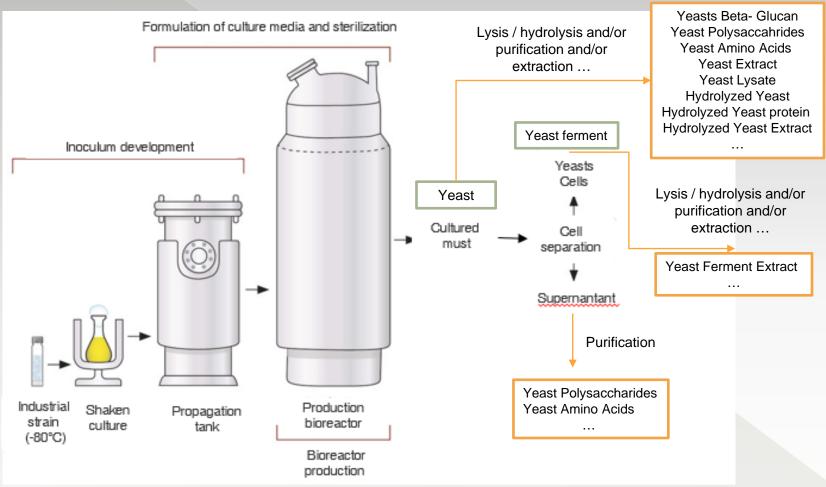


Process => culture of Yeast



Technologies des bioprocédés industriels, 2e édition, Louis Tessier

INCI names linked to Manufacturing process



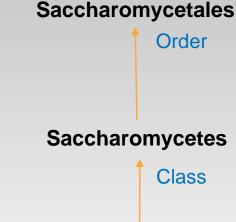
Hydrolyzed Candida Saitoana Extract Pichia Caribbica Ferment Pichia Ferment Lysate Filtrate Galactomyces Ferment Filtrate Yarrowia Lipolytica Extract Yarrowia Lipolytica Ferment Lysate Yarrowia Lipolytica Oil -Hydrolyzed Metschnikowia Agaves Extract Metschnikowia Agaves Polysaccharides Metschnikowia Agaves Extract Metschnikowia Henanensis Extract Hydrolyzed Metschnikowia Reukaufii Extract Metschnikowia Reukaufii Lysate Extract Hydrolyzed Metschnikowia Shanxiensis Extract Metschnikowia Viticola Extract Pichia Anomala Extract Pichia Ferment Extract Flltrate Pichia Pastoris Ferment Flltrate Pichia Ferment Lysate Filtrate Pichia Heedii Extract Pichia Extract Hydrolyzed Kluyveromyces Extract Kluyveromyces Extract Saccharomyces Cerevisiae Extract Saccharomyces Extract Saccharomyces Saccharomyces Ferment Filtrate Saccharomyces Ferment Lysate Filtrate Saccharomyces Ferment Saccharomyces Lysate Extract Filtrate Saccharomyces Lysate Extract Saccharomyces Lysate Saccharomyces Polypeptides Saccharomyces Saccharomyces Extract Saccharomyces Saccharomyces Ferment Filtrate Saccharomyces Ferment Lysate Filtrate Saccharomyces Ferment Saccharomyces Lysate Extract Filtrate Saccharomyces Lysate Extract Saccharomyces Lysate Saccharomyces Polypeptides SaccharomycesTorulaspora Delbrueckii Extract

Torulaspora Delbrueckii Ferment Hydrolyzed Torulaspora Delbruekii Extract

Hydrolyzed Candida Bombicola Extract

Identification - INCI names

Hydrolyzed Yeast
Hydrolyzed Yeast Extract
Hydrolyzed Yeast Protein
Yeast Amino Acids
Yeast Extract
Yeast Ferment Extract
Yeast Beta-Glucan
Yeast Polysaccharides



Yeast

Genenic INCI name

Ascoideaceae
Debaryomycetaceae
Dipodascaceae
Endomycetaceae
Metschnikowiaceae
Phaffomycetaceae
Pichiaceae
Saccharomycetaceae
Saccharomycetales incertae sedis
Saccharomycodaceae
Saccharomycopsidaceae
Trichomonascaceae

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INCI names - Conclusion

- All families belonging to the « Saccharomycetales » order from «Saccharomycetes » class are reviewed in this report
- From a taxonomy point of view, all yeast-related INCI names can be grouped into one generic INCI name: YEAST
- From a manufacturing process point of view : all yeast-related INCI names can be grouped into one generic INCI name: YEAST
- Great advantage of having only one generic INCI name:
 Yeast (in accordance with PCPC definition) rather than a
 multitude of INCI names with the scientific name which may
 regularly change due to taxonomy evolution

Process => media

 A generical culture media can be used for yeast growth; for example, a synthetic culture medium which allows very good repeatability because it is a standardized medium

Base medium	Quantity (g/L)
Ammonium sulphate	
Potassium phosphate	
Sodium phosphate di basic	
Magnesium sulphate	Confidential data
L-glutamic acid	
Sucrose or glucose or molasses	
Antifoam	
Ammoniac	

Oligo-elements	Quantity (mg/L)
EDTA	
ZnSO4. 7H2O	
MnCl2. 4H2O	
CuSO4. 5H2O	Confidential data
Na2MoO4. 2H2O	
CaCl2. 2H2O	
FeSO4. 7H2O	
KI	

Vitamins	Quantity (mg/L)
Biotin	
Pantothenic acid	
Nicotinic acid	
Myo-inositol	Confidential data
Thiamine-HCl	
Pyridoxine-HCI	
Para-amino-benzoic acid	

Process => Absence of impurities

Once the protocol has been established in R&D, this process is always applied in the same way to avoid any contamination / impurities or alteration (reproductible):



Revivification of the strain from the same secondary stock

The same inoculation rate is applied

The same culture medium is always used (preference for a standardized synthetic medium)

The chain of seeding and cultivation is always done over the same period

The sugar used is always the same and in the same quantity (glucose, sucrose or molasses)

The sugar supply is always the same

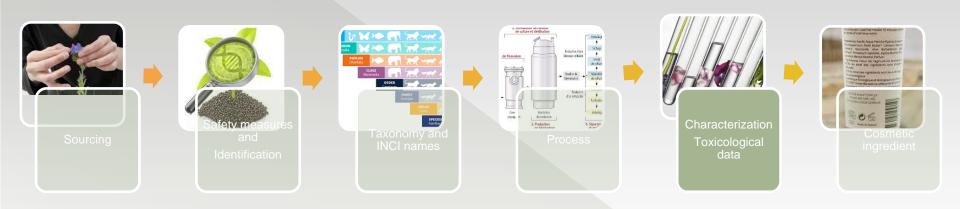
PH, temperature, oxygen, pressure, aeration are regulated

Many controls during culture: microscope, Optic Density, microbiology to ensure there is no contamination

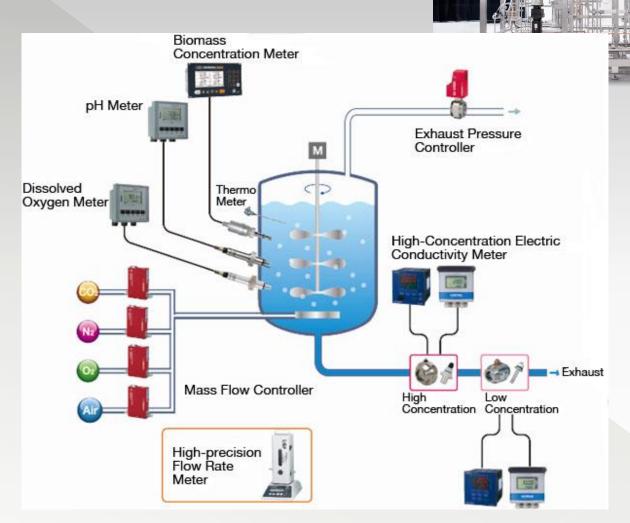
Cleaning in place is systematically controlled

=> All these controls allow: controlled, repeatable and reproducible culture

Characterization and Toxicological data

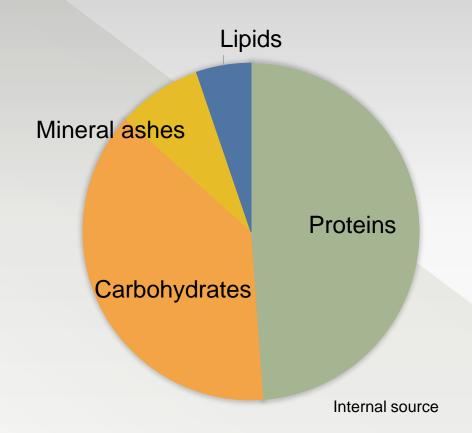


Controlled parameters



Analytical Characterization

Yeasts are always analytically characterized, in general:



Bibliographical characterization

Pathogenic Yeasts are well identified, 5 strains from Saccharomycetes class:

In Europe:

Candida albicans, Candida dubliensis, Candida glabrata, Candida parapsilosis, Candida tropicalis



In USA:

Candida auris (antibiotic resistance)

According to:

- Directive (EU) 2019/1833 of the commission of the October 24, 2019
- The US Centers for Disease Control (CDC) urgent threats list





 Bibliographical toxicological data has been found on the large majority of yeasts of each family belonging to Saccharomycetes class (complete data available on request)

NB: Data about Biocontrol were not taken into account since fruits and vegetables are supposed to be cleaned before consumption

Family	Food use – Bibliographical data			
Ascoideaceae	Listed in the publication : Diganta Narzary, Nitesh Boro et al. (2021), Community structure and metabolic potentials of the traditional rice beer starter 'emao'			
	Most of strains are :			
	- Listed in the publication: François Bourdichon, Serge Casaregola et al. (2011) "Food fermentations: Microorganisms with technological beneficial use"			
Debaryomycetaceae	 Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 			
	- Listed in publications about fruits fermentation for liquor (Camu-Camu, Agave)			
	- 1 strain notified for QPS status : Candida oleophila			
	- Listed in 1 patent : Method for producing beverages by acid removal (EP2866594A1)			

Family	Food use – Bibliographical data
Dipodascaceae	 Listed in the publication: François Bourdichon, Serge Casaregola et al. (2011) "Food fermentations: Microorganisms with technological beneficial use" Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 1 strain with QPS status: Yarrowia lipolytica
Endomycetaceae	Listed in the Patent US3296090A - Fermentation process for producing 1-tryptophane (one of the essential amino acids necessary for nutrition), 1984

Family	Food use – Bibliographical data
	Most of strains are :
Metschnikowiaceae	- Listed in publications about fermentations of beers and wines
	 Listed in publication: Hiroyuki Sasaharaa, Ken Izumori, (2005), "Production of L-talitol from L-psicose by Metschnikowia koreensis LA1 isolated from soy sauce mash", Journal of Bioscience and Bioengineering
	- Listed in the patent: EP 1 065 276 A1, (1999) Methods for producing D-arabitol, D-xylulose and xylitol using the yeast Metschnikowia
	 Listed in the publication: François Bourdichon, Serge Casaregola et al. (2011) "Food fermentations: Microorganisms with technological beneficial use"
	 Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022
	- 1 strain with GRAS status: Metschnikowia pulcherrima

Family	Food use – Bibliographical data
Phaffomycetaceae	 Listed in the publication: François Bourdichon, Serge Casaregola et al. (2011) "Food fermentations: Microorganisms with technological beneficial use" Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 1 strain with QPS status: Wickerhamomyces anomalus 1 strain notified for QPS status: Komagataella pastoris
Pichiaceae	 Most of strains are : Listed in publications about wine and distilled Agave beverages Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022

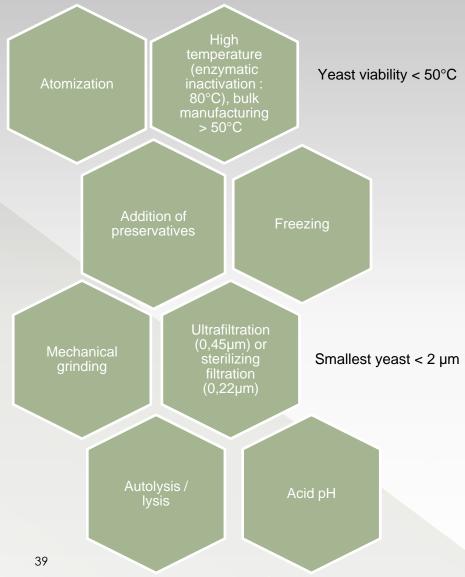
Family	Food use – Bibliographical data
Saccharomycetaceae	 Strains are: Listed in the publication: François Bourdichon, Serge Casaregola et al. (2011) "Food fermentations: Microorganisms with technological beneficial use" Listed in the bulletin of the IDF (International Dairy Federation), François Bourdichon, Andrea Budde-Niekiel et al. (2022), International Dairy Federation bulletin 514/2022 3 strains with QPS status: Kluyveromyces marxianus, Kluyveromyces lactis, Zygosaccharomyces rouxii 1 strain notified for QPS status: Eremothecium ashbyii 1 strain with GRAS status: Saccharomyces Cerevisiae
Saccharomycetales incertae sedis	- Listed in the publication: Roxane Detry, Noa Simon-Delso, (2020), "Specialisation of Yeast Genera in Different Phases of Bee Bread Maturation", Microorganisms

Family	Food use – Bibliographical data
Saccharomycodaceae	Listed in the Publication: Nuno Bourbon-Melo, Margarida Palma, (2021), "Use of Hanseniaspora guilliermondii and Hanseniaspora opuntiae to enhance the aromatic profile of beer in mixed-culture fermentation with Saccharomyces cerevisiae", Food Microbiology
Saccharomycopsidaceae	Listed in the Publication : Zai-Bin Xie, Kai-Zheng Zhang (2021), "Saccharomycopsis fibuligera in liquor production: A review", European Food Research and Technology
Trichomonascaceae	Listed in the Publication: Pradnya Chavan, Sarika Mane, Girish Kulkarn et al. (2009), "Natural yeast flora of different varieties of grapes used for wine making in India", Food Microbiology

Cosmetic ingredient manufacturing process

Manufacturing processes used to obtain cosmetic ingredients and products are incompatible with the viability of yeast.

Thus, no yeast can be alive in a cosmetic product



Conclusion

- Since the dawn of time, more and more yeasts have been used in food, especially in fermentation
- Identification and analytical characterization are key-information to guarantee the quality, stability and safety of the yeasts used
- As demonstrated before, from taxonomy and manufacturing process points of view, all yeast-related INCI names can be grouped into one generic INCI name YEAST
- Thanks to robust and well mastered manufacturing processes of cosmetic ingredients, the quality of yeasts is perfectly reproductible and stable

Conclusion

- Due to the existence of food use for the majority of strains from Saccharomycetes class, all can be grouped together in the "Yeast" INCI name and can be considered safe for use as a cosmetic ingredient
- Processes used to manufacture cosmetic products guarantee the absence of live organisms
- Even if the safety of the yeast is demonstrated, we strongly recommend a pre-market safety evaluation of the cosmetic ingredient, consistent with CIR reviews of other ingredients with a history of safe use in the diet, additional data concerning the potential for local effects, e.g., dermal irritation and sensitization, are needed

Thank you for attending Any questions?