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Review

Achievements of Autochthonous Wine Yeast Isolation and Selection in Romania—A Review

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Abstract: Winemaking in Romania has a long-lasting history and traditions and its viticulture dates back centuries. The present work is focused on the development of wine yeast isolation and selection performed in different Romanian winemaking regions during past decades, presenting the advanement of the methods and techniques employed, correlated with the impact on wine quality improvement. Apart from the historical side of such work, the findings will reveal how scientific advancement in the country was correlated with worldwide research in the topic and influenced local wines' typicity. To create an overall picture of the local specificities, the work refers to local grape varieties and the characteristics of the obtained wines by the use of local yeasts as compared to commercial ones. Numerous autochthonous strains of *Saccharomyces* were isolated from Romanian vineyards, of which several demonstrated strong oenological characteristics. Meanwhile, different non-*Saccharomyces* yeast strains were also isolated and are nowadays receiving the attention of researchers seeking to develop new wines according to wine market tendencies and to support wine's national identity.

Keywords: Romania; winemaking; autochthonous yeasts; non-*Saccharomyces* yeast; terroir

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1. Introduction

Winemaking in Romania has a long-lasting history and traditions and its viticulture dates back centuries [1]. With the EU accession in 2007, Romania started a journey with the final goal of putting Romania on the international high-quality wines map. Access to pre- and post-accession funds increased investment in wine making technology, the replacement of low-quality vines, and the replanting vineyards with improved genetic sources [2].

According to OIV (International Organization of Vine and Wine) 2022 statistics [3], Romania is nowadays the sixth largest wine producer in Europe and the thirteenth largest wine producer in the world ranking. The total wine production was estimated at around 4.45 million hl in 2021, increasing from around 3.63 million hl in 2015.

Meanwhile, the total area cultivated with vines decreased from 253.203 ha (1995) to 191.459 ha (2015). Since 2015, when Romania legally declared that wine is considered a food product [4], the area cultivated with vines has still shown some fluctuation, but it remained relatively balanced until 2021, when the number reached 188.891 ha [5].

The delimitation of Romanian viticultural areas was established by the National Office of Vine and Wine Products and is based on the climatic conditions determining the qualitative potential of the grapes and wines, the relief conditions, the applied technologies, the level of the obtained productions, and the qualitative characteristics of the resulting products [6]. Therefore, the Romanian viticultural space consists of 37 vineyards which comprise, in total, 120 viticultural centers and 46 independent viticultural centers, grouped in 8 regions and 3 viticultural areas, as presented in Table 1 and shown in Figure 1.

Table 1. The Romanian viticultural space.

Viticultural Area	Viticultural Region	Vineyards Denominations
Central area, inside the Carpathian arch	The Transylvanian plateau	Târnave, Alba, Sebeș-Apold, Lechința, Aiud
Peri-Carpathian hills	The hills of Moldova	Cotnari, Huși, Iași, Dealu Bujorului, Ivești, Nicorești, Panciu, Odobești, Cotești, Zeletin, Covurlui, Colinele Tutovei
	The hills of Muntenia and Oltenia	Dealu Mare, Sâmburești, Ștefănești, Drăgășani, Dealurile Craiovei, Dealurile Buzăului, Podgoria Severinului, Plaiurile Drancei
	Banat	6 independent centers
	Crișana and Maramureș	Diosig, Miniș-Măderat, Valea lui Mihai, Podgoria Silvaniei
Danube Pontic area	The Dobrogea hills	Murfatlar, Sarica-Niculitel, Istria-Babadag
	The Danube terraces	Ostrov, Greaca
	Region of sands and other favorable lands in the South of the country	Calafat, Sadova-Corabia, Podgoria Dacilor

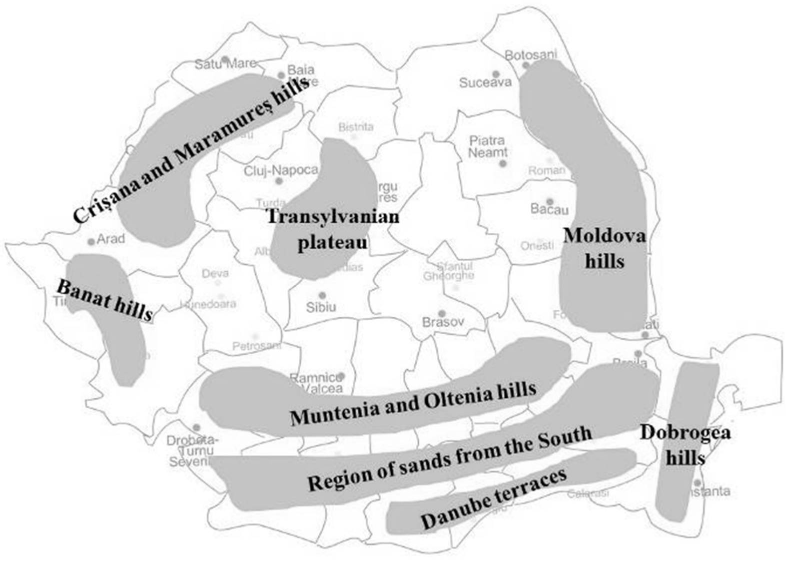

Figure 1. The Romanian viticultural regions and their geographical location.

The trend in Romanian winemaking is to maintain an uprising path in terms of total wine production volume, while also increasing the diversity of local wine types. These goals can be achieved starting from the use of local grape varieties, as well as via the isolation, selection, and then the use of autochthonous yeasts in the production of Romanian wines.

In recent decades, winemakers could choose from a wide variety of commercial yeasts provided by several well-known companies; these are yeasts that display a wide range of special characteristics, adapted to specific needs [7]. In line with the evolution of consumers' preferences and even with climate changes that bring about a higher-than-previous sugar concentration, finding yeasts with special traits was and is a continuous project [8].

Vineyard yeast biodiversity characterization and wine yeast selection are not new entries in wine-making research, but considering the history of wine, these approach can be considerred as young. In the history of winemaking, the use of selected starter cultures did not become widespread practice until the 1970s, and the vast majority of the industrial yeasts belong to *Saccharomyces cerevisiae*; however, currently, it is recongnized that non-*Saccharomyces* species may also be relevant for alcoholic fermentation [9]. It is generally recognized that the current set of the commercial *S. cerevisiae* strains or derived hybrids is not sufficient to provide new technological or organoleptic properties in wine; therefore, new strains are desired, if not essential [10]. Hybrid genomes of *Saccharomyces cerevisiae*/*Saccharomyces kudriavzevii* yeast strains used for wine making in France (Alsace), Germany, and Hungary have been characterized by the use of microsatellite markers [11]. Autochthonous strains represent alternative genetic resources by which the industry can overcome current challenges. The preservation of spontaneous microflora is essential to obtain the typical flavor and aroma of wines deriving from different grape varieties [12]. Meanwhile, the last two decades, practices of organic vine growing influenced fungi (yeast and molds) biodiversity. This was clearly proven in France, in the Bourgogne region, with respect to the Chardonnay variety [13]. In recent years, on the European level, researchers from different groups and countries have focused on yeast selection and biodiversity issues. Ecological and geographic studies have highlighted that unique strains are associated with particular grape varieties in specific geographical locations [14]. An example of such initiatives was provided by the European project, WILDWINE Project (EU contract 315065), focused on the selection of wild microorganism in five worldwide- recognized wine regions: Nemea and Crete (Greece), Piedmont (Italy), Bordeaux (France), and Priorat (Spain) [15]. In Italy, a wide range of vineyards were examined, covering most of the wine's Italian regions: in the northwest, in the Piedmont region and Monferrato vineyards concerning Barbera grapes [16]; the Barbera variety was also studied in the "Nizza" Barbera d'Asti DOC zone [17]. In Sicily, a wide study was conducted on hundreds of isolates and the superiority of the local strains over the commercial strains was proved [18]. Another focus was on Montepulciano d'Abruzzo "Colline Teramane" premium wine DOCG, produced in Teramo province; the presence of atypical *S. cerevisiae* strains only in a particular vineyard in a restricted area suggests the role of local selective pressure in the origin of distinctive *Saccharomyces* yeast populations [19]. In Spain, several groups conducted similar work, and screening results were reported for wine regions such as Douro, Extremadura, Galicia, La Mancha and Uclés, Ribera del Duero, Rioja, Sherry area, and Valencia [20]. Moreover, in the DOQ Priorat region, isolation was performed on varieties such as Grenache and Carignan [21]; in the northwest, in the Galicia region, biodiversity was studied, comparing organic and conventional culture [22]. Relatively recently, isolates from three appellations of Spanish origin were checked for fingerprinting of interdelta polymorphism; ancient vineyards managed with organic practices showed intermediate to low levels of strain diversity, indicating the existence of stable populations of *S. cerevisiae* strains [23]. In another European area, in the Greek island of Kefalonia, in the Mavrodafni wine region, at the end of the alcoholic fermentation, indigenous yeasts were isolated; selected strains are already in industrial use [24]. In the European eastern neighborhood, in Georgia, a traditional winemaking country, long-term biodiversity studies were conducted in the Dagestan region using various isolation techniques and various substrates [25].

The present work is focused on the development of wine yeast isolation and selection performed in different Romanian winemaking regions during recent decades, presenting the advance of the employed methods and techniques, correlated with the impact on wine quality improvement. Apart from the historical side of the importance of such work, our findings will reveal how scientific advancement in the country is correlated to worldwide research in the topic.

2. Materials and Methods

The current review is based on the available scientific articles that record research regarding the isolation and selection of local wine yeasts from different Romanian vineyards. Most of the sources approached are indexed in different international databases, such as

Google Scholar, ScienceDirect, Web of Knowledge-Clarivate, and CABI. However, the available records in the international databases start from 2005, while records in some native language (Romanian), available in different national libraries, go as far as the beginning of the century, in 1915 [26]. In addition, to create an overall picture of the local specificities, scientific reports were also used in relation to local grape varieties (Fetească regală, Fetească albă, Crâmpoșie, Băbească neagră, Fetească neagră, Grasă de Cotnari, Cadarcă, Tămâioasă românească, etc.) and the characteristics of the wines obtained by the use of local yeasts compared to the commercial ones.

3. Results and Discussion

This review took into account the reported work on wine yeast isolation and selection activities performed in the wine-growing areas of Romania from 1915 to the present.

From the chronological point of view, according to Brîndușe et al. [26], the first report, from 1915, comes from the doctoral thesis of Nițescu M.A. [27]. He made an ample physiological characterization of yeast isolated from different regions and local grape varieties, such as Cotnari (Grasă, Fetească albă), Iași (Fetească neagră), Pietroasele (Grasă), Drăgășani (Tămâioasă românească, Negru moale, Negru vârtos, Crâmpoșie), and Odobești (Tămâioasă românească). This study, conducted in Paris, was positively appreciated by Ribéreau Gayon and Peynaud in 1960, according to the same source [26]. Following this study, in the 1920s–1930s, Dr. Russ and his team (Dr. Moldovan and Dr. Mavromati) founded the national school of wine microbiology and the first Romanian wine yeast collection. In the years 1945–1965, different researchers focused on local wine yeast selection [28–34]. Beginning in the 1970s, isolation and selection work has increased, and the results are detailed below.

In terms of the vineyard region, yeast isolation and selection work was reported in several areas, covering most of the Romanian winemaking regions. For instance, in the Transylvanian plateau, Dănoaie [35] and Stamate et al. [36] focused on the yeast biodiversity in Târnave vineyard, while Oprean [37] studied several Sibiu wine-growing areas. In Moldova, such experiments were conducted by Sandu-Ville et al. [38,39], followed by Viziteu et al. [40] in Cotnari vineyard, by Vasile et al. [41] and by Nechita et al. [42] in the Iași-Copou vineyard, as well as by Găgeanu et al. [43] in Dealurile Bujorului vineyard. In the hills of Muntenia, the research started in Valea Călugărească center by Kontek and Kontek [44,45], followed by Matei Rădoi et al. [46] and Brîndușe et al. [47,48], and in the Buzău vineyard by Bărbulescu et al. [49]. In the Oltenia hills in Tamburești, Banu Mărăcine, Drăgășani, and Târgu Jiu, studies were conducted by Dragomir Tutulescu and Popa [50], while Beleniuc [51] isolated wine yeast from the Murfatlar vineyard in the Dobrogea hills.

3.1. Employed Techniques of Yeast Isolation, Identification and Selection

Different approaches were taken into account during the isolation work, starting from grape washing water [42,43,45,47,52], continuing with the juice from fresh crushed grapes [40,46,52] or must in different fermenting stages: respectively, at the beginning, middle, and end of fermentation [42,48,53]. The employed microbiological media were the classical ones, meaning Sabouraud medium or Yeast Extract Peptone Dextrose (YEPD) supplemented with chloramphenicol. Bărbulescu et al. also made use of a specific medium for yeast isolation (malt extract–peptone yeast extract agar), then another specific medium (yeast extract–malt extract sucrose agar) for the maintenance of the culture [49].

The selection work followed typical steps, i.e., respectively, by monitoring the parameters of the fermentations and the characteristics of the obtained wines. Classically, there were employed tests such as ethanol tolerance [7,42] or the refermentation capacity of the strains [42]. Of the yeasts tested by Nechita et al. from Iași-Copou, five strains proved to be tolerant of high concentrations of ethanol of about 14–15% [42]. Regarding their capacity to restart the stagnated fermentation at 11.5% ethanol and 70 g/L sugars, the strains managed to bring the fermentation to an end and produce dry wines. Dragomir, Tutulescu, and Popa used the standard methods accepted by OIV to isolate, identify, and described their strains' biological, physical, and oenological characteristics from the Oltenia area [50,54]. In the end, most of the authors reported the results of the physicochemical and organoleptical characteristics of the obtained wines after using the selected strains. Following this path, Vasile et al. isolated 86 local yeast strains from the Iași-Copou vineyard, followed by a final selection for the best fermentative characteristics and wine profiles [41,55]. In terms of the killer profile of the isolated yeast, only one report was identified in the databases, in which Matei and Găgeanu reported a killer positive strain isolated in Dealurile Bujorului county [56].

Less conventional methods were used in the characterization and wine yeast selection. For instance, Antoce and Nămoloșanu employed a calorimetric method using a multiplex batch micro-calorimeter (isothermal, conduction type) for the rapid yeast testing for ethanol tolerance in order to select strains that were useful for winemaking [57]. They demonstrated that the method could eliminate labor-intensive cell counting, as well as its high sensitivity and the possibility of measuring cultures grown in intense-colored or high-turbidity media, such as red wine. In addition, this method offers the benefit of simultaneously monitoring a large number of samples in a 48–72-h experiment.

The identification work, hand in hand with yeast biodiversity studies, had a slow evolution in terms of the employed techniques in past decades. Such work rquires know-how and specific tools, and the predominant methods were based on classical morpho-physiological tests, according to Barnett et al. [58,59], Krieger-van Rij [60], and Delfini [61]. Most authors reported studies on the macroscopic features of the colonies, pseudo-mycelium formation, and sporulation on a specific medium [43,44,46,47,52]. Tests such as fermentation and assimilation of different carbohydrates, nitrogen utilization, the use of ethanol as the sole carbon source, and arbutin split were taken into account [37,47,52]. Several authors were using rapid biochemical tests; that is, API galleries [40,46].

Some teams made use of MALDI-TOF mass spectrometry, especially that of Bărbulescu et al., wherein the isolated strains were prepared for the analysis after the extraction of peptides with formic acid, ethanol, and acetonitrile [49]. A similar approach was taken by Corbu and Csutak when studying yeast biodiversity in different traditional fermented foods, including wine [62]. For a more accurate physiological identification of the tested strains, phenotypic phylogeny analyses were also performed using Biolog Microbial ID System according to the manufacturers' specifications [63].

The molecular approach came later on in the country, when PCR-ITS RFLP techniques were employed by Gaspar et al. [64] in Sebeș vineyard (Apold-Blaj centre), followed by Găgeanu et al. [43] in Dealurile Bujorului vineyard, and Dumitrache et al. [53] in Pietroasa center (Dealu Mare vineyard); these results were also coupled with sequencing data. These teams performed conventional DNA extraction, followed by PCR amplification with ITS 1 and ITS 4 primers, continuing with *Hinf*I, *Hae*III, and *Hha*I digestion [43], or *Alu*I and *Taq*I [64], and comparing the obtained profiles with the existent databases.

The first PCR-RAPD approach was taken by Oprean, when different *Saccharomyces* and non-*Saccharomyces* strains, isolated from Sebeș-Apold vineyard, were identified [65]. Relatively recently, apart from using the ITS-RFLP technique of the ITS1-5.8S rDNA-ITS2 region, taking advantage of the restriction enzymes such as *Hinf*I, *Hae*III, *Cfo*I, and *Msp*I, Corbu and Csutak have also employed the RAPD method for the identification of yeast involved in wine spontaneous fermentation [62,63]. In their case, the intraspecific biodiversity (genetic relatedness) of the isolates was detected by analyzing the RAPD profile obtained for each strain and by calculating the similarity index using the Jaccard coefficient (Sij). Similarly, the interspecific biodiversity of the microbial communities from spontaneous fermented products was determined by comparing their profile to the RAPD profile of their co-fermenters; in the end, the dendograms were generated by PyElph, using the UPGAMA (unweighted pair group method with arithmetic mean) method.

3.2. Yeast Biodiversity and Identification Results

The wine yeast studies in Romania followed two different patterns. Most of the authors have isolated and selected different strains, followed by identification only for the strains proving special and/or demonstrating specific winemaking profiles and characteristics. Systematic studies were started only in later 1970s by Kontek et al. (1975–1977). Later on, a few studies took into account the study of the vineyard or fermented grape must yeast biodiversity as a whole [46,62].

A first ample biodiversity report study was performed by Kontek in 1977 [66], in Dealu Mare vineyard (Valea Călugărească centre), adopting the classification proposed by Lodder and Kreger-van Rij [67]. Among 244 isolates, the predominant genus was *Saccharomyces*, with the following species and var.: *S. ellipsoideus* (dominant), *S. bayanus*, *S. carlsbergensis*, *S. cerevisiae*, *S. exiguous*, *S. heterogenicus*, *S. florentinus*, *S. fructuum*, *S. italicus*, *S. oviformis*, *S. rosei*, *S. steinerii*, *S. uvarum*, and *S. logos*. In terms of non-*Saccharomyces* (NS) species, they reported *Candida mycoderma*, *Candida peliculosa*, *Kloeckera apicullata*, *Kloeckera africana*, *Torulopsis stellata*, *Pichia membranaefaciens*, and *Rhodotorula mucilaginosa*.

Later on, Matei Rădoi et al. performed a similar study in the Valea Călugărească center, Dealu Mare vineyard, comparing the data obtained by Kontek team in the 1970s in a double approach: classical morphophysiological study; and by API 20C AUX—Biomerieux [46]. The isolation was performed during 2007–2009 on Cabernet Sauvignon, Merlot, Fetească Neagră, and Pinot Noir varieties. A change in the yeast species profiles was noticed throughout the decades; specifically, the 1970s as compared to the 2000s. Among 262 isolates, the dominant species isolated in the vineyard belonged to the NS species, such as *C. famata*, *K. apiculata*, and *Debaryomyces hansenii*. One year later, a similar study was published in the same area [47], in which the dominant NS species were *C. utilis*, *K. apiculata*, *R. mucilaginosa*, and *D. hansenii*, with the employed method and the results being very close among the two teams. Other reported isolates belonged to *Candida lusitaniae*, *C. stellata*, *C. utilis*, *C. magnoliae*, *C. pelliculosa*, *Pichia anomala*, *P. jadinii*, *Torulaspora delbrueckii*, and *Hanseniaspora uvarum* (Table 2).

Multiple NS species were identified from the Cotnari vineyard by Viziteu et al., namely, *C. mycoderma*, *Hansenula anomala*, *H. uvarum*, *Kluyveromyces* spp., *P. membranafaciens*, and *T. stellata* [40].

Vasile et al. selected three *S. ellipsoideus* strains and determined their influence on the must of three grape varieties from Iași-Copou, namely, Fetească albă, Sauvignon blanc, and Chardonnay [41,55]. Other *Sacharomyces* spp. were reported by Găgeanu et al. in Dealurile Bujorului county (Table 3), such as *S. bayanus*, for instance [43].

The strains isolated and tested in Oltenia county by Dragomir Tutulescu and Popa in 2009–2010 were identified as *K. apiculata*, *P. membranafaciens*, *Rhodotorula glutinis*, *S. ellipsoideus* (the most abundant during must fermentation), and *S. oviformis*, but they also found few representatives of *S. rosei*, *Candida vinaria*, and *Metschnikowia reukaufii* [50,54].

In 2014, Oprean identified in Sebeș-Apold county, by molecular tools, *S. ellipsoideus* and *S. oviformis*, as well as NS yeasts such as *Candida vini* and *K. apiculata* [65]. Similarly, in Blaj centre, Stamate et al. reported as dominant, among 139 isolates, the species of *S. cerevisiae* var. *ellipsoideus*, *K. apiculata*, *S. oviformis*, and *S. bayanus* during must fermentation, while *K. apiculata*, *C. mycoderma*, and *T. stellata* were abundant on the grapes [36].

A general image on the *Saccharomyces* spp. isolated and selected in Romania is presented in Table 3. The main identified *Saccharomyces* species and varieties belong to *S. bayanus*, *S. cerevisiae*, *S. chevalieri*, *S. ellipsoideus*, *S. florentinus*, *S. oviformis* (synonym *S. cerevisiae*), or *S. uvarum*.

Table 2. The non-*Saccharomyces* (NS) yeasts isolated from various winemaking areas in Romania.

Genus	Species	Centre/Vineyard	References
Candida	*C. colliculosa*	Valea Călugărească, Dealu Mare	[46,47]
		Recaș	[68]
	C. famata	Valea Călugărească, Dealu Mare	[46,47]
	C. lusitaniae	Valea Călugărească, Dealu Mare	[46,47]
	C. magnoliae	Valea Călugărească, Dealu Mare	[46,47]
		Recaș	[68]
	C. mycoderma	Cernavodă, Murfatlar	[52]
		Cotnari vineyard	[40]
	C. pelliculosa	Valea Călugărească, Dealu Mare	[46,47]
	C. sphaerica	Valea Călugărească, Dealu Mare	[46,47]
	C. tropicalis	Recaș	[68]
	C. utilis	Valea Călugărească, Dealu Mare	[46,47]
	C. vini	Drăgășani, Tamburești	[50]
Clavispora	*C. lusitaniae*	Valea Călugărească, Dealu Mare	[47]
Debaryomyces	*D. hansenii*	Valea Călugărească, Dealu Mare	[46,47]
Dekkera	*D. anomala*	Pietroasa vineyard	[53]
Geotrichum	*G. penicillatum*	Valea Călugărească, Dealu Mare	[47]
Hanseniaspora	*H. uvarum*	Recaș	[68]
Hansenula	*H. anomala*	Cernavodă, Murfatlar	[52]
		Cotnari vineyard	[40]
Kloeckera	*K. apiculata*	Cernavodă, Murfatlar	[52]
		Valea Călugărească, Dealu Mare	[46,47]
		Drăgășani	[33]
		Recaș	[68]
Lachancea	*L. kluyveri*	Cotnari vineyard	[40]
Metschnikowia	*M. pulcherrima*	Drăgășani	[50]
		Pietroasa vineyard	[53]
Pichia	*P. angusta*	Recaș	[68]
	P. anomala	Valea Călugărească, Dealu Mare	[46,47]
	P. fermentans	Cernavodă, Murfatlar	[52]
		Recaș	[68]
	P. jadinii	Valea Călugărească, Dealu Mare	[46,47]
	P. kudriavzevii	Ilfov area	[63]
	P. membranaefaciens	Drăgășani, Tamburești	[50]
		Cotnari vineyard	[40]
	P. ohmeri	Valea Călugărească, Dealu Mare	[47]
Rhodotorula	*R. glutinis*	Valea Călugărească, Dealu Mare	[46,47]
		Recaș	[68]
	R. minuta	Valea Călugărească, Dealu Mare	[46,47]
	R. mucilaginosa	Cernavodă, Murfatlar	[52]
		Valea Călugărească, Dealu Mare	[47]
		Recaș	[68]
Torulaspora	*T. delbrueckii*	Valea Călugărească, Dealu Mare	[46,47]

Table 2. *Cont.*

Genus	Species	Centre/Vineyard	References
Torulopsis	*T. stellata*	Cernavodă, Murfatlar	[52]
		Cotnari vineyard	[40]
Zygosaccharomyces	*Z. bailii*	Cotnari vineyard	[40]
	Z. rouxii	Cotnari vineyard	[40]

Table 3. The *Saccharomyces* species and varieties isolated from various winemaking areas in Romania.

Species	Centre/Vineyard	References
S. bayanus	Cernavodă, Murfatlar	[52]
	Dealurile Bujorului	[43]
	Cotnari vineyard	[40]
S. cerevisiae	Buzău vineyard	[49]
	Pietroasa vineyard	[53]
	Recaș	[68]
	Valea Călugărească, Dealu Mare	[46]
	Cotnari vineyard	[40]
S. chevalieri	Cotnari vineyard	[40]
S. ellipsoideus	Cernavodă, Murfatlar	[52]
	Dealurile Bujorului	[43]
	Iași-Copou vineyard	[41]
	Cotnari vineyard	[40]
S. florentinus	Cotnari vineyard	[40]
S. oviformis (synonym *S. cerevisiae*)	Cernavodă, Murfatlar	[52]
	Dealurile Bujorului	[43]
	Cotnari vineyard	[40]
S. uvarum	Cotnari vineyard	[40]

3.3. Selected Yeast Properties and the Final Characteristics of Local Wines

From the available records, a wide range of grape varieties were tested, of which nine are registered as local varieties (Table 4), while the wines' characteristics (Table 5) were assessed for both red wines and white wines, though more attention have been given to the white wines.

In the case of white wines, the local selected yeasts were tested on local varieties (Fetească albă, Fetească regală, Tămâioasă românească), as well as on international varieties (Aligoté, Chardonnay, Sauvignon blanc, Pinot gris, Muscat ottonel).

Regarding Feteasca albă, this type of wine was obtained and tested in Dealu Bujorului, with 13.5% alcohol (*v*/*v*) and without residual sugar detected [69], and in Iași, with 11.6% alcohol (*v*/*v*) and 0.2 g/L sugars [55]. Colibaba et al. [70], Dobrei et al. [71], and Bora et al. [69] obtained Fetească regală wine from Iași, Miniș-Măderat, and Dealu Bujorului, with an average alcohol content of 13.7% (*v*/*v*). The residual sugar content was very different—from 1.9 g/L (Dealu Bujorului) and 3.9 g/L (Miniș-Măderat) to 6.63 g/L (Iași).

Aligoté wines showed some differences in terms of ethanol content from one location to another, but also within the same location. Thus, the Aligoté obtained in Dealu Bujorului had a content of 13.1% ethanol (*v*/*v*) with no residual sugars detected [69], while those obtained in Iași had, respectively, 10.08% ethanol (*v*/*v*) with 0.72 g/L sugars [70], and 11.33% ethanol without a mention of the residual sugars [72].

Colibaba et al. [73] and Bora et al. [69] also obtained Italian Riesling wines with around 11% ethanol, but the first author obtained a dry wine with 0.77 g/L residual sugar, while the second author obtained a sweet wine with 72 g/L residual sugar.

Table 4. Wine grape varieties from Romanian vineyards fermented with selected autochthonous yeast.

Grape Varieties	Vine Regions	References
Aligoté	Iași	[72]
	Iași	[70]
	Dealu Bujorului	[69]
Băbească gri	Dealu Bujorului	[69]
Cabernet sauvignon	Dealu Mare Miniș-Măderat Dobra (Satu Mare)	[73] [71] [74]
Cadarcă	Miniș-Măderat	[74]
Chardonnay	Iași	[55]
Feteasca albă	Dealu Bujorului Iași	[69] [55]
Feteasca neagră	Miniș-Măderat Panciu Ratești (Satu Mare) and Aliman (Constanța)	[74] [75] [74]
Fetească regală	Dealu Bujorului Iași Miniș-Măderat	[69] [70] [71]
Frâncușă	Iași	[70]
Grasa de Cotnari	Iași	[70]
Italian riesling	Dealu Bujorului Iași	[69] [70]
Merlot	Aliman (Constanța)	[74]
Muscat ottonel	Dealu Bujorului Iași	[69] [70,76,77]
Neuburger	Iași	[70]
Pinot gris	Iași Miniș-Măderat	[70] [71]
Pinot noir	Ratești (Satu Mare)	[74]
Rose traminer	Iași	[70]
Sarba	Dealu Bujorului	[69]
Sauvignon	Dealu Mare	[78]
Sauvignon blanc	Dealu Bujorului Iași	[69] [55,70]
Tamaioasă românească	Iași	[70]
Traminer	Miniș-Măderat	[71]

Muscat Ottonel wines were obtained in two Moldova areas, one from Dealu Bujorului and three from Iași. The wine obtained in Dealu Bujorului was a sweet wine, with 11% ethanol and 30.7 g/L residual sugar [69]. Colibaba et al. [70] and Vararu et al. [76] obtained dry wines from Iași, with less than 2 g/L sugar and 12.2%, respectively, and 13.6% ethanol. The glycerol content of Vararu et al. wine was almost 13 g/L. Focea et al. obtained a sparkling wine with 10.3% ethanol, but without mentioning the sugar content [77].

As for Pinot gris, two wines with an increased ethanol content of about 14% were obtained in Iași [70] and in Miniș-Măderat [71]. Vișan et al. [78] obtained three Sauvignon semi-dry wines from Dealu Mare, with an average of 12.5% ethanol, 11 g/L sugar, and about 8–10 g/L glycerol. Vasile et al. [55] and Colibaba et al. [70] each made a dry Sauvignon blanc from Iași, with 11.2–11.9% ethanol and approx. 1 g/L sugar; wine from 2010 had a content of 7.4 g/L glycerol. The Sauvignon blanc obtained from Dealu Bujorului [69] was semi-dry, with 12 g/L sugar and higher ethanol content of 14.4%.

On red wines' side, Cabernet sauvignon was tested in Dealu Mare [73], Miniș-Măderat [71], and in Dobra, Dealurile Silvaniei [74]. This type of wine had an alcohol content between 12% and 15% (*v*/*v*); the highest value was obtained in Miniș-Măderat. The residual sugar content was 3.8 g/L in the 2012 study, 10.05 g/L in the 2015 study, and not specified in the 2018 study. Vișan et al. also emphasized that the glycerol content was 9 g/L [73], which contributes to the wine's texture and body [79]. Manolache et al. [74,75] and Dobrei et al. [71] obtained and tested Feteasca neagră wine, with an average of 13.49% ethanol (*v*/*v*) and 3.48–3.9 g/L residual sugar.

Table 5. Wines obtained in Romanian winemaking areas after fermentation with local yeast and their physicochemical properties.

Grape Varieties	Vine Region	Alcohol Vol. (%)	Residual Sugars (g/L)	Total Acidity (g/L)	Volatile Acidity (g/L)	References
Aligoté	Iași	11.33	*	6.72	0.35	[72]
	Dealu Bujorului	13.1	nd	5.5	0.37	[69]
	Iași	10.08	0.72	9.14	0.33	[70]
Băbească gri	Dealu Bujorului	13.2	12.7	5.9	0.38	[69]
Cabernet Sauvignon	Dealu Mare	13.1	3.8	4.3	0.7	[73]
Cadarcă	Miniș-Măderat	15	10.05	5.5	0.43	[71]
	Dobra (Satu Mare)	12	*	5.42	0.47	[74]
	Miniș-Măderat	13.25	2.44	5.55	0.32	[71]
Chardonnay	Iași	12.4	nd	5.9	0.29	[55]
Fetească albă	Dealu Bujorului	13.5	nd	4	0.39	[69]
	Iași	11.6	0.2	5.6	0.28	[55]
Fetească neagră	Miniș-Măderat	13.97	3.48	5.93	0.42	[71]
	Panciu	13.5	3.9	5.32	0.88	[75]
	Ratești (Satu Mare)	13.06	*	5.98	0.57	[74]
	Aliman (Constanța)	13.43	*	5.41	0.73	[74]
Fetească regală	Dealu Bujorului	13.8	1.9	5.3	0.42	[69]
	Iași	13.94	6.63	6.92	0.43	[70]
	Miniș-Măderat	13.39	3.9	5.7	0.53	[71]
Frâncușă	Iași	11.87	0.63	8.54	0.41	[70]
Grasa de Cotnari	Iași	11.6	1.7	8.55	0.25	[70]
Italian Riesling	Dealu Bujorului	11	72	4.9	0.61	[69]
	Iași	11.83	0.77	7.07	0.29	[70]
Merlot	Aliman (Ostrov)	14.14	*	5.25	0.65	[57]
Muscat ottonel	Dealu Bujorului	11	30.7	4.4	0.54	[69]
	Iași	12.2	1.34	6.43	0.33	[70]
	Iași	13.6	1.67	6.4	0.35	[76]
Sparkling Muscat ottonel	Iași	10.3	*	6.2	0.33	[77]
Neuburger	Iași	12.44	10.63	7.71	0.45	[70]

Table 5. *Cont.*

Grape Varieties	Vine Region	Alcohol Vol. (%)	Residual Sugars (g/L)	Total Acidity (g/L)	Volatile Acidity (g/L)	References
Pinot gris	Iași	14.49	4.81	6.68	0.33	[70]
	Miniș-Măderat	13.39	2.04	5.93	0.47	[71]
Pinot noir	Ratești (Dealurile Silvaniei)	13.47	*	6.01	0.53	[74]
Rose Traminer	Iași	14.1	1.67	6.73	0.25	[70]
Șarba	Dealu Bujorului	14.1	23	5.8	0.54	[69]
Sauvignon	Dealu Mare	12.2	10	5.8	0.3	[78]
		13	12	5.4	0.4	
		12.5	12	5.2	0.4	
Sauvignon blanc	Dealu Bujorului	14.35	12	5.2	0.57	[69]
	Iași	11.24	1.1	5.94	0.29	[70]
	Iași	11.9	0.9	5.95	0.2	[55]
Tămâioasă românească	Iași	11.63	15.47	6.93	0.31	[70]
Traminer	Miniș-Măderat	12.3	50	5.9	0.47	[71]

*: the authors did not mention the residual sugar content in the respective wines; nd: not detected.

Special wines were also obtained in Dealu Mare, Valea Călugărească center by Kontek and Kontek (1976); specifically, Jerez type wines, made of pellicular autochthonous yeast isolates belonging to *S. bayanus* species. These wines reached 15–16% alcohol, a maximum of 4 g H_2SO_4/L acidity, and the most appreciated were the ones with residual sugar of 16–17 g/L. The same authors also reported a cryophilic yeast, identified by classical tools as *S. carslbergensis*, initially isolated from must fermenting at 5 °C; this strain led to rapid wine clarification and produced low volatile content and high glycerol content. Similarly, for the cryophilic property, Tudose et al. selected a *S. ellipsoideus* strain in Iași-Copou centre, which was also resistant to high sulphur hydrogen content [80].

For high-quality sparkling wines, isolates of *S. oviformis* and *S. carlsbergensis* were selected in Blaj county during the 1980s [35]; they were capable of complete sugar consumption, while not stimulating the malolactic fermentation and not producing high volatility.

In the 1980s–1990s, generally, special attention was given to high-alcohol, low-foaming, and high-glycerol wine yeast strains, e.g., in Valea Călugărească center [81] and Iași county [82].

Starting with the 2000s, attention was more focused on the aromatic profile of wines made of local grape varieties and local yeast, while less attention was given to the high alcoholic strength. For instance, Liță et al. reported different local strains of *S. cerevisiae* var. *ellipsoideus* as appropriate candidates for dry white wines made of local varieties, such as Fetească albă and Fetească regală [83]. Moreover, in 2017, Lengyel and Panaitescu reported a local yeast isolated from Gârbova area (Sebeș-Apold vineyard), which was capable of improving the terpene flavor compounds content in Muscat ottonel wines [84]. A deeper study and methodology was reported by Vararu et al. after analyzing the aromatic profile of Muscat ottonel variety fermented with commercial and local yeast from Copou Iași centre [76]; a visual and easy to understand foot-printing was also performed, based on a multiple variable analysis, which established differences in the fermentative volatilome.

3.4. New Selection Directions in the Terroir Concept Context

The conventional practice of producing wines on an industrial scale with the use of *Saccharomyces* species involves controlled fermentation from all points of view. The wines thus obtained can be denominated according to the geographical indication (GI) if certain legislative requirements are followed. However, for an even greater specificity, a possible direction might be the use of local yeasts from each geographical region, in addition to using grapes harvested from those areas.

On another note, one way to obtain local wines is the spontaneous fermentation of grapes, but there are multiple disadvantages. The obtained wines may have different characteristics from one vintage to another, depending on many environmental variables, such as climate (temperature, precipitation, sunlight, wind), biology (microbiota, flora, and fauna), relief (topographic coordinates, geomorphology), and geology (soil types, irrigation, fertilization), as well as human implications, namely, traditions, culture, applied technology, agronomic practices, and legislation [8,85,86]. All these are involved in the concept of *terroir*.

Knight et al. consider the possibility of the existence of the concept of "microbial *terroir*", which implies that the microbial consortia in a certain wine-growing area are specific to that certain area and are producing flavors typical of the area [85]. Their experiments showed that the organoleptic properties of wine are given by *S. cerevisiae* indigenous strains and their origin, which may sustain the microbial aspect of *terroir*; in addition, the biodiversity of the yeast in the vineyards is affected by the micro and macroclimatic conditions of the vine varieties and the geographical location of the vineyard, a fact that would explain why the yeast consortia are different between two different wine-growing regions [87].

A research direction that emerges from the above-mentioned data is the use of autochthonous yeast in the wine industry in order to produce specific wines for certain wine-growing areas. Spontaneous fermentation is an uncontrolled and complex biotechnological process, in which the alteration microorganisms could rapidly multiply and reach too-high levels quickly, which may negatively impact the quality of the finished products [88]; this, even if spontaneous fermentation is correlated with greater complexity, greater wine body, and uncommon flavors [89–91], and it could improve the qualities of the wine by creating unique regional fingerprints [92], it is a process to be avoided. Therefore, one could combine spontaneous fermentation with indigenous yeasts with the safety of controlled processes from the industrial environment [86]. This would imply the use of selected local yeasts as new starter cultures in the winemaking industry, which would be reflected in the specific fingerprint of the finished product [86,93].

The new selection directions regarding the local wine yeasts tend to follow different paths, i.e., obtaining new wines with predetermined properties (high glycerol content, low ethanol content, reduced acidity); creating new and specific technological flows for obtaining certain types of wines, especially in order to avoid the production of certain compounds (biogenic amines, volatile sulfur compounds) in the finished wines; obtaining new wines of controlled origin and with a geographical indication; and completing the oenological practices in the legal specifications.

Thus, the research could be divided into two different directions, namely, that with the use of *Saccharomyces* yeasts, and that with the use of non-conventional (non-*Saccharomyces*) yeasts, in different variations, such as simple cultures, co-fermentation, or in sequential fermentation with *Saccharomyces* yeasts in different proportions. As described above, already, several non-*Saccharomyces* (NS) local yeast were detected during the isolation work and are stored in the owners' collections. In this regard, the usefulness of unconventional yeasts and the need to isolate and select such wine yeasts is further emphasized.

Considering the existence of numerous studies [94–98] which confirm that NS wine yeasts are beneficial a very large proportion, and even essential to obtaining wines with extraordinary organoleptic and sensory properties (Table 6), the selection of these yeast species is desirable in the near future. Among the NS species, only *Dekkera* spp. was reported as having only spoilage impact on wines. In Europe, numerous studies have been registered that argue in favor of non-conventional yeasts for the fermentation of the grape must. It is well-known that numerous NS yeast genera, including, but not limited to, the ones mentioned in Table 6, possess desirable oenological properties, such as the production of glycerol and other higher alcohols [99–101], the decreased ethanol content in the finished wine [102], and also the production of extracellular enzymes [103–105], esters [101,106], or polysaccharides [107].

Table 6. Biotechnological role of some non-*Saccharomyces* yeasts.

Genus	Relevant Species	Initial Technological Significance	Real Biotechnological Role	References
Hanseniaspora/ Kloeckera	*H. uvarum/ H. apiculata*	Contamination /Spoilage	Higher alcohols, acetate, and ethyl esters production	[90,108]
Candida	*C. stellata*	Contamination	Glycerol production, fructophily	[109]
	C. zemplinina/ Starmerella bacillaris	Contamination	Glycerol, succinic acid production; decrease of alcohol content	[94,98]
Metschnikowia	*M. pulcherrima*	Contamination	Esters, terpenes, and thiols production, increase in aroma complexity	[91,98,107]
Pichia	*P. anomala*	Contamination /Spoilage	Increased production of volatile compounds, killer against *Dekkera/Brettanomyces*	[110]
	P. kluyveri		3-mercaptohexan-1-ol and 3-mercaptohexan-1-ol acetate production	[111]
Lachancea/ Kluyveromyces	*L. thermotolerans*	Contamination	Glycerol overproduction, reduction of volatile acidity	[112]
Torulaspora	*T. delbrueckii*	Spoilage	Succinic acid, polysaccharides production	[113]
Dekkera/ Brettanomyces	*D. bruxellensis*	Spoilage	Spoilage	[8,100]
Schizosaccharomyces	*S. pombe*	Spoilage	Malolactic deacidification; propanol and pyruvic acid production	[98,114]

Taking into account all the properties and real biotechnological roles of these NS yeasts in the production of wine, a new path for their use in grape must fermentation is open, which will avoid the production of certain chemical compounds in the final wines instead of desirable compounds such as esters and glycerol. However, due to the fact that NS yeasts are not able to finish the alcoholic fermentation (they are less efficient in the production of ethanol), the technology should be accmpanied by a sequential inoculation of the grape must [91]. Thus, the NS yeast may be inoculated at the beginning of the fermentation, and, after the fermented must reaches a content of approximatively 10% ethanol, a *Saccharomyces* yeast will be added. In this way, the fermentation will be concluded by the *Saccharomyces* species, while the NS species will produce the necessary metabolites to positively influence the aroma of the wine. A similar alternative involves the simultaneous inoculation of the two types of yeast. Finally, mixed or sequential fermentations with *Saccharomyces* and NS allow the developtment of local wines with a low alcohol content [91].

4. Conclusions

From a historical point of view, the first wine yeast selection work in Romania started in 1915 as part of the international research process started by French teams at the time, and the first local wine yeasts collection was delivered in years 1920s. After the 1970s and until the 1990s, the selection work reached almost all Romanian winemaking regions. The use of novel molecular identification and characterization tools followed the international trend, reaching the country later on (after 2010). The advancement in the past ten years was highly dependend on such techniques, and special selected yeast are nowadays in several local collections. However, their inclusion in international collection was not found in any report, and this is an aspect which should be taken into account in the near future.

Several autochthonous strains of *Saccharomyces* were isolated from Romanian vineyards, grapes, and musts, a part of which demonstrated oenological qualities that are desirable for Romanian local wines.

Moreover, numerous NS yeast strains, belonging to a multitude of different genera, have been isolated and identified from vineyards and wine research stations in Romania, but few Romanian authors have studied and published the use of local NS yeasts in winemaking.

The selection of local yeasts is of great interest for Romanian wine production due to the fact that there is the possibility of expanding the diversity of wines on the market, but also due to the high demand for local, unique products. Actually, it was reported recently [115] that a large majority of Romanian people prefer to consume only local wines. It is also worth mentioning the fact that a larger range of local yeasts used leads to developing a wider range of local wines, which supports Romanian gastronomic identity, culture, and tradition.

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