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# Production of benzaldehyde, benzyl alcohol and benzoic acid by yeasts and *Botrytis cinerea* isolated from grape musts and wines

by

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## Production d'aldéhyde benzoïque, d'alcool benzylique et d'acide benzoïque par des levures et *Botrytis cinerea* isolées de moûts et de vins

Résumé: La capacité de 100 souches de levures de la collection de l'Istituto Sperimentale per l'Enologia, isolées de moûts et de vins à produire aldéhyde benzoïque, alcool benzylique et acide benzoïque a été verifiée avec l'inoculation dans un milieu nutritif synthétique (MNS).

*Schizosaccharomyces* et *Zygosaccharomyces* ont été les genres les plus producteurs d'aldéhyde benzoïque (quantité maxima produite 1200 µg/l) et d'alcool benzylique (quantité maxima produite 523 µg/l). *Zygosaccharomyces* a été aussi le genre le plus producteur d'acide benzoïque (quantité maxima produite 536 µg/l), suivi par *Saccharomyces*, *Cryptococcus*, *Kloeckera* et *Torulaspora*.

L'hypothèse que les levures peuvent être une source exogène de l'enzyme qui oxyde l'alcool benzylique en aldéhyde benzoïque dans les moûts et les vins a été vérifée.

Des souches de levures, appartenant aux genres *Saccharomyces*, *Zygosaccharomyces* et *Schizosaccharomyces*, en fermentant en MNS contenant 150 g/l de glucose et de l'alcool benzylique ajouté, ont transformé ceci en acide benzoïque seulement quand le glucose a disparu, mais ils ne l'ont pas transformé en aldéhyde benzoïque. Aucune différence n'a été observée entre les conditions aérobiques et anaérobiques de fermentation.

L'utilisation d'alcool benzylique a été rapide dans les essais faits en présence de moins de 10 g/l de glucose et dans les essais d'assimilation avec 'yeast nitrogen base broth' et des composés carboniques assimilables. L'existence d'une répression catabolique par le glucose est vraisemblable.

*Botrytis cinerea* a été capable de transformer l'alcool benzylique en aldéhyde benzoïque en se développant sur Czapek-Dox broth avec 30 g/l de saccharose.

Key words: benzaldehyde, benzyl alcohol, benzoic acid, phenol, flavour, yeast, Botrytis cinerea, culture medium, fermentation, aerobiosis, anaerobiosis, glucose, enzyme.

## Introduction

Benzaldehyde is an aroma compound responsible for the bitter almond taste of wines. DELFINI and DI STEFANO (1984) found it in sparkling wines in concentrations up to 7 mg/l, about twice the olfactory threshold concentration of that in white wines (3.0—3.5 mg/l) (DELFINI 1987). BLAISE and BRUN (1986) reported large amounts of benzaldehyde for wines kept in cement tanks coated with epoxy resins that released benzyl alcohol. This should be oxidized by an enzyme, not yet well known, that would be present in grape musts and wines. In Italy, where epoxy resins are used without benzyl alcohol, this compound, instead, originated from some commercial liquid gelatines for oenological use (up to 1.68 mg/g; DELFINI 1987). It is interesting that after the communication of this discovery no sparkling wine with strong odor of bitter almond was found in Italy. The source of the oxidizing enzyme (grape or yeast or other microorganism) is not yet clear (DELFINI 1987). Yeasts are able to utilize benzaldehyde in the presence of glucose (KIRCHHOFF 1962). Some strains of *Saccharomyces cerevisiae* and *Schizosac-*

*charomyces japonicus* transformed benzaldehyde into benzyl alcohol, benzoic acid and other substances not well defined, but they did not accumulate benzaldehyde in a synthetic nutritive medium (MNS) with 10 ppm of benzyl alcohol, benzoic acid, 4-hydroxybenzoic acid, phenylacetic acid and phenylalanine added (DELFINI 1987).

This paper describes experiments performed to ascertain the capability of production of benzaldehyde, benzyl alcohol and benzoic acid during the alcoholic fermentation in synthetic media by several wine yeasts and *Botrytis cinerea* in presence or absence of benzyl alcohol.

## Materials and methods

Yeasts: Several yeast strains were used, belonging to different genera and species isolated from grape musts and wines, from the I.S.E. (Istituto Sperimentale per l'Enologia) collection (the strains are listed in the tables; the names of the genera are abbreviated according to KREGER-VAN RIJ (1984); in the strain number code, b is for *bayanus*, c for *cerevisiae*, u for *uvarum* and i for *italicus* varieties).
*Botrytis cinerea* isolated from Pinot grapes was also used.

Media: In the different experiments the following media were used:

- Experiment 1: Synthetic nutrient medium (MNS), pH 3.5, according to DELFINI (1982), composed of D-glucose 150 g, tartaric acid 3 g, L(+)-malic and acid 2 g, $(NH_4)_2SO_4$ 0.45 g, $(NH_4)_2PO_4$ 0.45 g, casamino acid (Difco) 0.5 g, $KH_2PO_4$ 1 g, $MGSO_4 \cdot 7\ H_2O$ 0.5 g, NaCl 0.1 g, $CaCl_2$ 0.1 g, inositol 25 mg, microelements, vitamins, water 1000 ml, desired pH adjusted with KOH; without benzyl alcohol.
- Experiment 2: MNS, pH 3.5, with 10 mg/l of benzyl alcohol added.
- Experiment 3: MNS, pH 3.18, with 25 mg/l of benzyl alcohol added.
- Experiment 4: MNS, pH 3.18, containing initially 10 g/l of glucose. During this experiment further 5 fractions of 10 g/l glucose each were added to the cultures (up to a total of 60 g/l) when the consumption of the sugar was verified by weighing the cultures according to DELFINI (1982).
- Experiment 5: AB (assimilation broth). Yeast Nitrogen Base (Dico) 60 g/l, glucose 2 g/l, cellobiose 20 g/l, trehalose 20 g/l, D-mannitol 20 g/l, glycerol 20 g/l and benzyl alcohol 20 mg/l.
- Experiment 6: Czapek-Dox broth. Sucrose 30 g, $NaNO_3$ 3 g, $K_2HPO_3$ 1 g, $MGSO_4$ 0.5 g, KCl 0.5 g, $FeSO_4$ 0.01 g, water 1000 ml, pH 3.5 by adding $H_2SO_4$; with 15 mg/l of benzyl alcohol added.

Preparation of the cultures: The yeast strains were streaked on malt extract agar slant. After 3 d the growing cells were directly resuspended in 5 ml of medium which was used for inoculation. Two drops of cellular suspensions were added to each 100 ml of medium. The cultures were incubated at 25 °C.

In Experiments 1 and 2 the cultures were prepared in 150 ml of MNS and incubated in 200 ml flasks which were closed with Pasteur pipettes. After 30 d of growing, verified visually from the cell mass or $CO_2$ production, the cultures were centrifuged and the supernatant extracted and analyzed by gas chromatography.

In Experiment 3, each 1000 ml flask contained 800 ml of MNS and was closed with cotton plugs (cultures 'a') or Muller valves (cultures 'an').

In Experiment 4, each 500 ml flask contained 400 ml of MNS and was closed with Pasteur pipettes.

In Experiment 5, the cultures were prepared in 500 ml flasks containing 200 ml of assimilation medium and closed with cotton plugs.

In Experiment 6, *Botrytis cinerea* was grown in 600 ml of Czapek-Dox broth in 2000 ml flasks closed with cotton plugs.

Preparation of the sample for gas chromatography analysis: 250 ml of the centrifuged media were supplied with 1 % of an internal standard solution (342 mg/l of 1-heptanol in water-ethyl alcohol 1:1) and then extracted with 90 ml of dichloromethane. The separation of the solvent from the aqueous phase was completed by centrifugation. The organic phase was then concentrated by distillation.

Gas chromatographic conditions: Gas chromatograph Varian 3600 mounted with a capillary column DB-WAX, 30 m, 0.252 mm. Initial column temperature 60 °C for 2 min; from 60 to 200 °C increased by 2 °C/min; final hold time 10 min.

Mass spectrometry (mass detector HP 5970 coupled with gas chromatograph HP 5890) was applied to 5 samples in 3 different determinations to ascertain the identification of benzaldehyde, benzyl alcohol and benzoic acid peaks on gas chromatograms.

## Results and discussion

Experiment 1: The Pasteur pipettes allowed to maintain conditions of semian-aerobiosis during the fermentation, passing in fact gradually from initial aerobiosis,

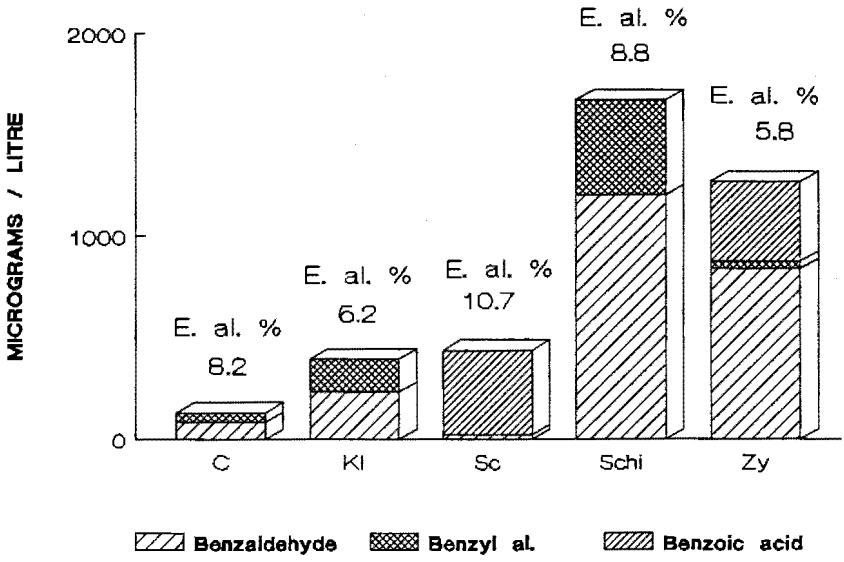


Fig. 1: Production of benzaldehyde, benzyl alcohol, benzoic acid and ethyl alcohol by some typical wine yeast strains employed in Experiment 1. — C = *Candida stellata* I 2/72, Kl = *Kloeckera apiculata* R17, Sc = *Saccharomyces cerevisiae* 6, Schi = *Schizosaccharomyces pombe* 2, Zy = *Zygosaccharomyces bailii* b 14.

Production d'aldéhyde benzoïque, d'alcool benzylique, d'acide benzoïque et d'alcool éthyliques par quelques souches de levures employées dans l'Essai 1.

because the medium was saturated with air, to semianaerobiosis by the endogenous $CO_2$ production.

The results obtained are summarized in Table 1 while in Fig. 1 individual data of some typical wine yeast strains are shown. *Schizosaccharomyces* and *Zygosaccharomyces* were the strongest genera in producing benzaldehyde (maximal amount found 1200 µg/l) and benzyl alcohol (maximally 523 µg/l). *Zygosaccharomyces* was also most effective in the production of benzoic acid (maximally 536 µg/l), followed by *Saccharomyces, Cryptococcus, Kloeckera* and *Torulaspora.*

An uninoculated sample of MNS without benzyl alcohol was also analyzed and no benzaldehyde, benzylalcohol or benzoic acid were detected.

Considering that some strains produced large amounts of benzaldehyde and very low amounts of benzyl alcohol and benzoic acid (e.g. *Zygosaccharomyces bailii* 14s with 839.9 µg/l of benzaldehyde and only 36.6 µg/l of benzyl alcohol and 390.5 µg/l of benzoic acid), the hypothesis that yeasts can be an exogenous source of the benzyl alcohol oxidizing enzyme in grape musts and wines appears interesting and worthy of further verification.

Experiment 2: The production of benzaldehyde (Table 2) was not significantly different from that obtained in Experiment 1 or in the control cultures without benzyl alcohol. The major amount (327 µg/l) was produced by *Schiz. pombe.* The benzyl alcohol found at the end of fermentation in the cultures with benzyl alcohol added, varied from 932 µg/l (strain S51u) to 7224 µg/l (strain Zy9bi), while very low amounts were produced in the control cultures. Individual data of some typical wine strains are given in Fig. 2.

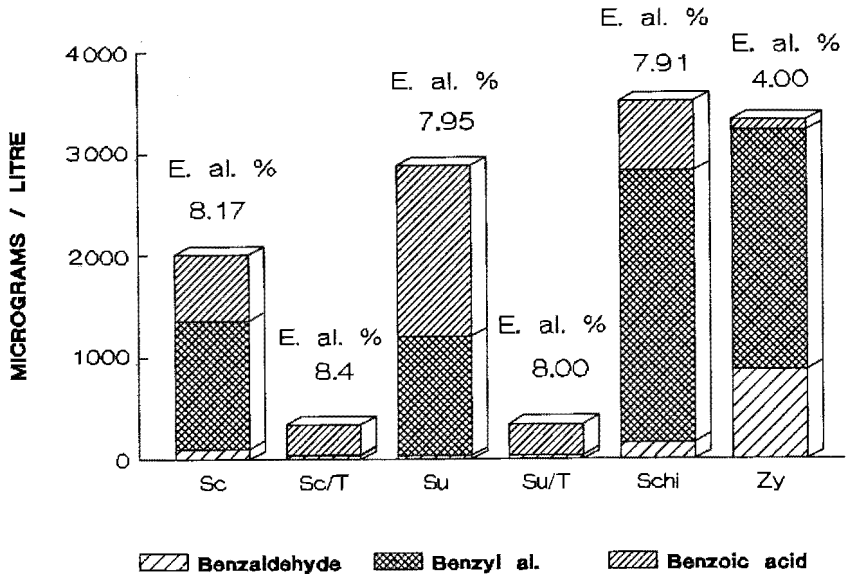


Fig. 2: Production of benzaldehyde, benzyl alcohol, benzoic acid and ethyl alcohol by some typical wine yeast strains employed in Experiment 2. Sc = *Saccharomyces cerevisiae* 189c, Sc/T = control sample without benzyl alcohol added, Su = *Saccharomyces cerevisiae* var. *uvarum* S30u, Su/T = control sample, Schi = *Schizosaccharomyces pombe* 2, Zy = *Zygosaccharomyces bailii* 14.

Production d'aldéhyde benzoïque, d'alcool benzylique, d'acide benzoïque et d'alcool éthylique par quelques souches de levures employées dans l'Essai 2.

Table 1

Production of benzaldehyde, benzyl alcohol, benzoic acid and ethyl alcohol by different yeast strains in MNS containing 150 g/l glucose, without benzyl alcohol added · Names of genera are abbreviated according to KREEGER-VAN RIJ (1984) · T.s. = total number of strains assayed; F = frequency in the quantitative class

Production d'aldéhyde benzoïque, d'alcool benzylique, d'acide benzoïque et d'alcool éthylique en MNS contenant 150 g/l de glucose et sans ajouter d'alcool benzylique · Les noms de genres sont abrégés selon KREEGER-VAN RIJ (1984) · T.s. = Nombre total de souches essayées; F = fréquences dans les classes

| Species | T.s. | Benzaldehyde µg/l | F. | Benzyl alc. µg/l | F. | Benzoic acid µg/l | F. | Ethyl alc. % |
|---|---|---|---|---|---|---|---|---|
| *C. guillermondii* | 7 | 0.0—2.6 | 7 | 0.0—21.9 | 7 | 0.0—27.3 | 7 | 3.1—4.8 |
| *C. melinii* | 1 | 0.0 | 1 | 27.9 | 1 | 0.0 | 1 | 0.6 |
| *C. stellata* | 1 | 86.7 | 1 | 45.9 | 1 | 0.0 | 1 | 8.2 |
| *C. valida* | 1 | 2.3 | 1 | 1.8 | 1 | 0.0 | 1 | 0.8 |
| *C. vini* | 1 | 1.2 | 1 | 0.0 | 1 | 11.1 | 1 | — |
| *Cr. albidus* | 1 | 0.0 | 1 | 10.5 | 1 | 378.5 | 1 | — |
| *Kl. apiculata* | 19 | | | | | | | 3.9—6.2 |
| *Kl. apiculata* | | 6.2—31.2 | 11 | 18.0—41.5 | 9 | 0.0—54.8 | 9 | |
| *Kl. apiculata* | | 50.1—72.1 | 4 | 42.3—161.5 | 4 | 55.0—127.4 | 7 | |
| *Kl. apiculata* | | 142.6—232.2 | 4 | 248.2—351.2 | 6 | 140.6—393.9 | 3 | |
| *Kl. corticis* | 1 | 0.0 | 1 | 5.8 | 1 | 47.3 | 1 | 9.1 |
| *Tr. cutaneum* | 1 | 0.0 | 1 | 0.0 | 1 | 0.0 | 1 | — |
| *H. anomala* | 1 | 10.7 | 1 | 8.8 | 1 | 16.5 | 1 | 6.3 |
| *H. jadinii* | 1 | 0.0 | 1 | 4.8 | 1 | 0.0 | 1 | 0.7 |
| *H. petersonii* | 1 | 8.0 | 1 | 10.6 | 1 | 75.0 | 1 | 0.9 |
| *H. saturnus* | 1 | 9.4 | 1 | 7.5 | 1 | 0.0 | 1 | 4.9 |
| *M. pulcherrima* | 1 | 83.5 | 1 | 84.4 | 1 | 69.5 | 1 | 5.2 |
| *P. carsonii* | 3 | 0.0 | 3 | 0.0—22.4 | 3 | 0.0 | 3 | — |
| *Pichia sp.* | 3 | 0.0—54.3 | 3 | 0.0—77.4 | 3 | 0.0—202.6 | 3 | 0.6—4.5 |
| *S. cerevisiae* | 28 | | | | | | | 8.8—10.7 |
| *S. cerevisiae* | | 0.0—54.8 | 22 | 0.0—12.8 | 24 | 0.0—103.2 | 20 | |
| *S. cerevisiae* | | 59.0—80.6 | 4 | 16.5—47.3 | 3 | 216.9—268.9 | 4 | |
| *S. cerevisiae* | | 90.7—155.0 | 2 | 304.1 | 1 | 282.4—416.8 | 4 | |
| *S. exiguus* | 1 | 45.8 | 1 | 0.0 | 1 | 0.0 | 1 | 8.5 |
| *S. codes ludwigii* | 1 | 35.0 | 1 | 81.5 | 1 | 0.0 | 1 | 9.0 |
| *Schiz. japonicus* | 8 | | | | | | | 5.0—8.4 |
| *Schiz. japonicus* | | 71.8—93.7 | 5 | 133.3—158.9 | 4 | 0.0—0.0 | 7 | |
| *Schiz. japonicus* | | 96.4—120.7 | 3 | 167.9—523.4 | 4 | 167.2 | 1 | |
| *Schiz. pombe* | 2 | 497.1—1,200.5 | 2 | 303.8—464.5 | 2 | 0.0 | 2 | 8.8—9.4 |
| *T'spora delbrueckii* | 8 | | | | | | | 7.6—9.3 |
| *T'spora delbrueckii* | | 21.8—52.1 | 5 | 0.0—4.8 | 7 | 0.0—131.3 | 6 | |
| *T'spora delbrueckii* | | 61.6—158.1 | 3 | 15.7 | 1 | 170.7—320.9 | 2 | |
| *Zygosacch. bailii* | 6 | | | | | | | 3.5—8.1 |
| *Zygosacch. bailii* | | 45.4—64.0 | 2 | 0.0—36.6 | 4 | 0.0—155.2 | 3 | |
| *Zygosacch. bailii* | | 111.8—243.6 | 3 | 86.1—136.1 | 2 | 390.5—536.0 | 3 | |
| *Zygosacch. bailii* | | 839.9 | 1 | | | | | |
| *Zygosacch. florentinus* | 1 | 34.1 | 1 | 59.0 | 1 | 120.0 | 1 | 8.9 |
| *Zygosacch. rouxii* | 1 | 68.5 | 1 | 135.8 | 1 | 271.0 | 1 | 4.5 |

An uninoculated sample of MNS, with 10 mg/l benzyl alcohol added, showed the presence of 59 µg/l benzaldehyde, 8235 µg/l benzyl alcohol and no benzoic acid. These results probably annul the meaning of very low amounts of benzaldehyde produced in MNS containing benzyl alcohol, but we have also to consider that benzaldehyde is consumed by yeasts in the presence of glucose (KIRCHHOFF 1962; DELFINI 1987). So, it is difficult to know which amount of benzaldehyde was actually produced by yeasts.

The benzoic acid, on the other hand, increased generally with all strains tested but there was no significant correlation between high amounts of benzyl alcohol disappeared and high amounts of benzoic acid formed. E.g. *Zygosaccharomyces bailii* Zy9bi left only 748 µg/l benzyl alcohol without producing significative amounts of benzoic acid; *S. cerevisiae* S46c and S191c behaved similarly. These results, not easy to explain at first, suggested to look for some metabolic factors which could influence the uptake and the transformation of benzyl alcohol into benzaldehyde and benzoic acid, such as aerobiosis rate and catabolic repression by glucose.

Experiment 3: The results of alcoholic fermentations did not show significant differences (Table 3) in the production of benzaldehyde, benzyl alcohol and benzoic acid between the cultures subjected to aerobic or anaerobic conditions. Benzoic acid was produced in major quantities only by *S. cerevisiae* S46c and S51u, but without a corresponding strong decrease of benzyl alcohol, even if it was found in cultures (a) and (an).

The amounts of benzaldehyde produced by *Schiz.* 2p and *Zygosacch.* 9bi strains were relatively high compared with those produced in Experiment 1 (Table 1), thus confirming that these strains are effective producers of benzaldehyde.

The results of these experiments demonstrate that the aerobiosis rate is not one of the factors controlling the uptake and the transformation of benzyl alcohol into benzaldehyde and benzoic acid, at least under our alcoholic fermentation conditions, which are similar to those of winemaking.

Experiment 4: The successive additions of glucose to the medium allowed us to maintain the concentration of glucose constantly under 10 g/l to avoid eventual catabolic repression by glucose.

The results obtained in the fermenting cultures in the presence of less than 10 g/l glucose (Table 4) showed a production of benzoic acid significantly correlated to the disappearance of benzyl alcohol. The production of benzaldehyde, on the other hand, was not significantly different from that found in the previous experiment. Further, in many cultures the production of benzoic acid was higher 48 d after inoculation, the moment in which the major amount of glucose was consumed, than after 24 d.

Thus, the glucose concentration of the medium is one of the most important factors controlling the uptake of benzyl alcohol by yeasts and its transformation into benzoic acid. However, this does not explain the large amounts of benzaldehyde found in sparkling wines by DELFINI and DI STEFANO (1984). Other technological factors, such as the addition of sulfur dioxide or the secondary fermentation performed with sucrose for making sparkling wines, can be hypothesized to play a very important role in the transformation of benzyl alcohol into benzaldehyde.

Experiment 5: The AB medium allowed to verify the uptake of benzyl alcohol under aerobiotic conditions in the presence of a minimum of glucose and a high amount of assimilable carbon compounds.

The data (Table 5) showed a strong production of benzoic acid and the disappearance of benzyl alcohol by *S. cerevisiae* S46c, *C. guillermondii* C10g and *Zygosacch. bailii* Zy9bi.

Table 2

Production of benzaldehyde and benzoic acid in MNS containing 150 g/l glucose, with 10 mg/l benzyl alcohol added, during alcoholic fermentation under semianaerobic conditions · a, b = Repetitions in presence of benzyl alcohol; c = repetitions without benzyl alcohol

Production d'aldéhyde benzoïque et d'acide benzoïque en MNS contenant 150 g/l de glucose, avec l'addition de 10 mg/l d'alcool benzylique, en conditions semi-anaérobiques pendant la fermentation alcoolique · a, b = Répétitions en présence d'alcool benzylique; c = répétitions sans d'alcool benzylique

| Strains | | Benzaldehyde µg/l | Benzyl alc. µg/l | Benzoic acid µg/l | Ethyl alc. % |
|---|---|---|---|---|---|
| *Kl. apiculata* | a | 0 | 2,844 | 1,092 | 2.9 |
| R17 | b | 68 | 2,725 | — | 2.7 |
| *S. cerevisiae* | a | 0 | 319 | 708 | 8.0 |
| S26b | b | 0 | 254 | 791 | 8.1 |
| *S. cerevisiae* | a | 0 | 3,021 | 488 | 8.0 |
| S27b | b | 25 | 2,356 | 570 | 8.1 |
| | c | 70 | 181 | 453 | 8.1 |
| *S. cerevisiae* | a | 34 | 2,695 | 1 474 | 8.2 |
| S39b | b | 0 | 2,534 | 833 | 8.2 |
| *S. cerevisiae* | a | 57 | 1,573 | 291 | 7.9 |
| S46c | b | 21 | 1,043 | 430 | 8.0 |
| | c | 26 | 12 | 388 | 8.0 |
| *S. cerevisiae* | a | 84 | 1,419 | 353 | 8.2 |
| S189c | b | 119 | 1,097 | 931 | 8.2 |
| | c | 38 | 12 | 293 | 8.1 |
| *S. cerevisiae* | a | 0 | 1,518 | 1,679 | 8.0 |
| S191c | b | 78 | 4,218 | 607 | 8.0 |
| | c | 43 | 16 | 0 | 8.1 |
| *S. cerevisiae* | a | — | — | — | 8.2 |
| S10u | b | 52 | 1,074 | 1,953 | 8.0 |
| | c | 138 | 45 | 454 | 8.1 |
| *S. cerevisiae* | a | 44 | 1,124 | 1,317 | 7.9 |
| S30u | b | 28 | 1,212 | 2,040 | 8.0 |
| | c | 40 | 0 | 306 | 8.0 |
| *S. cerevisiae* | a | — | — | — | 8.1 |
| S51u | b | 290 | 932 | 607 | 7.9 |
| | c | 42 | 0 | 382 | 8.0 |
| *S. cerevisiae* | a | 0 | 2,509 | 387 | 8.0 |
| S1i | b | 0 | 2,704 | — | 8.0 |
| *Schiz. pombe* | a | 0 | 2,652 | 453 | 7.9 |
| Schi2p | b | 327 | 2,684 | 908 | 7.9 |
| *Schiz. japonicus* | a | 0 | 2,498 | 380 | 7.2 |
| Schi.11 | b | 81 | 2,771 | 365 | 7.2 |
| *Schiz. japonicus* | a | 67 | 2,388 | 270 | 4.8 |
| Schi41 | b | 107 | 2,740 | 430 | 5.1 |
| *T'spora delbrueckii* | a | 75 | 2,974 | 0 | 5.4 |
| Tor10r | b | 0 | 2,398 | 0 | 5.8 |
| *Zygosacch. bailii* | a | 2,002 | 7,224 | 0 | 1.9 |
| Zy9bi | b | 48 | 748 | 0 | 2.1 |
| *Zygosacch. bailii* | a | 127 | 3,089 | 0 | 4.4 |
| Zy14bi | b | 48 | 1,605 | 199 | 3.6 |

Table 5

Production of benzaldehyde and benzoic acid in AB broth with 2 g/l glucose and 20 g/l of each assimilable carbon compound - a, b = Repetitions

Production d'aldéhyde benzoïque et d'acide benzoïque en AB broth avec 2 g/l de glucose et 20 g/l de chaque composé carbonique assimilable - a, b = Répétitions

| Strains | d of incubation | | Benzaldehyde µg/l | Benzyl alcohol µg/l | Benzoic acid µg/l |
|---|---|---|---|---|---|
| Control flask not inoculated | 0 | | 9 | 8,750 | 24 |
| *S. cerevisiae* S46c | 25 | a | 0 | 514 | 4,510 |
| | | b | 0 | 376 | 4,734 |
| | 49 | a | 0 | 255 | 3,851 |
| | | b | 0 | 301 | 4,673 |
| | 50 | Addition of further 10 mg/l benzyl alc. | | | |
| | 90 | a | 0 | 325 | 11,494 |
| | | b | 0 | 101 | 7,610 |
| *Zygosacch. bailii* Zy9bi | 25 | a | 0 | 5,886 | 747 |
| | | b | 0 | 5,936 | 1,169 |
| | 49 | a | 44 | 5,369 | 962 |
| | | b | 0 | 5,610 | 721 |
| *C. guillermondii* C10g | 25 | a | 35 | 3,143 | 3,385 |
| | | b | 0 | 3,475 | 2,584 |
| | 49 | a | 0 | 2,654 | 3,923 |
| | | b | 0 | 2,849 | 3,885 |
| *Kl. apiculata* Kl1a | 25 | a | 64 | 7,363 | 196 |
| | | b | 0 | 7,023 | 66 |
| | 49 | a | 139 | 7,414 | 114 |
| | | b | 0 | 7,064 | 264 |
| *Schiz. pombe* Schi2p | 25 | a | 59 | 7,463 | 56 |
| | | b | 0 | 7,187 | 48 |
| | 49 | a | 47 | 7,828 | 145 |
| | | b | 42 | 7,064 | 91 |

*Kl. apiculata* Kl1a and *Schiz. pombe* Schi2p, on the other hand, did not transform benzyl alcohol nor produced benzoic acid.

No production of benzaldehyde was found for all strains tested.

These results, confirming those of Experiment 4, demonstrate that *S. cerevisiae*, the most important species in enology, can utilize benzyl alcohol in grape musts or in wines when it has consumed large amounts of glucose, or while it is utilizing substances with metabolic pathways different from that of glucose. This fact confirms also the existence of a catabolic repression by glucose.

Experiment 6: This experiment showed clearly the production of large amounts of benzaldehyde by *Botrytis cinerea* growing on a medium with sucrose as carbon source (Table 6). This in spite of the fact that only a small amount of benzalde-

Table 3

Production of benzaldehyde and benzoic acid in MNS containing 150 g/l glucose, with 35 mg/l benzyl alcohol added, under aerobic (a) and anaerobic (an) conditions

Production d'aldéhyde benzoïque et d'acide benzoïque en MNS contenant 150 g/l de glucose, avec l'addition de 25 mg/l d'alcool benzylique, en conditions aérobiques (a) et anaérobiques (an)

| Strains | | Benzaldehyde µg/l | Benzyl alc. µg/l | Benzoic acid µg/l | Ethyl alc. % |
|---|---|---|---|---|---|
| Control flasks not inoculated | | 94 | 25,718 | 103 | |
| *Zygosacch. bailii* | a | 132 | 22,358 | 0 | 8.6 |
| Zy9bi | an | 116 | 23,090 | 0 | 4.5 |
| *S. cerevisiae* | a | 33 | 19,151 | 1,900 | 8.9 |
| S46c | an | 41 | 20,906 | 2,056 | 8.8 |
| *S. cerevisiae* | a | 30 | 19,648 | 3,376 | 9.1 |
| S51u | an | 41 | 21,252 | 4,650 | 8.4 |
| *Schiz. pombe* | a | 500 | 24,241 | 485 | 9.0 |
| Schi2p | an | 670 | 23,172 | 159 | 8.5 |

Table 4

Production of benzaldehyde (B.de) and benzoic acid (B.ac.) in MNS containing initially 10 g/l glucose, with 15 mg/l benzyl alcohol (B.alc.) added, under semianaerobic conditions · E.alc. = Ethyl alcohol; a, b = repetitions

Production d'aldéhyde benzoïque (B.de) et d'acide benzoïque (B.ac.) en MNS contenant, au départ de l'experiment, 10 g/l de glucose, avec l'addition de 15 mg/l d'alcool benzylique (B.alc.) en conditions semi-anaérobiques · E.alc. = Alcool éthylique; a, b = répétitions

| Strains | | 1st Sampling (24 d after inoculation) | | | | 2nd Sampling (48 d after inoculation) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | B.de µg/l | B.alc. µg/l | B.ac. µg/l | E.alc. % | B.de µg/l | B.alc. µg/l | B.ac. µg/l | E.alc. % |
| *Kl. apiculata* | a | 38 | 14,218 | 370 | 1,9 | 61 | 13,437 | 905 | 2.6 |
| Kl17a | b | | — | — | — | 147 | 13,504 | 194 | 2.9 |
| *S. cerevisiae* | a | 106 | 12,252 | 1,849 | 2,3 | 18 | 6,864 | 6,744 | 3.0 |
| S22b | b | 24 | 11,888 | 1,873 | 2,1 | 23 | 11,817 | 2,583 | 2.8 |
| *S. cerevisiae* | a | 0 | 11,353 | 1,351 | 2,3 | 18 | 8,024 | 4,413 | 2.9 |
| S46c | b | 184 | 13,070 | 475 | 2,3 | 124 | 11,601 | 1,163 | 3.1 |
| *S. cerevisiae* | a | 50 | 9,030 | 3,234 | 2,3 | 95 | 7,140 | 7,001 | 3.0 |
| S191c | b | 46 | 12,064 | 978 | 2,0 | 19 | 11,402 | 749 | 2.7 |
| *S. cerevisiae* | a | 49 | 12,956 | 1,196 | 2,0 | 51 | 11,834 | 1,442 | 2.7 |
| S10u | b | 33 | 11,688 | 2,219 | 2,2 | 57 | 8,458 | 4,610 | 2.8 |
| *Schiz. pombe* | a | 167 | 14,911 | 0 | 2,2 | 195 | 13,013 | 400 | 2.9 |
| Schi2p | b | 186 | 14,429 | 109 | 2,2 | 21 | 12,014 | 2,974 | 3.0 |
| *Zygosacch. bailii* | a | 41 | 14,176 | 194 | 2,1 | 33 | 14,021 | 285 | 2,9 |
| Zy9bi | b | 35 | 15,046 | 150 | 2,0 | 26 | 13,858 | 3,919 | 2.5 |

Table 6

Production of benzaldehyde and benzoic acid by *Botrytis cinerea* in Czapek-Dox broth containing 30 g/l sucrose, with 15 mg/l benzyl alcohol added

Production d'aldéhyde benzoïque et d'acide benzoïque par *Botrytis cinerea* en Czapek-Dox broth contenant 30 g/l saccharose, avec l'additon de 15 mg/l d'alcool benzylique

| d of incubation | Benzaldehyde µg/l | Benzyl alcohol µg/l | Benzoic acid µg/l | Sucrose found g/l |
|---|---|---|---|---|
| | Sample without benzyl alc. added | | | |
| 30 | 7 | 0 | 32 | |
| 48 | 32 | 21 | 63 | |
| | Sample with benzyl alc. added | | | |
| 0 | 94 | 12,216 | 0 | 30.0 |
| 17 | 205 | 8,105 | 42 | |
| 23 | 261 | 8,106 | 35 | |
| 34 | 451 | 6,320 | 45 | |
| 41 | 938 | 4,882 | 22 | |
| 57 | 600 | 4,297 | 194 | 26.7 |
| 72 | 4,285 | 563 | 96 | |
| 79 | Further addition of 15 mg/l benzyl alc. | | | |
| 112 | 4,895 | traces | traces | |

hyde, as impurity of the commercial benzyl alcohol product, was found in the uninoculated control sample with benzyl alcohol added (Table 6).

Thus, *Botrytis cinerea* can be a source of the enzyme that transforms benzyl alcohol into benzaldehyde in wines. But this hypothesis has to be demonstrated as well as it must be demonstrated that *Botrytis cinerea* can free the enzyme in musts coming from affected grapes and/or that it does not precipitate and maintains its activity during the alcoholic fermentation and vinification process.

It appears also worthy to verify the hypothesis that grape can be a source of this enzyme and it is active only when the vinification process creates favorable conditions.

## Summary

The capacity of 100 yeast strains — isolated from grape musts and wines from the Istituto Sperimentale per l'Enologia collection — to produce benzaldehyde, benzyl alcohol and benzoic acid was verified by inoculation into a synthetic nutrient medium (MNS).

*Schizosaccharomyces* and *Zygosaccharomyces* were strongest in producing benzaldehyde (maximal amount found 1200 µg/l) and benzyl alcohol (maximally 523 µg/l). *Zygosaccharomyces* was also most effective in the production of benzoic acid (maximally 536 µg/l), followed by *Saccharomyces, Cryptococcus, Kloeckera* and *Torulaspora.*

The hypothesis was verified that yeasts can be an exogenous source of the benzyl alcohol oxidizing enzyme in grape musts and wines. Wine yeast strains of *Saccharomyces* spp., *Zygosaccharomyces* spp. and *Schizosaccharomyces* spp. fermenting MNS containing 150 g/l glucose, with benzyl alcohol added, transformed this into benzoic acid only when glucose was disappearing, but not into benzaldehyde. No difference was

observed between aerobic and anaerobic fermentation conditions. The uptake of benzyl alcohol was rapid in fermentation essays in presence of only 10 g/l glucose and in assimilation essais performed in yeast nitrogen base broth with assimilable carbon compounds added. A catabolic repression by glucose appears likely.

*Botrytis cinerea* was able to transform benzyl alcohol into benzaldehyde and benzoic acid on Czapek-Dox broth with 30 g/l sucrose added.

Benzyl alcohol was transformed by wine yeasts into benzoic acid when the concentration of glucose in the mineral medium was less than 10 g/l, but no production of benzaldehyde was observed. A catabolic repression of this transformation by glucose is likely.

*Botrytis cinerea* was able to produce benzaldehyde in a mineral medium with benzyl alcohol and sucrose added.

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