# GROWTH IN SPENT SULPHITE LIQUOR AND BIOTRANSFORMATION OF VANILLIN BY YEASTS FROM DECAYING WOOD

Jonas RÖNNANDER[1]*, Joel LJUNGGREN[2], Sandra A. I. WRIGHT[1]
[1]University of Gävle, Gävle, Sweden
[2]Mid Sweden University, Sundsvall, Sweden
Email: jonas.ronnander@hig.se

## ABSTRACT

Wood is a challenging environment for microorganisms. Spent sulphite liquor (SSL), which derives from acid hydrolysis of lignocellulosic biomass is an even greater challenge, due to the presence of inhibitors e.g. toxic phenolic compounds[1,2]. One of these is vanillin (VA), a lignin monomeric derivative, which can be biodegraded to vanillyl alcohol (VAL)[3] and possibly to the corresponding acid (Figure 1). In the present study, yeasts were isolated from decaying wood and identified by ITS and D1/D2 sequence comparisons. The strains were cultivated on media containing SSL and VA (Figure 2). The strains that grew best on SSL, *Candida argentea* and *Debaryomyces* spp., also grew well in VA (Table 1). VAL was the major biodegradation product of VA (Figure 3).

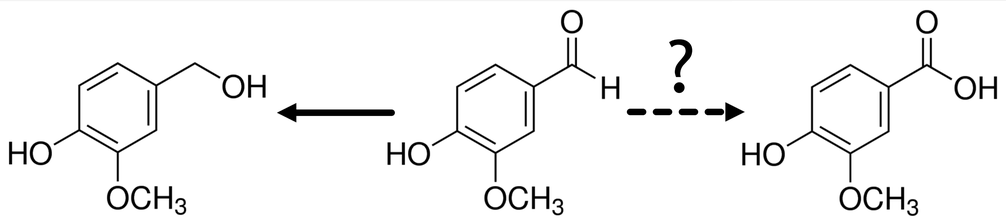


**Figure 1** Biodegradation of vanillin into vanillyl alcohol and possibly into vanillic acid.

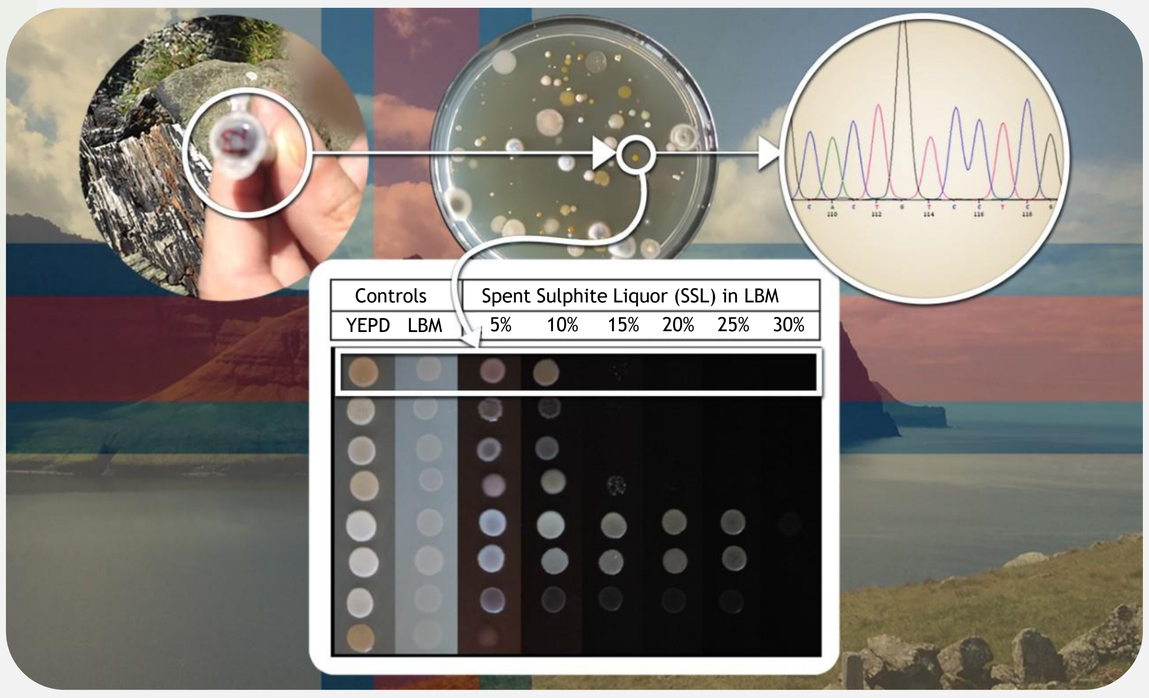


**Figure 2** Flow chart describing the sampling, identification and cultivation of the yeast strains in lignin basal medium (LBM) supplemented with SSL.

## MATERIALS & METHODS

The yeast strains used in the present study were isolated from decaying wood from the Faroe Islands and identified by ITS1-5.8S-ITS2 and D1/D2 sequences comparisons. Tolerance to spent sulphite liquor (SSL) was evaluated by cultivation in LBM supplemented with distinct volumes of SSL, resulting in concentrations of 5%, 10%, 15%, 20%, 25% and 30% of SSL. Vanillin (VA) tolerance was evaluated by cultivating the yeasts in Lilly-Barnett (LiBa) medium supplemented with 1 mM VA[3]. Growth in VA was assessed after 48 h by visual determination of cell mass of centrifuged samples. VA biodegradation products in supernatants were determined by LC/Q-TOF MS.

## CONCLUSIONS

Many of the yeast species described herein are associated with wood and/or maritime cold environments. The strains that grew at the highest concentration of SSL also grew well in the presence of vanillin, from which they rapidly produced large amounts of vanillyl alcohol, and many other biodegradation products. Conversely, the isolates with poor or no growth in vanillin had extremely low or no tolerance to SSL. Thus, high tolerance to vanillin appears to be a prerequisite for growth in SSL-based medium. The presence of specific inhibitors can be limiting to growth of specific yeasts.

## RESULTS

A comprehensive analysis of growth in VA and SSL, and of vanillin biodegradation ability resulted in five groups (**I** - **V**), containing specific yeast genera. Strains of *Debaryomyces* sp., *Candida sake* and *Candida argentea* (**Group I**), *Goffeauzyma* sp., *Naganishia albidosimilis* and *Naganishia onofrii* (**Group II**), *Cystofilobasidium infirmominiatum* and *Rhodotorula* sp. (**Group III**), *Cystobasidium laryngis* (**Group IV**), *Holtermanniella takashimae* and *Nadsonia starkeyi-henricii* (**Group V**) were identified. Most strains were able to grow in LBM supplemented with SSL or in LiBa supplemented with 1 mM VA. Species of *Candida* and *Debaryomyces* were the most tolerant, able to grow at 25-30% SSL, whereas species of *Nadsonia* and *Holtermanniella* grew poorly. Two strains of *Naganishia* grew well on 1 mM VA but did not tolerate high concentrations of SSL (Table 1). The major biodegradation product was vanillyl alcohol (VAL) followed by vanillic acid (VAA), as evidenced by LC/QTOF MS. Several other biodegradation products were also observed (Figure 3). $^{1}$H-NMR was previously used to identify the biodegradation product VAL in *C. laryngis* FMYD002[3].

**Table 1** Subset of yeasts tested for biotolerance to Spent Sulphite Liquor (SSL) and vanillin (VA).

| | | Biotolerance (BT) to SSL[a] | | | | | | | BT to VA[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **Strain** | LBM | 5% | 10% | 15% | 20% | 25% | 30% | LiBa | 1 mM | **Gr.** |
| ***Candida argentea*** | FGAA004 | w | + | + | + | + | w | – | ++++ | +++ | **I** |
| ***Debaryomyces sp.*** | FTJA004 | w | + | + | + | + | + | w | +++ | +++ | **I** |
| ***Debaryomyces sp.*** | FLYA002 | w | + | + | + | + | + | – | ++++ | +++ | **I** |
| ***Naganishia albidosimilis*** | FXXA004 | w | + | w | – | – | – | – | +++++ | ++++ | **II** |
| *Naganishia onofrii* | FMYH004b | w | + | w | – | – | – | – | ++ | ++ | **II** |
| *Holtermanniella takashimae* | FBOC004 | w | + | – | – | – | – | – | + | – | **V** |
| *Nadsonia starkeyi-henricii* | FAEA002 | w | w | – | – | – | – | – | + | – | **V** |
| *N. starkeyi-henricii* | FAEA004 | w | w | – | – | – | – | – | + | – | **V** |

[a]Growth on LBM agar plates containing 2.0 g glucose $l^{-1}$; LBM agar plates supplemented with spent sulphite liquor (SSL) at increasing concentrations; [b]LiBa supplemented with 0.15 g vanillin $l^{-1}$; growth visually evaluated from pelletted 48 h culture; BT, Biotolerance; (SSL experiment): +, Growth; w, Weak Growth; –, No Growth; (VA experiment): Growth in descending order with +++++ is maximum cell volume of pellet; Yeast in bold had high tolerance to VA and/or SSL. Each Roman numeral corresponds to a seperate biotolerance/biodegradation group.

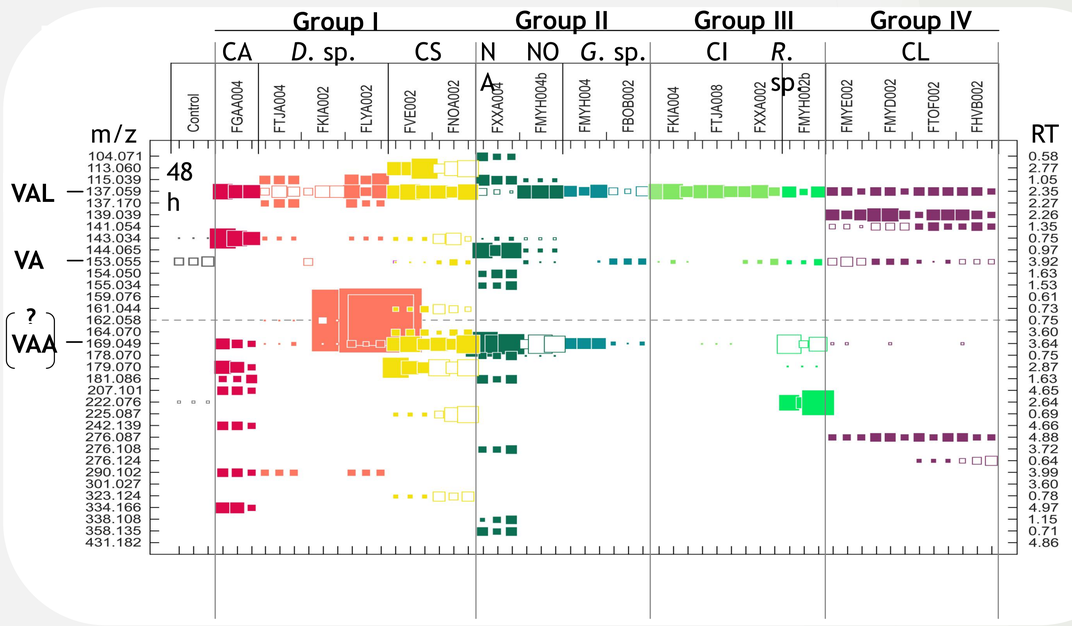


**Figure 3** Biodegradation product profiles, detected by LC/Q-TOF MS, which were produced by yeasts cultured for 48 h in vanillin-supplemented LiBa medium. CA, *Candida argentea*; *D* sp., *Debaryomyces* sp.; CS, *Candida sake*; NA, *Naganishia albidosimilis*; NO, *Naganishia onofrii*; *G.* sp., *Goffeauzyma* sp.; CI, *Cystofilobasidium infirmominiatum*; *R.* sp., *Rhodotorula* sp.; CL, *Cystobasidium laryngis*. Roman numerals corresponds to seperate biodegradation/biotolerance groups. Group V (not included) did not grow in vanillin-supplemented LiBa. VAL, vanillyl alcohol; VA, vanillin; VAA, vanillic acid.

## ACKNOWLEDGEMENTS

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