



BOOK OF ABSTRACTS

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Weine. Diese Vielfalt der Datenquellen erhöhte die Robustheit und Zuverlässigkeit der Studie erheblich. Der Referenzwert für präzipitierbare Tannine wurde mittels der MCP-Methode bestimmt.

'Machine Learning' wie multiple lineare Regression mit Regularisierungsmethoden (z. B. ELASTIC NET, RIDGE) wurden angewendet, um die Beziehung zwischen den Variablen und der MCP-Methode zu identifizieren und zu beschreiben. Das endgültige Vorhersagemodell erreichte eine R^2 von 0,72 im Testdatensatz und zeigte eine starke Übereinstimmung mit der Referenzmethode, trotz der inhärenten Variabilität zwischen Laboren.

Das vorgeschlagene statistische Modell erweist sich als zuverlässige, schnelle, automatisierte, zugängliche und kosteneffiziente Lösung zur Tanninquantifizierung. Seine Robustheit unter realen Analysebedingungen gewährleistet eine breite Anwendbarkeit in verschiedenen Laborumgebungen, Weintypen und Vinifikationsprozessen und macht es zu einem wertvollen Instrument zur Optimierung der Weinqualität.

2025-753: LARGE-SCALE APPLICATION OF LACTIPLANTIBACILLUS PLANTARUM IN THE WINEMAKING PROCESS OF NERO D'AVOLA AND PETIT VERDOT GRAPES IN SICILY

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Malolactic fermentation represents a distinctive phase in the vinification process. Indeed, this process contributes substantially to enhancing microbial stability and improving certain sensory attributes of the final wines. The use of non-Oenococcus Lactic Acid Bacteria (LAB) strains presents novel and auspicious prospects for innovation in winemaking. After the completion of Alcoholic Fermentation (AF), yeast activity decreases, and only LAB that are resistant to the stressful conditions of grape must, are able to survive. These LAB are crucial in winemaking as they drive malolactic fermentation (MLF), a secondary biological process that converts L-malic acid into L-lactic acid and CO₂. Within this experimentation, MLF was conducted with the *Lactiplantibacillus plantarum* MLPK45HTM strain, inoculated into grape musts before AF took place. Moreover, *Saccharomyces cerevisiae* yeast NF213, belonging to the oenological yeasts collection of the Department of Agricultural, Food and Forest Sciences (SAAF) (University of Palermo, Italy), was added to conduct AF. For each variety a trial without MLF starter addition was also included in the experimentation and considered as control (spontaneously fermented) trial. *L. plantarum* MLPK45H™ strain inoculation occurred 24 hours after the addition of the starter yeast culture in order to promote MLF. The fermentations took place at 20 °C. Each trial was conducted in triplicate. The following analyses were performed on the collected samples: microbiological analysis, dominance of yeasts and bacteria strains, physicochemical analysis (glucose, fructose, ethanol, glycerol, acetic, tartaric, malic, lactic acids, pH, acidity, ethanol, free and total sulphur dioxide), volatile organic composition (VOC) and sensory evaluation. After inoculation, *Saccharomyces* spp. populations increased significantly, reaching 7.2 - 7.8 Log CFU/mL, whereas non-*Saccharomyces* spp. (NS) showed lower cell densities. LAB populations, particularly *L. plantarum* MLPK45H™, reached high levels post-inoculation (about 8 Log CFU/mL), demonstrating robust growth and stability throughout the fermentation process. At the end of AF, *Saccharomyces* levels decreased in all trials, while LAB counts remained 7-8 log cycles up to day 8. In all controls, LAB counts were consistently lower than in trials inoculated with *L. plantarum* MLPK45H™.

Chemical analysis indicated that glucose and fructose levels decreased steadily during AF, with all trials completing AF in less than 12 days. In the co-inoculated trials, MLF trend was different. In Petit Verdot, the complete degradation of malic acid occurred after 7 d of MLF, reaching lactic acid values of 1.38 g/L, while in Nero d'Avola MLF occurred after 10 d. Indeed, the highest values of lactic acid (1.78 g/L) were recorded at the end of AF. In the control trials, spontaneous MLF was carried out after the end of AF. Co-inoculation with MLPK45H™ resulted in wines with more pronounced floral attributes compared to those undergoing spontaneous fermentation. This effect was driven by the production of VOCs linked to floral sensory characteristics. Sensory analysis revealed that wines produced with MLPK45H™ received higher scores for overall acceptability and different sensory attributes. In addition, interesting differences were found between cultivars. In conclusion, our study underscores the potential of MLPK45H™ to optimize malolactic fermentation and enhance the sensory characteristics of wines. The investigation also clarified the existence of relationship between cultivar and LAB in the

expression of wine aromas. Further investigation through characterisation of the exometabolome will be necessary to clarify the relationships between cultivar and LAB.

APPLICATION A GRANDE ECHELLE DE LACTIPLANTIBACILLUS PLANTARUM DANS LE PROCESSUS DE VINIFICATION DES RAISINS NERO D'AVOLA ET PETIT VERDOT EN SICILE

La fermentation malolactique représente une phase distinctive du processus de vinification. En effet, ce processus contribue considérablement à renforcer la stabilité microbienne et à améliorer certains attributs sensoriels des vins finaux. L'utilisation de souches de Bactéries Lactiques (BL) non-Oenococcus présente des perspectives nouvelles et prometteuses pour l'innovation dans la vinification. Après l'achèvement de la Fermentation Alcoolique (FA), l'activité de la levure diminue et seules les bactéries lactiques résistantes aux conditions stressantes du moût de raisin sont capables de survivre. Ces BL sont cruciales pour la vinification car elles conduisent la fermentation malolactique (FML), un processus biologique secondaire qui convertit l'acide L-malique en acide L-lactique et en CO₂. Dans le cadre de cette expérimentation, la FML a été réalisée avec la souche *Lactiplantibacillus plantarum* MLPK45H™, inoculée dans les moûts de raisin avant que la FA n'ait lieu. En outre, la levure *Saccharomyces cerevisiae* NF213, appartenant à la collection de levures œnologiques du département des Sciences Agricoles, Alimentaires et Forestières (SAAF) (Université de Palerme, Italie), a été ajoutée pour réaliser la FA. Pour chaque variété, un essai sans ajout de starter de FML a également été inclus dans l'expérimentation et considéré comme un essai de contrôle (fermentation spontanée). L'inoculation de la souche *L. plantarum* MLPK45H™ a eu lieu 24 heures après l'ajout de la culture de levure de départ afin de promouvoir la FML. Les fermentations ont eu lieu à 20 °C. Chaque essai a été réalisé en trois exemplaires. Les analyses suivantes ont été effectuées sur les échantillons collectés : analyse microbiologique, dominance des souches de levures et de bactéries, analyse physicochimique, composition organique volatile (COV) et évaluation sensorielle. Après inoculation, les populations de *Saccharomyces* spp. ont augmenté de manière significative, atteignant 7,2 - 7,8 Log CFU/mL, alors que les spp. non-*Saccharomyces* (NS) ont montré des densités cellulaires plus faibles. Les populations de BL, en particulier *L. plantarum* MLPK45H™, ont atteint des niveaux élevés après l'inoculation, démontrant une croissance robuste et une stabilité tout au long du processus de fermentation. À la fin de la FA, les niveaux de *Saccharomyces* ont diminué dans tous les essais, tandis que les numérations de BL sont restées à des cycles de 7-8 logs jusqu'au jour 8. Dans tous les contrôles, les numérations de BL étaient systématiquement inférieures à celles des essais inoculés avec *L. plantarum* MLPK45H™. L'analyse chimique a indiqué que les niveaux de glucose et de fructose diminuaient régulièrement pendant la FA, tous les essais ayant terminé la FA en moins de 12 jours. Dans les essais co-inoculés, la tendance de la FML était différente. Dans le Petit Verdot, la dégradation complète de l'acide malique s'est produite après 7 jours de FML, atteignant des valeurs d'acide lactique de 1,38 g/L, tandis que dans le Nero d'Avola, la FML s'est produite après 10 jours. Dans les essais de contrôle, la FML spontanée a eu lieu après la fin de la FA. La co-inoculation avec MLPK45H™ a permis d'obtenir des vins aux attributs floraux plus prononcés que ceux soumis à la fermentation spontanée. L'analyse sensorielle a révélé que les vins produits avec MLPK45H™ ont reçu des notes plus élevées pour l'acceptabilité globale et les différents attributs sensoriels. En outre, des différences intéressantes ont été constatées entre les cultivars. En conclusion, notre étude souligne le potentiel de MLPK45H™ pour optimiser la fermentation malolactique et améliorer les caractéristiques sensorielles des vins. L'enquête a également clarifié l'existence d'une relation entre le cultivar et la BL dans l'expression des arômes du vin. Des recherches plus approfondies par la caractérisation de l'exométabolome seront nécessaires pour clarifier les relations entre le cultivar et les BL.

APPLICAZIONE SU LARGA SCALA DI LACTIPLANTIBACILLUS PLANTARUM NEL PROCESSO DI VINIFICAZIONE DI UVE NERO D'AVOLA E PETIT VERDOT IN SICILIA

La fermentazione malolattica rappresenta una fase peculiare del processo di vinificazione. Infatti, questo processo contribuisce in modo sostanziale ad aumentare la stabilità microbica e a migliorare alcuni attributi sensoriali dei vini finali. L'uso di ceppi di Batteri Lattici (BL) non-Oenococcus presenta nuove e promettenti prospettive di innovazione nella vinificazione. Dopo il completamento della fermentazione alcolica (FA), l'attività dei lieviti diminuisce e solo i BL resistenti alle condizioni di stress del mosto d'uva sono in grado di sopravvivere. Questi BL sono fondamentali nella vinificazione in quanto guidano la fermentazione malolattica (FML), un processo biologico secondario che converte l'acido L-malico in acido L-lattico e CO₂. Nell'ambito di questa sperimentazione, la FML è stata condotta con il ceppo *Lactiplantibacillus plantarum* MLPK45HTM, inoculato nei mosti d'uva prima che avvenisse la FA. Inoltre, per condurre la FA è stato aggiunto il lievito *Saccharomyces cerevisiae* NF213, appartenente alla collezione di lieviti enologici del Dipartimento di Scienze Agrarie, Alimentari e Forestali (SAAF) (Università di Palermo, Italia). Per ogni varietà è stata inclusa nella sperimentazione anche una prova senza aggiunta di FML starter, considerata come prova di controllo (fermentazione spontanea). L'inoculo del ceppo L.

plantarum MLPK45H™ è avvenuto 24 ore dopo l'aggiunta della coltura di lievito starter per promuovere la FML. Le fermentazioni sono avvenute a 20 °C. Ogni prova è stata condotta in triplo. Sui campioni raccolti sono state eseguite le seguenti analisi: analisi microbiologiche, dominanza di ceppi di lieviti e batteri, analisi fisico-chimiche, analisi dei composti organici volatili (VOC) e valutazione sensoriale. Dopo l'inoculo, le popolazioni di *Saccharomyces* spp. sono aumentate in modo significativo, raggiungendo 7,2-7,8 Log CFU/mL, mentre i non-*Saccharomyces* spp. (NS) hanno mostrato densità cellulari inferiori. Le popolazioni di BL, in particolare *L. plantarum* MLPK45H™, hanno raggiunto livelli elevati dopo l'inoculo (circa 8 Log CFU/mL), dimostrando una crescita robusta e una stabilità durante tutto il processo di fermentazione. Alla fine della FA, i livelli di *Saccharomyces* sono diminuiti in tutte le prove, mentre la conta dei BL è rimasta di 7-8 cicli log fino all'ottavo giorno. In tutti i controlli, la conta dei BL era costantemente più bassa rispetto alle prove inoculate con *L. plantarum* MLPK45H™. Le analisi chimiche hanno indicato che i livelli di glucosio e fruttosio sono diminuiti costantemente durante la FA, con tutte le prove che hanno completato la FA in meno di 12 giorni. Nelle prove con coinoculo, l'andamento della FML è stato diverso. Nel Petit Verdot, la completa degradazione dell'acido malico si è verificata dopo 7 d di FML, raggiungendo valori di acido lattico di 1,38 g/L, mentre nel Nero d'Avola la FML si è verificata dopo 10 d. Infatti, i valori più alti di acido lattico (1,78 g/L) sono stati registrati alla fine della FA. Nelle prove di controllo, la FML spontanea è avvenuta dopo la fine della FA. Il coinoculo con MLPK45H™ ha prodotto vini con attributi floreali più pronunciati rispetto a quelli sottoposti a fermentazione spontanea. Questo effetto è stato determinato dalla produzione di COV legati alle caratteristiche sensoriali floreali. L'analisi sensoriale ha rivelato che i vini prodotti con MLPK45H™ hanno ricevuto punteggi più alti per l'accettabilità complessiva e per i diversi attributi sensoriali. Inoltre, sono state riscontrate interessanti differenze tra le varie cultivar. In conclusione, il nostro studio sottolinea il potenziale di MLPK45H™ per ottimizzare la MLF e migliorare le caratteristiche sensoriali dei vini. L'indagine ha inoltre chiarito l'esistenza di una relazione tra cultivar e LB nell'espressione degli aromi del vino. Ulteriori indagini attraverso la caratterizzazione dell'esometaboloma saranno necessarie per chiarire le relazioni tra cultivar e BL.

2025-759: MONITORING AND IDENTIFICATION OF BRETTANOMYCES BRUXELLENSIS IN YOUNG PORTUGUESE RED WINES

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This study focused on a key topic in the wine industry: the impact of alternative yeasts on the organoleptic quality and microbiological stability of wine. A comprehensive assessment of the presence, growth and phenolic production of *Brettanomyces bruxellensis* in wine, allows a deeper knowledge on wine quality and microbiological stability. The aim of the present study was to identify novel strategies to limit *B. bruxellensis* proliferation and mitigate the formation of volatile compounds that negatively affect wine aroma and flavour.

The study objectives were pursued through the following steps: (i), collection of samples from wineries affiliated to the University of Lisbon (School of Agriculture University of Lisbon, ISA); (ii), isolation and identification of volatile phenol-producing *B. bruxellensis* both in young wines and in matrices that reproduce wine characteristics, such as synthetic wine medium (SWM) and sterilised young red wines; (iii), analysis of volatile organic compounds (VOCs) by gas chromatography; (iv), identification and quantification of yeasts by RT-PCR.

During alcoholic fermentation, the kinetics of the yeast population were monitored. The production of 4-ethylphenol (4-EP) was frequently monitored throughout the experiment, as this compound is an index of *Brettanomyces* spp. contamination (Malfeito Ferreira, 2011).

Among commercial red wines, three samples showed *B. bruxellensis* concentration at significant values that was detected only on DBDM (Dekkera/*Brettanomyces* Differential Medium), showing 4-EP levels above the perception threshold (481 ppb). After seven days, the viability of the yeast decreased, indicating a transition to the VBNC (vital but not cultivable) state. This transition to the VBNC state may have been an adaptive response to stressful environmental conditions, such as limited oxygen or nutrient availability. The detection of cells in the VBNC state is crucial to understand the growth kinetics of *B. bruxellensis* surviving cells in wines, since the presence of these cells is not detectable by traditional culture methods, cells continue to proliferate under favourable conditions and influence the organoleptic characteristics of the wine.

In the SWM growth medium, three strains showed significant growth ($OD_{640\text{ nm}} \geq 0.5$), with an initial lag phase followed by population expansion. After 28 days, a decline phase was observed, characterised by surface film formation and increased production of acetic acid. Competition between *B. bruxellensis* and acetic acid bacteria, suggested by the decrease in CFU/mL.



CERTIFICATE OF SCIENTIFIC PRESENTATION

On behalf of the Scientific Committee

I hereby certify that

Antonino Pirrone, Vincenzo Naselli, Valentina Craparo, Enrico Viola, Filippo Amato, Azzurra Vella, Irene Dolce, Davide Alongi, Giulio Perricone, Venera Seminerio, Micaela Carusi, Raimondo Gaglio, Daniele Oliva, Luca Settanni, Giancarlo Moschetti, Nicola Francesca, Antonio Alfonzo

has/have presented the paper in the form of

Poster

entitled

Large-scale application of Lactiplantibacillus plantarum in the winemaking process of Nero d'Avola and Petit Verdot grapes in Sicily

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Gheorghe Arpentin

President of The Scientific Committee



**International Organisation
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