EXECUTIVE SUMMARY

This report summarises research activities in the selection of wine yeasts and malolactic bacteria for desirable beta-glycosidase activity and sensory enhancement of wines.

Glycosidase activity of wine yeasts and wine bacteria was found to be principally due to β-glucosidase. Some α-arabinosidase and α-rhamnosidase activity was also detected but this was relatively minor compared to the activity of β-glucosidase. A series of wine microorganisms (87 yeast and 105 bacterial strains) were screened for β-glucosidase activity using a β-glucoside analogue (4-methylumbelliferyl-β-D-glucopyranoside) incorporated into agar plates. This method gave a visual indication of β-glucosidase activity. The majority of the organisms tested exhibited some β-glucosidase activity.

The agar plate method using 4-methylumbelliferyl-β-D-glucopyranoside as a substrate only provides a qualitative estimation of β-glucosidase activity. A quantitative assay using the β-glucoside analogue, ρ-nitrophenyl-β-D-glucopyranoside, (pNPG) was therefore developed in liquid culture. Lactic acid bacteria generally had higher activities of β-glucosidase than the wine yeasts examined although the fastidious nutritional requirements of lactic acid bacteria prevented direct comparisons to be made.

β-glucosidase activity was found to be pH dependent, with little activity below a pH of 3.0 and maximum activity between pH 3.5 and 4.0. β-Glucosidase activity of all the wine yeasts screened with the exception of *Saccharomyces cerevisiae* was affected by ethanol in the incubation medium.

Glucose in the incubation medium inhibited the expression of β-glucosidase activity in all of the organisms studied. This effect was particularly evident in cultures of *Saccharomyces cerevisiae.*

Grape glycosides were purified from grape juice by passage down a C18 column and added to a chemically defined grape juice medium. Fermentations were then carried out and the glycosidase activity of wine yeasts assessed by determining the decrease in the glycosyl-glucose content of the juice using the GG assay. *H. anomala* and *S cerevisiae* had the greatest glycosidase activity in this medium, followed by *K. apiculata*. *C. pulcherima* had the weakest glucosidase activity.

Differences in glycosidase activity may have the potential to be exploited by the wine industry for the release of flavour and aroma compounds of wine that are glycosidically bound. The potential to use these non-*S. cerevisiae* enzymes-systems in wine production techniques requires further investigation.

PROJECT OVERVIEW

Research Venue:
National Wine and Grape Industry Centre, Wagga Wagga and Australian Wine Research Institute, Adelaide.

Research staff:

Dr Hong N Jin, Charles Sturt University	1992 – 1993
Assoc. Prof. B. Freeman, Charles Sturt University	1992 – 1993
Dr Bryan Todd, Charles Sturt University	1995 – 1997
Mr Charoen Charoenchai, Charles Sturt University	1995
Ms Mation Kater, Charles Sturt University	1996
Ms Jo Hatfield, Charles Sturt University	1997 – 1998
Dr Chris Steel, Charles Sturt University	1998
Dr Leigh Francis, AWRI	1997 – 1998
Dr Paul Henschke, AWRI	1992 – 1998

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Project Dates and funding:

Funding commenced in July 1992 and concluded in December 1998. During this period, there were several interruptions to the project due to staff departures in 1993 and 1997.

Funds provided by the GWRDC were:

1992/93	$15,000
1993/94	$28,170
1994/95	$15,000
1996/97	$31,848

Project Objectives:

- Survey of β-glycosidase activity in wine yeasts and malolactic bacteria originating from commercial sources and the culture collections of CSU, AWRI, UNSW and UA.
- Evaluate selected wine microorganisms for their potential to enhance wine flavour and aroma.
- Examination of the factors affecting β-glycosidase activity in selected microorganisms,
- Evaluate desirable glycosidases from selected wine microorganisms and assess their potential for releasing glycosidically bound flavour components during the wine making process.

Outcomes:

- Development of an agar based assay to screen wine yeast and lactic acid bacteria glycosidase activity.
- Development of a quantitative assay for determining glycosidase activity in wine yeast and lactic acid bacteria.
- Identification of non-*Saccharomyces* yeasts with high β-glucosidase activity.
- Identification of lactic acid bacteria with high β-glucosidase activity.
- β-Glucosidase identified as the main glycosidase activity detected in all wine microorganisms that were examined.
- Effects of pH, ethanol and glucose content on β-glucosidase activity determined.
- Glycosidase activity of selected wine yeasts was determined in model grape juice media.

DETAILED PROJECT REPORT

Background:
It is now well-established that many flavour and aroma-active compounds (such as monoterpenes, C_{13}-norisoprenoids and shikimic acid-derived metabolites) exist in grape juice and wine as glycosidically-bound precursors with no sensory properties. These precursors may be hydrolysed by various methods resulting in the liberation of volatile components that have been shown to improve both the flavour and aroma of wines. The close relationship between the glycoside content of grapes and resulting wine quality has led to the development of techniques to assess grape quality by determining the total glycoside content of the juice. This potential for enhancement of wine quality has therefore generated considerable industry interest in grape glycosides.

Hydrolysis of grape glycosides may be achieved by acid-hydrolysis, thermal processing, and treatment with commercial glycosidase enzymes. Acid-hydrolysis and thermal processing of wines represent added processing steps with associated add-on costs. Furthermore, acid-hydrolysis of wine glycosides can readily give aroma compounds different to those occurring as natural free volatiles. Alternatively wine microorganisms can cleave glycosidic linkages enzymatically. The glycosidase activity of both wine yeasts and wine bacteria is the subject of this study.

Screening of wine microorganisms for β-glucosidase activity using an agar plate assay method:
Culture collections of wine microorganisms were screened for β -glucosidase activity using indicator agar plates containing a β-glucoside analogue (4-methylumbelliferyl-β-D-glucopyranoside) as a carbon source. The hydrolysis of this compound via β-glucosidase activity results in the formation of 4-methylumbelliferone, a fluorescent compound that can be observed under long wave UV light. Using this medium, 87 yeast and 105 bacterial strains were assayed for β-glucosidase activity (Tables 1 and 2, Appendix 1). In general, it was found that *Saccharomyces cerevisiae* appeared to require glucose in the medium before appreciable enzyme activity was observed. Notably, several non-*Saccharomyces* yeasts such as *Candida* and *Hansenula* appeared to be higher producers of β-glucosidase activity than *Saccharomyces cerevisiae*. Selected lactic acid bacteria were also found to possess β-glucosidase activity, although the presence of glucose in the medium appeared to inhibit expression of the enzyme.

β -glucosidase activity of wine yeasts and wine bacteria using (ρ-nitrophenyl-β-D-glucopyranoside, (pNPG) as a substrate:
In order to confirm the qualitative data obtained using the plate assay described above, methods were developed to quantify β-glucosidase activity. Initially, attention was directed towards isolating the enzyme for quantitative assays. However, the enzyme was found to be extremely unstable upon isolation, so the focus was changed towards relating β-glucosidase activity to hydrolysis of a β-glucoside analogue (ρ-nitrophenyl-β-D-glucopyranoside, pNPG) on a dry cell weight basis in a chemically defined fermentation medium. To compare the enzyme activity in yeasts and bacteria, it was considered necessary to ensure that both types of microorganisms were assayed in the same medium. Unfortunately this was not successful in this project due to the fastidious growth requirements of lactic acid bacteria. However, results indicated that wine bacteria express higher levels of β-glucosidase activity on a per dry cell weight basis than wine yeasts.

β -glucosidase activity of wine yeasts:
Strains of non-*Saccharomyces* yeasts (including *Candida pulcherimma*, *Kloeckera apiculata*, and *Hansenula anomala*) expressed significantly higher levels of β -glucosidase activity than the strains of *Saccharomyces cerevisiae* studied (on a per dry cell weight basis). This brings into

question the role that such yeasts play in flavour and aroma development through β -glucosidase activity during the early stages of wine fermentation where they are most active. A further consideration is the
significant concentrations of glucose present early in fermentation which could affect expression of β -glucosidase activity.

β -glucosidase activity of lactic acid bacteria:
Quantitative assays of β -glucosidase activity in wine lactic acid bacteria were initially problematic because of the fastidious nutritional requirements of the organisms involved. However, after trialing a number of different formulations, strains of *Leuconostoc oenos*, *Pediococcus cerevisiae*, *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Lactobacillus hilgardii* were assayed for expression of β -glucosidase activity in the presence and absence of glucose. As observed with yeasts, glucose inhibited expression of β -glucosidase activity in all wine bacteria examined. In the absence of glucose, however, several bacterial strains (belonging to *Lactobacillus plantarum*, *Pediococcus cerevisiae*, and *Leuconostoc oenos*) exhibited approximately 5 to 10 times the level of β- glucosidase activity compared to strains of *S. cerevisiae* (on a per dry cell weight basis). It can be seen, therefore, that these bacteria have the potential to play a significant role in flavour and aroma development via β-glucosidase activity, particularly in MLF conducted after the primary fermentation when the concentration of glucose is relatively low.

Effect of glucose concentration on β-glucosidase activity:
The effect of glucose on β-glucosidase activity was evaluated in a series of wine fermentations in a defined grape juice formulation. Liquid culture assays for the yeast showed that some organisms, (particularly *S. cerevisiae*), exhibited excessive amounts of growth in the presence of sugar; this growth is associated with a mediocre level of β -glucosidase activity. Analysis of this data where activity is related to growth indicated that the presence of glucose in fact inhibits β -glucosidase activity in all the wine yeasts examined. This inhibition of ß-glucosidase activity is significant at a glucose concentration of 5% (w/v). This needs to be considered in view of the fact that there are significant concentrations of glucose present during the early stages of fermentation which could prevent expression of β -glucosidase activity.

Effect of pH and ethanol on β-glucosidase activity:
The expression of β-glucosidase activity by wine microorganisms increases between pH 2.8 and 4.5. Trials conducted with *Saccharomyces cerevisiae* showed that β-glucosidase activity was virtually non-existent at pH values less than 3.0, while the greatest increase in activity was observed between pH 3.5 and 4.0. A similar effect was observed in selected strains of non-*Saccharomyces* yeasts (*Hansenula anomala*, *Kloeckera apiculata*, and *Candida pulcherimma*) and lactic acid bacteria (*Leuconostoc oenos*, *Pediococcus cerevisiae*, and *Lactobacillus plantarum*)

The effect of ethanol (5% v/v) on β-glucosidase activity of wine yeasts varied according to the species. In *S. cerevisiae*, the effect of ethanol on β-glucosidase activity was negligible. For non-*Saccharomyces* yeasts, however, the results were more variable with approximately 40% of activity lost with strains of *H. anomala* and *Kl. apiculata* while a strain of *C. pulcherimma* exhibited a 90% loss in β-glucosidase activity. Nevertheless, the activity expressed by these yeasts in ethanol was still greater than that produced by *S. cerevisiae* under the same conditions.

This suggests that the β-glucosidase activity of non-*Saccharomyces* yeasts could play a more significant role in flavour release during fermentation than previously thought. Fermentations were therefore conducted with these yeasts (*H. anomala*, *Kl. apiculata* and *C. pulcherimma*) and *S.cerevisiae* in a simulated grape juice containing a β-glucoside analogue. The results of these fermentations confirmed the trends indicated by the assays. Even under winemaking conditions (where the greatest limiting factor is the presence of reducing sugars) the non-*Saccharomyces* yeasts were still able to express β-glucosidase activity in significant quantities. For example,

strains of *H. anomala* and *Kl. apiculata* hydrolysed up to 1.5 times the quantity of the β-glucoside analogue compared to *S. cerevisiae* after seven days of fermentation.

Unfortunately, obtaining similar data for lactic acid bacteria during malolactic fermentation proved extremely problematic due to difficulties in formulating a defined medium (that closely resembles wine) in which the selected strains exhibit satisfactory growth, so this aspect of the project was not completed.

Assay of glycosidase activity other than β-glucosidase:
Quantitative assay of other glycosidase activities in both wine yeasts and bacteria also included determination of α-arabinosidase and α-rhamnosidase activity. In both groups of organisms, α-arabinosidase and α-rhamnosidase were found to be expressed in relatively low levels compared to β-glucosidase activity. The only exception to this general observation were two strains of *Kloeckera apiculata* that possessed α-arabinosidase activity in the same order of magnitude as their β-glucosidase activity. However, the presence of glucose in the assay medium was found to reduce any α-arabinosidase and α-rhamnosidase activity to negligible levels in all wine microorganisms studied. It would appear, therefore, that β-glucosidase activity is the major glycosidase activity in the wine microorganisms studied.

Hydrolysis of grape glycosides:
Glucosidase activity was assayed in a number of wine yeasts using grape glycosides, purified from grape juice and incorporated into a chemically defined grape juice (CDGJ) medium. Glucosidase activity was determined on the basis of a decrease in the G-G (μmoles) content of the juice. The glycosidic fraction from grape juice was purified by passing a known volume of juice through a C18 reverse phase extraction column. Grape glycosides were eluted from the column using ethanol and the ethanol collected taken to dryness by rotary evaporation. The glycosides were dissolved in CDGJ medium using a volume equivalent to the original starting juice volume. Fermentations were carried out using 200 ml volumes of this medium at 25 ^{o}C. The cultures were periodically sampled (20ml volume removed aseptically and filter sterilised) and analysed at the AWRI for Brix and G-G.

The glucosidase activity of two strains of *S. cerevisiae*, and one strain each of *H. anomala, Kl. apiculata* and *C. pulcherima* were compared using grape glycosides in the artificial grape juice medium. Three separate fermentations were carried out, and on each occasion the two strains of *S. cerevisiae* (V1118 & HB 350) had the greatest glucosidase activity reducing the G-G content by 23% and 39% respectively during the growth phase with a further reduction of approximately 5% during the stationary phase.

The glucosidase activity of *H. anomala* 209 was comparable with that of *S. cerevisiae*, the G-G content was reduced by 30% during the growth phase with no further reduction during the stationary phase. *Kl. apiculata* 401 had moderate glucosidase activity, the G-G content decreased by 8% during the growth phase, but during the stationary phase the G-G content was further reduced by 19%. The G-G content of the *C. pulcherima* 2000 culture remained largely unchanged, however the change in Brix was comparable with that of the *Kl. apiculata* and *H. anomala* cultures.

As discussed above, glucosidase activity is dependent upon the concentration of free sugar present in the medium. Despite this differences in glycosidase activity were still apearant in the artificial grape juice medium, even though the glucose concentration was 20% w/v.

Scientific Publications – See appendix 2.
C. Charoenchai, G. H. Fleet, P. A. Henschke and B. E. N. Todd 1997.
Screening of non-*Saccharomyces* wine yeasts for the presence of extracellular hydrolytic enzymes.
Australian Journal of Grape and Wine Research **3**, 2 – 8.

Appendix 1 Survey of β-glucosidase activity in wine yeasts and bacteria

This Appendix contains experimental data detailing the results of a survey of β-glucosidase activity in wine yeasts (Table 1) and bacteria (Table 2).

Table 1 Detection of β-glucosidase activity in wine yeasts using an agar plate based system

Species	**Strain**	**Glucose in medium (%)**	
		0	**0.5**
Sacch. cerevisiae	70-13	+	++
Sacch. cerevisiae	704000	-	+
Sacch. cerevisiae	706900	-	+
Sacch. cerevisiae	707600	-	+
Sacch. cerevisiae	707900	-	+
Sacch. cerevisiae	Assmanshauser	-	+
Sacch. cerevisiae race *chevalieri*	AWRI 5A	-	+
Sacch. cerevisiae race *bayanus*	AWRI 1A	-	+
Sacch. cerevisiae	AWRI 727	+	+
Sacch. cerevisiae	AWRI 729A	-	+
Sacch. cerevisiae	AWRI 3A	+	++
Sacch. cerevisiae	AWRI 797	-	+
Sacch. cerevisiae	Beerenauslesse	-	++
Sacch. cerevisiae	Chandon	-	++
Sacch. cerevisiae	Enoferm Bordeaux Red	-	+
Sacch. cerevisiae	Enoferm Burgundy	-	+
Sacch. cerevisiae	Enoferm Simi white	+	++
Sacch. cerevisiae	EP	-	+
Sacch. cerevisiae	Geisenheim EP-XB	+	+
Sacch. cerevisiae	82/WI	-	++
Sacch. cerevisiae	83/WI	-	+
Sacch. cerevisiae	L2226	+	+
Sacch. cerevisiae race *bayanus*	Lalvin EC1118	+	++
Sacch. cerevisiae	Lalvin ICV D47	-	+
Sacch. cerevisiae	Lalvin M2	-	+
Sacch. cerevisiae	Lalvin V-1116	-	+
Sacch. cerevisiae	Lalvin Zymaflore VL1	-	+
Sacch. cerevisiae	LW 692	+	++
Sacch. cerevisiae	N96	-	++
Sacch. cerevisiae	NS2	-	++
Sacch. cerevisiae	Pasteur Red	-	++
Sacch. cerevisiae	R104	-	+
Sacch. cerevisiae	R108	-	+
Sacch. cerevisiae	R109	-	+
Sacch. cerevisiae	R93	-	+
Sacch. cerevisiae	Rahoul-2	-	+
Sacch. cerevisiae	Red Star Epernay 2	-	+
Sacch. cerevisiae	Red Star Montrachet	-	+
Sacch. cerevisiae	SIHA-3	-	+
Sacch. cerevisiae race *bayanus*	SIHA-4	-	+
Sacch. cerevisiae race *bayanus*	SIHA-5	-	++

Table 1 Detection of β-glucosidase activity in wine yeasts using a agar plate based system (continued)

Species	Strain	Glucose in medium (%)	
		0	0.5
Sacch. cerevisiae	SIHA-7	-	+
Sacch. cerevisiae	SIHA-8	-	+
Sacch. cerevisiae	Steinberg (German)	-	++
Sacch. cerevisiae	Steinberg-12	-	+
Sacch. cerevisiae	TY 508	-	++
Sacch. cerevisiae	UCD 505	-	+
Sacch. cerevisiae	V8-6	-	++
Sacch. cerevisiae	Weinburg	-	++
Sacch. cerevisiae	Zinfandel	-	+
Sacch. cerevisiae	CUB Brewer's yeast	-	++
Sacch. cerevisiae	SB-1	-	+
Sacch. cerevisiae	3002	-	++
Sacch. cerevisiae	806	-	++
Sacch. cerevisiae	914	+	++
Sacch. cerevisiae	411	-	++
Sacch. cerevisiae	921	-	++
Sacch. cerevisiae	663	-	-
Sacch. cerevisiae	100	+	++
Sacch. cerevisiae	5A	-	++
Sacch. cerevisiae	TYR 303	-	+
Sacch. cerevisiae	2H-Y1	-	++
Sacch. cerevisiae	516	-	+
Sacch. cerevisiae	303	-	+
Sacch. cerevisiae	HB350	+	+
Sacch. cerevisiae	V1118	+	+
Kl. Apiculata	401	+	-
Kl. Apiculata	610	+	-
Kl. Apiculata	4011	++	++
Kl. Apiculata	8004	+	++
C. famata	6010	+	+
C. famata	618	-	+
C. famata	817	+	++
C. colliculosa	207	-	+
C. colliculosa	9003	-	+
C. colliculosa	3007	+	+
C. colliculosa	502		
C. stellata	800	+	+
C. stellata	8008	-	+
C. stellata	504	+	++
C. pulcherrima	1000	++	+
C. pulcherrima	2000	+	+
H. anomala	209	++	+++
H. anomala	703300	++	+++
T'spora delbrueckii	6005	-	+
T'spora delbrueckii	9512	-	+
C. kruseii	304	-	+

Table 2 Detection of β-glucosidase activity in wine bacteria using an agar plate based system

Species	**Strain**	**Glucose in medium (%)**	
		0	**0.5**
Leuconostoc oenos	273	++	+
Leuconostoc oenos	CSU B921	+	-
Leuconostoc oenos	CSU B923	+	-
Leuconostoc oenos	CSU B924	++	+
Leuconostoc oenos	CSU B925	-	-
Leuconostoc oenos	CSU B926	+	-
Leuconostoc oenos	Lalvin MCW	++	+
Leuconostoc oenos	Lalvin MT01	-	-
Leuconostoc oenos	Lalvin OSU	+	-
Leuconostoc oenos	LW 921	++	+
Leuconostoc oenos	LW 936	++	+
Leuconostoc oenos	LW 977	+	-
Leuconostoc oenos	LW 980	++	-
Leuconostoc oenos	Oenos	++	+
Leuconostoc oenos	L042	+	-
Leuconostoc mesenteroides	Lclc	++	+
Leuconostoc mesenteroides	Lc1d	-	-
Leuconostoc mesenteroides	Lc1e	+	+
Leuconostoc mesenteroides	Lc1g	+	-
Leuconostoc mesenteroides	Lc1h	++	-
Leuconostoc oenos	Lc5d	+	-
Leuconostoc oenos	Lc5e	++	+
Leuconostoc oenos	Lc5f	++	++
Leuconostoc oenos	Lc5h	++	+
Leuconostoc oenos	Lc5k	+	-
Leuconostoc oenos	Lc5l	++	+
Leuconostoc oenos	Lc5o	++	-
Leuconostoc oenos	Lc5s	+	+
Leuconostoc oenos	Lc5t	-	-
Leuconostoc oenos	Lc5u	+	-
Leuconostoc oenos	Lc5v	+	+
Leuconostoc oenos	Lc5w	++	+
Leuconostoc oenos	Lc5x	+	+
Leuconostoc oenos	Lc5y	+	+
Leuconostoc oenos	Lc5z	++	++
Leuconostoc oenos	Lc5aa	++	+
Leuconostoc oenos	Lc5ba	++	+
Leuconostoc oenos	Lc5ca	+	-
Leuconostoc oenos	Lc5da	++	-
Leuconostoc oenos	Lc5ea	+	-
Leuconostoc oenos	Lc5fa	+	-
Leuconostoc oenos	Lc5ga	++	+
Leuconostoc oenos	Lc5ia	-	-
Leuconostoc oenos	Lc5ja	++	+
Leuconostoc oenos	Lc5ka	++	-
Leuconostoc oenos	Lc5na	++	+
Leuconostoc oenos	Lc5oa	++	+
Leuconostoc oenos	Lc5pa	++	+
Leuconostoc oenos	Lc5qa	+	+
Leuconostoc oenos	Lc5ra	++	+
Leuconostoc oenos	Lc5sa	+	-

Table 2 Detection of β-glucosidase activity in wine bacteria using an agar plate based system (continued)

Species	**Strain**	**Glucose in medium (%)**	
		0	**0.5**
Leuconostoc oenos	Lc5ta	+	-
Leuconostoc oenos	Lc5ua	+	-
Leuconostoc oenos	Lc5va	+	-
Leuconostoc oenos	Lc5wa	+	+
Leuconostoc oenos	Lc5xa	-	-
Leuconostoc oenos	Lc5cb	+	-
Leuconostoc oenos	Lc5db	++	+
Leuconostoc oenos	Lc5gb	++	+
Leuconostoc oenos	Lc5hb	++	+
Leuconostoc oenos	Lc5ib	++	+
Leuconostoc oenos	Lc5jb	++	+
Leuconostoc oenos	Lc5nb	++	+
Leuconostoc oenos	B203	++	+
Leuconostoc oenos	B202	++	+
Leuconostoc oenos	Er1a	++	+
Leuconostoc oenos	Lc023-R	++	+
Leuconostoc oenos	Lc-S1.1-R	++	+
Leuconostoc oenos	Ey2d	+	+
Leuconostoc oenos	Lc8303.2	++	-
Leuconostoc oenos	Lc8323	++	-
Leuconostoc spp	Lc5kb	+	+
Leuconostoc spp	Lc5lb	++	+
Leuconostoc spp	Lc5mb	++	+
Pediococcus cerevisiae	P1a	++	+
Pediococcus cerevisiae	P1b	+	-
Pediococcus cerevisiae	P1c	++	+
Pediococcus parvulus	P6a	++	-
Pediococcus parvulus	P6b	++	+
Pediococcus parvulus	P6c	++	-
Pediococcus parvulus	P6d	+	-
Pediococcus parvulus	P6f	++	-
Pediococcus parvulus	P6g	++	+
Pediococcus parvulus	P6h	++	-
Pediococcus parvulus	P6i	+	-
Pediococcus parvulus	P6k	+	-
Pediococcus parvulus	P6l	+	-
Pediococcus parvulus	P6m	++	+
Lactobacillus plantarum	L11a	++	+
Lactobacillus plantarum	L11a1	+	+
Lactobacillus fermentum	L15b	-	-
Lactobacillus brevis	L17d	-	-
Lactobacillus brevis	L17a1	-	-
Lactobacillus hilgardii	L21b	+	-
Lactobacillus hilgardii	L21c	-	-
Lactobacillus hilgardii	L21e	-	-
Lactobacillus hilgardii	L21f	-	-
Lactobacillus hilgardii	L21g	-	-
Lactobacillus hilgardii	L21h	++	+
Lactobacillus spp	L26a	+	-

Table 2 Detection of β-glucosidase activity in wine bacteria using an agar plate based system (continued)

Species	**Strain**	**Glucose in medium (%)**	
		0	**0.5**
Lactobacillus spp	L26b	+	-
Lactobacillus spp	L26c	-	-
Lactobacillus spp	L26d	-	-
Lactobacillus spp	L26e	-	-
Lactobacillus spp	L8249	++	+

Appendix 2 -Publication

C. Charoenchai, G. H. Fleet, P. A. Henschke and B. E. N. Todd 1997.

Screening of non-*Saccharomyces* wine yeasts for the presence of extracellular hydrolytic enzymes.

Australian Journal of Grape and Wine Research **3**, 2 – 8.