Industry allocated project number	PHI allocated project number

SATI	CFPA	SAAPPA/SASPA	DFTS	Winetech
tarryn@satgi.co.za Tel: 021 863-0366	inmaak@mweb.co.za Tel: 021 872-1501	theresa@hortgro.co.za Tel: 021 882-8470	dappies@dtd.co.za Tel: 021 870 2900	andraga@winetech.co.za Tel: 021 276 0499
				X

Indicate (X) client(s) to whom this concept project proposal is submitted. Replace any of these with other relevant clients if required.

NB: The instructions in red, throughout the template, should be omitted from the final document.

FINAL REPORT
(2017)

1. PROGRAMME AND PROJECT LEADER INFORMATION

	Research Organisation Programme leader	ARC Research Team Manager	Project leader
Title, initials, surname	Prof. Maret du Toit		Prof. Maret du Toit
Present position	Professor		Professor
Organisation, department	IWBT, US		IWBT, US
Tel. / Cell no.	021 808 3772		021 808 3772
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	mdt@sun.ac.za		mdt@sun.ac.za

2. PROJECT INFORMATION

Research Organisation Project number	SU **IWBT-B 11-02**
Project title	Understanding citric acid and ester metabolism – producing fruitier wines
Short title	MLF- Fruity and buttery wines

Fruit kind(s)	Wine		
Start date (mm/yyyy)	01/01/2012	**End date** (mm/yyyy)	31/12/2016

Key words	Malolactic fermentation; citric acid metabolism; diacetyl; ester; wine aroma

Approved by Research Organisation Programme leader (tick box) ☐

THIS REPORT MUST INCLUDE INFORMATION FROM THE **ENTIRE** PROJECT

3. EXECUTIVE SUMMARY

*This must report on the **ENTIRE** project. Address the objectives and milestones of the project as well as the impact of the study on the industry, **not exceeding 500 words.** You can overtype the example.*

Objectives & Rationale

We assessed the impact of different wine parameters related to MLF on the production of buttery and fruity aromas. The ultimate aim is to generate knowledge in order to promote the production of diacetyl if a buttery style is needed or if not to enhance the fruity character of wine using different MLF inoculation scenarios or different MLF starter species.

Methods

The effect of different wine parameters (glucose and fructose concentration, and pH) on the metabolism of citrate in *Lactobacillus plantarum* and *Oenococcus oeni* by focussing on the concentration of end products from the citrate pathway using GC-MS, the transcriptional response of the citrate pathway genes using qPCR, as well as the relationship between malic and citric acid utilization using spectrophotometric automated analysis. The two species were also tested in Chardonnay using different MLF inoculation strategies. The two species were also screened for the presence of esterase genes and also the enzyme activity using different wine parameters.

Key Results

- Genetic screening of the presence of three different esterase genes in *L. plantarum* and *O. oeni* showed that there was huge strain diversity with some strains containing all three genes and other containing only 1. The impact of pH on esterase activity showed that pH 5 was the most optimal with pH 3 showing strain dependent activity for *L. plantarum*. In contrast *O. oeni* displayed the highest activity at pH 7 and 8. The impact of temperature indicated that *L. plantarum* had higher activity at 30-40°C while *O. oeni* was between 20-30°C. Higher ethanol levels induced the esterase activity in both species. Co-inoculation yielded more esters and higher alcohols, especially ethyl lactate and 2-phenylethanol, but less diacetyl.
- From the citrate gene expression results obtained for all the treatments showed that glucose had the smallest influence on citrate metabolism. In the glucose treatments, the cit^- *O. oeni* strain did not consume citrate and therefore produced trace amounts of diacetyl and acetoin. Both the *Lb. plantarum* strains partially consumed citrate, however, the cit^+ *Lb. plantarum* strain produced more diacetyl and acetoin in all the glucose treatments. In the fructose treatments, the cit^- *O. oeni* strain did not consume any citrate and the cit^- *Lb. plantarum* strain partially consumed citrate. The cit^+ *O. oeni* strain consumed more citrate and produced more diacetyl and acetoin in the pH 3.0 and 3.5 treatments than in the pH 4.0 and 5.0 treatments. The *Lb. plantarum* strains consumed more citrate and therefore produced more diacetyl and acetoin in the pH 3.5, 4.0 and 5.0 treatments than in the pH 3.0 treatment.
- The citrate negative strains did not degrade citrate and had lower D-lactate and acetic acid concentrations than the citrate positives LAB strains. The different LAB strains had different diacetyl and acetoin concentrations with the two *L. plantarum* producing higher concentrations of these compounds than the two *O. oeni* strains in co-inoculation of Chardonnay.

Conclusion/Discussion

It can be conclude from the esterase screening and activity assay that many *L. plantarum* and *O. oeni* strains have some of the genes and that the activity of the gene differs. Therefore if this function is important from a starter culture perspective the evaluation on a strain level is crucial and this could explain the great variance in starter culture ability to produce esters and diacetyl. MLF co-inoculation therefore yields more fruitier wines. The differences from a fruit and buttery perspective was LAB strain dependent but also the yeast strain played a crucial role.

4. PROBLEM IDENTIFICATION AND OBJECTIVES

State the problem being addressed and the ultimate aim of the project.

Lactic acid bacteria have the potential to impact wine aroma and flavour compounds through the production of secondary metabolites. Two of the metabolic pathways that have an indirect or direct impact on fruity characteristics of wine are citric acid metabolism and the production of esters. In a previous project, we have identified LAB strains that have different combinations of enzyme-encoding genes related to these two metabolic pathways. Since the production of diacetyl can negatively impact on the perception of fruitiness in wine, it is important to understand the functioning of these enzymes and how we can reduce the production of diacetyl. Moreover, it is not clear whether and/or in which conditions extracellular esterase produce or hydrolyse esters.

The ultimate aim is to generate knowledge on the functioning of the genes and corresponding enzymes of the citric acid metabolic pathway and the production or hydrolysis of esters under various winemaking conditions in order to promote a more favourable wine aroma. Understanding the regulation of the genes involved in the citric acid metabolic pathway and the factors that influence the functioning of the enzymes will allow us to manipulate the production of diacetyl and therefore enhance the fruity character of wine. This is especially relevant in the production of Chardonnay where MLF is needed to enhance fruitiness, but where high levels of a buttery character are not always wanted. If more esters can be produced by the esterase activity of LAB during MLF, this can enhance the fruitiness of white and red wines.

5. DETAILED REPORT

Completion of a, b, c and d is obligatory.

a. PERFORMANCE CHART (for the duration of the project)

Milestone	Target Date	Extension Date	Date completed
1. Gene expression of the citrate pathway in *Lactobacillus plantarum* and *Oenococcus oeni* as a function of citrate concentration, pH, ethanol and oxygen to control diacetyl production in wine	2013	2015	Dec 2016
2. Elucidate differences in diacetyl production by *Oenococcus oeni* and *Lactobacillus plantarum* in Chardonnay vinified by sequential alcoholic/malolactic fermentation and simultaneous alcoholic/malolactic fermentation	2014		Dec 2014
3. Correlate the expression esterase genes under winemaking conditions with ester hydrolysis and synthesis in wine	2014	2015	Dec 2015
6. Journal publication(s) – final milestone *(list intended Peer Reviewed and Popular/Semi-scientific publications.*			

Completed publications to be listed in detail under point 6d) Pretorius et al. 2018 Evaluation of citrate metabolism in *Oenococcus oeni* and *Lactobacillus plantarum* strains under different conditions. SAJEV Botma et al. Influence of wine parameters on ester production. WineLand			2018 Jul 2018

b) WORKPLAN (MATERIALS AND METHODS)

List trial sites, treatments, experimental layout and statistical detail, sampling detail, cold storage conditions and examination stages and parameters.

Objective 1: Expression of the genes involved in the citrate pathway of *Oenococcus oeni* as a function of citrate and malate concentration, pH, ethanol, temperature and oxygen to control diacetyl production in wine (year 1 and 2).

Milestone 1: The experiments will be performed in synthetic wine (cFT80) consisting of different pH values (3.2 and 3.6), citric acid concentrations (0.0, 0.3 and 0.8 g/L) and ethanol concentrations (11 and 14%). The effect of temperature and oxygen will also be investigated. One *O. oeni* strain will be selected and analysed in the synthetic wine medium. The experiment will start off by using a constant malic acid concentration (2.0 g/L), ethanol content (13%) and temperature (20°C), whereas the citric acid (0.0 g/L, 0.3 g/L, 0.8 g/L) and pH (3.2, 3.6) parameters will be altered.

Milestone 2: Sampling will occur regularly until citric acid is completely degraded. The viable cell numbers will be monitored with plate assays. Organic acid (citric, L-(-)-malic, L and D-(-)-lactic, acetic and pyruvic acid) concentrations will be measured with enzymatic assays.

Milestone 3: Samples will be taken throughout the fermentations for the analysis of carbonyl compounds (diacetyl and acetoin) with gas chromatography-mass spectrometry (GC-MS).

Milestone 4: Samples will be taken throughout the fermentations for RNA extractions for gene expression analysis with quantitative real-time PCR. Expression of the genes such as *citE* will be determined.

Milestone 5: Finally, data analysis will be carried out, that will provide a platform to establish a model. The model can be used to illustrate a relationship between substrate concentration (citric acid), rate of citrate consumption, rate of diacetyl production, rate of malolactic fermentation and cell growth under different oenological conditions.

Milestone 6: The experiment will be repeated in real must to confirm the results obtained in the synthetic wine.

Objective 2: Elucidate differences in diacetyl production by *Oenococcus oeni* and *Lactobacillus plantarum* in Chardonnay vinified by sequential alcoholic/malolactic fermentation and simultaneous alcoholic/malolactic fermentation (year 2)

Saccharomyces cerevisiae, *L. plantarum* and *O. oeni* strains will be selected to perform fermentations. The Chardonnay juice will be fermented either by consecutive alcoholic/malolactic fermentation where the bacteria are inoculated after the completion of alcoholic fermentation or

simultaneous alcoholic/malolactic fermentation where the bacteria are inoculated together with the yeast. Standard wine parameters will be monitored using infrared spectroscopy. Growth will be monitored using selective plating. Wines will be analysed for malic, lactic, citric, pyruvic acids using enzymatic assays. GC-MS will be used to profile the carbonyl compounds produced during fermentation. If significant differences are observed the wines will be sensorially evaluated. All data generated will be integrated to assess the impact of the different strains and MLF scenarios.

Objective 3: Correlate the expression esterase genes under winemaking conditions with ester hydrolysis and synthesis in wine (year 2 and 3)

The same work plan will be followed as for milestone 1 to assess the expression of esterase genes from *O. oeni* and *L. plantarum.* Different esters will be used as substrate to assess hydrolyses and different acids and alcohols will be used to determine if the same genes can also hydrolyse. Once the mechanism is understood, small-scale fermentations can be done to assess the impact of MLF on yeast derived esters. The obtained genes can be sub-cloned into yeast or bacteria to studied the function and activity of the genes or enzymes respectively.

c) RESULTS AND DISCUSSION

State ALL accumulated results from the start of the project, including a short discussion where applicable.

Objective 1:

In the first objective of this study, it was shown that glucose, fructose, pH and the LAB strains used to induce MLF in synthetic wine can influence citrate consumption, the gene expression of the citrate lyase β subunit (*citE*) and the production of metabolites associated with citrate metabolism, specifically D-lactate, acetate, diacetyl and acetoin. Of the factors tested, glucose had the smallest impact on citrate metabolism. The citrate positive (cit+) *O. oeni* strain was the only strain that completely consumed citrate. This strain consumed citrate faster in the 115 g/L glucose treatment than in the 50 g/L and 2.5 g/L treatments. However, the lowest relative expression was seen in the 115 g/L glucose treatment where malate and citrate were consumed the fastest which the bacteria might have incorporated as a survival mechanism for when malate has been depleted. The 115 g/L and 50 g/L glucose treatments did not influence the D-lactate, acetate, diacetyl and acetoin concentrations produced by all the strains. The 2.5 g/L glucose treatment inoculated by most strains had the lowest D-lactate and acetate concentrations which might be due to the limited substrate in this treatment. The cit+ *Lb. plantarum* strain was the only strain that produced diacetyl concentrations above the minimum quantification limit in the 2.5 g/L glucose treatment. This treatment inoculated with cit+ *Lb. plantarum* strain had a final diacetyl concentration above the 0.2 mg/L to 2.8 mg/L sensory threshold value which could have contributed to a desirable buttery aroma. The cit+ *Lb. plantarum* strain produced more acetate, diacetyl and acetoin than the citrate negative (cit-) *Lb. plantarum* strain, but similar amounts of D-lactate. The cit+ *O. oeni* strain produced more D-lactate and acetate than the cit- *O. oeni* strain, but both strains produced trace amounts of diacetyl and acetoin. The *Lb. plantarum* strains

produced more D-lactate, acetate, diacetyl and acetoin than the *O. oeni* strains in all the treatments.

The fructose treatments had a more pronounced effect on the citrate metabolism of LAB than the glucose treatments. The fructose treatments only influenced the citrate consumption by the cit+ strains. The cit+ *Lb. plantarum* strain was able to consume more citrate in the 115 g/L and 50 g/L fructose treatments than in the glucose treatments, but there was still not a relative transcriptional response. The citrate consumption by the cit+ *O. oeni* strain was slightly delayed in the 115 g/L fructose treatment which had the highest relative expression of *citE*. As mentioned previously, it seems as if the relative expression of *citE* might be linked to the malate degradation period. Furthermore, it seems as if high fructose concentrations stimulated the production of diacetyl and acetoin by the cit+ strains, since the highest diacetyl and acetoin concentrations were produced in the 115 g/L treatment. The diacetyl concentrations in the 115 g/L fructose treatment inoculated with the cit+ strains were above the 5 mg/L limit of desirable diacetyl and could have spoiled the wine by giving rise to an overwhelming buttery aroma which could have masked the fruity and vegetative aromas in wine. These results indicate that co-inoculated wines with a cit+ strain might cause wine spoilage due to the high diacetyl and acetoin concentrations produced. This is however in contrast with previous studies which indicated that co-inoculated wines, where LAB are inoculated simultaneous with the yeast, have been described as being more fruity than sequential wines, where the LAB are inoculated after the completion of alcoholic fermentation. This contradiction might be due to the fact that during this study there were no yeast present to reduce the diacetyl produced by the LAB strains to the less sensory active acetoin and 2,3-butanediol. The cit+ *O. oeni* strain produced more D-lactate, acetate, diacetyl and acetoin than the cit- *O. oeni* strain. The cit+ *Lb. plantarum* strain produced more diacetyl and acetoin than the cit- *Lb. plantarum* strain, but similar amounts of D-lactate and less acetate. As was seen for the glucose treatment, the *Lb. plantarum* strains produced more D-lactate, diacetyl and acetoin than the *O. oeni* strains. However, the *O. oeni* strains produced more acetate than the *Lb. plantarum* strains in most of the treatments.

Lastly, the pH treatments influenced the LAB species differently which is mainly coupled to the optimum growth pH of the individual species. *Lb. plantarum* has a growth optimum pH above 3.5 and the *Lb. plantarum* strains were therefore able to survive longer, consume more citrate and thus produced more D-lactate, acetate, diacetyl and acetoin in the pH 3.5, 4.0 and 5.0 treatments. On the other hand, *O. oeni* is better adapted than *Lb. plantarum* to a low pH and are able to survive in wines with a pH equal to or below 3.5. The cit+ *O. oeni* strain was therefore able to consume more citrate in the lower pH treatments, pH 3.0 and 3.5 treatments, and produced more diacetyl and acetoin than in the pH 4.0 and 5.0 treatments. The relative expression of *citE* in the cit+ *O. oeni* strain was also the highest in the pH 3.0 treatment where the malate and citrate were

consumed over a longer period as was seen in the sugar treatments. The D-lactate and acetate concentrations produced by this cit+ *O. oeni* strain were however not more in the lower pH treatments than in the higher pH treatments. The cit+ *O. oeni* strain produced diacetyl above the 5 mg/L limit in the pH 3.0 and 3.5 treatments and could have contributed to a buttery off-flavour in wine. The cit+ *Lb. plantarum* strain produced diacetyl concentrations below 5 mg/L but higher than the 0.2 mg/L to 2.8 mg/L diacetyl sensory threshold value in the pH 3.5, 4.0 and 5.0 treatments and could have contributed to a desirable buttery aroma and thus wine complexity. This cit+ *Lb. plantarum* strain was the only strain that produced acetoin concentrations above the 150 mg/L threshold value in the pH 3.5, 4.0 and 5.0 treatments and could have caused an overwhelming creamy aroma. The cit+ strains only produced more diacetyl and acetoin than the cit strains, but similar amounts of D-lactate and acetate. As was seen in the sugar treatments, the *Lb. plantarum* strains produced more D-lactate and acetoin than the *O. oeni* strains in all the pH treatments, except in the pH 3.0 treatment where the *O. oeni* strains produced more D-lactate than the *Lb. plantarum* strains. However, the *O. oeni* strains produced more acetate than the *Lb. plantarum* strains in all the pH treatments, except in the pH 5.0 treatment where the *Lb. plantarum* strains produced more acetate than the *O. oeni* strains.

From the results obtained in this study, it was evident that diacetyl and acetoin concentrations increased with a low glucose and a high fructose concentration. A low pH stimulated the production of diacetyl and acetoin by the cit+ *O. oeni* strain, whereas a high pH stimulated the diacetyl production of these compounds by the cit+ *Lb. plantarum* strain. To avoid the production of an overwhelming buttery, creamy aroma in wine, winemakers can induce MLF with a cit- LAB strain such as Viniflora® CiNe™ from Chr. Hansen. This strain was the only strain that did not consume any citrate and therefore produced the lowest D-lactate, acetate, diacetyl and acetoin concentrations in most of the treatments. Although the factors tested during this study influenced the concentrations of these compounds produced by this strain, the concentrations were never above their sensory threshold values and could have only contributed to wine complexity.

Objective 2:

Four different LAB strains, two citrate positive (*O. oeni* 136.2 and *L. plantarum* 7) and two citrate negative (*O. oeni* CiNe and *L. plantarum* 42), were used for MLF in Chardonnay must. The sequential inoculation scenario will not be discussed, since the MLF was unsuccessful in all the treatments. The different LAB treatments did not influence the pH or ethanol concentration of the Chardonnay wine, since these parameters were similar between all the treatments. MLF was completed for most strains before AF. The degradation rate of L-malic acid, within the first 3 days, was slower for the citrate negative strains, *O. oeni* CiNe and *L. plantarum* 42, than for the citrate positive strains, *O. oeni* 136.2 and *L. plantarum* 7. The *L. plantarum* strains degraded the malic

acid faster than the *O. oeni* strains within 3 days after inoculation. The citrate concentrations on day 3 were similar between all the different treatments.

The citrate positive LAB strains (*O. oeni* 136.2 and *L. plantarum* 7) started to degrade citrate after 3 days. Citrate was completely degraded by the citrate positive strains on day 7, while the citrate negative strains together with the control treatment did not degrade the citrate.

Pyruvate concentrations increased in all treatments on day 3 and then pyruvate concentrations decreased in all treatments from day 3 to 7.

D-lactate concentrations in most of the treatments were similar on day 3, except for *L. plantarum* 7 had higher D-lactate concentrations. The citrate positive strains (*O. oeni* 136.2 and *L. plantarum* 7) produced higher concentrations of D-lactate than the citrate negative strains on day 7. Acetic acid concentrations increased in all treatments during MLF with *O. oeni* having slightly higher levels.

Diacetyl on day 3 showed differences between the strains but on day 7 all levels were similar. The acetoin levels on day 7 showed that *L. plantarum* had higher levels in the wine. Unfortunately, the measurement of 2,3-butanediol could not be conducted as this would have given the final insight into the pathway as the last metabolite.

Objective 3:

Screening of esterase genes in *L. plantarum* and *O. oeni* yielded strains difference as seen in the Tables.

Table 3 Summary of screening results of *L. plantarum*

Strains	lp_0796	est_1092	Putative esterase	Potential esterase genes
B 094	X	√	X	1
B 095	X	√	√	2
B 096	X	√	√	2
B 098	√	√	√	3
B 100	X	√	√	2
B 101	√	√	√	3
B 102	X	√	√	2
B 103	X	√	X	1
B 105	X	X	X	0
B 106	√	√	√	3
B 107	√	√	√	3
B 114	X	√	√	2
B 115	√	X	√	2
B 117	X	X	X	0
B 121	X	X	√	1
B 122	X	X	√	1
B 144	X	X	√	1
W 77A2	X	X	X	0
W 80A2	X	X	X	0
W 80B1A	√	X	√	2
W 81c2b	X	X	X	0
W 83A2	√	X	X	1
W 86A2	X	X	√	1
W 87 A1b	X	X	X	0

Table 4 Summary of screening results of *O. oeni*

*S*trains	Est_Co08	Est_A2	Est_B28	Potential esterase gene
Viniflora oenos	X	X	X	0
Viniflora CiNe	X	√	X	1
B 20	X	√	X	1
B21	√	√	X	2
B 24	X	√	X	1
B 26	√	√	X	1
B 27	X	√	X	1
B 30	X	√	X	1
B 35	√	√	X	2
B 36	X	√	X	1
B 37	√	√	X	2
B 38	X	X	X	0
B 41	√	√	X	2
B 50	X	√	X	1
B 54	√	√	X	2
B 55	√	X	X	1

From the results of the screening we chose to work with 5 strains of *L. plantarum* and 3 strains of *O. oeni*. The effect of pH on esterase activity of the 8 LAB strains toward *p*-nitrophenyl octanoate showed that all the lactobacilli strains showed maximal activity at pH 5 however the maximal activity was not consistant amoungst the strains. B 115 for example has 50% higher activity at pH 5 than the rest of the *L. plantarum* strains. The activity of pH 7 and 8 was consistant amoung the five strains. In contrast, *O. oeni* had a higher activity toward esters at a neutral pH (8). Lower pH (3 and 5) did not seem to favor the degradation of esters.

L. plantarum strains showed greater activity at intermediary temperatures (30-40°C) for all the strains tested. 50 and 60°C had very low esterase activity toward *p*-nitrophenyl octanoate. All

strains exhibited approximately the same esterase activity to each temperature treatment and expressed their activity quantitatively at the same level relative to the rest. B 105, the negative control, had slightly higher activity. The highest degree of esterase activity was observed between 20 and 30°C for all 3 *O. oeni* strains. *O. oeni* B 21 had shown the highest activity amongst the strains. Higher temperature (40, 50 and 60°C) inhibited the *O. oeni* strains activity.

Ethanol is a very important factor to consider for the degradation of esters in wine since malolactic fermentation is conducted mostly after alcoholic fermentation when the ethanol concentration is equal or exceeds 12% (v/v). For both the *Lactobacillus* and the *Oenococcus* strains it was observed that ethanol induced esterase activity. At a 14% ethanol concentration *L. plantarum* had the highest esterase activity. *O. oeni* strains had the highest esterase activtiy 8% and 14% ethanol concentration.

In the Chardonnay wine the only esters that were significantly impacted are ethyl butyrate, isoamyl acetate, hexyl acetate, ethyl lactate, and 2-phenylethyl acetate. For most of the esters *L. plantarum 42* produced the highest levels. The exception was ethyl lactate were *L. plantarum* 7 produced double the amount of any other strain and this correlations with the highest D-lactate levels.

d) CONCLUSIONS

Objective 1:

From the results obtained in this study, it seems as if a high fructose concentration stimulated the production of diacetyl and acetoin by cit+ strains of the species *O. oeni* and *Lb. plantarum*. Furthermore, a low pH increased the diacetyl and acetoin concentrations by the cit+ *O. oeni* strains, while a high pH increased the D-lactate, acetate, diacetyl and acetoin production by the cit+ *Lb. plantarum* strain. Thus, grape must from warm climate regions with a higher pH must rather be inoculated with an *O. oeni* starter culture when a buttery aroma is not wanted. Lastly, the use of the cit+ LAB strains would not be encouraged to induce MLF in wines where the buttery aroma is not desired as the cit+ LAB strains used in this study produced much higher diacetyl and acetoin concentrations than the cit- strains.

Objective 2:

The degradation of citric acid by MLF starter cultures can influence wine aroma and is therefore important to consider during winemaking. The buttery aroma from diacetyl produced during citric acid degradation can be managed by certain procedures to create a buttery wine style. The MLF

starter culture, inoculation dosage and timing of inoculation are the most important factors to be considered for making a buttery style wine.

Objective 3:

The results obtained showed that there are different esterase genes in the two species and that the combination of the genes and enzyme activity differs tremendously between the strains and this could translate into differences in ester production. The function of the different enzymes also show that pH, ethanol and temperature tested in this study affect the activity differently but the induction function of ethanol is interesting as this correlates that the bacteria is detoxifying the environment for sequential MLF to be more successful. Therefore, if fruitiness is an important characteristic for a specific strain screening for the gene presence alone is not optimal. The ester analyses in the wine showed that *L. plantarum* and *O. oeni* as well as co-inoculation vs. sequential MLF yielded different ester levels in the wines.

7. ACCUMULATED OUTPUTS

List ALL the outputs from the start of the project. The year of each output must also be indicated.

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

Indicate the commercial potential of this project, e.g. Intellectual property rights or commercial product(s).

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

Provide steps taken to ensure the transfer of the gained/new information/knowledge to ultimately benefit the South African fresh fruit industry.

Popular articles will be written, different presentation at conferences and industry workshops have been given.

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

Complete the following table, adding more lines if necessary.

Student Name and Surname	Student Nationality	Degree (e.g. MSc Agric, MComm)	Level of studies in final year of project	Graduation date	Total cost to industry throughout the project
Honours students					
S de Koker	SA	HonsBSc		2013	
N Pretorius	SA	HonsBSc		2014	
A van Heerden	SA	HonsBSc		2014	
IJ Botma	SA	HonsBSc		2015	
Masters Students					
N Pretorius	SA	MSc		2016	24 000

PhD students					
Postdocs					
Support Personnel (not a requirement for HORTGRO Science)					

PERSONS PARTICIPATING IN THE PROJECT (Excluding students)

Initials & Surname	Highest Qualification	Degree/ Diploma registered for	Race (1)	Gender (2)	Institution & Department	Position (3)	Cost to Project R
L Engelbrecht	MSc		W	F	IWBT	TA	150 000
E Lerm	MScAgric		W	F	IWBT	TA	50 000
H Schoeman	PhD		W	F	IWBT	TA	100 000
D Jacobson	PhD		W	M	IWBT	RA	100 000
B Divol	PhD		W	M	IWBT	Co	0

[(1)]Race
B = African, Coloured or Indian
W = White

[(2)]Gender
F = Female
M = Male

[(3)]Position
Co = Co-worker (other researcher at your institution)
Coll = Collaborator (participating researcher that does not receive funding for this project from industry)
PF = Post-doctoral fellow
PL = Project leader
RA = Research assistant
TA = Technical assistant/ technician

d) PUBLICATIONS (POPULAR, PRESS RELEASES, SEMI-SCIENTIFIC, SCIENTIFIC)

Please list using the format illustrated in the example below. ***ATTACH PDF COPIES OF ANY PAPERS ALREADY PUBLISHED***

Pretorius N, Engelbrecht L, Du Toit M. (2017). Using MLF to create a buttery wine or not. WineLand May.

e) PRESENTATIONS/PAPERS DELIVERED

Please list using the format illustrated in the example below.

Engelbrecht, L., Du Toit, M. & De Koker, S. **2013**. Unravelling the regulation of the citrate pathway genes under wine conditions. 15th Australian Wine Industry Technical Conference, Sydney, Australia.
Du Toit, M. **2013**. Inoculation times for MLF. Lallemand ML School, Hblaba Klosterneuburg, Austria.
Du Toit, M. **2013**. Impact of MLF on white wine aroma. Lallemand ML School, Hblaba Klosterneuburg, Austria.
Du Toit, M. **2014**. Using timing as a tool to maximise MLF – the early bug gets the prize. Thales workshop: Bio-protection and other bugging issues, Joostenberg Conference Venue, Joostenberg Farm.
Du Toit, M. **2014**. MLF in white wine: impact in the glass. Lallemand seminar: Controlled MLF – more than just a simple biological de-acidification step, US-IWBT, Stellenbosch.
Du Toit, M. **2015**. Chasing wine aroma: impact of different lactic acid bacteria & MLF scenarios. Lallemand ML School, Toulouse, France.
Pretorius N, Engelbrecht L, Du Toit M. (2016). Comparison of the effects of glucose and fructose on citrate metabolism of *Oenococcus oeni* and *Lactobacillus plantarum*. Poster presentation at SASEV conference at Lord Charles Hotel, Somerset West, 23-25 Augustus 2016.

8. BUDGET *(PHI projects to complete separate Excel annexure)*

TOTAL COST SUMMARY OF THE PROJECT

YEAR	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
2012	0	0	0	0	145 350	63 750	0	209 100
2013	0	0	0	0	188 100	82 500	0	270 600
2014	0	0	0	0	282 560	89 100	0	371 660

EVALUATION BY INDUSTRY

This section is for office use only

Project number	

Project name	

Name of Sub-Committee*	

Comments on project

Committee's recommendation (Review panel in the case of PHI)

- Accepted. ☐
- Accepted provisionally if the sub-committee's comments are also addressed. Resubmit this final report by_____ ☐
- Unacceptable. Must resubmit final report. ☐

Chairperson_____ Date_____

*SUB-COMMITTEES

Winetech
Viticulture: Cultivation; Soil Science; Plant Biotechnology; Plant Protection; Plant Improvement;
Oenology: Vinification Technology; Bottling, Packaging and Distribution; Environmental Impact; Brandy and Distilling; Microbiology

Deciduous Fruit
Technical Advisory Committees: Post-Harvest; Crop Production; Crop Protection; Technology Transfer
Peer Work Groups: Post-Harvest; Horticulture; Soil Science; Breeding and Evaluation; Pathology; Entomology