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NUMERICAL TAXONOMIC STUDY OF *OENOCOCCUS OENI* ISOLATES ON THE BASIS OF SUGAR METABOLISM*

ESTUDO POR TAXONOMIA NUMÉRICA DE ALGUNS ISOLAMENTOS DE *OENOCOCCUS OENI* COM BASE NO METABOLISMO DE AÇÚCARES

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SUMMARY

The present work deals with the sugar metabolism profile of 48 isolates of *Oenococcus oeni* (Garvie, 1967) Dicks *et al.*, 1995 obtained from wines of different origins. The degradation of 5 sugars (hexoses and pentoses) usually present in the wine, and the respective acetic acid and ethanol formation, were the characters studied. The overall similarities of these isolates were determined by average taxonomic distance and correlation coefficients, followed by clustering using UPGMA algorithm. The results were expressed as phenogram. Ordination of the same isolates in a space of reduced dimensionality was done by principal component analysis. Based on the phenetic data obtained several isolates could be selected that may be usefull in wine industry for inducing or accelarating malolatic fermentation.

Key words: Numerical taxonomy; ***Oenococcus oeno***; sugar metabolism; screening.

Palavras chave: Taxonomia numérica; ***Oenococcus oeni***; metabolismo de açúcares; selecção.

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INTRODUCTION

The species *Oenococcus oeni* (Garvie, 1967) Dicks *et al.*, 1995 was proposed by Garvie (1967) as *Leuconostoc oenos*, based on the study of 19 isolates obtained from wines of different origins. This species currently includes isolates which cells usually occur in pairs and chains and are Gram positive. Growth is slow having complex growth factors and aminoacids requirements (Garvie, 1986). Recently, Dicks *et al.* (1995) assigned *Leuconostoc oenos* Garvie, 1967 to a new genus as *Oenococcus oeni* (Garvie, 1967) Dicks *et al.*, 1995. Among lactic acid bacteria (LAB) *O. oeni* is the main species present in the wine and the one best adapted to carry out the malolactic fermentation (MLF) at the low pH of wine (Lafon-lafourcade), 1983, and Wibowo *et al.*,1985).

Malolactic fermentation is concomitant with the fermentation of the sugars remaining in the medium after alcoholic fermentation. These sugars provide the required energy for biomass formation with acetic acid accumulation which will affect the wine sensorial properties, with an appreciable loss of quality. Radler (1967) referred to the fact that only 0.1 g/L of carbohydrates are necessary to from enough biomass to degrad 30% of the malic acid content of the medium.

In order to induce or to accelerate MLF wine industry has used several pure cultures of selected acid bacteria in freeze dried, liquid or frozen form (Gallander, 1979, Lafon-Lafourcade *et al.*, 1983, King, 1984). However, despite the progresses obtaind these preparations are not yet fully reliable (Kunkee, 1991).

The main fermentable sugars in wine are glucose and fructose, the former being present at concentrations of 0.2 to 0.8 g/L and the latter in the range of 1.0 to 1.5 g/ L; pentoses (arabinose, xylose and ribose) are also present but at concentrations less than 1.0 g/L (Ribéreau-Gayon *et al.*, 1976 and Curvelo-Garcia, 1988). Although, with the use of well adapted starter cultures, less growth should de required to metabolise malic acid in wine, LAB can always utilise the sugars present with subequent accumulation of acetic acid.

The aim of the present work was to determine, taking

into account their sugar metabolism patterns, the phenetic relationship of 48 isolates. of *O. oeni* from wines of different origins and to select some isolates with relevant characteristics for the wine industry. The degradation of the five sugars (hexoses and pentoses) usually present in wine, as well as the respective acetic acid and ethanol formed by the isolates studied, were the characters used in the present study.

As a successful result of collaboration work, from which this paper is only a small contribution, is now possible to purpose the use of direct inoculation of wine with a new freeze-dried culture of *Oenococcus oeni* (Nielsen *et al.*, 1995, Prahl, *et al.*, 1995).

MATERIAL AND METHODS

Bacteria

The 48 isolates (=OTUs, Operational Taxonomic Units) of *O. oeni* used in our study were obtained from the red wines, collected in different geographic origins, which were undergoing malolatic fermentation.

The 48 isolates used in our study were previously selected from a large collection using their growth potencial, malolactic activity, citric acid and sugar metabolism, according to criteria followed by the members of the EC-ECLAIR AGRE 0012 project. Degradation of five sugars (hexoses and pentoses) and the respective acetic acid and ethanol formation by each isolate were the characteristics used in the present study (Table 1).

The identification of the 48 isolates was made by microscopic observation using Gram staining technic and by DNA/DNA hybridization (Lonvaud-Funel et al., 1989) carried out at Institut d'Oenologie de Bordeaux. The isolates were stored at +4 °C in Dubois medium (Lafon-Lafourcade and Joyeux, 1979), supplemented with 10% (v/v) of sterilized wine.

Inoculum preparation and culture conditions

For the preparation of the inocula the stock cultures were first grown, during two days, at 25 °C, in a modified Carr

TABLE I

List of characters used in the study[1]
Lista dos caracteres usados no estudo[2]

A	degraded arabinose
F	degraded fructose
G	degraded glucose
R	degraded xylose
X	degraded arabinose
aA	acetic acid formed from arabinose
aF	acetic acid formed from fructose
aG	acetic acid formed from glucose
aR	acetic acid formed from ribose
aX	acetic acid formed from xylose
eA	ethanol formed from arabinose
eF	ethanol formed from fructose
eG	ethanol formed from glucose
eR	ethanol formed from ribose
eX	ethanol formed from xylose

[1] Characters A - X, percentage of degraded sugar; aA - aX, percentage of acetic acid produced by unit of consumed sugar; eA - eX, idem for ethanol.

[2] Caracteres A a X, percentagem de açúcar degradado; aA a aX, percentagem de ácido acético produzido por unidade de açúcar degradado; eA a eX percentagem de etanol produzido por unidade de açúcar degradado.

medium containing (in distilled water): yeast extract, 4 g/L; casaminoacids, 5 g/L; KH_2PO_4, 0.6 g/L; KCl, 0.45 g/L; CaC_{12}, 0.13 g/L; MnSO4, 0.13 g/L; DL-malic acid, 5 g/L and tomato juice, 50 mL/L. The pH was adjusted to 3.8; sterilization did not appreciably changed this value. The medium was poured into 150 mL Erlenmeyer flasks containing 100 mL of the medium referred to above. After sterilization for 15 min, at 121 °C, 2.5 g/L of arabinose, fructose, glucose, ribose or xylose were added to the medium from previously sterilized solutions. Sugar concentrations were chosen according to those currently present in wine. The cells were centrifuged at 2000*g* for 10 min, washed and re-suspended in a small volume of culture medium and inoculated at 1 % (v/v) in the same medium. All experiments were carried out at 25 °C, under semi-anaerobic conditions achieved by tightly closing the flasks with

a rubber stopper. As slight differences in the medium and environmental conditions lead to differences in specific metabolisms and, consequently, to different end products (Thomas *et al.* 1979, Fordyce *et al.*1984, Firme *et al.* 1994) all the experiments were carried out under the same media or culture conditions, only the sugars changed according to each specific experiment.

Analytical procedures

After cell growth (followed by the absorbance change at 600 nm) until stationary phase was achieved, the remaining sugars, acetic acid and ethanol produced were determined. Concentration of arabinose, fructose, glucose, ribose and xylose were evaluated by ionic chromatography using a ion exchange column dionex HPIC-AS6 10 μm (P/N 35 391) and a solution of $Ba(OH)_2$ 1.0 mM and acetic acid 0.125 mM as eluent. The detector used was a Dionex amperometric pulse detector. Quantification was achieved by external standard calibration. Acetic acid and ethanol were determined in the culture medium, after growth, by head-space gas chromatography analysis using a flame ionisation detector and a Permabond glass (FFAP-DF 0.25, of 25 m x 0.32 mm i.d.). Quantification was achieved by external standard calibration, initial and final concentration in the medium were always compared.

All the experimentes were repeated at least twice.

Data analysis

Fifteen characters (listed in Table 1) were scored for the 48 isolates or OTUs (=Operational Taxonomic Units). Table 2 lists the OTUs studied and their code number. Characters were standardized so that each would have mean zero and a standard deviation of one.

Correlation and taxonomic distance coefficients were calculated for all pairs of OTUs. Cluster analyses, utilizing the unweighted-pair group method using arithmetic averages (UPGMA), were performed on both correlation and distance matrices, and the results summarized in phenograms. The distortion of each phenogram was computed as a cophenetic correlation coefficient.

The 15 standardized characters by 48 isolates (=OTUSs) original data matrix was also studied by principal component analysis (PCA). This matrix was used for computation of the correlation matrix among the 15 characters and the 48 OTUs were projected onto the planes defined by the 1st and 2nd and 1st and 3rd principal axes. The original characters were also projected onto the same planes (Sneath and Sokal, 1973).

All computations were carried out using NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) package, version 1.7, of computer programs (Rohlf, 1992).

RESULTS AND DISCUSSION

Cluster analysis

To explore the phenetic relationships among OTUs (isolates), in order to detect potential groups or subgroups of isolates, clustering techniques were chosen, using two different resemblance coefficients, namely correlation and average taxonomic distance. The resulting phenograms, using the UPGMA clustering technique, are illustrated in Fig. 1 and 2. The cophenetic correlation between the original resemblance matrices and those implied by the phenogram are r=0.64796 and r=0.73934, respectivelly, showing that there is some distortion.

The phenogram based on correlations among OTUs revealed 7 major groups, named A, B, ..., G (Fig. 1). No clear cut clustering is shown in the phenogram based on distances, with the exception of group F (Fig. 2). This is further confirmed by the results of the principal components analysis (see below). The cophnetic matrix correlation between the matrices of correlation and average taxonomic distance coefficients is r=-0.54798, showing that these two matrices revealed a different structure of the data.

For the further discussion we used the correlation phenogram of OTUs since it gave more defined cluster groups and is more interpretable than the distance phenogram, despite its lower cophenetic correlation coefficient (r=0.64796).

In order to better understand the relationships among OTUs, within and between cluster groups, and to characterize and differentiate the several groups the original data matrix was rearranged

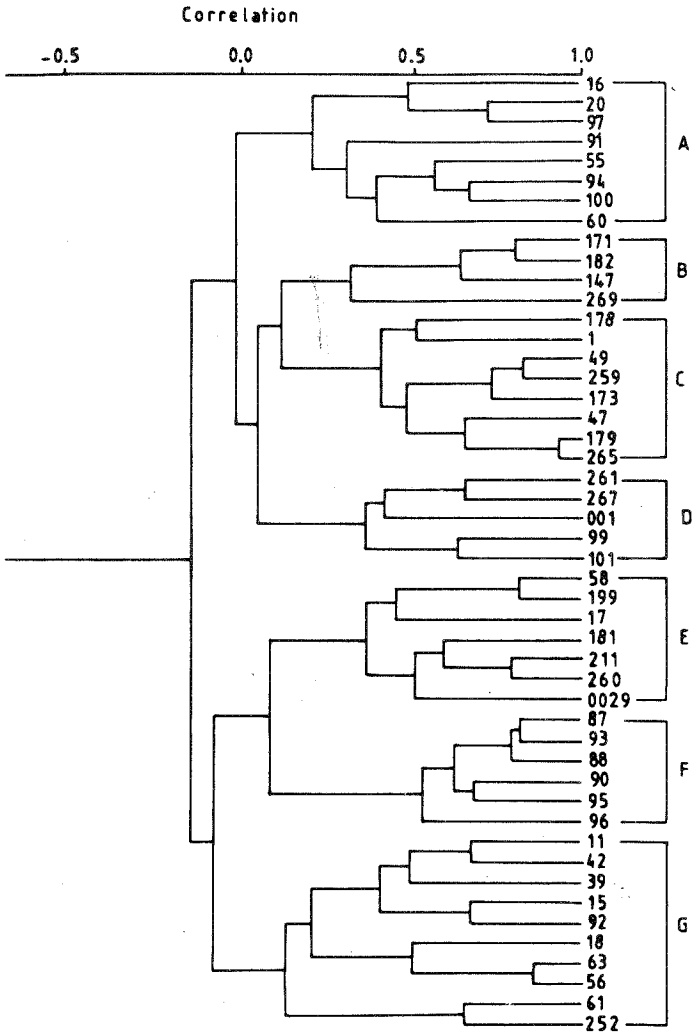

Fig. 1 - Correlation phenogram of the 48 isolates or OTUs based on the UPGMA method (cophenetic correlation r=0.64796).
Fenograma de correlação dos 48 isolamentos ou OTUs baseado no método UPGMA (coeficiente de correlação cofenética r=0,64796).

(Table 2), so that the OTUs were stored in the order shown in the correlation phenogram (Fig. 1). A more precise analysis of the interrelationship characters/isolates will be better done after the discussion of PCA results.

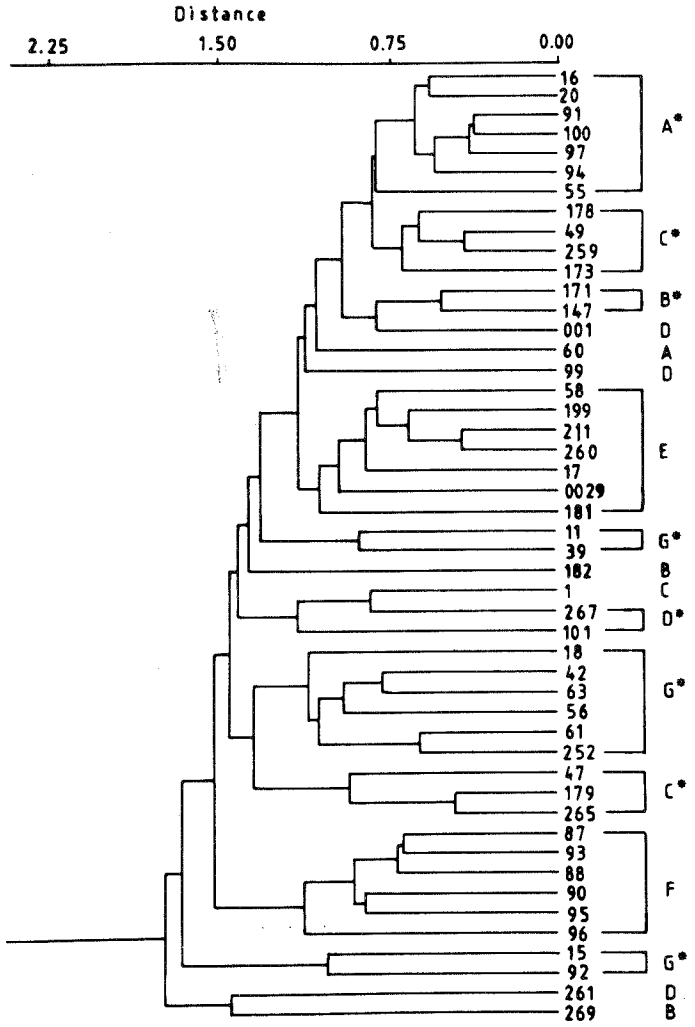

Fig. 2 - Distance phenogram of the 48 isolates or OTUs based on the UPGMA method (cophenetic correlation r=0.73934).
Fenograma de distâncias dos 48 isolamentos ou OTUs baseado no método UPGMA (coeficiente de correlação cofenética r=0,73934).

Cluster-group A contains 8 OTUs [16-60] characterized by low degradation of arabinose and low formation of acetic acid from arabinose and glucose; and high degradation of glucose

TABLE II

Original data matrix rearranged in the order shown in the correlation phenogram

Matriz de dados originais rearranjados pela ordem apresentada no fenograma de correlação

OTU	Character														
	G	F	R	A	X	aG	aF	aR	aA	aX	eG	eF	eR	eA	eX
16	44.0	42.5	6.0	17.3	27.0	6.8	10.0	7.3	8.9	4.4	1.2	0.9	1.4	2.4	5.9
20	35.5	29.5	21.7	8.1	18.0	5.2	12.0	8.1	15.9	5.7	2.1	1.0	1.7	1.7	4.6
97	43.8	43.5	26.5	23.8	14.0	3.9	8.5	5.7	12.6	7.6	5.7	1.7	1.3	2.5	2.8
91	33.1	71.7	35.4	11.5	14.0	5.2	6.0	6.1	10.5	4.3	5.6	0.9	1.4	2.1	1.8
55	55.4	86.0	26.0	14.6	35.7	2.3	6.9	9.1	4.5	3.0	0.6	2.5	1.0	0.9	0.6
94	42.8	26.4	36.9	15.4	22.6	5.7	5.6	9.2	7.1	4.1	0.8	6.3	1.0	0.7	1.0
100	47.0	40.7	44.2	6.2	19.9	4.2	5.4	6.3	10.8	4.8	2.1	2.9	1.3	1.9	2.1
60	46.8	0.0	94.0	26.2	22.6	3.1	0.0	11.3	8.5	3.5	3.8	0.0	4.0	4.1	4.5
171	37.5	9.7	17.7	15.4	2.3	3.7	16.2	16.7	8.5	4.9	2.4	6.3	2.4	2.3	2.0
182	44.9	6.9	28.1	6.2	0.0	2.6	19.1	8.5	4.9	0.0	1.6	14.6	0.5	1.1	0.0
147	31.8	15.3	24.1	10.0	9.5	4.1	10.4	21.1	8.6	3.5	1.7	8.5	2.2	2.5	1.3
269	100.0	17.7	17.4	30.9	0.0	8.4	10.4	19.0	13.0	0.0	21.0	6.5	1.1	2.8	0.0
178	37.6	43.5	49.4	18.5	5.0	2.2	9.5	8.3	4.7	2.9	4.4	3.1	1.4	1.2	9.4
1	43.0	37.8	77.0	4.4	4.9	5.2	8.7	11.3	7.9	27.5	1.9	3.7	1.3	5.3	12.0
49	44.0	41.4	94.0	13.8	20.8	1.8	8.4	7.1	4.7	5.5	1.3	2.7	2.6	1.9	2.2
259	47.9	36.4	75.1	6.9	12.2	3.6	8.0	11.7	6.4	7.7	1.4	3.1	2.4	3.4	4.6
173	38.3	23.6	57.9	0.0	22.6	1.9	10.2	10.9	0.0	4.1	0.2	6.5	0.9	0.0	0.8
47	42.0	41.9	86.0	15.9	19.8	5.5	8.8	9.0	5.4	8.3	7.9	11.6	3.4	8.9	4.8
179	75.0	53.2	95.0	9.1	17.1	4.4	13.2	11.9	8.7	4.9	2.7	24.4	3.2	8.5	14.5
265	62.8	53.2	96.8	2.0	22.3	5.4	11.3	13.4	9.1	4.4	8.9	19.5	3.2	7.0	13.3
261	100.0	100.0	24.0	29.9	0.3	11.0	7.8	26.5	11.7	25.4	19.8	15.2	1.3	3.1	13.8
267	66.9	33.8	44.0	48.4	2.4	6.5	6.6	15.0	7.8	32.6	9.0	6.4	1.1	1.8	4.5
001	19.6	13.1	28.0	49.8	0.8	8.3	9.9	12.3	7.8	15.0	2.0	1.5	1.4	1.8	4.1
99	0.0	100.0	18.7	45.6	5.5	0.0	5.1	15.5	7.0	14.6	0.0	0.8	0.9	1.2	1.9
101	9.8	100.0	100.0	38.2	6.5	12.9	5.5	20.6	9.7	37.9	3.1	2.6	3.8	2.1	4.4
58	42.1	100.0	94.0	99.2	0.0	3.8	4.7	18.5	10.5	0.0	3.4	6.4	3.3	4.9	0.0
199	36.9	46.3	48.2	41.5	0.0	3.7	6.1	13.3	8.4	0.0	2.3	2.8	2.6	5.5	0.0
17	46.8	100.0	13.0	100.0	0.0	3.9	6.8	8.7	13.9	0.0	3.5	5.7	3.1	7.6	0.0
181	22.4	100.0	100.0	23.6	0.0	12.6	4.1	9.4	9.9	0.0	9.7	10.8	7.1	2.5	0.0
211	34.2	100.0	74.4	34.8	0.0	7.0	7.7	10.8	8.7	0.0	6.9	3.6	0.6	2.6	0.0
260	42.9	100.0	35.0	31.1	0.0	7.4	7.5	14.3	10.4	0.0	10.8	3.7	1.3	2.3	0.0
0029	19.9	100.0	58.9	20.3	0.0	8.6	4.1	19.3	18.4	0.0	0.8	0.6	0.4	1.6	0.0
87	22.9	100.0	19.7	100.0	28.2	19.6	10.5	10.2	16.1	7.9	2.6	3.7	0.7	10.4	2.4
93	27.5	100.0	37.4	75.8	36.3	10.4	9.3	10.7	16.0	7.9	0.9	1.3	1.3	10.4	0.7
88	8.1	100.0	70.0	100.0	37.7	14.2	10.7	7.1	16.0	6.4	4.3	5.6	4.6	10.2	6.1
90	12.2	100.0	11.1	75.7	28.5	14.6	7.4	12.3	15.4	6.5	3.2	10.6	8.5	13.8	8.3
95	29.6	100.0	45.0	86.3	28.5	9.5	7.8	7.9	12.8	6.9	2.6	1.1	10.2	10.8	1.5
96	11.4	100.0	51.9	8.6	0.0	14.3	9.7	9.0	15.6	0.0	3.2	1.6	2.2	11.7	0.0
11	31.6	68.9	0.0	100.0	2.1	4.6	9.4	0.0	12.5	7.4	2.2	1.4	0.0	5.6	10.9
42	51.5	60.6	36.8	100.0	7.4	3.9	9.2	9.3	12.2	7.6	7.3	8.8	5.5	7.8	10.0
39	34.3	25.2	0.0	100.0	0.0	5.0	14.9	0.0	13.0	0.0	2.0	1.2	0.0	8.2	0.0
15	81.4	66.7	0.0	100.0	19.2	9.7	13.5	0.0	14.7	7.4	18.9	1.9	0.0	7.0	8.9
92	38.9	100.0	0.0	100.0	28.5	8.6	9.3	0.0	13.1	4.4	25.3	16.5	0.0	8.3	6.1
18	84.1	27.0	8.2	100.0	3.9	4.2	16.8	10.5	11.2	8.4	12.2	4.1	11.5	3.8	11.2
63	68.6	53.2	23.3	71.0	17.9	4.2	10.8	7.7	12.4	4.6	2.8	8.8	12.5	10.0	5.5
56	100.0	100.0	26.3	100.0	24.3	7.9	10.1	13.4	17.3	6.3	2.4	10.9	14.0	9.8	9.9
61	75.0	60.9	95.0	100.0	16.1	5.4	9.7	10.8	15.1	3.6	4.3	3.7	4.8	4.7	9.7
252	89.0	32.9	100.0	100.0	22.1	5.0	14.2	13.2	12.9	6.4	10.7	4.7	4.1	5.6	5.9

Note - See Table I for character codes.

Nota - Ver os códigos na Tabela I.

and low ethanol formation. This group, with the exception of OTU no. 60, is recovered in the distance phenogram.

Cluster-group B, consisting of 4 OTUs [171-269], completely splits apart in the distance phenogram. OTUs 147 and 171 clustered with OTU 001 (group D), the other two (182 and 269) remaining quite isolated. There is some degradation of xylose and fructose. Additionally there is a high production of acetic acid from fructose. All the other characters vary widely within the group (Table 2).

Cluster-group C accommodates 8 OTUs [178-265]. Despite some variation all the isolates of this group degraded very little arabinose. Production of ethanol from fructose is moderatetly high (<11%) for 3 out of the 8 OTUs.

Cluster-group D, composed of 5 OTUs [261-101], completely splits apart in the distance phenogram. Three out of the 5 isolates completely degraded fructose. Degradation of arabinose is moderately high and of xylose is very reduced. There is a relatively high proportion of acetic acid from xylose and ribose.

Cluster-group E includes 7 OTUs [58-0029] and is characterized by completely degrading fructose (except OTU 199) and not degrading xylose, and by not forming, of course, acetic acid and ethanol from xylose. This group is recovered intact in the distance phenogram.

Cluster-group F consists of 6 OTUs [87-96], which are fully recovered in the distance phenogram. This group is characterized by completely degrading fructose and a high proportion of arabinose and moderately xylose (except OTU 96). Additionally this is the group with the highest production of acetic acid from glucose.

Cluster-group G is formed by 10 OTUs [11-252], and is characterized by completely degrading arabinose (except OTU 63) and by a relatively high level of acetic acid formation from arabinose. This group is further subdivided in 3 subgroups. Subgroup G1, including OTUs 11 to 92, and, with the exception of OTU 42, completely degraded ribose and had no production of acetic acid and ethanol from ribose. Subgroup G2, including OTUs 18 to 56, with relatively high degradation of ribose, relatively high production of acetic acid from ribose and high production of ethanol from ribose. Subgroup G3, including OTUs 61 to

252, with almost no degradation of ribose, and relatively high production of acetic acid from ribose. In the distance phenogram (Fig. 2) group G is not recovered intact. However, 6 [18-252] of the 10 OTUs were regrouped, resulting from the fusion of groups G2 and G3 plus OTU 42. The remaining four OTUs from subgroup G1 were clustered in two quite isolated subgroups, [11-39] and [15-92].

Summing up, comparison of the structure recovered by the two phenograms shows that groups E and F are recovered intact in both phenograms, groups B and D split apart, groups C and G split in 3 subgroups, and group A, with the exception of OTU 60, is recovered in both phenograms.These results show that there is a reasonable agreement between the structure depicted by both clustering strategies, as some of the groups arre concerned, despite the low matrix cophenetic correlation between the correlation and distance matrices coefficients (r=0.54798).

Principal component analysis

The projections of the 48 OTUs and the 15 original characters onto the planes defined by the 1st and 2nd and 1st and 3rd principal axes are shown in Fig. 3A and 3B.

Table 3 shows the loadings of each character for the first 5 principal axes. This Table also includes the eigenvalues and the percentage of the total variance explained by each principal component. If we squared the score of character *i* for the component *j* we have the proportion of the total variance for character *i* accounted by the component *j*. It can also be shown that each element of this eigenvector matrix is the correlation between character *i* and component *j* (Hope, 1968).

The characters contributing most to the total variance in principal axis 1 are, in decreasing order of importance, ethanol and acetic acid formation from arabinose (eA and aA) and arabinose comsumption (A), that is this axis is strongly related to arabinose. The characters contributing greatly to the ordination of the OTUs onto the principal axis 2 are, also in descending order of importance, glucose consumption (G), ethanol formed from xylose and fructose degradation (eX and eF), acetic acid formation from fructose

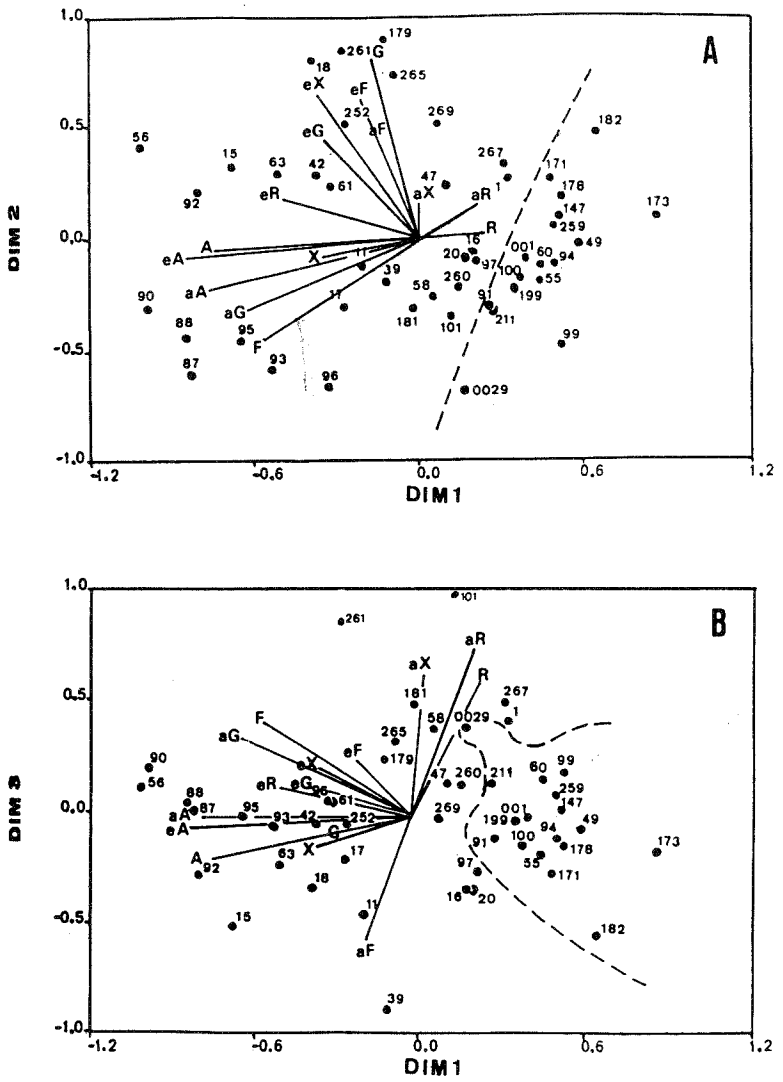

Fig. 3 - Projections of the 48 isolates or OTUs and of the 15 characters onto the plane defined by the first (22.9%) and the second (16.3%) (A) and the first (22.9%) and the third (12.8%) (B) principal components. See Table I for character codes.

Projecções dos 48 isolamentos ou OTUs e dos 15 caracteres no plano definido pela primeira (22.9%) e pela segunda (12.8%) (A) e pela primeira (22.9%) e pela terceira (12.8%) (B) componentes principais. Ver Tabela I para os códigos dos caracteres.

TABLE III

Correlations of original characters with the first five principal components (Analysis based on the correlation matrix calculated from 15 characters for 48 isolates of *Oenococcus oeni*)

Correlações dos caracters originais com as cinco componentes principais (Análise baseada na matriz de correlações calculada apartir de 15 caracteres para 48 isolamentos de Oenococcus oeni)

	Eigenvectors				
Character	1	2	3	4	5
A	-0.753	-0.053	-0.200	-0.139	0.053
F	-0.562	-0.465	0.394	-0092	-0.151
G	-0.162	0.806	-0.027	-0107	-0.166
R	0.221	0.031	0.560	10.511	-0.194
X	-0.363	-0.089	-0.147	0.585	0.263
aA	-0.785	-0.233	-0.020	-0.249	-0.093
aF	-0.174	0.520	-0.528	-0.069	0.078
aG	-0.639	-0.333	0.341	-0.199	0.078
aR	0.209	0.148	0.732	-0.176	-0.225
aX	0.018	0.163	0.579	-0.168	0.724
eA	-0.839	-0.091	-0.056	0.275	-0.035
eF	-0.215	0.632	0.234	0.197	-0.291
eG	-0.340	0.465	0.102	-0.551	-0.152
eR	-0.499	0.187	0.116	0.447	-0.184
eX	-0.364	0.656	0.187	0.167	0.396
eigenvalue	3.437	2.451	1.916	1.453	1.055
% variation	22.9	16.3	12.8	9.7	7.0
% cumulative variation	22.9	39.2	52.0	61.7	68.7

Note - See Table I for character coding.
Nota - Ver os códigos dos caracteres na Tabela I.

degradation (aF) and ethanol formed from glucose (eG). Finally, principal axis 3 represents the contrast of acetic acid formation from fructose degradation (aF) *vs.* acetic acid formation from ribose and xylose degradation (aR and aX) and ribose consumption (R).

Fig. 3A shows that there are two almost independent sets

of characters: 1) fructose and arabinose consumption (F and A), acetic acid formation glucose and arabinose degradation (aG and aA) and ethanol formation from arabinose degradation (eA); and 2) glucose consumption (G), ethanol formed from glucose and xylose degradation (eG and eX) and acetic acid and ethanol formation from fructose degradation (aF and eF).

Characters belonging to each of these sets are highly correlated among themselves. Ethanol formation from ribose degradation (eR) shows an intermediate position between these two sets.

Fig. 3B shows, as stated above, the contrast between the acetic acid formation from fructose *vs.* the degradation of ribose and the acetic acid formation from ribose and xylose.

The projections of the 48 OTUs onto the plane defined by the first two principal axes are in Fig. 4. Six (A, B, C, E, F and G) of seven groups shown in the correlation phenogram

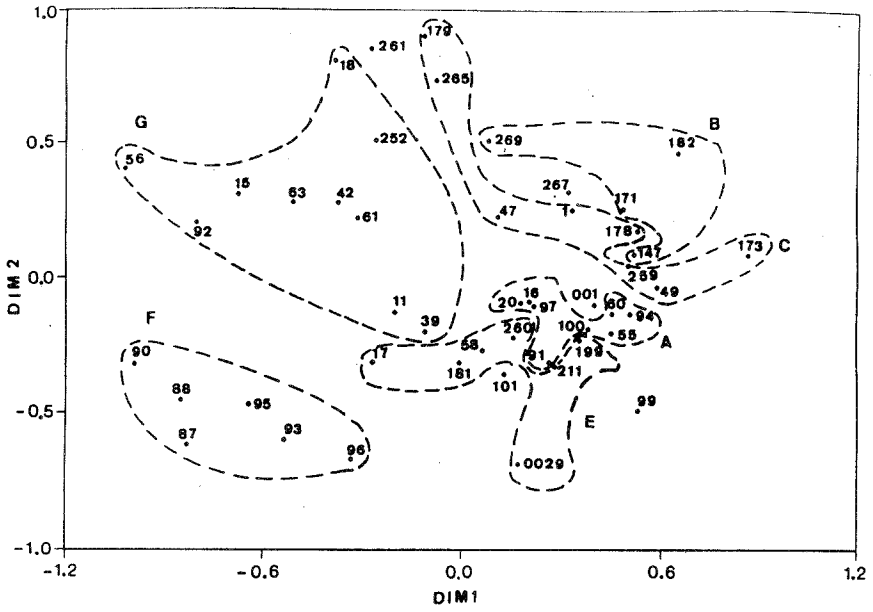

Fig. 4 - Projections of the 48 isolates or OTUs onto the plane defined by the first and the second principal components. The groups (A, B, ... G) defined by the correlation phenogram are marked by dashed lines.
Projecções dos 48 isolamentos ou OTUs e dos 15 caracteres no plano definido pelas primeira e segunda componentes principais. Os grupos (A, B, ... G) definidos pelo cenograma de correlação são marcados por linhas tracejadas.

(Fig. 1) are marked by dashed lines. A dashed line was not used for the seventh group (D, formed by OTUs 001, 99, 101, 261 and 267) due to its great diversity and in order to keep Fig. 4 reasonably clear. Only group F is clearly separated from the other groups, despite the great diversity showned by the OTUs ascribed to these groups.

It should be pointed out that ordination techniques, such as PCA, have the advantage of better summarizing the results of a phenetic analysis when there are not clear cut groups of OTUs, as no assumptions are made that OTUs must fall into a series of nested clusters or that clusters even exist.

Cluster analysis and PCA proved to be useful techniques to study the phenetic relationships of a large number of isolates of *O. oeni*, characterized by a large number of characteristics. Additionally these techniques gave an objective way for depicting sets of isolates possessing certain useful properties for special purposes. In the present study the most interesting strains, according to our objectives, were, therefore, those having a negative correlation with the acetic acid production and or sugar consumption specially the glucose, fructose and arabinose consumption as they are the main residual sugar normally present in the wine, and consenquently the substracts first degraded.

The selection of the isolates to be used for further studies, taking into account the trend of variation exhibited by the isolates studied, and the initial special purpose objectives, should, therefore, be done based on the projections of the characters (Fig. 3A and 3B). The OTUs more relevant are, Therefore, those on the right hand of the dashed lines of Fig. 3A and 3B: 182, 171, 178, 173, 147, 259, 49, 001, 94 60, 100, 55, 199, 91 211, 99 and 0029.

It is interesting to point out that cluster-groups F and G, based on the correlation and distance coefficients (Fig. 1 and 2), do not include any of these OTUs.

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RESUMO

Estudo por taxonomia numérica de alguns isolamentos de *Oenococcus oeni* com base no metabolismo de açúcares

No presente trabalho apresenta-se o perfil do metabolismo de cinco açúcares (glucose, frutose, arabinose, xilose e ribose) de 48 isolamentos de *Oenococcus oeni* (Garvie, 1967) Dicks *et al.*, 1995 obtidos de vinhos de diferentes origens. Os caracteres estudados foram a degradação de cinco açúcares (hexoses e pentoses) geralmente presentes no vinho e a correspondente formação de ácido acético e etanol. As semelhanças globais entre os diferentes isolamentos foram determinadas usando as distâncias euclidianas médias e os coeficientees de correlação. A análise em grupos destas duas matrizes foi feita pelo método UPGMA, sendo os resultados apresentados sob forma de fenogramas. A ordenação dos isolamentos num espaço definido pelas três primeiras componentes foi feita recorrendo à análise em componentes principais. Com base nos resultados obtidos foi possível diferenciar vários grupos de bactérias caracterizadas por um perfil açúcar consumido/produto formado próprio. As técnicas de taxonomia numérica permitem a selecção das bactérias mais interessantes para estudos subsequentes de controlo de fermentação malolática em vinhos: bactérias formando baixos teores de ácido acético a partir dos vários açúcares e/ou bactérias com baixa capacidade de degradação desses açúcares.

RÉSUMÉ

Étude par taxonomie numérique de quelques isolements do *Oenococcus oeni* basée sur le métabolisme des sucres

Ce travail montre le profile du métabolisme de cinq sucres (glucose, fructose, arabinose, xylose et ribose) par 48 isolements de *Oenococcus oeni* (Garvie, 1967) Dicks *et al.*, 1995 obtenues à partir de vins de différentes

origines. Les caractères choisis lors de cette étude sont l' utilization du sucre et la formation correspondante de certains produits tels que l' acide aceptique et l' étanol. Les ressemblances entre les différents isolements ont été calculées en utilisant la distance euclidienne moyenne et les coefficients de corrélation. L' analyse en groupes de ces deux matrices a été efféctuée par la technique désignée par UPGMA et les résultats présentés sous la forme de phenogrammes. L' ordination des isolements dans un éspace dimensionel réduit a été efféctuée par analyse en composantes principales. Les résultats obtenus ont permis de différencier plusieurs groupes de bactéries caractérisés par un profil sucre dégradé/produits formés. Les techniques de taxonomie numérique ont permis de sélectionner des bactéries susceptibles d' être choisies pour des études envisageant le contrôle de la fermentation malolactique des vins.

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