Is WL (Wallerstein Laboratory) Nutrient Agar of any use in yeast colony differentiation from table olives?

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Background

Yeasts are important in table olives quality and spoilage, and their isolation and characterization are of interest for both academia and industries. Media used for yeast enumeration are not typically differential and colony morphologies are quite similar. On the other hand, WL (Wallerstein Laboratory) nutrient agar (WL), has been specifically developed for the differentiation of yeasts in winemaking and brewing (doi.org/10.1002/j.2050-0416.1971.tb03413.x) but, to our knowledge, its value in differentiating colony morphologies of yeasts typically isolated from olives and olive environments has never been tested systematically.

Methods

261 isolates from olives, olive mill wastewater, olive oil and wine or must were used. For 199 of these, identification by sequencing of the ITS1-5.8S-ITS2 region and by MALDI-ToF spectrometry assigned them to 17 species mostly belonging the genera *Pichia, Candida, Lachancea, Nakazawaea, Saccharomyces, Rhodotorula*, and *Wickerhamomyces.* After streaking on WL, colony and cell morphology was evaluated after 5 days of incubation at 25°C. A set of binary characters for colony shape, elevation, surface, and colour and cell morphology was used for unsupervised and supervised classification.

Results

Exploratory data analysis (cluster analysis and MDS of the Jaccard distance matrix, Kohonen Self Organizing Maps) were used to obtain a preliminary grouping. For some species (*S. cerevisiae, N. molendiolei, L. fermentati*) relatively homogeneous clusters were found with all methods. Random forest classifiers resulted in excellent results for the same species, but relatively poor with other.

Conclusions

Colony morphology on WL is only partially useful for the classification of yeasts from table olives. This is not surprising since this medium was developed for other ecosystems. However, optimization of the descriptors may improve the results.

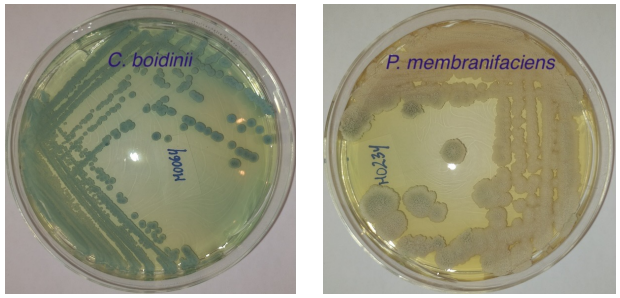

Fig 1. Colony morphology of two typical species

Effectiveness of colony morphology in identification, training set, model 3 (simple colors + cell morphology)

class	accuracy	kappa	sensitivity	specificity	pos_pred_value	neg_pred_value
NA	0.6666667	0.6011213	NA	NA	NA	NA
Ca_bo	NA	NA	0.2500000	0.9937107	0.7500000	0.9461078
Kl_la	NA	NA	0.0000000	1.0000000	NaN	0.9649123
La_fe	NA	NA	1.0000000	0.9696970	0.5454545	1.0000000
Me_pu	NA	NA	0.2000000	1.0000000	1.0000000	0.9764706
Na_mo	NA	NA	1.0000000	1.0000000	1.0000000	1.0000000
Pi_ku	NA	NA	0.0000000	1.0000000	NaN	0.9532164
Pi_ma	NA	NA	0.7647059	0.8613139	0.5777778	0.9365079
Pi_me	NA	NA	0.5405405	0.8358209	0.4761905	0.8682171
Sa_ce	NA	NA	0.9642857	0.9510490	0.7941176	0.9927007
Wi_an	NA	NA	0.8260870	0.9797297	0.8636364	0.9731544

Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani

Advances in the microbiology of table olives – Matera 1-2/9/2025

Acknowledgements

This work was carried out within the PRIN 2022 Project METAOlive 2022NN28ZZ and received funding from Ministero dell'Università e della Ricerca (Rome) and the European Union Next-GenerationEU, CUP C53D23005460006 (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1048 14/07/2023).