**The yeast Starmerella bacillaris (synonym Candida zemplinina) shows high genetic diversity in winemaking environments**

# The yeast *Candida zemplinina* (*Starmerella bacillaris*) shows high genetic diversity in winemaking environments

Running title: microsatellite analysis of Candida zemplinina

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**Abstract**

The yeast *Candida zemplinina* (*Starmerella bacillaris*) is frequently isolated from grape and wine environments. Its enological use in mixed fermentation with *S. cerevisiae* has been extensively investigated these last years, and several interesting features including low ethanol production, fructophily, glycerol and other metabolites production, have been described. In addition, molecular tools allowing the characterization of yeast populations have been developed, both at the inter- and intraspecific levels. However, most of these fingerprinting methods are not compatible with population genetics or ecological studies. In this work, we developed ten microsatellite markers for the *C. zemplinina* species that were used for the genotyping of 163 strains from nature or various enological regions (28 vineyards/wineries from seven countries). We show that the genetic diversity of *C. zemplinina* is shaped by geographical localisation and displays no evidence of domestication. Populations isolated from winemaking environments are quite diverse at the genetic level: neither clonal-like behaviour nor specific genetic signature was associated with the different vineyards/wineries. Altogether, these results suggest that *C. zemplinina* is not under selective pressure in winemaking environments.

## Introduction

Yeast taxonomy is continuously evolving and new species are frequently described or their phylogenetic position resolved. This is the case of *Candida zemplinina* (synonym *Starmerella bacillaris* (Duarte*, et al.*, 2012)), that was firstly described in 2003 by Matthias Sipiczki. For a long time, *C. zemplinina* has been confounded with its sister species *C. stellata* that shares similar ecological niches, particularly grape and wine environments (Sipiczki, 2004, Sipiczki*, et al.*, 2005, Csoma & Sipiczki, 2008, Duarte*, et al.*, 2012). Indeed, *C. zemplinina* is almost systematically found in grape must, whatever the region or the grape variety, usually at relatively high population level of $10^{e4}$-$10^{e6}$ cells/ml (Nisiotou*, et al.*, 2007, Zott*, et al.*, 2008, Tristezza*, et al.*, 2013, Pfliegler*, et al.*, 2014, Sun*, et al.*, 2014). Like *Saccharomyces* species and *Hanseniaspora uvarum*, *C. zemplinina* is also detected at lower levels on winery surfaces prior to harvest, and may be the source of repeated inoculation in successive batches (Bokulich*, et al.*, 2013). Then, its presence during subsequent grape fermentation is frequently reported, even if *S. cerevisiae* dominates yeast microbiota at that point (Nisiotou*, et al.*, 2007, Cordero-Bueso*, et al.*, 2013). Indeed, only some *Saccharomyces* species are able to complete alcoholic fermentation (AF) in enological conditions (i.e. to consume all sugars present in grape must), explaining why *C. zemplinina* and other non-*Saccharomyces* species are less identified during AF. However, some NS species, of which *C. zemplinina*, can be isolated even at the end of AF, which is congruent with the fact that some strains can produce and tolerate relatively high levels of ethanol (Rantsiou*, et al.*, 2012). *C. zemplinina* occurrence is particularly high within sweet wines whose musts have high initial sugar concentration (Sipiczki, 2003, Urso*, et al.*, 2008, Tofalo*, et al.*, 2009, Magyar & Toth, 2011, Rantsiou*, et al.*, 2012, Rantsiou*, et al.*, 2013). *C. zemplinina* is more rarely isolated from other substrates, such as local fermentations, fruits (usually rotting fruits), fruit-associated insects or soil (Nielsen*, et al.*, 2005, Stamps*, et al.*, 2012). This suggests that *C. zemplinina* primary ecological reservoir is alcoholic fermentation of fruit juice and particularly of grapevine, with occasional colonisation of other favourable niches.

These last 10 years, several authors have highlighted the enological potentials of *C. zemplinina* species (Ciani & Comitini, 2015). In mixed fermentation with *S. cerevisiae* (necessary to complete AF), it produces wine with reduced ethanol levels (Di Maio*, et al.*, 2012, Bely*, et al.*, 2013, Giaramida*, et al.*, 2013, Englezos*, et al.*, 2015). Such modifications of sugar/ethanol yield may be due, at least partially, to an increased content of glycerol (Di Maio*, et al.*, 2012, Giaramida*, et al.*, 2013, Zara*, et al.*, 2014). This feature is particularly interesting since global warming and the evolution of viticulture practices have led to grape

must with increased sugar content and thus increased potential ethanol content. *C. zemplinina* species has also been investigated for its fructophilic character (Magyar & Toth, 2011, Tofalo, *et al.*, 2012, Englezos, *et al.*, 2015), an interesting characteristic in winemaking. Other promising metabolic features include modification of anthocyanin profiles (Mangani, *et al.*, 2011), higher level of some terpenes and lactones (Sadoudi, *et al.*, 2012), the release of mannoproteins (Domizio, *et al.*, 2014), malic acid metabolization (Tofalo, *et al.*, 2012), or the production of some organic acid (Magyar, *et al.*, 2014). Finally, mixed cultures including *S. cerevisiae* and *C. zemplinina* were associated with increased production of some aromatic compounds (Andorrà, *et al.*, 2012), and tools are now developed in order to follow thoroughly the different populations in mixed cultures (Wang, *et al.*, 2014). However, sensory evaluation of mixed cultures were not fully satisfying (Bely, *et al.*, 2013). Thus, *C. zemplinina* species appears as an interesting non-*Saccharomyces* (NS) yeast in winemaking to limit the production of some metabolites (ethanol), or to increase the production of other ones (glycerol, mannoproteins, etc.). However, several efforts must be performed in order to improve the species and propose strains with neutral impact on the organoleptic properties of wine. To date, *C. zemplinina* improvement remains difficult as the biology of the species is poorly known and due to the limited amount of molecular methods developed for non-*Saccharomyces*.

At the interspecific level, some tools are available to characterize yeast populations during alcoholic fermentation process. Besides culture-dependent methods, molecular methods can be used to discriminate the different species in enological environments and to monitor their growth, such as PCR-DGGE (Urso, *et al.*, 2008), quantitative PCR (Andorra, *et al.*, 2010, Zott, *et al.*, 2010) or High-Throughput Sequencing that has been recently proposed (Bokulich, *et al.*, 2013). These tools are particularly valuable to describe yeast dynamics in various AF context, but also to study the impact of oenological practices, the consequences of farming practises, etc. (Andorrà, *et al.*, 2008, Milanovic, *et al.*, 2013, Albertin, *et al.*, 2014, Martins, *et al.*, 2014). At the intra-specific level, few methods are described. The mitochondrial genome of *C. zemplinina* has been fully sequenced and intra-specific variations were described within intronic sequences, allowing the description of two different mitochondrial patterns (Pramateftaki, *et al.*, 2008). RAPD-PCR fingerprinting (Tofalo, *et al.*, 2012, Pfliegler, *et al.*, 2014) and tandem repeat-tRNA (TRtRNA) PCR method (Barquet, *et al.*, 2012) allow higher intra-specific discrimination, yet do not allow accurate population genetics or ecological studies. Multi-locus microsatellite typing has been successfully developed for yeast species of enological interest, such as *Saccharomyces cerevisiae* (Legras, *et al.*, 2007), *S. uvarum*

(Masneuf-Pomarede, et al., 2007), *Torulaspora delbrueckii* (Albertin*, et al.*, 2014) or the spoilage species *Brettanomyces bruxellensis* (Albertin, et al., 2014). Microsatellite analysis provided new insights into the genetic variability and population structure of wine yeasts, and also provided valuable data regarding the life-cycle of the species (Albertin*, et al.*, 2014). In this work, we developed 10 microsatellite markers for the *C. zemplinina* species that were used for the genotyping of 163 strains from nature and various winemaking regions. We show that the genetic diversity of *C. zemplinina* is shaped by geographical localisation and displays no evidence of domestication. Populations isolated from winemaking environments are quite diverse and no specific genetic signature were associated with the different vineyards/wineries.

## Material and Methods

### Yeast strains and culture conditions

163 strains of *C. zemplinina* were sampled from different collections (Table 1) and were mostly isolated from different vineyards or wineries in Europe (France, Greece, Hungary, Italy, Spain, Switzerland), and New Zealand. Six strains from nature were included (11-479, 11-60, 11-9, UWOPS 07-402.2, UWOPS83-775.2, UWOPS 91-743.1). In addition, the type strain of *C. stellata* CBS $157^T$ was used to test the specificity of the microsatellites markers. All strains were grown at 24°C in YPD medium containing 1% yeast extract (w/v, Difco Laboratories, Detroit, MI), 1% Bacto peptone (w/v, Difco), and 6% glucose (w/v), supplemented or not with 2% agar (w/v).

### Genomic DNA extraction and species assessment

For genomic DNA extraction, cells grown on YPD medium were lysed using a FastPrep-24 instrument (MP Biomedicals, Illkirch, France): 100 µL of glass beads (acid-washed, 425-600 µm, Sigma, Lyon, France) were added to cells pellet as well as 300µl of Nuclei Lysis solution (Wizard Genomic DNA purification Kit, Promega). Cells were crushed through 2 cycles of 20s (max. speed). Subsequent DNA extraction was performed with the Wizard Genomic DNA purification Kit (Promega) following the manufacturer's protocol. A second step of protein precipitation solution, as well as subsequent precipitation using isopropanol and ethanol was performed in order to ensure high purity DNA extraction.

For the rapid genotyping of *B. bruxellensis* strains, we used a punch-based method using FTA CloneSaver card (Whatman, BioScience, USA). Eight µl of cells grown on YPD medium were loaded on a CloneSaver card, then before PCR, 2.0-mm disks were punched, washed twice with 50µl of TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) and once with 50µl of ultrapure water.

For each *C. zemplinina* strain, a PCR-RFLP method (ITS-5.8S rDNA amplification followed by *Mbo*I restriction) was used as described by Sipiczki (2004) in order to confirm species identity and exclude strains from the sister species *C. stellata*.

### Genome sequencing and *de novo* assembly of the *C. zemplinina* type strain CBS9494

A draft genomic sequence was produced using Ion Torrent technology. Briefly, genomic library of CBS 9494 was produced using the Ion Xpress™ Plus Fragment Library Kit (Life Technologies, Carlsbad, USA), with an enzymatic shearing of 10min at 37°C. DNA was sequenced on an Ion Torrent PGM (Life Technologies, Carlsbad, USA). After trimming on

quality threshold (Phred-type quality score of $Q20$, $Q_{Phred}=20$) and length threshold (50pb) using CLC Genomics Workbench (CLC bio, Boston, USA), a total of 5,698,579 reads (mean sequence: 200pb) were used for de novo assembly using Newbler (454 Life Sciences). The 268 assembled contigs (mean: 108,648pb, max: 649,352pb) formed a 9,3Mb sequence assembly for an estimated genome size of 9.8Mb (Sipiczki, 2004).

**Microsatellite loci identification and primers design**

Trinucleotide repeats were searched within the *de-novo* genome assembly of the type strain CBS 9494. In order to exclude possible telomeric and subtelomeric repeats, we did not considered microsatellites located within 3Kb of the 5'-end and 3'-end of the contigs. Primers were designed using the 'Design primers' tool on the SGD website (http://www.yeastgenome.org/cgi-bin/web-primer). To reduce the cost associated with primers fluorescent labelling, the forward primers were tailed on 5'-end with M13 sequence (19nt) as described by Schuelke, 2000 (Schuelke, 2000), and universal M13 primers labelled with different fluorescent dyes were added (see below). Amplified fragment sizes varied from 101 to 361 bp, allowing subsequent multiplexing of the amplicons (Table 2).

**Microsatellites amplification**

PCR reactions were performed in a final volume of 15µl containing one washed punch from FTA CloneSaver card, 0.05 µM of forward primer, 0.5 µM of reverse primer and labelled primer, 1X Taq-&GO (MP Biomedicals, Illkirch, France). Universal M13 primers were labelled with either FAM-, HEX-, PET- or NED-fluorescent dyes (Eurofins MWG Operon, Les Ulis, France).

Touch-down PCR were carried out using iCycler (Biorad, Hercules, CA, USA) thermal cycler. The program encompassed an initial denaturation step of 1 min at 94°C followed by 10 cycles of 30 s at 94°C, 30 s at Tm+10°C (followed by a 1°C decrease per cycle until Tm is reached) and 30 s at 72°C, then 20 cycles of 30 s at 94°C, 30 s at Tm and 30 s at 72°C, and a final extension step of 2 min at 72°C.

Amplicons were initially analysed by a microchip electrophoresis system (MultiNA, Shimadzu) and the optimal conditions for PCR amplifications were assessed. Then, the sizes of the amplified fragments were measured on an ABI3730 DNA analyzer (Applied Biosystems). For that purpose, PCR amplicons were diluted (1800-fold for FAM, 600-fold for HEX, 1200-fold for PET and 1800-fold for NED-labelled amplicons respectively) and multiplexed in formamide. LIZ 600 molecular marker (ABI GeneScan 600 LIZ Size Standard,

Applied Biosystem) was 100-fold diluted and added for each multiplex. Before loading, diluted amplicons were heated 4 min at 94°C. Allele size was recorded using GeneMarker Demo software V2.4.0 (SoftGenetics).

**Data analysis**

Microsatellite analysis was used to investigate the genetic relationships between strains. A dendrogram was built using Euclidean distance and Neighbor-Joining's clustering, by means of R (R Development Core Team, 2010) and package phyclust version 0.1-14 (Chen & Dorman, 2013). In order to assess the robustness of the tree nodes, multiscale bootstrap resampling associated with an approximately unbiased test (Shimodaira, 2002) was performed by means of R and the pvclust package v1.2-2 (Suzuki & Shimodaira, 2006, R Development Core Team, 2010).

In addition to dendrogram drawing, the software STRUCTURE (v2.3.4) was used to delineate clusters of individuals on the basis of their microsatellite genotypes using a Bayesian approach (Pritchard, et al., 2000). The parameters were as followed: 10000 Burn-in period, 1000 Repetitions. Models with number of populations (K) ranging from K=3 to K=20 were tested, and models with and without admixture gave similar results (the model with no admixture was thus conserved for the graphical representation of the population).

To test for population differentiation, analysis of molecular variance (AMOVA) was performed by means of the pegas package (Paradis, 2010) with n=1000 permutations. We tested whether the genetic distance was significantly explained by geographical localisation (i.e. the country of isolation was used as grouping factor) or substrate origin ('Enology' versus 'Wild' origins). The relationship between genetic distance and geography was furthermore confirmed by Mantel's test (Mantel, 1967) using ade4 package (Chessel*, et al.*, 2004). Mantel's test allows correlating two distance matrices, in that case we used the genetic distance matrix computed from microsatellite data, and a kilometric distance matrix (computed using latitude and longitude of strain location).

## Results

### Development of microsatellite markers for *Candida zemplinina*

Ion Torrent technology was used to produce a raw sequence (268 contigs) of the genome sequence of CBS 9494T, the type strain of *C. zemplinina*. Microsatellite loci were searched within this draft genome, and we considered dinucleotide and trinucleotide repeats that were not located within the 5'-end and 3'-end of the contigs, in order to exclude possible telomeric or subtelomeric positions. Primers were designed to amplify ten microsatellite loci (Table 2), none of them being located in coding sequence.

The amplicons were separated using a microchip electrophoresis system (MultiNA), and the optimal conditions for microsatellites amplifications were assessed on a panel of twenty strains of *C. zemplinina* (data not shown). After optimisation, the microsatellites markers were tested on *C. stellata*, the sister species of *C. zemplinina*. No amplification was observed for CBS 157T, indicating that the microsatellite markers developed were specific of *C. zemplinina* species.

The 10 microsatellites markers were then used to genotype 157 *C. zemplinina* strains isolated from various oenological regions (Figure 1, Table 1). Six strains from non-enological environments (soil, insect, other fruits) were also genotyped: 11-479, 11-60, 11-9, UWOPS 07-402.2, UWOPS83-775.2, UWOPS 91-743.1. All microsatellites were polymorphic, with 3 different alleles for CZ13 and up to 19 alleles for CZ54 (Table 2). Over the 163 strains, 121 different genotypes were observed, confirming the discriminant power of microsatellite analysis. Interestingly, only one strain displayed heterozygosity for two loci (CZ15, CZ59), while all other 162 strains showed only one allele per locus.

### Establishment of the genetic relationships between *C. zemplinina* strains

The genetic relationships between the 163 strains of *C. zemplinina* were further examined using the Euclidean distance and Neighbor-Joining clustering. The resulting dendrogram tree showed 4 main clusters: the first one included most Spanish strains (9 upon 15) as well as many French strains (23 upon 83) and was quite robust (bootstrap value of 93). One group contained most Italian (11/19) and Greek (10/21) strains, while another one harboured several French strains (33 upon 83), these two groups being less robust (bootstrap value of 58). Finally, the last group, although robust (bootstrap value of 90), contained strains from France (11) as well as Greece (6), Spain (4) or a few other countries.

Another complementary analysis, using Bayesian approach, was applied to assess the significance of these four clusters. STRUCTURE found an optimum of K= 4 populations that

captured most of the genetic structure of *C. zemplinina* species, and was congruent with the dendrogram tree. In particular, the two groups with moderate bootstrap values were clearly related to two distinct ancestral groups as determined by STRUCTURE (Figure 1).

In order to definitively determine whether, and to what extent, the genetic variation of *C. zemplinina* was related to geographical origin, an analysis of molecular variance (AMOVA) was performed. We used the country of isolation as grouping factor. The geographical origin was significantly related to genetic data (pvalue $<<10^{e-6}$) and explained 29.71% of the total variation of the microsatellite dataset. The relationship between genetic distance and kilometric distance between strains was also confirmed by Mantel's test (pval=0.013, Ho= incongruence of genetic/geographic matrices). This indicated that geographical origin shaped significantly, yet not completely, the genetic diversity of *C. zemplinina* species. By contrast, the substrate origin appeared to impact poorly the genetic diversity of the species: the 6 strains from non-enological environments (11-479, 11-60, 11-9, UWOPS 07-402.2, UWOPS83-775.2, UWOPS 91-743.1) were distributed throughout the dendrogram tree, while AMOVA using ecosystem ('Enology' versus 'Wild' origins) as grouping factors was non-significant. Although the 'wild' panel was low (only 6 strains), these data suggested that substrate origin did not significantly shaped the genetic diversity of *C. zemplinina* species.

**Population diversity in oenological conditions**

*C. zemplinina* occurrence is particularly high within sweet wines. Indeed, our collection included several strains isolated from high sugar grape musts (38 strains), as well as 112 strains from non-sweet wines. We thus performed an AMOVA using sugar concentration (sweet musts/wines versus non-sweet musts/wines) as grouping factors. The AMOVA was non-significant, indicating that the sugar concentration of the medium was not related to genetic diversity.

We studied strains from different European vineyards/wineries (France, Greece, Hungary, Italy, Spain, and Switzerland) and from New Zealand. Strains isolated from the same vineyard/winery usually displayed quite different genotypes and were frequently distributed throughout the dendrogram tree (Figure 2). For example, 10 strains were isolated from Winery 8 near Bordeaux (different samples and different years). Nine upon ten different genotypes were evidenced (L0629 and L0653 sharing the same genotype), belonging to all four groups (Figure 2), suggesting that no specific genotype showed persistence within a

given winery across tanks and vintages. The absence of 'genetic signature' at the vineyard/winery level was observed for most of the vineyards/wineries we tested (Figure 2).

In addition, some strains were also isolated from one unique sample (see Table 1), as it is the case of the NZ strains (NZ2, NZ6, NZ8, NZ11 and NZ12), all coming from one unique harvest of Chardonnay fermenting must (Sample 10). A total of 71 strains coming from 19 unique samples were genotyped. Few clonal populations were evidenced (samples 7, 9, 14). In some cases, strains isolated from one unique sample clustered in the same group as evidenced on the dendrogram tree (samples 7, 9, 11, 13, 14, 15 and 18). However, in most cases, strains isolated from one unique sample clustered in two different groups (samples 1, 2, 3, 5, 8, 16, 17 and 19) or in three different groups (samples 4, 10 and 12). An extreme case was for sample 6 for which 16 strains were isolated, showing 15 different genotypes distributed on the four clusters of the dendrogram tree. Globally these data indicated that *C. zemplinina* populations associated with winemaking were not clonal populations, and that no specific genetic signature were associated with the different samples and vineyards/wineries.

## Discussion

### Microsatellite genotyping, a discriminant tool for population genetics studies of *C. zemplinina*

Non-*Saccharomyces* yeast species are currently studied for their potential oenological interest (Jolly, *et al.*, 2014). Thus, some tools are being developed to allow their genetic characterization, at the interspecific level but also at the intra-specific level in order to discriminate and evaluate different strains of the same species. Few methods were described for *C. zemplinina* species: Pfliegler et al. (2014) observed moderate diversity using PCR-fingerprinting methods with 14 patterns for 35 tested strains. Tofalo et al. obtained quite discriminant patterns from 36 strains and suggested an important genetic heterogeneity of the *C. zemplinina* species. Tandem repeat-tRNA (TRtRNA) PCR method also appeared as a promising discriminant approach (Barquet, *et al.*, 2012). However, all these methods are unsuitable for population genetics, and may yield different dendrograms and clusters (Pfliegler, *et al.*, 2014).

The microsatellite tool is extremely popular for population and ecological studies of many species. In yeast, it has been successfully applied to several wine species: *S. cerevisiae* (Legras, *et al.*, 2005, Legras, *et al.*, 2007), *S. uvarum* (Masneuf-Pomarede, *et al.*, 2007, Zhang, *et al.*, 2015), *Torulaspora delbrueckii* (Albertin, *et al.*, 2014), *Brettanomyces bruxellensis* (Albertin, *et al.*, 2014). One main advantage of the microsatellite tool lies in its portability, meaning that genotyping across different laboratories can be compared. This is not the case for fingerprinting approaches, which are prone to interlaboratory variation. In this work, ten microsatellites markers were developed and successfully applied to 163 strains of *C. zemplinina*. Microsatellite genotyping appeared highly discriminant, with 121 different patterns. Moreover, population structure inferred from microsatellite data appeared reliable as classical clustering and Bayesian approaches yielded similar results. The microsatellite tool will be of interest for subsequent ecological analysis, and also in applied research for the checking of strain implantation in mixed-cultures for example.

### *Candida zemplinina* species shows no evidence for domestication

The genetic diversity of *C. zemplinina* species showed no specific clustering depending on the substrate origin of the strains (‘Enology’ versus ‘Wild’ origins). Although the number of ‘wild’ strains was relatively low (6 strains), no specific clustering was observed as these wild strains sorted in two of the four clusters on the dendrogram tree. In addition, as *C. zemplinina* is known to be particularly associated with high sugar musts or wines, we tested whether

initial sugar concentration could be related to genotype selection. We did not find any relationship between the genetic diversity and sweet/non-sweet wines, suggesting that high sugar concentration in winemaking has no impact on *C. zemplinina* selection and adaptation. Indeed, *C. zemplinina* species showed no evidence for domestication event. By contrast, previous studies using microsatellite data demonstrated the domestication of other yeasts for winemaking: *S. cerevisiae* was the first wine yeast shown to be domesticated for human application, including winemaking, bakery, brewery, etc. (Legras*, et al.*, 2005, Legras*, et al.*, 2007). Approaches using comparative genomics later confirmed these results (Liti*, et al.*, 2009) and *S. cerevisiae* was established as a relevant model of domesticated microorganism (Sicard & Legras, 2011). Among the *Saccharomyces sensu stricto* complex, *S. uvarum*, sometimes used in winemaking and cidermaking, seems to be selected for human application (Almeida*, et al.*, 2014), and the presence of introgressed genome portion could be a molecular mechanisms underlying domestication in that species. Recently, a non-*Saccharomyces* species, related to wine, was also studied using microsatellite markers. *T. delbrueckii* has been associated with winemaking and other bioprocesses (bakery, dairy products, etc.) for decades (Albertin*, et al.*, 2014). The genetic diversity of *T. delbrueckii* was congruent with two domestication events associated with winemaking from one hand, and other bioprocesses on the other hand. Indeed, winemaking and other human application have strongly shaped the genetic diversity of several yeasts, including *Saccharomyces* and non-*Saccharomyces* ones. By contrast, *C. zemplinina* shows no evidence for such domestication events. Two opposite hypotheses could be congruent with those results: first, *C. zemplinina* could be a fully domesticated species, meaning fully associated with oenology, without actual wild relatives. In that case, the few strains isolated from natural environments would come from dispersion from nearby enological environments. However, one would expect such yeast populations to be selected in winemaking environments, and thus to be more clonal-like. The second hypothesis is that *C. zemplinina* is not a domesticated species. This last hypothesis would be congruent with the fact that no clonal behaviour is observed, that no genetic evidence of domestication is found and, conversely, that geographical origin significantly shaped the genetic diversity of the species (which is expected for non-domesticated species). Even if *C. zemplinina* possess interesting oenological properties (low ethanol production, fructophily, high glycerol production, etc.), more efforts should be put into the study of physiology and metabolism of this non-*Saccharomyces* especially in relation to its impact on the organoleptic characteristics of the wine.

**The life cycle of *Candida zemplinina*:**

In addition to population structure, microsatellite analysis may be useful to raise the curtain on the life-cycle of the species (Paolocci*, et al.*, 2006, Albertin*, et al.*, 2014). Here, we showed that all 163 strains bar one showed only one allele per locus. Under the assumption of a diploid species, almost complete homozygosity could be explained by a high level of sporulation leading to fully homozygous diploid representative. However, no ascospores formation was evidenced, even after several weeks of incubation on traditional sporulation medium (Sipiczki, 2003). Indeed, since *C. zemplinina* strains showed no evidence of sporulation ability, it can be hypothesized that this species has a mostly haploid life-cycle, with essentially haploid (homozygous) individuals and rare diploid (heterozygous) representative. Further experiments, like various assays of sporulation and breeding between strains will elucidate definitively the life-cycle of *C. zemplinina* species.

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546 **Tables**

547

548 **Table 1. Origin of *Candida zemplinina* and *C. stellata* strains used in this study.**

549 [a] Strains having the same sample code in brackets are strains isolated from the same sample.

550 [b] **CBS-KNAW**: Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre,
551 institute of the Royal Netherlands Academy of Arts and Sciences (Koninklijke Nederlandse
552 Akademie van Wetenschappen), Utrecht, the Netherlands; **CRB Oeno**: Centre de Ressources
553 Biologiques Œnologie (Isabelle Masneuf-Pomarede), Villenave d'Ornon, France; **CRPR**:
554 Centre de Recherche Pernod-Ricard (Benoit Colonna-Ceccaldi), Creteil, France; **Debrecen**:
555 University of Debrecen (Matthias Sipiczki), Hungary; **DEMETER**: Hellenic Agricultural
556 Organisation (Aspasia Nisiotou), Wine Institute of Athens, Greece; **DISAFA**: University of
557 Torino (Luca Cocolin), Italy; **Foggia**: University of Foggia (Giuseppe Spano), Italy; **IUVV**:
558 Institut Universitaire de la Vigne et du Vin "Jules Guyot" (Hervé Alexandre), Dijon, France;
559 **ISVV**: Institut des Sciences de la Vigne et du Vin (Marina Bely), Villenave d'Ornon, France;
560 **URV**: Universitat Rovira i Virgili (Albert Mas), Tarragona, Spain; **UWOPS**: Culture
561 collection of the University of Western Ontario (Marc-André Lachance), Department of
562 Biology (formerly Plant Sciences), London, Canada.

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| Species | Strain | Geographical origin | Substrate | Winery (sample) | Collection/ Laboratory | Reference |
|---|---|---|---|---|---|---|
| C. zemplinina | 10-373 | Hungary, Tolcsva | Enology - fermenting sweet botrytized musts, 2001 | Winery 1 | Debrecen | Sipiczki, 2003 |
| C. zemplinina | 10-374 | Hungary, Tolcsva | Enology - fermenting sweet botrytized musts, 2001 | Winery 1 | Debrecen | Sipiczki, 2003 |
| C. zemplinina | 10-375 | Hungary, Tolcsva | Enology - fermenting sweet botrytized musts, 2001 | Winery 1 | Debrecen | Sipiczki, 2003 |
| C. zemplinina | 10C | Italy, San Severo | Enology - fermenting must (Uva di Troia), 2011 | | Foggia | |
| C. zemplinina | 11-1 | Spain, Almeria | Enology - grape must | | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-101 | Hungary, Tarcal | Enology - botrytized grape, 2002 | | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-124 | Hungary, Tarcal | Enology - botrytized grape, 2003 | | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-128 | Hungary, Tarcal | Enology - botrytized grape, 2003 | | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-145 | Hungary, Erdobénye | Enology - botrytized must | Winery 2 | Debrecen | |
| C. zemplinina | 11-149 | Hungary, Tarcal | Enology - wine | Winery 3 | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-150 | Hungary, Tarcal | Enology - wine | Winery 3 | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-18 | Switzerland, Waedenswill | Enology - fermenting wine | Winery 4 | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-19 | Switzerland, | Enology - fermenting wine | Winery 4 | Debrecen | Pfliegler et al., 2014 |

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|---|---|---|---|---|---|---|
| | | Waedenswill | | | | |
| C. zemplinina | 11-20 | Switzerland, Waedenswill | Enology - fermenting wine | Winery 4 | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-4 | Slovakia | Enology - grape treated with Kaptan | | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-479 | Philippines, Manila | Wild - fruit (rotting banana) | | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-6 | Italy, Verona | Enology - fermenting must | | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-60 | USA | Wild - fly (Drosophila pinicola) | | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 11-9 | South Africa | Wild - soil | | Debrecen | Pfliegler et al., 2014 |
| C. zemplinina | 13C | Italy, Castelluccio dei Sauri | Enology - fermenting must (Uva di Troia), 2011 | | Foggia | |
| C. zemplinina | 1C | Italy, Lucera | Enology - fermenting must (Uva di Troia), 2011 | | Foggia | |
| C. zemplinina | 2C | Italy, Barletta | Enology - fermenting must (Uva di Troia), 2011 | | Foggia | |
| C. zemplinina | 6C | Italy, San Severo | Enology - fermenting must (Uva di Troia), 2011 | | Foggia | |
| C. zemplinina | 7C | Italy, Barletta | Enology - fermenting must (Uva di Troia), 2011 | | Foggia | |
| C. zemplinina | BA1-7 | France, Bourgogne | Enology - grape must (Pinot noir), 2010 | | IUVV | |
| C. zemplinina | BBM4VFA1 | France, Bourgogne | Enology - grape must (Chardonnay), 2010 | Winery 5 | IUVV | |
| C. zemplinina | BBMV5FA17 | France, Bourgogne | Enology - grape must (Chardonnay), 2010 | Winery 5 | IUVV | |
| C. zemplinina | BBMV6-3 | France, Bourgogne | Enology - grape must (Chardonnay), 2010 | Winery 5 | IUVV | |
| C. zemplinina | BBS1FA3 | France, Bourgogne | Enology - grape must (Chardonnay), 2010 | Winery 5 | IUVV | |
| C. zemplinina | BBS2FA17 | France, Bourgogne | Enology - grape must (Chardonnay), 2010 | Winery 5 | IUVV | |
| C. zemplinina | BC60 | Italy, Friuli–Venezia Giulia | Enology - dried grapes must (Picolit) | | DISAFA | Urso et al., 2008 |
| C. zemplinina | BT3C11 | France, Bourgogne | Enology - fermenting must (Chardonnay), 2011 | Winery 6 (Sample 1) | IUVV | |
| C. zemplinina | BT3C16 | France, Bourgogne | Enology - fermenting must (Chardonnay), 2011 | Winery 6 (Sample 1) | IUVV | |
| C. zemplinina | BT3C18 | France, Bourgogne | Enology - fermenting must (Chardonnay), 2011 | Winery 6 (Sample 1) | IUVV | |
| C. zemplinina | BT3C6 | France, Bourgogne | Enology - fermenting must (Chardonnay), 2011 | Winery 6 (Sample 1) | IUVV | |
| C. zemplinina | BTOC39 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 2) | IUVV | |
| C. zemplinina | BTOC40 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 2) | IUVV | |
| C. zemplinina | BTONSC44 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 2) | IUVV | |
| C. zemplinina | BTONSC49 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 2) | IUVV | |
| C. zemplinina | BTONSC50 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 2) | IUVV | |
| C. zemplinina | BTONSC52 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 2) | IUVV | |
| C. zemplinina | BTONSC56 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 2) | IUVV | |
| C. zemplinina | CBS 9494 | Hungary, Tolcsva | Enology - fermenting sweet botrytized musts, 2001 | Winery 1 | CBS | |

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|---|---|---|---|---|---|---|
| C. zemplinina | CZ01 | Italy, Asti wine area | Enology - fermenting must (Barbera) | Vineyard 1 (Sample 3) | DISAFA | |
| C. zemplinina | CZ02 | Italy, Asti wine area | Enology - fermenting must (Barbera) | Vineyard 1 (Sample 3) | DISAFA | |
| C. zemplinina | CZ03 | Italy, Asti wine area | Enology - fermenting must (Barbera) | Vineyard 1 (Sample 3) | DISAFA | |
| C. zemplinina | CZ04 | Italy, Asti wine area | Enology - fermenting must (Barbera) | Vineyard 2 (Sample 4) | DISAFA | |
| C. zemplinina | CZ05 | Italy, Asti wine area | Enology - fermenting must (Barbera) | Vineyard 2 (Sample 4) | DISAFA | |
| C. zemplinina | CZ06 | Italy, Asti wine area | Enology - fermenting must (Barbera) | Vineyard 2 (Sample 4) | DISAFA | |
| C. zemplinina | CZ07 | Italy, Asti wine area | Enology - fermenting must (Barbera) | Vineyard 3 (Sample 5) | DISAFA | |
| C. zemplinina | CZ08 | Italy, Asti wine area | Enology - fermenting must (Barbera) | Vineyard 3 (Sample 5) | DISAFA | |
| C. zemplinina | CZ09 | Italy, Asti wine area | Enology - fermenting must (Barbera) | Vineyard 4 | DISAFA | |
| C. zemplinina | DT3NS1 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS11 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS12 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS13 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS14 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS16 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS17 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS18 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS2 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS3 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS4 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS5 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS6 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS7 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS8 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | DT3NS9 | France, Bourgogne | Enology - grape must (Chardonnay), 2011 | Winery 6 (Sample 6) | IUVV | |
| C. zemplinina | E21NL17 | Greece, Peloponnesus, Nemea | Enology - fermenting must (Agiorgitiko) | Vineyard 5 | DEMETER | |
| C. zemplinina | E222PL2 | Greece, Crete, Peza | Enology - fermenting must (Kotsifali) | Vineyard 6 (Sample 7) | DEMETER | |
| C. zemplinina | E222PL5 | Greece, Crete, Peza | Enology - fermenting must (Kotsifali) | Vineyard 6 (Sample 7) | DEMETER | |
| C. zemplinina | E228NL16 | Greece, Peloponnesus, Nemea | Enology - fermenting must (Agiorgitiko) | Vineyard 7 (Sample 8) | DEMETER | |
| C. zemplinina | E228NL8 | Greece, Peloponnesus, | Enology - fermenting must (Agiorgitiko) | Vineyard 7 (Sample 8) | DEMETER | |

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|---|---|---|---|---|---|---|
| | | Nemea | | | | |
| C. zemplinina | E244PL8 | Greece, Crete, Peza | Enology - fermenting must (Kotsifali) | Vineyard 8 | DEMETER | |
| C. zemplinina | E245PL51 | Greece, Crete, Peza | Enology - fermenting must (Kotsifali) | Vineyard 8 | DEMETER | |
| C. zemplinina | E27NL2 | Greece, Peloponnesus, Nemea | Enology - fermenting must (Agiorgitiko) | Vineyard 9 | DEMETER | |
| C. zemplinina | E2NL510 | Greece, Peloponnesus, Nemea | Enology - fermenting must (Agiorgitiko) | Vineyard 10 | DEMETER | |
| C. zemplinina | E312NL11 | Greece, Peloponnesus, Nemea | Enology - fermenting must (Agiorgitiko) | Vineyard 11 | DEMETER | |
| C. zemplinina | E326NL7 | Greece, Peloponnesus, Nemea | Enology - fermenting must (Agiorgitiko) | Vineyard 9 | DEMETER | |
| C. zemplinina | E348PL7 | Greece, Crete, Peza | Enology - fermenting must (Mavroliatis) | Vineyard 6 | DEMETER | |
| C. zemplinina | E35PL2 | Greece, Crete, Peza | Enology - fermenting must (Kotsifali) | Vineyard 12 | DEMETER | |
| C. zemplinina | E427PL20 | Greece, Crete, Peza | Enology - fermenting must (Vilana) | Vineyard 13 | DEMETER | |
| C. zemplinina | E437PL9 | Greece, Crete, Peza | Enology - fermenting must (Mavroliatis) | Vineyard 14 | DEMETER | |
| C. zemplinina | E438PL20 | Greece, Crete, Peza | Enology - fermenting must (Mandilaria) | Vineyard 14 | DEMETER | |
| C. zemplinina | E43PL1 | Greece, Crete, Peza | Enology - fermenting must (Kotsifali) | Vineyard 8 | DEMETER | |
| C. zemplinina | E510PL2 | Greece, Crete, Peza | Enology - fermenting must (Vilana) | Vineyard 15 | DEMETER | |
| C. zemplinina | E52PL2 | Greece, Crete, Peza | Enology - fermenting must (Vilana) | Vineyard 16 (Sample 9) | DEMETER | |
| C. zemplinina | E52PL3 | Greece, Crete, Peza | Enology - fermenting must (Vilana) | Vineyard 16 (Sample 9) | DEMETER | |
| C. zemplinina | E6PL30b | Greece, Crete, Peza | Enology - fermenting must (Kotsifali) | Vineyard 12 | DEMETER | |
| C. zemplinina | FC54 | Italy, Friuli–Venezia Giulia | Enology - dried grapes must (Picolit) | | DISAFA | Urso et al., 2008 |
| C. zemplinina | L0311 | France, Sauternes | Enology - grape must, 2003 | Winery 7 | CRB Oeno | |
| C. zemplinina | L0471 | France, Mérignac | Enology - grape must (Merlot), 2004 | Winery 8 | CRB Oeno | |
| C. zemplinina | L0472 | France, Mérignac | Enology - grape must (Merlot), 2004 | Winery 8 | CRB Oeno | |
| C. zemplinina | L0473 | France, Mérignac | Enology - grape must (Merlot), 2004 | Winery 8 | CRB Oeno | |
| C. zemplinina | L0629 | France, Mérignac | Enology - grape must (Merlot), 2006 | Winery 8 | CRB Oeno | |
| C. zemplinina | L0650 | France, Mérignac | Enology - grape must (Merlot), 2006 | Winery 8 | CRB Oeno | |
| C. zemplinina | L0651 | France, Mérignac | Enology - grape must (Merlot), 2006 | Winery 8 | CRB Oeno | |
| C. zemplinina | L0653 | France, Mérignac | Enology - grape must (Merlot), 2006 | Winery 8 | CRB Oeno | |
| C. zemplinina | L0656 | France, Mérignac | Enology - fermenting must (Merlot), 2006 | Winery 8 | CRB Oeno | |
| C. zemplinina | L0670 | France, Mérignac | Enology - grape must (Merlot), 2006 | Winery 8 | CRB Oeno | |
| C. zemplinina | L0740 | France, Mérignac | Enology - grape must (Merlot), 2007 | Winery 8 | CRB Oeno | |

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|---|---|---|---|---|---|---|
| C. zemplinina | L1405 | France, Saint-Christophe-des-Bardes | Enology - grape must (Merlot), 2013 | Winery 9 | CRB Oeno | |
| C. zemplinina | L14105 | France, Barsac | Enology - high sugar grape must (Semillon), 2013 | Winery 10 | CRB Oeno | |
| C. zemplinina | L14117 | France, Barsac | Enology - high sugar grape must (Sauvignon), 2013 | Winery 10 | CRB Oeno | |
| C. zemplinina | L14123 | France, Sauternes | Enology - | Winery 11 | ISVV | |
| C. zemplinina | L14132 | France, Sauternes | Enology - high sugar grape must (Semillon and Muscatelle), 2013 | Winery 12 | CRB Oeno | |
| C. zemplinina | L14151 | France, Sauternes | Enology - high sugar grape must, 2013 | Winery 7 | CRB Oeno | |
| C. zemplinina | L1429 | France, Lussac | Enology - Pied de cuve' (Merlot), 2013 | Winery 13 | CRB Oeno | |
| C. zemplinina | L1457 | France, Sauternes | Enology - high sugar grape must (Sauvignon), 2013 | Winery 11 | CRB Oeno | |
| C. zemplinina | L1464 | France, Sauternes | Enology - | Winery 14 | ISVV | |
| C. zemplinina | L1479 | France, Sauternes | Enology - high sugar grape must (Semillon), 2013 | Winery 7 | CRB Oeno | |
| C. zemplinina | L1485 | France, Sauternes | Enology - high sugar grape must (Sauvignon), 2013 | Winery 7 | CRB Oeno | |
| C. zemplinina | MCR9 | France, Bourgogne | Enology - grape must (Pinot noir), 2010 | | IUVV | |
| C. zemplinina | NZ11 | New Zealand, Napier | Enology - fermenting must (Chardonnay), 2009 | Winery 15 (Sample 10) | CRPR | |
| C. zemplinina | NZ12 | New Zealand, Napier | Enology - fermenting must (Chardonnay), 2009 | Winery 15 (Sample 10) | CRPR | |
| C. zemplinina | NZ2 | New Zealand, Napier | Enology - fermenting must (Chardonnay), 2009 | Winery 15 (Sample 10) | CRPR | |
| C. zemplinina | NZ6 | New Zealand, Napier | Enology - fermenting must (Chardonnay), 2009 | Winery 15 (Sample 10) | CRPR | |
| C. zemplinina | NZ8 | New Zealand, Napier | Enology - fermenting must (Chardonnay), 2009 | Winery 15 (Sample 10) | CRPR | |
| C. zemplinina | PE 102 | France, Barsac | Enology - high sugar grape must | Winery 10 | ISVV | |
| C. zemplinina | PE 153 | France, Villenave d'ornon | Enology - grape must (Merlot), 2012 | Vineyard 17 | ISVV | |
| C. zemplinina | PE 159 | France, Villenave d'ornon | Enology - grape must (Merlot), 2012 | Vineyard 17 | ISVV | |
| C. zemplinina | PE 215 | France, Villenave d'ornon | Enology - grape must (Merlot), 2012 | Vineyard 17 | ISVV | |
| C. zemplinina | PE 261 | France, Sauternes | Enology - high sugar grape must | Winery 11 | ISVV | |
| C. zemplinina | PE 265 | France, Sauternes | Enology - high sugar grape must | Winery 11 | ISVV | |
| C. zemplinina | PE 269 | France, Sauternes | Enology - high sugar grape must | Winery 11 | ISVV | |
| C. zemplinina | PE 272 | France, Sauternes | Enology - high sugar grape must | Winery 12 | ISVV | |
| C. zemplinina | PE 276 | France, Sauternes | Enology - high sugar grape must | Winery 12 (Sample 11) | ISVV | |
| C. zemplinina | PE 278 | France, Sauternes | Enology - high sugar grape must | Winery 12 (Sample 11) | ISVV | |
| C. zemplinina | PE 279 | France, Sauternes | Enology - high sugar grape must | Winery 12 (Sample 12) | ISVV | |
| C. zemplinina | PE 281 | France, Sauternes | Enology - high sugar grape must | Winery 12 (Sample 12) | ISVV | |
| C. zemplinina | PE 282 | France, Sauternes | Enology - high sugar grape must | Winery 12 (Sample 12) | ISVV | |
| C. zemplinina | PE 303 | France, Razac de | Enology - grape must | Winery 16 | ISVV | |

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|---|---|---|---|---|---|---|
| | | Saussignac | (Merlot) | | | |
| C. zemplinina | PE 387 | France, Sauternes | Enology - high sugar grape must | Winery 14 | ISVV | |
| C. zemplinina | PE 399 | France, Ladaux | Enology - grape must (Merlot), 2012 | Vineyard 18 (Sample 13) | ISVV | |
| C. zemplinina | PE 400 | France, Ladaux | Enology - grape must (Merlot), 2012 | Vineyard 18 (Sample 13) | ISVV | |
| C. zemplinina | PE 401 | France, Ladaux | Enology - grape must (Merlot), 2012 | Vineyard 18 (Sample 13) | ISVV | |
| C. zemplinina | PE 455 | France, Cadaujac | Enology - grape must (Merlot), 2012 | Vineyard 19 (Sample 14) | ISVV | |
| C. zemplinina | PE 458 | France, Cadaujac | Enology - grape must (Merlot), 2012 | Vineyard 19 (Sample 14) | ISVV | |
| C. zemplinina | PE 460 | France, Puisseguin | Enology - grape must (Merlot), 2012 | Vineyard 20 (Sample 15) | ISVV | |
| C. zemplinina | PE 461 | France, Puisseguin | Enology - grape must (Merlot), 2012 | Vineyard 20 (Sample 15) | ISVV | |
| C. zemplinina | PE 49 | France, Barsac | Enology - high sugar grape must | Winery 10 | ISVV | |
| C. zemplinina | PE 494 | France, Ladaux | Enology - grape must (Merlot), 2012 | Vineyard 18 (Sample 16) | ISVV | |
| C. zemplinina | PE 495 | France, Ladaux | Enology - grape must (Merlot), 2012 | Vineyard 18 (Sample 16) | ISVV | |
| C. zemplinina | PE 89 | France, Sauternes | Enology - high sugar grape must | Winery 11 | ISVV | |
| C. zemplinina | PE 97 | France, Sauternes | Enology - high sugar grape must | Winery 11 | ISVV | |
| C. zemplinina | R5 | Italy, Friuli–Venezia Giulia | Enology - dried grapes (Ramandolo) | | DISAFA | |
| C. zemplinina | Spain1 | Spain, Poboleda | Enology - grape must (Garnacha), 2012 | Winery 17 (Sample 17) | URV | |
| C. zemplinina | Spain10 | Spain, Porrera | Enology - fermenting must (Garnacha), 2012 | Winery 18 (Sample 18) | URV | |
| C. zemplinina | Spain11 | Spain, Porrera | Enology - fermenting must (Garnacha), 2012 | Winery 18 (Sample 18) | URV | |
| C. zemplinina | Spain12 | Spain, Porrera | Enology - fermenting must (Garnacha), 2012 | Winery 18 (Sample 19) | URV | |
| C. zemplinina | Spain13 | Spain, Porrera | Enology - fermenting must (Garnacha), 2012 | Winery 18 (Sample 19) | URV | |
| C. zemplinina | Spain14 | Spain, Porrera | Enology - fermenting must (Garnacha), 2012 | Winery 18 (Sample 19) | URV | |
| C. zemplinina | Spain15 | Spain, Constantí | Enology - grape must (Xarel.lo), 2013 | Winery 19 (Sample 20) | URV | |
| C. zemplinina | Spain2 | Spain, Poboleda | Enology - grape must (Garnacha), 2012 | Winery 17 (Sample 17) | URV | |
| C. zemplinina | Spain3 | Spain, Poboleda | Enology - grape must (Garnacha), 2012 | Winery 17 (Sample 17) | URV | |
| C. zemplinina | Spain4 | Spain, Poboleda | Enology - grape must (Garnacha), 2012 | Winery 17 (Sample 17) | URV | |
| C. zemplinina | Spain6 | Spain, Poboleda | Enology - grape must (Garnacha), 2012 | Winery 17 (Sample 17) | URV | |
| C. zemplinina | Spain7 | Spain, Morera del Montsant | Enology - fermenting must (Cariñena), 2012 | Winery 20 | URV | |
| C. zemplinina | Spain8 | Spain, Escaladei | Enology - fermenting must (Cariñena), 2012 | Winery 21 | URV | |
| C. zemplinina | Spain9 | Spain, Porrera | Enology - fermenting must (Garnacha), 2012 | Winery 18 (Sample 19) | URV | |
| C. zemplinina | UWOPS 07-402.2 | Canada, London | Wild - fruit (Osage Orange) | | UWOPS | |
| C. zemplinina | UWOPS 83-775.2 | Bahamas | Wild - fruit (Opuntia stricta) | | UWOPS | |

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|---|---|---|---|---|---|---|
| C. zemplinina | UWOPS 91-743.1 | USA, Hawaii | Wild - fly (Sapindus) | | UWOPS | |
| C. stellata | CBS 157 | N/A | Enology - wine | | CBS | |

564 **Table 2. Microsatellite loci for *Candida zemplinina* genotyping.**

565 Allele size in pb. Forward primers were tailed on 5'-end with M13 sequence (CACGACGTTGTAAAACGAC). Tm is the melting temperature

566 used for microsatellite amplification (see Materials and Methods).

| Microsatellite name | Motif | Fluorescent dye | Primers | Tm | Alleles size (repeats number) for CBS 9494T | Alleles size (repeats number) range |
|---|---|---|---|---|---|---|
| CZ13 | TCA/TCC/TCG | FAM | F: TTGCGAATGTGTTTCGGA;<br>R: ATGAGAAGGCCGAGGACGAT | 55 | 125 (21) | 101-125 (13-21) |
| CZ45 | CTT/CCT | PET | F: TCCAGCTCGGCAATATCAAT;<br>R: TGACGAGGAGAACAGTGAAGA | 55 | 298 (21) | 289-304 (18-23) |
| CZ11 | GT/GA/TA | FAM | F: TGCGATTATACTATTTTGCGA;<br>R: TGCGAAAAGAACGACAGGAA | 55 | 339 (43) | 271-361 (9-54) |
| CZ33 | GAC/GAA | HEX | F: TGGCTATACCGATTTTGGTGA;<br>R: TGTCCTAATTCCTCTCTCGTC | 55 | 115 (10) | 109-118 (8-11) |
| CZ1 | GT | HEX | F: AAGAACGTTGGTAGGCCTGAA;<br>R: GGGTTCAATTCAATGTTCGG | 55 | 168 (15) | 152-172 (7-17) |
| CZ15 | CAA | HEX | F: AACTTGCGCAACAAGTGTTGA;<br>R: TGATTCTGCATTTGTCCTGG | 55 | 299 (13) | 278-299 (6-13) |
| CZ20 | ACA/GCA | NED | F: ATACCTGGTAGCCCGAATGC;<br>R: TTTGATTGTTGCTGTTGCTG | 52 | 130 (20) | 112-133 (14-21) |
| CZ54 | AGA | NED | F: AAAATAAACCGGCTAGCGGTG;<br>R: TCCTTTCTCCATCCTGAGACA | 55 | 301 (19) | 265-319 (7-25) |
| CZ59 | TA/CA | PET | F: ATATAAACACCCACCGCCACA;<br>R: TTGCAGATTGAGCATTGCAC | 55 | 170 (23) | 154-170 (15-23) |
| CZ4 | TCT | PET | F: CCATATGCGCATCAACATCA;<br>R: ATGGTAGCTGACGCTACTGGT | 55 | 248 (15) | 236-251 (11-16) |

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**Figure legends**

**Figure 1. Genetic relationships between 163 *C. zemplinina* strains using ten microsatellite markers.**

A: Dendrogram tree built using Euclidean distance and Neighbor-Joining's clustering. The robustness of the node was assessed using multiscale bootstrap resampling and approximated unbiased test (n = 1000 boots).

B: Barplot representing STRUCTURE results (K = 4). The posterior probability (y-axis) of assignment of each strain (vertical bar) to ancestral groups is shown by colors (green, yellow, orange and pink represent each 4 ancestral populations).

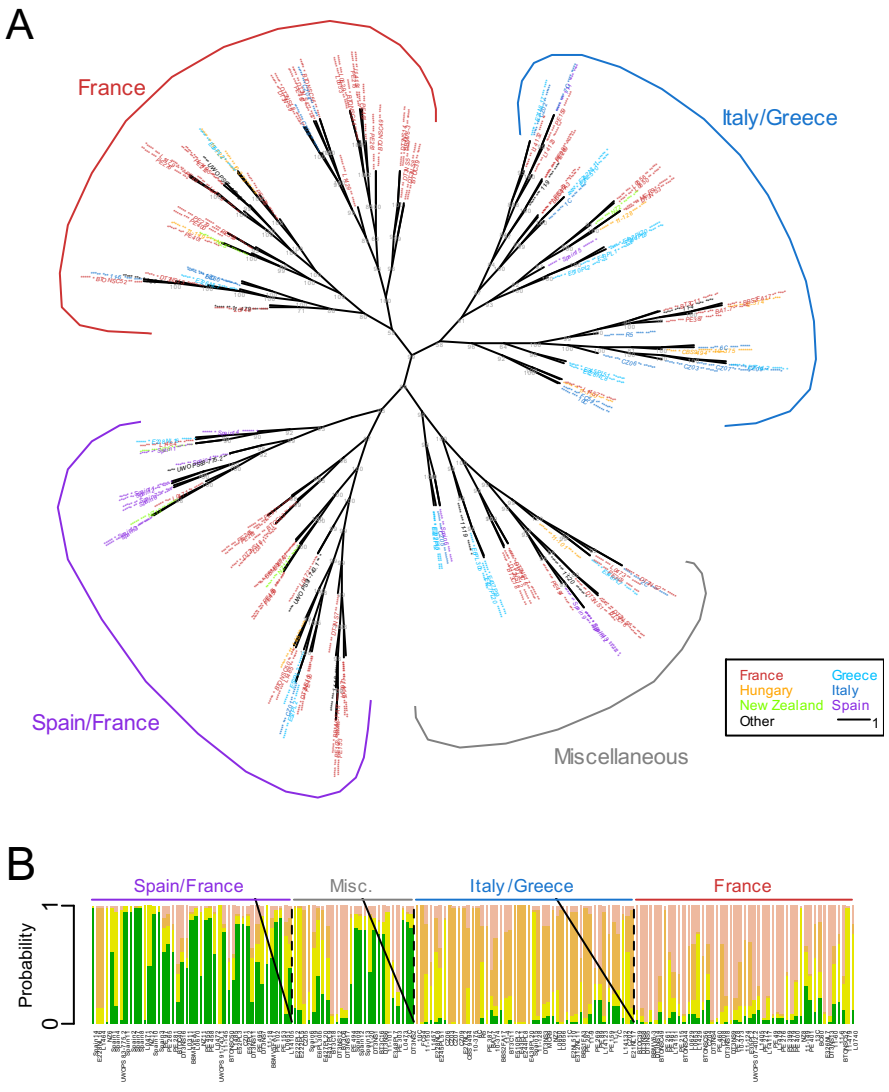

**Figure 2. Genetic relationships between isolates from the same vineyard/winery.**

Strains isolated from 7 vineyards/wineries (in 7 different countries) were localised on the dendrogram tree.