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REVIEW ARTICLE

Non-*Saccharomyces* Commercial Starter Cultures: Scientific Trends, Recent Patents and Innovation in the Wine Sector

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Abstract: For 15 years, non-*Saccharomyces* starter cultures represent a new interesting segment in the dynamic field of multinationals and national companies that develop and sell microbial-based biotechnological solutions for the wine sector. Although the diversity and the properties of non-*Saccharomyces* species/strains have been recently fully reviewed, less attention has been deserved to the commercial starter cultures in term of scientific findings, patents, and their innovative applications.

Considering the potential reservoir of biotechnological innovation, these issues represent an underestimated possible driver of coordination and harmonization of research and development activities in the field of wine microbiology. After a wide survey, we encompassed 26 different commercial yeasts starter cultures formulated in combination with at least one non-*Saccharomyces* strain. The most recent scientific advances have been explored delving into the oenological significance of these commercial starter cultures. Finally, we propose an examination of patent literature for the main yeasts species commercialised in non-*Saccharomyces* based products.

We highlight the presence of asymmetries among scientific findings and the number of patents concerning non-*Saccharomyces*-based commercial products for oenological purposes. Further investigations on these microbial resources might open new perspectives and stimulate attractive innovations in the field of wine-making biotechnologies.

Keywords: Wine, alcoholic fermentation, non-*Saccharomyces*, starter cultures, yeasts, patents.

1. INTRODUCTION

For thousands of years, wine and fermented alcoholic beverages have been made without understanding the biological, biochemical and chemical basis responsible of this kind of productions. Thanks to the progresses in oenological microbiology, we know that a complex microbial consortium is associated with the conversion of grape juice/must in wine, with a dominance of yeasts than can carry out the Alcoholic Fermentation (AF). During AF, the main bioprocess is the conversion of sugars in ethanol. However, complex ranges of primary and secondary yeast metabolites are susceptible to influence deeply wine quality [1, 2]. Following AF, a secondary bioconversion of oenological interest can take place in wine. It is called Malolactic Fermentation (MLF) and consists of the decarboxylation of malic acid in lactic acid mediated by Lactic Acid Bacteria (LAB) [1]. Nowadays, it is common to start the MLF during the first two days of AF and drive both fermentations simultaneously. *Saccharomyces cerevisiae* and *Oenococcus oeni* are generally recognized as the main microorganisms responsible for the AF and the MLF, respectively. However, the spontaneous fermentations in the traditional wine making process do not rely only on these two species [3]. Several studies have shown that the microbial population originally present in the must is very diverse in terms of eukaryotic microbes, and during the early steps of the spontaneous AF, *S. cerevisiae* does not represent the dominant species of the microbial population [2, 4, 5] . Indeed, it is possible to find high concentrations of yeasts of oenological interest that do not belong to the *Saccharomyces* genera. For this reason, this heterogeneous group of microorganisms is generally mentioned as ‘non-*Saccharomyces*’. Non-*Saccharomyces* species drive the first steps of the AF, followed by the dominance of *S. cerevisiae*, which is responsible for the core and final phases of the AF. Indeed, only *Saccharomyces* species (*S. cerevisiae* or more rarely *S. uvarum*) are able to complete AF in

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oenological conditions, meaning to consume all sugar initially present in grape must. For this reason, non-*Saccharomyces* species are essentially used as mixed or sequential starters. All the microorganisms naturally present on the grapes, in the vineyard or in the wine cellars, can be genotypically and phenotypically different, influencing wine quality and sensory perception, contributing to making it unique [1].

Considering the general management of microbial resources, like for other food industries dealing with the manufacture of fermented foods and beverages, the adoption of starter cultures technology as a winemaking practice represented an outstanding progress in the wine making practices in the last decades. Among the different description of starter cultures reported in the scientific literature [6], a general definition has been provided by Hansen in the Encyclopaedia of Food Microbiology; "Commercial starter cultures are standardized inoculum to be used for the production of fermented foods. Starter cultures are produced by specialized manufactures. Rigorous quality assurance and quality control are conducted to ensure performance, composition, and safety of the culture" [7]. The starters can help winemakers to achieve several benefits such as the accomplishment of AF and MLF, the reduction of time needed for the fermentation/productions (with a corresponding reduction of production costs) process and the control of negative effects due to indigenous microorganisms. Moreover, microbial starters may allow the manufacture of specific productions (*e.g.* sparkling wines) [8-10]. Hence, commercial starter cultures allowed wine makers to produce stable and sustainable products, mainly through the exploitation of the great diversity of *S. cerevisiae* and *O. oeni* strains genotypically and technologically characterized [11, 12]. In other terms, this biotechnological solution has changed the way of producing wine, minimizing the role of the spontaneous indigenous wine by steering and accelerating microbial-based bioprocesses of interest in oenology.

Together with the continuous interest in the selection of new and suitable *S. cerevisiae* and *O. oeni* strains and in the design of new microbial combinations, in the last decade, an increasing attention has been focused on the selection of non-*Saccharomyces* species/strains for the formulation of new starter cultures able to pilot the AF. According to different studies, several species showed interesting properties in aromatic molecules production and in the field of natural bio-protection of the wine against spoilage yeasts or bacteria. Some examples of the consequences due to the use of selected non-*Saccharomyces* species are: production of mannoproteins, lower production of volatile acidity, reduced alcohol content, production of esters, terpenes and thiols, production of polysaccharides, stabilisation of colour and consumption of malic acid [13-19]. Non-*Saccharomyces* yeasts represent an important reservoir of biotechnological innovation in the field of wine microbiology that requires a major coordination of research and development actions. While the diversity and the properties of non-*Saccharomyce*s species/strains have been recently fully reviewed [15, 20-22], less attention has been paved to the commercial starter cultures in term of scientific findings, patents, and innovations. For this reason, the aim of this review is to provide and sum up the most recent studies, innovations and patents concerning commercialised non-*Saccharomyces* starters, their use, and their properties, in order to improve coordination and harmonization of the research and development activities.

2. NON-*SACCHAROMYCES* SPECIES COMMERCIALIZED IN THE WINE SECTOR AND THE REGULATORY ASPECTS

Seven species, used to design the complete panel of commercial starter cultures, were considered in this survey (Table **1**): *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, *Metschnikowia fructicola, Kluyveromyces thermotolerans*, *Kluyveromyces wickerhamii*, *Candida zemplinina*, *Schizosaccharomyces pombe,* and *Pichia kluyveri*. Fig. (**1**) provides information on the first introduction on the market of relevant and representative commercial starter cultures that include in the formulation of a non-*Saccharomyces* strain. The timeline highlights the first release on the market in 2004 by Christian Hansen, followed by Lallemand (2007) and Laffort (2010). Furthermore, it clearly testifies the continuous interest of the market in new products tailored to offer innovative solutions to the wine industry. *T. delbrueckii* is one of the most studied non-*Saccharomyces* yeasts, probably due to its contribution to the quality of the volatile fraction of wine (with low production of volatile acidity and other undesirable volatile compounds) [66, 67]. Other aspects of wine quality that are susceptible to be influenced by *T. delbrueckii* metabolism in wine (also in combination with *S. cerevisiae* strains) are volatile thiols [18], esters [57], vinylphenols [68], higher alcohols [68], pigments [45] and mannoproteins [69]. While the interaction with *S. cerevisiae* has been explored to evaluate the performances in AF, recent studies explored the impact of *T. delbrueckii* inoculation on the subsequent MLF [29-31].

Two species represent *Metschnikowia* genus: *M. pulcherrima* and *M. fructicola*. The antagonistic activity of *M. pulcherrima* on other wine yeast species is considered a key property for the design of protective cultures and biocontrol applications [70]. This species has also been found susceptible to modulate sensory profile/volatile aroma composition and/or exploitable to reduce the final alcohol content in wine [21, 71, 72]. The scientific literature offers few studies, but similar effects for *M. fructicola*, with a pronounced biocontrol efficiency for viticultural applications [73-76].

K. thermotolerans can improve wine quality at different levels. Together with its influence on the sensory perception and its role in bioprotection [13, 77], less attention has been paid on lactic acid bio-production [13, 78], in order to avoid excessive deacidification of musts, and the enhanced content of polymeric pigments [79]. The effect on mannoproteins, polysaccharides, and amino acids has also been evaluated [13]. On the opposite, *K. wickerhamii* has been used for specific biotechnological application connected with the antagonistic activity exerted by the killer toxin produced by one of its strain [80].

Table 1. Commercial starter cultures containing non-*Saccharomyces* species/strains. The table provides information concerning product names, companies, yeast species, strain names. For each product, we report exemplificative references of scientific works that tested the oenological performances of the starter cultures.

Product	Providing Company	Species	Strains	Advantages Features of Interest in Winemaking	Refs.
Atecream 11H	BioEnologia	*Candida zemplilina*	-	Indicated for high alcohol content wines. Increased production of glycerine and low alcohol rate.	-
Atecream 12H	BioEnologia	*Schizosaccharomyces pombe*	-	Used for the MLF. High production of glycerol. Low production of volatile acidity, sulphites and acetic acid.	-
FROOTZEN®	Christian Hansen	*Pichia kluyveri*	-	Secure the fermentation and enhance fruit flavors.	[23-26]
Biodiva	Lallemand	*Torulaspora delbrueckii*	TD241	Increases perception of some esters without masking the typicity. low volatile acidity.	[15, 19, 24-32]
CONCERTO™	Christian Hansen	*Kluyveromyces thermotolerans*	-	Ideal for hot climate. It increases total acidity, and it produces fresh strawberry aroma.	[13, 15, 23, 24, 33-36]
ENARTIS FERM BRETT OUT K	Enartis	*Kluyveromyces wickerhamii*	-	Production of a mycotoxin inhibiting the growth of spoilage microorganisms.	-
ENARTIS FERM BRETT OUT W	Enartis	*Wicheranomyces anomalus*	-	Production of a mycotoxin inhibiting the growth of spoilage microorganisms.	-
Excellence® BIO-NATURE	Lamothe-Abiet	*Metschnikowia pulcherrima*	-	Production and control of the indigenous microbiota.	-
Flavia	Lallemand	*M. pulcherrima*	MP346	Releases of thiols and terpenic compounds during AF, favorising the expression of red and white wines.	[13-16, 25-27, 29, 37]
Gaïa	Lallemand	*Metschnikowia fructicola*	-	Protection from spoilage yeasts, reducing the risk of early pre-fermentation.	[38-40]
LAKTIA™	Lallemand	*Lachancea thermotolerans*	-	Production of high amount of lactic acids during AF, and complex aromas at the beginning of the AF.	-
Level^{2}TD	Lallemand	*S. cerevisiae* *T. delbrueckii*	 E491	Increased aromatic complexity thanks to terpenes and esters production and reduction of the volatile acidity.	[41-45]
Levulia® Alcomeno	AEB	*K. thermotolerans*	-	High production of lactic acid. Indicated for hot climates or overripe grapes.	-
VINIFLORA RYTHM	Christian Hansen	*K. thermotolerans,* *S. cerevisiae*	-	Production of aromatic red wines rich in blackberry and blackcurrant odors, and preservation of the acidity in wine.	[41]
VINIFLORA HARMONY	Christian Hansen	*K. thermotolerans,* *T. delbrueckii* *S. cerevisiae*	-	Enhanced mouthfeel and palate weight. Generation of sweet fruit intense aromas.	[41, 46]
VINIFLORA SYMPHONY	Christian Hansen	*K. thermotolerans,* *S. cerevisiae*	-	Clear floral aroma and bright, tropical fruity notes in white wines. Complex and round flavors in red wines.	[47]
MELODY™	Christian Hansen	*K. thermotolerans,* *T. delbrueckii* *S. cerevisiae*	-	For red and white wines. Generation of fruity and spicy aromas.	[13, 48]
Oenoferm® wild & pure	Erbslòh	*T. delbrueckii*	[HR23]	It brings a creamy texture with a pleasant lasting mouthfeel.	[42]
Oenovin Torulaspora Bio	Oeno	*T. delbrueckii*	-	It increases the olfactory notes of red fruit and it improves the softness and roundness of wines.	-

(Table 1) Contd…

Product	Providing Company	Species	Strains	Advantages Features of Interest in Winemaking	Refs.
PRELUDE™	Christian Hansen	*T. delbrueckii*	-	It guarantees flavor complexity by producing medium-chain (stable) fatty acid esters and by promoting MLF. Production of high concentration of mannoproteins that give a fuller and smoother mouth feel.	[13, 42, 49, 50]
PRIMAFLORA®	AEB GROUP	*T. delbrueckii*	-	Protection of the must from spoilage microorganisms by competitive selection. It brings some aromatic complexity and it improves mouthfeeling.	[45, 51]
ProMalic®	Proenol	*Schiz. pombe*	-	It allows malo-alcoholic deacidification.	[13, 36, 52]
Qτ	Enartis	*T. delbrueckii*	-	Production of high amounts of esters and terpenoids that create fresh, red fruits aromas. Production of low volatile acidity. It increases smoothness.	-
viniferm NSTD	AgroVin	*T. delbrueckii*	-	Intensification of the perception of floral aromas by producing β-phenyl ethanol. Production of high amounts of mannoproteins.	[53-55]
ZYMAFLORE® Alpha TD n. sacch	Laffort	*T. delbrueckii*	-	Production of varietal thiols. Low production of volatile acidity.	[16, 18, 41, 45, 56, 57]
ZYMAFLORE® ÉGIDE^TDMP	Laffort	*T. delbrueckii M. pulcherrima*	-	Pre-fermentative control and bio-protection.	-

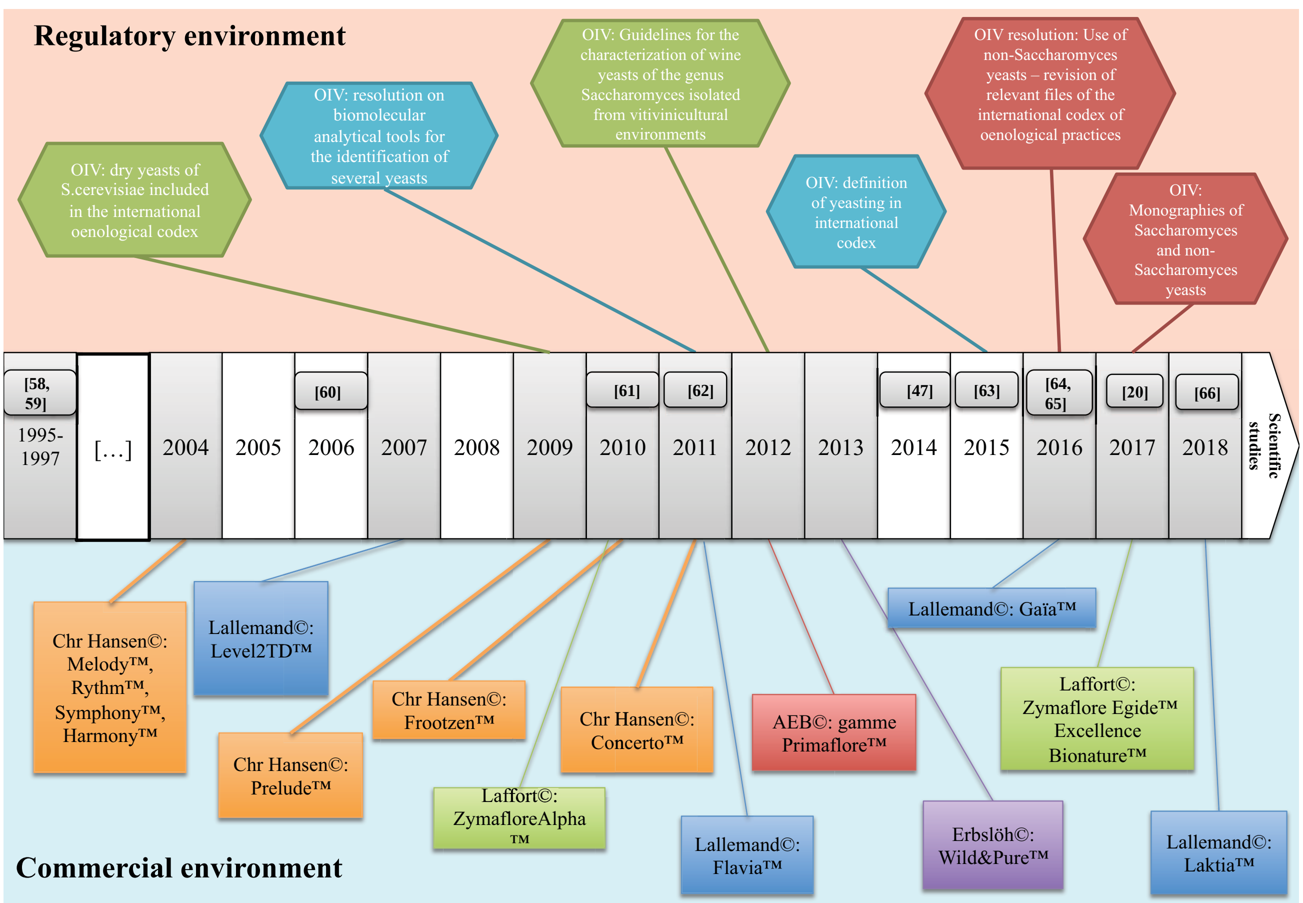

Fig. (1). Timeline of relevant non-*Saccharomyces* starter cultures introduction on the market, together with exemplificative information on the regulatory environment and on scientific studies.

The major interests in the application of *C. zemplinina* in wine are related to various metabolic features such as low ethanol production, fructophily, and glycerol production [63, 81]. A positive effect on the acetic acid content in sweet wine fermentations has been reported [82]. Furthermore, the oenological interest of *S. pombe* is mainly connected with its ability to convert L-malic acid into ethanol [83], providing an alternative to classic MLF, in terms of reduction in malic acid content. Compared to *S. cerevisiae* in pure culture, *S. pombe* led to a significant reduction in the biogenic amines content and in the ethyl carbamate precursors concentration [84], probably due to the modulation of the malolactic consortium. Besides, compared to *S. cerevisiae*, *S. pombe* displays also higher capacity to release polysaccharides [85].

Even if we stress the positive significance of this heterogeneous eukaryotic class in oenology, it is to be mentioned that for a long time, the only attention deserved to this microbial class was due to their spoilage potential [86]. Several genera/species continue to represent a source of spoilage phenomena [87, 88], with a particular mention for the yeast *Brettanomyces bruxellensis* due to the simultaneous presence of peculiar phenotypes, such as the high production of volatile phenols [89], the aptitude to enter in a VBNC state [90, 91], and the resistance to sulphites [92, 93], all aspects deeply connected to the genetic intraspecific diversity highlighted within the species [89, 91, 92]. In addition, we cannot neglect that some non-*Saccharomyces* strains were found capable of bio-producing biogenic amines during alcoholic fermentation implying safety risks associated with their development [94, 95].

To conclude this introductory paragraph, we propose a brief update on the international regulatory environment, using information from recently approved resolutions from the principal internationally recognized intergovernmental organization dealing with scientific and technical aspects of viticulture and winemaking, the International Organization of Vine and Wine (OIV). In 2016, the OIV revised the international codex of oenological practices according to the emergence of non-*Saccharomyces* among relevant microbial resources use in the field, OIV-OENO 546-2016. Furthermore, in 2017, the OIV released a document entitled « Monography of non-*Saccharomyces* yeasts » to the International Oenological codex (OIV-OENO 576B-2017), testifying that the non-*Saccharomyces* species have now reached an important role in the oenological sector. To point out the international relevance of these specifications, it is mentioned that, in 2017, the European Union, represents important countries in terms of wine production, recommended to the members of the Union, the compliance and the OIV resolutions concerning non-*Saccharomyces* species COM/2016/0579 final - 2016/0273 (NLE). First, it is important to note that these yeasts «must be isolated from grapes, musts or wine or result from hybridisation of grape/must/wine strains, or they have been derived from other wine yeasts» (OIV-OENO 576B-2017). Additionally, the document state that it must be indicated on the packaging: *i)* the genus name, the species name, the name of the strain(s) and all elements that can guarantee the traceability of the product, *ii)* the name of the selector, *iii)* operating instructions recommended by the manufacturer, *iv)* a recommended rate of inoculation, *v)* the minimum number of viable cells (CFU, colony forming unit) per gram of product guaranteed by the manufacturer, with a recommended storage temperature, and where relevant, *vi)* the indication that the yeast strain(s) were obtained through genetic modifications and their modified character(s) (OIV-OENO 576B-2017). Non-*Saccharomyces* selected yeasts can be commercialized as Active Dry Yeast (minimum dry matter of 92%), Active Frozen Yeast (dry matter from 40 to 85%), Compressed Yeast (dry matter from 30 to 35%), Cream Yeast (dry matter from 18 to 25%). In all these cases, the level of viable yeasts should be equal to or above 10^{10} CFU/g of dry matter (OIV-OENO 576B-2017). In addition, OIV admitted encapsulation (beads) and/or immobilisation (*e.g.* in alginate) (minimum of dry matter of 86%), with a level of viable yeasts equal to or above 10^9 CFU/g of dry matter. With the concern of admitted microbial contaminants, the monographs of *Saccharomyces* (OIV-OENO 576A-2017) and non-*Saccharomyces* (OIV-OENO 576B-2017) give the same limits of contaminations (Table **2**). Starting from 2011, the OIV described analytical tools for the identification of several oenological yeasts including non-*Saccharomyces* species (OIV-OENO 408-2011). DNA sequencing of the variable domain D1/D2 of 26S ribosomal region obtained by PCR amplification is indicated as the reference method, even if several molecular approaches have been successfully developed in order to profile yeasts diversity associated with oenological fermentation [96, 97]. On the opposite, the intraspecific characterization within the main non-*Saccharomyces* species of pro-technological interest is a subject more recently explored. Several molecular techniques for genetic clustering were conceived and tested, such as RAPD-PCR fingerprinting, pulsed-field electrophoresis, tandem repeat-tRNA and RFLP [98]. From this point of view, the design of suitable primers for microsatellites genotyping tailored for each species represents a promising approach in order to provide a measure also of the intraspecific genetic diversity [98]. This kind of molecular

Table 2. Limits of microbial contaminations given by the OIV monographies of *Saccharomyces* (OIV-OENO 576A-2017) and non-*Saccharomyces* (OIV-OENO 576B-2017).

Microbial Contaminants	Limit
Yeasts of species different from the species indicated on the label	Less than 10^5 CFU/g
Mould	Less than 10^3 CFU/g
Lactic acid bacteria	Less than 10^5 CFU/g
Acetic acid bacteria	Less than 10^4 CFU/g
Salmonella	Absence should be checked on a 25 g sample
Escherichia coli	Absence should be checked on 1 g sample
Staphylococci	Absence should be checked on 1 g sample
Coliforms	Less than 10^2 CFU/g

biomarker has been developed for *T. delbrueckii* [99], *C. zemplinina* [63] and *H. uvarum* [100]. Finally, it is to be mentioned that the design of tailored probes can have a fundamental role in the rapid detection of spoilage non-*Saccharomyces* yeasts in order to avoid consistent economic losses [101, 102].

3. NON-*SACCHAROMYCES* BASED COMMERCIAL STARTER CULTURES IN THE WINE SECTOR AND THE SCIENTIFIC LITERATURE

For 15 years, non-*Saccharomyces* starter cultures have represented important innovative products in the field of microbial solutions for the oenological sector. In this work, following a wide search, we encompass 26 different commercial yeasts starter cultures formulated with at least one non-*Saccharomyces* strain (Table **1**). These yeast products belong to 11 different companies well known in the market of oenological starters and they can be sorted according to their oenological function/significance, the species/biotypes of the yeasts, and their employment for the production of red, white or rosé wines [27, 31, 103]. Christian Hansen, Lallemand and Enartis are the companies producing the wider panel of commercial products with respectively 7, 5, and 3 products, accounting for the 57% of the analysed products. They are followed by Bioenologia, AEB Group and Laffort with 2 products for each company. The other corporations selected, designed and commercialised only one non-*Saccharomyces* starter cultures. However, as shown in Table **1**, scientific works testing the oenological performances of non-*Saccharomyces* starter cultures have been performed only for few commercial formulations actually available on the market, indicating that the employment of non-*Saccharomyces* species/strains for wine-making is still an open field that needs to be supported by further scientific evidence. Fig. (**2**) summarizes this information reporting the percentages of the number of non-*Saccharomyces* based products sold by dynamic companies in the field of oenological starter cultures. The main non-*Saccharomyces* yeasts species of oenological interest are *T. delbrueckii* (46% of the analysed products), *K. thermotolerans* (27% of the analysed products), and *M. pulcherrima* (12% of the analysed products). Indeed, commercial starter formulations including one of these three species are commercialised by the majority of the companies. Concerning the oenological significance of these commercial starter cultures, in this mini-review, we considered formulations that offer solutions for specific technological issues (*e.g*. reduction of volatile acidity, bio-protection), and microbial products susceptible to enhance the sensorial and organoleptic wine quality. In particular, 11 commercial products claiming to improve the aromatic complexity of the wine *bouquet*. The second category of products encompasses five formulations providing bio-protection activities. Few products (4 out of 26) deliver solutions for specific technical issues as well as the acidity increase due to the bio-production of lactic acid. This approach is tailored for wines produced from wine grapes grown in hot climatic regions, in order to balance the acidity and alcoholic perception [92, 100, 104], and it could become more and more exploited due to global warming. Another objective explored by some commercial products [4] is to improve the textural properties of the wine based on the production of mannoproteins, which lead to a smoother mouth feeling [105]. Interestingly, a clear relationship among the microbial species and the function of the oenological products can be identified. While *Metschnikowia* genus is sometimes exploited for bio-protection purposes and *K. thermotolerans* for the production of lactic acid [13, 106], *T. delbrueckii* has been generally found useful to both, enhance aromas and improve wine texture [18, 27, 31, 32, 44, 45]. Considering the technological interest of the analysed products, 62% of the starter cultures above reported have been revised in the scientific literature (Table **1**). The products that received the highest interest are Biodiva®, Flavia®

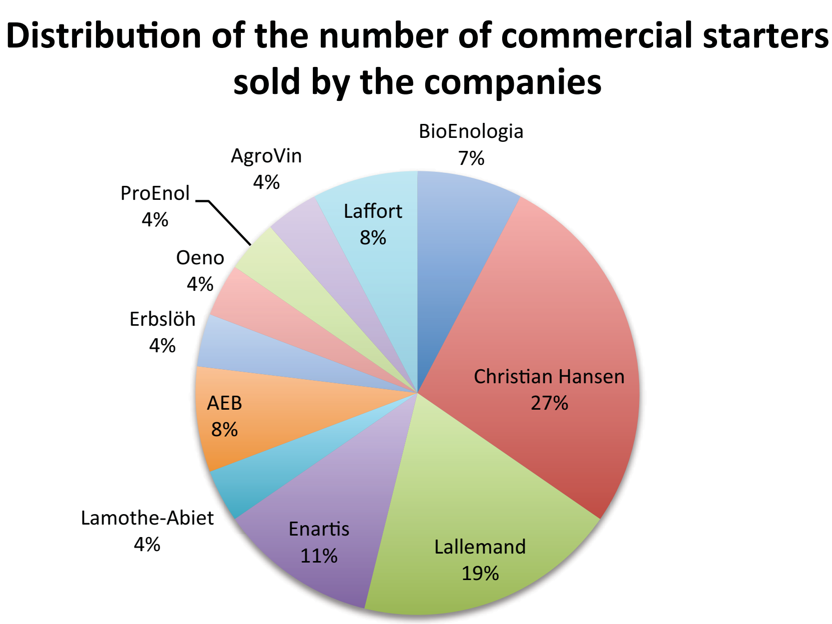

Fig. (2). Percentages of the number of non-*Saccharomyces* based products sold by companies active in the field of oenological starter cultures.

and Concerto®, corresponding respectively to the species *T. delbrueckii*, *M. pulcherrima* and *K. thermotolerans*. The main advantages of Biodiva are the decrease of volatile acidity and the enhancement of the aroma complexity of both red and white wines [18, 27, 31, 32, 45]. Recent findings also suggest an increase of foam persistence in case of Biodiva application in the production of sparkling wines and, a major content of glycerol when this starter is added to the must, with a pronounced coexistence in the case of mixed fermentations with *S. cerevisiae* strains [10, 27, 31]. Whitener *et al.* [15] pointed out that the release of aroma compounds by Biodiva® depends on the grape must, and the total ester production was lower than when *S. cerevisiae* was only used [15]. Biodiva® has also been found to produce various sulfuric and thiol compounds which can generate off-odours when they are found in high concentrations. Nevertheless, no sensorial analysis detected any aromatic faults usually linked to these compounds, which means that their concentrations are usually not high enough to produce these faults [25]. On the opposite, Flavia® enhance the aroma complexity, producing a high amount of esters, thiols and terpenes compounds [15, 24, 27, 37]. The main aromas claimed by sensory and chemical analyses are smoky, flowery, citrus and pineapple [15, 24, 25]. For Concerto®, scientific studies focused on its possible use to increase the wine acidity by producing lactic acid and malic acid [13]. As reported for hot climate regions, this property is very interesting also in future perspectives related to climate change. Indeed, the recorded increase in temperature leads to an important rise of sugar concentrations and a reduction of organic acids content in musts. Consequently, it will be important to develop new strategies to balance the flavours and the wine taste by increasing its acidity [106, 107]. It is interesting to underline that Concerto® possesses the capacity to fully ferment beer and improves the mouthfeel of wines [13, 35]. Nevertheless, this starter doesn't survive at a high concentration of ethanol (10%) and must be inoculated before *S. cerevisiae* to assure optimal coexistence/performances [26]. Other starter cultures like Promalic® (*S. pombe*) and Frootzen® (*Pichia kluyveri*) have shown different properties. *S. pombe* is a species used for deacidification of the wine, hence Promalic is employed to realise AF and MLF at the same time, reducing the time necessary to complete both fermentations steps in the winemaking process [36]. This property, transposed to the industrial context is very interesting to gain time of the wine production and marketability with a consequent reduction of the corresponding costs. Frootzen® can produce high quantities of acetaldehyde, acetic acid, methyl esters, butyl octanol, and other fruity aromas esters [26]. However, this formulation can also produce molecules responsible of off-odours such as 3-methyl-butanoic acid, associated with cheesy and sour aromas, or phenethylamine, even if the concentrations of these undesired compounds are usually below the threshold of perception by human sense.

Although this review provides a background of the most interesting properties of the main commercial starters for wine-making, it should be highlighted that they are often not supported by scientific evidence. However, some of these products have been released only recently, which can partially explain the absence of scientific research.

4. NON-*SACCHAROMYCES* SPECIES COMMERCIALIZED IN THE WINE SECTOR AND RECENT PATENTS

Table **3** [108-124] reports the patents found on the database 'Google Patent' (accessed at website https://patents.google.com/) using as queries, the non-*Saccharomyces* species used in commercial starter design/formulation. Accordingly to above reported, the most representative species is *T. delbrueckii* followed, in the patent literature, by *S. pombe* and *P. kluyveri*. In general, for every species present in the formulation of commercial starters, at least one patent in the database can be found. The main topics of these patents are related to the production of alcoholic beverages, including the inoculation of these species in grape or barley must (6 patents) [110, 113, 115-117, 120], the production of interesting yeasts for starter formulations, and the analysis or detection of microbial species/strains (5 patents) [108, 109, 114, 118, 121]. Some patents related to the production

Table 3. Non-*Saccharomyces* species used in the formulation of commercial starter cultures. For each species, we report exemplificative patents in the field of oenology, viticulture and alcoholic beverages.

Species	Patent Title	Classification	Abstract Extract
K. thermotolerans	Yeast compositions and starter cultures [108]	C12G1/0203 Preparation of must from grapes; Must treatment and fermentation by microbiological or enzymatic treatment	The present invention aims at solving the problem of how to provide a starter culture, including a mixed starter culture, capable of fermenting *e.g.* grape juice without losing the desirable characteristics of less competitive non-*Saccharomyces* yeast species early on in the fermentation process.
-	A method to discriminate and quantify *T. delbrueckii* in mixture with *K. thermotolerans* and *S. cerevisiae* [109]	C12Q1/06 Quantitative determination	A method useful for the discrimination and quantification of *T. delbrueckii*, which can be present in a mix together with *K. thermotolerans* and *Saccharomyces* in a starter culture or in cultures in general.
L. thermotolerans	Methods for the production of fermented beverages and other fermentation products [110]	C12C12/006 Yeasts	The present invention is directed to methods for making fermented beverages and other fermentation products including lactic acid and ethanol for fuel and the products produced therefrom.

(Table 3) Contd…

Species	Patent Title	Classification	Abstract Extract
M. fructicola	Combinations of biological control agents and insecticides [111]	A01N63/04 Microbial fungi or extracts thereof	Compositions are provided that improve overall plant vigor and yield by combining agriculturally effective amounts of at least one environmentally friendly biological control agent and at least one chemical insect control agent.
-	A novel antagonistic yeast useful in controlling spoilage of agricultural produce, methods of use thereof and compositions containing same [112]	C12N1/14 Fungi; Culture media therefor	The present invention relates to the yeast *M. fructicola* and to use thereof to inhibit growth of unwanted microorganisms on a portion of a plant, for example, foliage, flowers, fruit, roots or vegetables.
M. pulcher-rima	Production of a low-alcohol fruit beverage [113]	C12G1/0203 Preparation of must from grapes; Must treat-ment and fermentation by microbiological or enzymatic treatment	The present invention relates to the production of fermented fruit beverages, such as wine and cider, with a reduced level of alcohol.
P. kluyveri	*P. kluyveri* strain and uses thereof [114]	C12R1/84 Pichia	The invention relates to a *P. kluyveri* strain that can ferment the reducing sugars present in orange juice, to a method for growing said microorganism, to the use thereof in the production of a drink having a low alcohol content and optimum organoleptic properties for consumption, and to the product derived from fermentation using the *P. kluyveri* strain.
-	Enhancement of beer flavor by a combination of *Pichia* yeast and different hop varieties [115]	C12C11/003 Fermentation of beerwort	The invention relates to a method of brewing beer comprising a step of fermentation of a hopped wort with a *P. kluyveri,* where there is an interaction of the hops with the *P. kluyveri* yeast strain to en-hance the flavor of beer.
-	Production of low-alcohol or alcohol-free beer with *P. kluyveri* yeast strains [116]	C12C12/04 Beer with low alcohol content	The invention relates to a method of preparing a low-alcohol or alcohol-free beverage with an alcohol content of no more than 1.2% (vol/vol) and an isoamyl acetate content of at least 0.5 ppm com-prising the steps of a) providing a wort; and b) fermenting the wort with at least one *P. kluyveri* yeast strain to obtain a low-alcohol or alcohol-free beverage under sterile conditions and with *P. kluyveri* as the only species of yeast strain added.
-	Method for preparing a fermented beverage and beverage thus produced [117]	C12C5/026 Beer flavouring preparations	The present invention concerns a process for the production of a beverage.
T. del-brueckii	Hybrids obtained by the fusion of saccharomyces cerevisiae and *T. delbrueckii*, their use to conduct red and white wine fermentations and/or to restart stuck or sluggish fermenta-tions, and process of obtaining such hybrids [118]	C12N1/16 Yeasts; Culture media therefor	This invention relates to a process of obtaining hybrid strains ob-tained by protoplast fusion of *S. cerevisiae* and *T. delbrueckii* and their use in fully conducting alcoholic fermentations of red and white wine as well as their use as restarter strains in the rectification of stuck or sluggish fermentations.
-	A method to discriminate and quantify *T. delbrueckii* in mixture with *K. thermotolerans* and *S. cerevisiae.* [109]	C12Q1/06 Quantitative deter-mination	A method useful for the discrimination and quantification of *T. delbrueckii*, which can be present in a mix together with *K. thermotolerans* and *Saccharomyces* in a starter culture or in cultures in general.
-	Yeast compositions and starter cul-tures [108]	C12G1/0203 Preparation of must from grapes; Must treat-ment and fermentation by microbiological or enzymatic treatment	The present invention aims at solving the problem of how to provide a starter culture, including a mixed starter culture, capable of fer-menting *e.g.* grape juice without losing the desirable characteristics of less competitive non-*Saccharomyces* yeast species early on in the fermentation process.
-	Processes for increasing the fermenta-tive capacity of non-*Saccharomyces* yeasts [119]	C12N1/16 Yeasts; Culture media therefor	The present invention relates to processes for increasing the fermen-tative capacity of non-*Saccharomyces* yeasts in the fermentation of juice from fruit, vegetables, tubers, and cereal macerates.

(Table 3) Contd…

Species	Patent Title	Classification	Abstract Extract
-	Process for obtaining a sparkling alcoholic beverage where wine macerated in hops is mixed with vegetable water and/or water, and addition of wine concentrate and concentrated fruit and/or vegetable juice [120]	C12G1/0203 Preparation of must from grapes; Must treatment and fermentation by microbiological or enzymatic treatment	The present invention relates to a process for obtaining or making a wine- based sparkling alcoholic beverage resulting from a preparation process, wherein wine macerated in hops is mixed with vegetable water and/or water, to which wine concentrate and concentrated fruit and/or vegetables juice are added.
Schizosaccharomyces pombe	L-malic acid degrading yeast for wine making [121]	C12R1/645 Processes using microorganisms using fungi	This invention refers to yeasts obtained by fusion of spheroplasts capable of maintaining in a single genotype the demalifying trait of a *S. pombe* parental strain and the growth rate of the parental strain *S. cerevisiae*, 3 new yeasts obtained from protoplast fusion between the fusion product initially obtained (MB7TC alpha) and the parental strain, and two other *S. cerevisiae* yeasts, and the use of these yeasts for obtaining demalification of acid wines.
-	Compositions and methods for reducing H2S levels in fermented beverages [122]	C12N15/81 Vectors or expression systems specially adapted for eukaryotic hosts for fungi for yeasts	The present invention provides compositions and methods for reducing H2S levels in fermented beverages.
-	Functional enhancement of yeast to minimize production of ethyl carbamate *via* modified transporter expression [123]	C07K14/395 Peptides having more than 20 amino acids; Gastrins; Somatostatins; Melanotropins; Derivatives thereof from fungi from yeasts from *Saccharomyces*	A yeast strain transformed to reduce nitrogen catabolite repression of gene expression of a urea transporter protein under fermenting conditions.
-	Method of deacidifying wine and composition therefor [124]	C12N11/04 Enzymes or microbial cells being immobilised on or in an organic carrier entrapped within the carrier, *e.g.* gel, hollow fibre	Deacidifying wine by passage through an alginate gel containing living cells of *Leuconostoc oenos* therein.

of alcoholic beverages display a general description [110, 117] but others are more specific, and deal with the production of low-alcoholic beverages [113, 116], the enhancement of beer flavours [115] or the production of sparkling wine from vegetable [120]. Starter yeasts patents are also mainly general [108, 114, 121] but some of them discuss more specific topics, like for example, the increase of the fermentative capacity of non-*Saccharomyces* strains [119], the hybridization of different strains [118] or specific methods to discriminate *T. delbrueckii* strains [109]. Another patent [122] provides solutions concerning a ‘hot’ topic in the field of winemaking: how to reduce the use of sulphur dioxide levels in fermented beverages. This invention uses non-*Saccharomyces* cells, which contain a polynucleotide encoding a MET10 polypeptide that does not catalyze the conversion of sulfite into sulfide [122]. The bio-protection issue is discussed in the patents on *M. fructicola*, revealing interesting applications in the sector of viticulture/oenology [111, 112].

CONCLUSION

We highlight the presence of asymmetries among scientific findings and the number of patents concerning non-*Saccharomyces*-based commercial products for oenological purposes. Further investigations on these microbial resources might open new perspectives and stimulate attractive innovations in the field of wine-making biotechnologies.

CURRENT & FUTURE DEVELOPMENTS

In this review, we offer a new perspective on the oenological applications of the heterogeneous group of yeasts called non-*Saccharomyces*. In particular, we want to emphasize some asymmetries among scientific findings and patents on commercial products testifying the potential interest in the field for future patenting purposes. As evidenced by an increased presence on the market, non-*Saccharomyces* resources play an important role in current wine-making biotechnologies. New studies exploring their potential applications could stimulate attractive innovations and the corresponding commercial exploitation.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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