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RESEARCH ARTICLE

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Foliar application of nitrogenous compounds and elicitors to Tempranillo grapevines: Microbiological implications

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Abstract

This study was aimed to analyze the effects of the nitrogen foliar application, and elicitation on microbial populations of musts. The nitrogenous compounds were raw and treated wastewater from mushroom industry and a commercial yeast derivate; the elicitors were methyl jasmonate, chitosan and one commercial elicitor from a yeast source. Those six products were sprayed as pre-harvest treatment over the leaves of Tempranillo grapevines for improving quality and maturity of grapes. The applications of raw and treated wastewaters from mushroom industry, chitosan and methyl jasmonate elicitation did not cause changes in microbial diversity of must samples. In contrast, spraying commercial yeast derivate products made the must microbiota reach a high richness of species that would be positive in ecological terms. This research succeeded in establishing the impacts of foliar applications in the grapevines on the microbial community, and in any case, negative impacts were observed so that, presumably, these foliar applications can be perfectly implemented as agronomic practice.

Additional keywords: vine leaves; elicitation; grape; diversity; nitrogen.

Abbreviations used: AAB (acetic acid bacteria); AF (alcoholic fermentation); MLF (malolactic fermentation); EB (environmental bacteria); LAB (lactic acid bacteria); VP (viable population). **Media:** DBDM (*Dekkera/Brettanomyces* differential medium); GYP (glucose yeast peptone); MRS (Man, Rogosa and Sharpe); M30 (MRS + 30 g/L agar); Mann (mannitol); ZPP (*Zymomonas* Pymaricine penicillin).

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Introduction

The operations developed in the vineyard before the harvest are important to obtain sound and quality grapes. Some of these agronomical operations are focused on reaching the correct nitrogen content in grapes.

Nitrogen (N) is an important element for adequate plant growth and for alcoholic fermentation (AF) completion (Bell & Henschke, 2005). The N fertilizers have been traditionally added to the soil for being absorbed by plants´ roots but, taking into account an increase in the pollution of soils derived from their use (Galloway *et al.*, 2003), new ways of fertilization as foliar application of N sources have been developed (Haytova, 2013). Those N sources are thought to increase the amino acid content of must (Bell & Henschke, 2005). Some of these amino acids are precursors of the aroma synthesis routes so that they can improve the aromatic profiles of wines. Some studies have been conducted on the foliar application of different N sources such as urea and phenylalanine which caused an increase in the amino acid composition of musts (Ancín-Azpilicueta *et al.*, 2013; Portu *et al.* 2015a,b). Other substances can be sprayed over leaves. Thus, some companies as Lallemand S.L. have commercialized products composed by specific fractions of the inactive, dry and non-genetically modified organism (non-GMO) oenological yeast *Saccharomyces cerevisiae* (LalVigne® Aroma) that are

applied over the plants´ leaves like a N fertilizer in order to enhance wine aromatic characteristics. Moreover, the N concerned in oenology joins to the objective of performing an eco-friendlier agriculture (He *et al.*, 2017). The mushroom industry is a quite relevant industry in La Rioja (Spain), for this reason, two types of mushroom wastewaters were considered in the current study. Those wastewaters are thought to be rich in nitrogenous compounds (confidential information pending of patent) so that an improvement of the aromatic features of wines could be achieved with its application.

Additionally, increasing the phenolic compounds in wine is other important aim of the viticulture and oenology. These compounds are known for collaborating in the color, mouthfeel properties and even increasing the stability during ageing what could directly improve wine organoleptic properties (Villangó *et al.*, 2015). Submitting the plants to an external biotic or abiotic threat is referred to as "elicitation". The elicitation could be an interesting tool to make the phenolic content of grapevines be enhanced because it increases the natural biosynthesis of phenolic compounds – by the phenylpropanoid pathway – as a biological response of surviving (Suzuki *et al.*, 2004; Farouk *et al.*, 2017). The chitosan is a biopolymer synthesized by the alkaline deacetylation of chitin that has been employed in agriculture for elicitation. The methyl jasmonate is a plant metabolite to manage the defense responses against stress situations; therefore, it can be also considered an elicitor. The main application in agriculture has been aimed to induce resistance against fungal infection to reduce the postharvest diseases of fruits. As other authors have recognized, the products proceeding from yeast extracts and cell walls such as mannoproteins, lipids, glucans and even chitin can act as elicitors for the plant (Walker, 2009). The company Lallemand S.L. also commercialized an elicitor based on specific fractions of the inactive, dry and non-GMO oenological yeast *S. cerevisiae* (LalVigne® Mature) that improves the phenolic content and consequently the phenolic maturity of grapes (Villangó *et al.*, 2015).

The application of nitrogenous compounds and elicitors may produce a variation in the must amino acid content and in phenolic compounds concentration. Little research about the impact of those physical and chemical shifts on microbial communities – yeasts, molds, lactic acid bacteria (LAB), acetic acid bacteria (AAB) and environmental bacteria (EB) – has been performed until now. In effect, only one previous study performed with two nitrogenous compounds applied to leaves and based on culture-independent methods has been recently published with interesting results, specifically for urea foliar application effect on must microbiota (González-Arenzana *et al.*, 2017). The present research was intended to analyze for the first time the microbial diversity of must samples from grapevines where nitrogenous compounds and elicitors had been foliar applied.

Material and methods

Treatment products

Three nitrogenous compounds [raw and treated wastewater from mushroom industry and a commercial yeast derivate (LalVigne® Aroma = YE1)], and three elicitors [methyl jasmonate, chitosan and one commercial elicitor from a yeast source (LalVigne® Mature = YE2)] were sprayed as preharvest treatment over the leaves of Tempranillo grapevines.

Commercial substances such as YE1 and YE2 were provided by Lallemand S.L. enterprise. Both compounds are composed of specific fractions of the inactive, dry and non-GMO oenological yeast *S. cerevisiae* but detailed composition is confidential (patented trademark). The "Research Centre of the Mushroom in La Rioja" provided two types of wastewaters from mushroom industry. One type was the raw wastewater and the other type was enriched with microorganisms for improving the N uptake previously to its employment; both are pending of patent. Methyl jasmonate and chitosan were purchased from Sigma-Aldrich (Madrid, Spain).

Grapevine treatments and sampling

The study was conducted in a commercial Tempranillo vineyard located in the Rioja qualified Designation of Origin (DOCa Rioja) that had been under conventional soil tillage management system.

The foliar treatments were sprayed on the grapevines at the veraison moment and one week later using a knapsack sprayer. Treatments were applied in triplicate and were arranged in a complete randomized block design with 10 vines per replicate. The YE1 and YE2 solutions were individually prepared following the manufacturer's instructions (Lallemand S.L.) at a concentration of 2.5 g L^{-1} and with doses of 400 mL per plant. Moreover, 475 mL of each type of wastewater (~ 316 mg N L^{-1}) were also sprayed per plant to carry out the treatments made with these sub-products. The methyl jasmonate and chitosan solutions were prepared at 10 mM and 0.03% (w/v), respectively, according to the protocol described by Portu *et al.* (2015c, 2016). In all treatments, Tween 80

(Sigma-Aldrich, Madrid, Spain) was used as wetting agent (0.01 v/v). A control treatment only sprayed with the wetting agent was also included.

Tempranillo grapes from the control and the treated vines were harvested, destemmed, crushed and then placed individually into the vats. Then, 50 mL of each of each must replicate (n=3) were aseptically taken before sulphiting the remaining must.

Culture dependent methods: colony counts and species identification

The six treated must samples and control must sample and their biological triplicates were diluted in sterile saline solution and spread on plates of six different culture media what meant 42 plates for each culture media.

– For yeast counts and identifications, GYP culture medium (20 g L^{-1} of glucose, 5 g L^{-1} of yeast extract, 5 g L^{-1} of peptone, 20 g L^{-1} agar, and 100 mg L^{-1} chloramphenicol) was used.

– For detecting *Brettanomyces/Dekkera* yeast genera, DBDM culture medium was employed (Rodrigues *et al.*, 2001).

– For counting and classifying LAB, MRS culture medium (De Man *et al.*, 1960) supplemented with 50 mg L^{-1} of pymaricine, and the M30 (MRS supplemented with 0.1 v/v of tomato juice, 6 gL^{-1} of fructose, 0.5 g L^{-1} of cysteine - HCl, 5 g L^{-1} of D,L-malic acid and 50 mg L^{-1} pymaricine) were used.

– A medium containing mannitol (Mann) (25 g L^{-1} of D-mannitol, 3 g L^{-1} of peptone, 5 g L^{-1} of yeast extract and 15 g L^{-1} of agar with 50 mg L^{-1} of pymaricine and 30 U L^{-1} of penicillin) was used for isolating AAB.

– Finally, the *Zymomonas* pymaricine penicillin (ZPP) culture medium (Coton & Coton, 2003) was employed for counting and identifying the environmental bacteria referred as EB.

Plates of GYP, ZPP and Mann culture media were incubated at 25 ºC for at least two days. DBDM plates were incubated during 15 days at 25 ºC under anaerobic conditions (GasPak System, Oxoid Ltd., Basingstoke, England). Eventually, MRS and M30 plates were incubated also under anaerobic conditions but at 28 ºC during at least 10 days.

After incubation, the colony forming units per milliliter (CFU mL^{-1}) were counted and five colonies from each replicate plates with representative population (from 30 to 300 colonies) were isolated for identification. Genera and species identification was carried out by molecular biology methods. In case of yeast species identification, partial 26S rRNA genes were amplified using the primers NL1 and LS2 (Cocolin, 2000). For LAB species identification, PCR was performed with primer pairs WLAB1 and WLAB2 targeted the V4 and V5 16S rDNA regions as previously described López *et al.* (2003). Finally, AAB and EB species identification was performed by amplification of the V7 to V8 region of 16S rDNA gene with WBAC1 and WBAC2 primers (López *et al.*, 2003). Macrogen Inc. (Seoul, South Korea) sequenced the PCR amplicons and then sequences were compared to GenBank database using the Basic Local Alignment Search Tool (BLAST) to obtain the correct identification.

Measurement of diversity and structure population. Statistical analysis

Alpha diversity parameters were assessed by the software PAST 3.10 P.D. (Ryan *et al.*, 1995) analyzing the correctly identified species (more than 98% of similarity in BLAST) in each of the replicates (n=3) of the samples. For each replicate, the number of detected species (S), the Margalef index (Margalef, 1958), and the Menhinick index (Karydis & Tsirtsis, 1996) were calculated for describing the richness of species of each sample. The Margalef´s index supposes a functional relation between the number of species and the total number of individuals (Margalef, 1958; Death, 2008) and Menhinick index is the ratio of the number of taxa to the square root of the sample size.

The structure of the studied communities was determined by a non-parametric index such as Chao1 and by parametric indexes of dominance such as Berger-Parker index, Simpson index and Shannon-Weiner.

– The Chao1 is an estimator of the species number based on the odd species (Portillo *et al.*, 2016).

– The Berger-Parker index measures the dominance of the predominant taxon in individuals (Harper, 1999).

– The Simpson index, opposite in meaning, measures the possibility of two randomly chosen individuals belong to different or to the same species (respectively) (Fedor & Spellerberg, 2013).

– Finally, the entropy of the community was measured by the Shannon-Wiener index (H) that takes into account the number of individuals as well as the number of species (Death, 2008; Karydis & Tsirtsis, 1996).

Data of counts and diversity indices were processed using the variance analysis (ANOVA) with the Tukey tests (at $p \leq 0.01$) using the software IBM SPSS Statistic 20.0 (Chicago, USA). Hierarchical cluster with all the information regarding each sample was constructed with the same software.

Results

Cell population and genera after foliar application and elicitation

In Figure 1, data of the cell population (CFU mL^{-1}) in logarithmic (log) units observed in the six culture media are displayed. Regarding GYP, counts on every must sample were higher than 4.5 log units and lower than 5.1 log units, without encountering significant differences between the control sample and the sprayed samples. Regarding DBDM, the counts on control sample were of 1.9 log unit that was not statistically different to the treated samples. Nonetheless, significant differences were found among the highest viable population (VP) found after the wastewater foliar application and the lowest described after the chitosan and the YE2 foliar applications.

Referring to VP detected on the MRS plates, significant differences were not observed after the foliar treatments compared to the control. The control sample had 0.7 log units growing on MRS, being the lowest count after the foliar application of YE1 and the highest after the foliar application of treated wastewater from mushroom industry. On the M30 plates cultivated after the foliar application YE1 and the elicitation of methyl jasmonate and chitosan, VP was not detected. In the case of control sample, the VP was not significantly different to the other samples with LAB population in the wastewater samples from the mushroom industries.

The VP of control sample on Mann plates was 2.8 log units, and significant differences were not found after the foliar application of nitrogenous compounds and elicitation. Statistical differences were encountered between the highest VP after the foliar application of wastewater from mushroom industry (W) and the lowest after the elicitation of YE2. The VP growing on ZPP plates was not higher than 2 log units for the wastewater treatment (W) as shown in Figure 1.

Data about the genera detected with each culture media are shown in Figure 2. Figure 2A contains the genera detected with the GYP and DBDM media. Seven genera of yeasts [*Candida (C.), Hanseniaspora (H.), Hypopichia (Hy.), Metschnikowia (M.), Pichia*

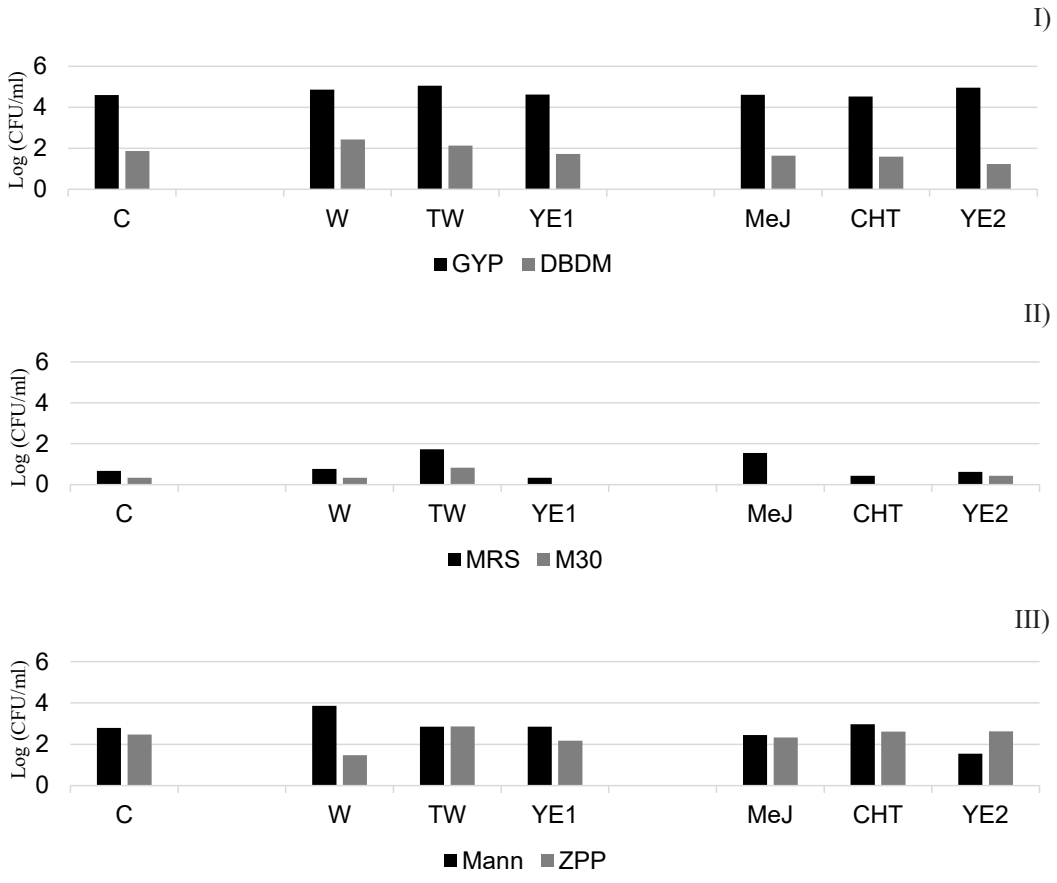

Figure 1. Cell population in logarithmic units counted on six culture media: (I) GYP, DBDM; (II) MRS, M30; (III) Mann and ZPP. Seven treatments were used: control (C); nitrogenous compounds [treated wastewater (TW), wastewater (W), and LalVigne® Aroma (YE1)]; and elicitors [methyl jasmonate (MeJ), chitosan (CHT) and LalVigne® Mature (YE2)]. Different letters mean significant differences (p<0.05) between cell population of different samples on the same culture media.

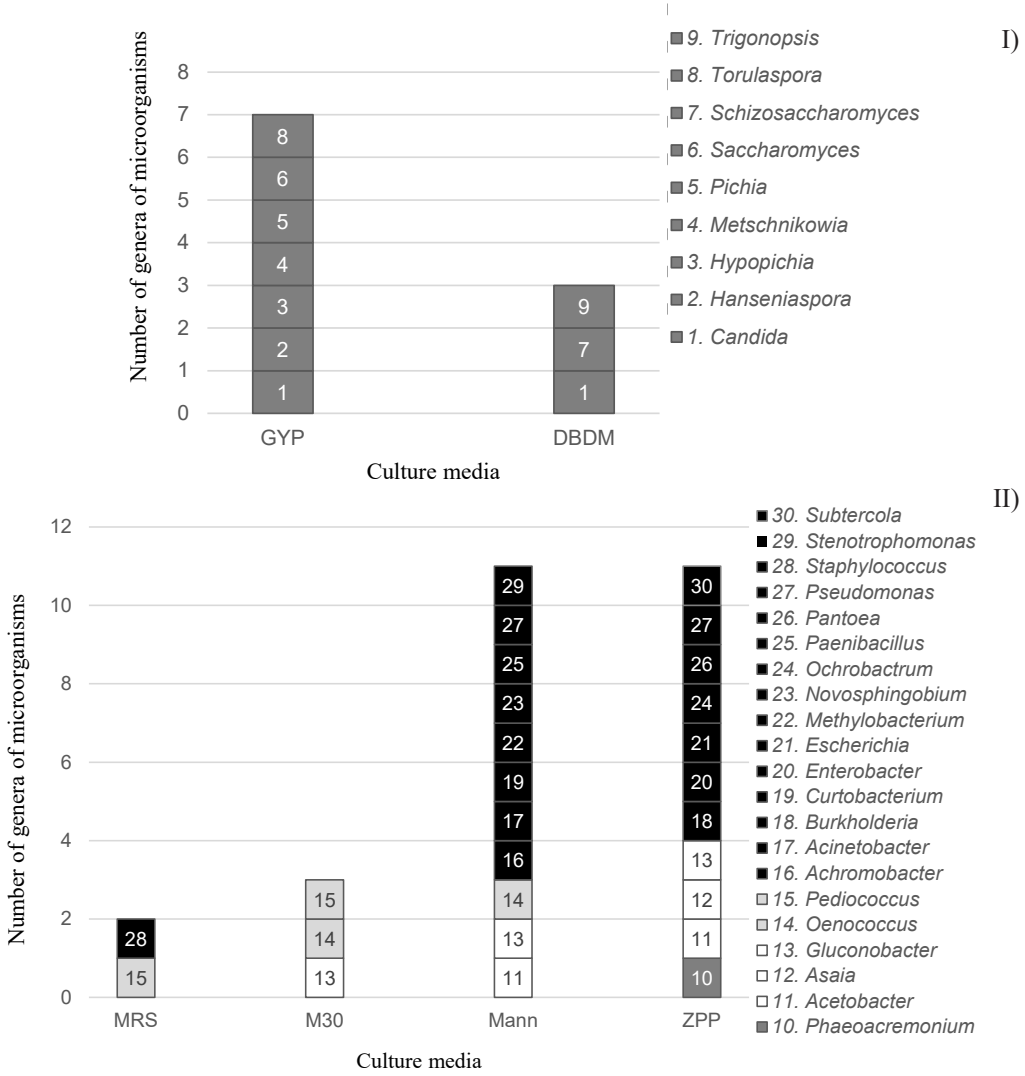

Figure 2. Genera of microorganisms detected on each culture media: (I) GYP and DBDM; (II) MRS, M30, Mann and ZPP. Dark grey slices were yeasts/molds, light grey slices were LAB, empty slices were AAB genera and black slices were EB.

(P.), Saccharomyces (S.) and *Torulaspora (T.)*] grew and were identified on the GYP plates, whereas on DBDM plates three genera were found [*Candida, Schizosaccharomyces (Sch.)* and *Trigonopsis (Tr.)*]. Figure 2B shows the bacteria detected on MRS, M30, Mann and ZPP plates. In the medium MRS, *Pediococcus (Pd.)* and *Staphylococcus (St.)* were identified. On the M30 plates, *Oenococcus (O.)*, *Pediococcus* and *Gluconobacter (G.)* were described. Regarding Mann mediium, 11 genera were found [*Acetobacter (A.) Gluconobacter, Oenococcus, Achromobacter (Ach.), Acinetobacter, Curtobacterium (Cur.), Methylobacterium, Novosphigobium, Paenibacillus, Pseudomonas (Ps.)* and *Stenotrophomonas*]; in ZPP other 11 genera were detected: the fungi *Phaeocremonium, Acetobacter, Asaia (As.), Gluconobacter* and *Burkholderia (Bur.)*, *Enterobacter, Escherichia (Es.), Ochrobactrum, Pantoea (Pa.), Pseudomonas* and *Subtercola*.

Species of microorganisms detected

In Figure 3 the species of yeasts LAB, AAB and EB detected at each sample are displayed. In the control sample 11 species were found: *H. uvarum, Hy. pseudoburtonii, P. kluivery, S. cerevisiae, Sch. pombe, Pd. acidilactici, A. indonesiensis, G. cerinus* and *G. oxydans, B. mimosarium* and *Ochrobactrum* sp.

After the foliar application of the treated wastewater from mushroom industry, 13 species were found, eight were the same than the identified in the control sample and three were different (*C. zemplinina, O. oeni* and *Ach. xylosoxidans*). When the wastewater was sprayed over leaves, 15 species were identified, being eight species common to the control sample and seven different (*T. delbrueckii, T. cantarelli, O. oeni, Ach. xylosoxidans, Methylobacterium* sp., *Paenibacillus* sp. and *Pantoea* sp.). After the foliar application of the YE1, 15 species were found, seven species were

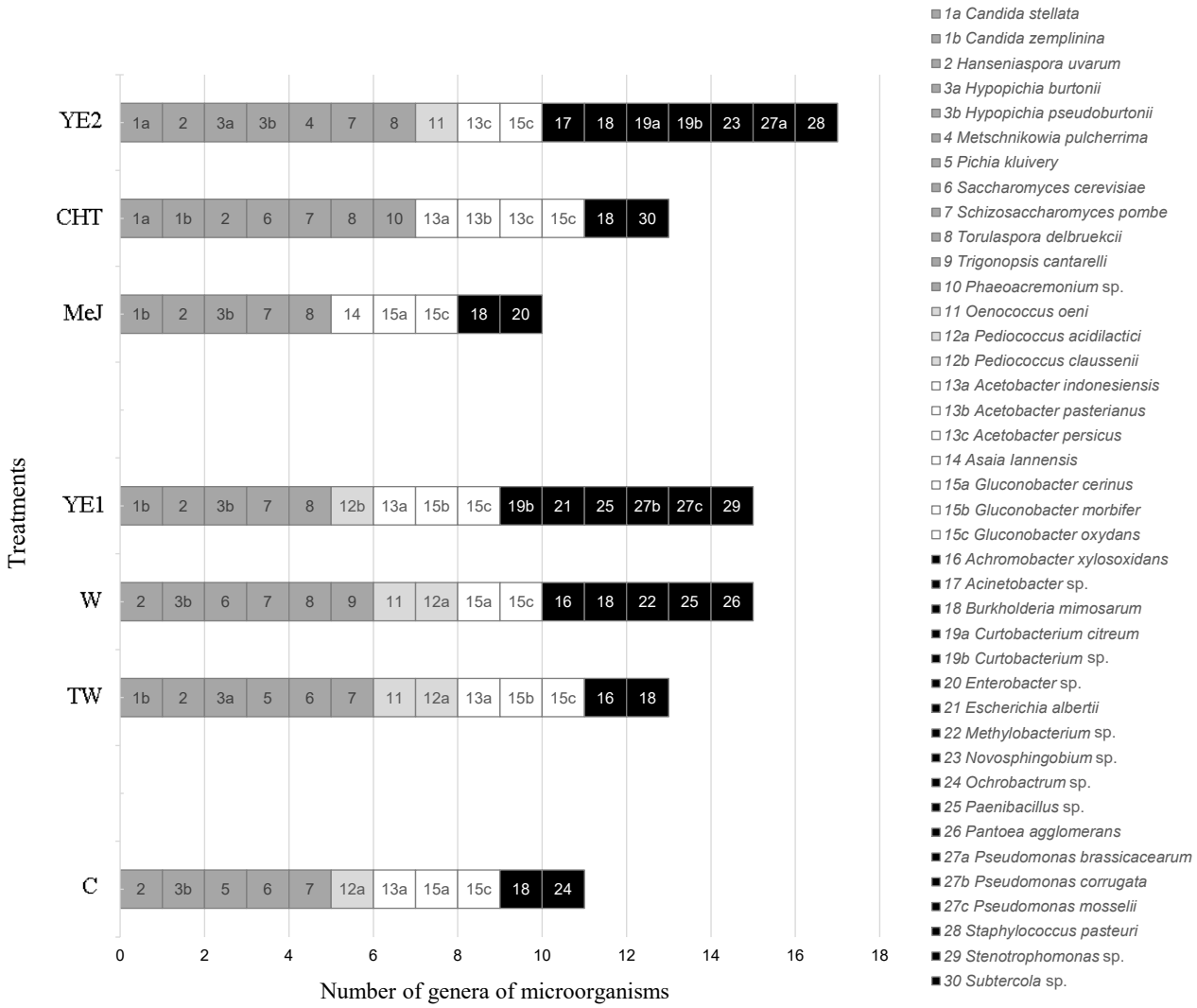

Figure 3. Species detected in the samples that were the control (C), the nitrogenous compounds; treated wastewater (TW), wastewater (W), LalVigne® Aroma (YE1) and the elicitors; methyl jasmonate (MeJ), chitosan (CHT) and LalVigne® Mature (YE2). Dark grey slices were yeasts/molds, light grey slices were LAB, empty slices were AAB genera and black slices were EB.

shared with the control sample and eight were different (*C. zemplinina, T. delbrueckii, Pd. claussenii, G. morbifer, Cur. citreum, Es. albertii, Paenibacillus* sp., *Ps. corrugata, Ps. mosselii* and *Stenotrophomonas* sp.).

Referent to the elicitation, 10 species were found after spraying methyl jasmonate, six species were common to the control sample and four distinct (*C. zemplinina, T. delbrueckii, As. lannensis* and *Enterobacter* sp.). After the elicitation with chitosan, 13 species were identified. In this case, the species common to the control sample were six whereas seven were different (*C. stellata, C. zemplinina, T. delbrueckii, Phaeocremonium* sp., *A. pasteurianus, A. persicus* and *Subtercola* sp.). Finally, the elicitation with the YE2 allowed the identification of 17 species, among them five were also found in the control sample and twelve were different (*C. stellata, Hy. burtonii, M. pulcherrima, T. delbrueckii, O. oeni, A. persicus, Acinetobacter* sp., *Curtobacterium* sp., *Cur. citreum, Novosphigobium* sp., *Ps. brassicacearum*, and *St. pasteuri*).

Microbial alpha diversity of the samples

Data about microbial alpha-diversity are shown in Table 1. The indexes were assessed with the data of the species. The average number of species (S) was significantly lower in control sample and methyl jasmonate samples than the number for the YE2. The Menhinick index control samples was significantly lower than the one assessed with YE1.

The hierarchical cluster built with the diversity indices of the samples generated a dendrogram with two branches (Fig. 4). Cluster A was composed by the control

Table 1. Microbial alpha biodiversity of the samples control (C), the nitrogenous compounds [treated wastewater (TW), wastewater (W), LalVigne® Aroma (YE1)] and the elicitors [methyl jasmonate (MeJ), chitosan (CHT) and LalVigne® Mature (YE2)]

Samples	Richness of species			Structure			
	S	Margalef index	Menhinick index	Chao 1	Simpson index	Berger -Parker	H
C	10.3 a	2.65	1.77 a	14.8	0.85	0.25	2.10
TW	12.0 ab	3.46	2.45 ab	21.7	0.87	0.22	2.27
W	13.3 ab	3.93	2.78 ab	34.0	0.88	0.22	2.39
YE1	13.3 ab	3.99	2.84 b	23.0	0.90	0.18	2.45
MeJ	9.67 a	2.80	2.06 ab	14.1	0.86	0.23	2.10
CHT	11.7 ab	3.56	2.61 ab	26.3	0.88	0.22	2.29
YE2	16.0 b	4.22	2.70 ab	21.5	0.91	0.16	2.62

In each column, different letters mean significant differences between samples (p<0.01). No letters means no significant effect. S= number of species. H= Shannon-Wiener index.

sample and the sample of the methyl jasmonate elicitation, whereas cluster B was composed by the remaining samples, being the samples of the Lallemand products clustered together and the samples from the mushroom industries and chitosan clustered also together.

Discussion

The microbial diversity of must samples of Tempranillo grapes was analyzed from different approaches to determine the possible effects of pre-harvest applications of three nitrogenous compounds and three elicitors to grapevine leaves. The study was based on culture-dependent methods that allow the detection of viable, cultivable and metabolically active microorganisms.

The application of these different substances was focused on leaves, although these products have probably reach the grape surface by dripping. In general, biofilms in their mature stage might be disrupted by external or internal factors and microbial cells can then reach

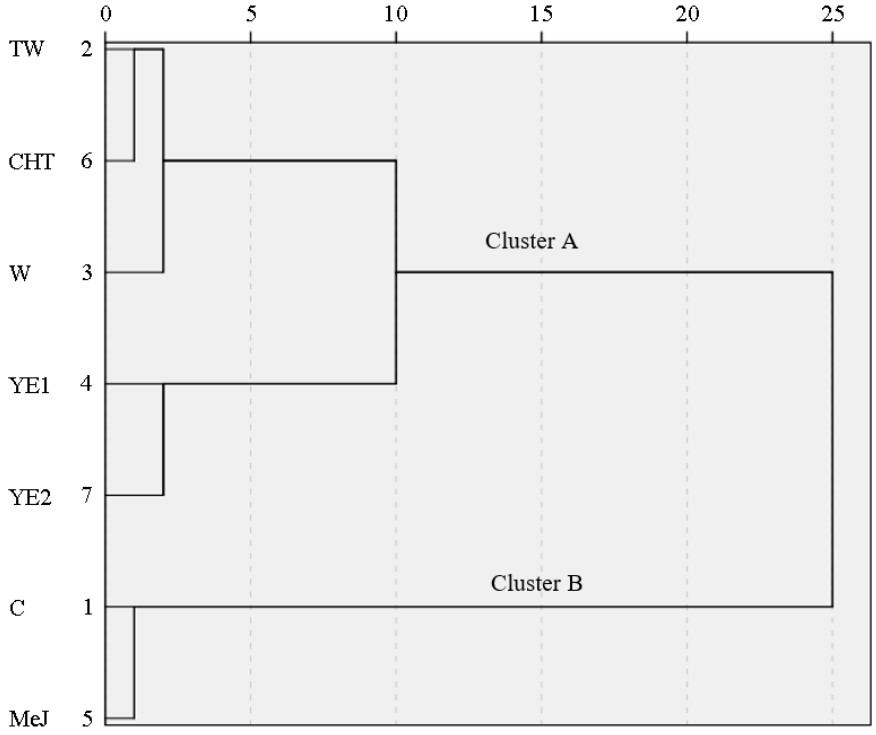

Figure 4. Hierarchical cluster of samples that were the control (C), the nitrogenous compounds; treated wastewater (TW), wastewater (W), LalVigne® Aroma (YE1) and the elicitors; methyl Jasmonate (MeJ), chitosan (CHT) and LalVigne® Mature (YE2).

other locations or surfaces (Taraszkiewicz *et al.*, 2013). Indeed, foliar application could indirectly cause changes in the grape microbial biofilm integrity. Furthermore, these substances are thought to cause variations in the amino acidic and polyphenolic composition of must, therefore both aspects could have influenced the size, diversity and structure of the microbial communities of must samples. These changes could have an impact on the development and completion of AF and malolactic fermentation (MLF).

In the must samples, the microbial groups traditionally described are yeasts, AAB and LAB (Ribéreau-Gayon *et al.*, 2007), but ubiquitous EB were also identified in must samples of the current study. Therefore, the inherent microbial diversity of samples was considerable. The counts of GYP plates were in concordance with the described by other authors (Santamaría *et al.*, 2005; Garijo *et al.*, 2011) and, apparently, the foliar applications did not cause significant modification of the size of the yeast population and of the genera detected on GYP plates. A slight difference was detected with the DBDM culture media, which consisted of significantly highest population after the foliar application of the two types of wastewater from the mushroom industry in comparison to the population after the YE2 application. Despite DBDM was aimed to detect *Brettanomyces/ Dekkera* genus (Rodrigues *et al.*, 2001), this species was not found probably because it was not present in the must samples. In contrast, the DBDM allowed the identification of spoilage yeast genera such as *Candida, Schizosaccharomyces* and *Trigonopsis*. The high population of microorganisms isolated in this DBDM medium after the foliar application of wastewater could be detrimental for must quality. A case in point was the presence of *Schizosaccharomyces* on DBDM plates. This species produce negative metabolites for wine quality and also degrades malic acid (Mylona *et al.*, 2016), consequently its occurrence may not be desirable.

Regarding bacterial community size, the LAB detected during this study in every sample with both culture media (MRS and M30) was quite small and significant differences did not exist between treatments. Small LAB populations are quite common from grapes to the completion of AF (Bae *et al.*, 2006). None of the employed culture media was specific, so that other bacteria genera, different to LAB, were encountered. Therefore, in the MRS medium, besides *Pediococcus, Staphylococcus*, which is considered a novel histamine producer (Benavent-Gil *et al.*, 2016), was detected. On M30, employed for enhancing *Oenococcus* genus (González-Arenzana *et al.*, 2012), this genus, *Pediococcus* and the genera of AAB *Gluconobacter* were also found.

The size of the population found on Mann and ZPP culture media were between yeasts and LAB. On Mann plates, the wastewater foliar application provided higher bacteria population in comparison to the foliar elicitation of YE2. Nevertheless, on ZPP plates – culture media used for promoting EB – differences in EB sizes were not registered. Some of the genera detected on Mann plates were AAB, but other bacteria such as the LAB *Pediococcus* or several EB were also found. Certainly, ZPP provided similar information about the genera than Mann. The majority of the EB genera detected in both media (Mann and ZPP) has been sometimes described as opportunistic pathogen (Osdaghi *et al.*, 2015; Cao *et al.*, 2017) but their role in oenological industries is not well-known although, for instance, some interesting results have been reported about *Enterococcus* from wines by Pérez-Martín *et al.* (2014). The higher population of the microorganisms isolated in Mann media after the foliar application of wastewater might not be beneficial for must quality. Considering the raw wastewater from mushroom industry an important source of EB, the detection of those genera in the must should not be surprising if a probable transference of those microorganisms from leaves to grapes in the vineyard were supposed to occur. Nevertheless, detecting such an enormous diversity of viable EB in must samples is a result that should be taken into consideration for future research.

Analyzing the species detected in control samples regarding the species found after the foliar application of nitrogenous compounds and the elicitation provided very interesting results. An obvious example was that the control sample and the methyl jasmonate elicitation samples had the lowest number of yeast species while the foliar elicitation of YE2 and chitosan were featured by the presence of a higher number of yeasts species. Among yeasts, the genera *Candida* and *Hypopichia* were the only two represented by two species. Most of the species detected matched with non-*Saccharomyces* species that are precisely more frequently found in the early stages of the vinification. Some of those yeasts species have been traditionally thought to be negative for wine quality (Hutzler *et al.*, 2012); in contrast, they are being currently studied owing to their ability of enhancing some color and aromatic characteristics of wines (González-Arenzana *et al.*, 2016). Curiously the mold *Phaeocremonium* sp. that is considered a pathogen for some plants (Gramaje *et al.*, 2015), only was found after the elicitation of chitosan in spite of being used in food and beverages industries as a fungicide (Rinaudo, 2006). Furthermore, the foliar application of chitosan to Tempranillo grapevines did not cause a significant improvement on the grape and wine phenolic compounds (Portu *et al.*, 2016) and its

application did not seem positive due to the yeast species composition of the sample.

Considering the LAB species, in general, they were underrepresented and, in some samples, such as elicitation of chitosan and methyl jasmonate, they were not found. Regarding the AAB species observed, they were in many cases quite similar among samples and three representative species of the genera *Gluconobacter* and *Acetobacter* were noticed. Apparently, the LAB and AAB present in samples were not really influenced by the foliar applications. In relation with the EB species detected, it was found that only two species were noticed in the control sample and just after the foliar application of the treated wastewater from the mushroom industry and the methyl jasmonate and chitosan elicitations. In contrast, the foliar application of wastewater and the elicitation of the two LalVigne products were characterized by holding a quite high number of EB species. The genera *Pseudomonas* and *Curtobacterium* were represented by at least two species, while the other genera were represented by no more than one species what could indicate a very diverse group of bacteria. Curiously, after the foliar application of raw mushroom wastewater two species of EB, *Methylobacterium* sp. and *Pantoea agglomerans*, were found; these species were not present in other samples, what could be related to the nature of this mushroom wastewater that could contain higher diversity of EB than the treated version of the wastewater.

Although the species information is important, it is only a qualitative information useful for making an exhaustive description of the species richness of each sample. Precisely with the aim of obtaining some interesting measurable information, a bioinformatic software for biodiversity quantification was used. The software takes into account the number of species and the representative colonies of each species in the different replicates from a sample to asses diversity indices. These indices meant a quantitative description of the alpha diversity of samples. According to this fact, significant differences observed between samples were scarce; in effect, only indices regarding the richness of species were different after some treatments while indices linked to structure of community were not described. The ANOVA statistical analysis showed that control samples had similar richness of species (S and Menhinick index) to the methyl jasmonate elicitation. The richness of species of YE1 was significantly higher than the described for the other samples based on the number of species. In the same way, the richness of species after the elicitation of the YE2 was significantly higher than the described for the other samples, in this case according to the Menhinick index.

Aimed to obtain a more general approach of results, a hierarchical cluster was constructed with average and standardized diversity indices. Similar to the ANOVA results, the control sample and the methyl jasmonate elicitation were clustered together, what means that both treatments were quite similar having a minimal impact on microbial community. Albeit, Portu *et al.* (2015c) described a positive effect of the methyl jasmonate foliar application to grapevines during two different vintages, increasing the grape and wine phenolic compounds; and Garde-Cerdán *et al.* (2016) observed an increase of some amino acids content in musts after the foliar application of methyl jasmonate. The methyl jasmonate elicitation might improve some oenological characteristic (Portu *et al.*, 2015c) and its application would not greatly modify the microbial community. Curiously, the YE1 and YE2 were clustered together, so that both could have a similar effect on the microbial population being applied for reaching different aims. Overall, considering the diversity alpha after application of LalVigne products did not cause any important impact on the structure of microbial community. In contrast, they were responsible for a significant increase of the richness of species what could be linked to its nutritive composition of fractions of the inactive and dry yeast *S. cerevisiae*. Generally, threaten environments lack diversity of species (Flores-Rentería *et al.*, 2016) so that observing the opposite effect might indicate that the application of both products might have influenced positively the microbial community of musts. The remaining samples stayed together in the hierarchical dendrogram what means that had not an evident impact on microbial alpha diversity of musts.

Generally, the treatments did not cause significant changes nor in the population size of yeasts and bacteria neither in the observed genera and species. Furthermore, dramatic or negative changes were not noticed in any case regarding to the diversity alpha of the microbial communities of the samples. The methyl jasmonate elicitation had similar values of microbial diversity than the control sample what means that its application could be advisable aimed to improve wine organoleptic characteristics. The LalVigne® Aroma and Mature products caused an increase of the richness of species what might be positive for microbial community depending on the species springing up after treatments. Assuming that only one vintage was tested, this study showed interesting indicative results for future research aimed at establishing some possible patterns of microbial community response to foliar treatments.

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