



Australian Government

Department of Health, Disability and Ageing
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan (Consultation version) for

DIR 231

Fermentation of plant material-derived sugars using genetically modified yeast for ethanol production

Applicant: Ethanol Technologies Pty Ltd

30 October 2026

This RARMP is open for consultation until 11 December 2026.

Written comments on the risks to human health and safety and the environment posed by this proposed release are invited. You may make your submission

Via the consultation hub: <https://consultations.health.gov.au> (search for DIR 231)

via email to: ogtr@health.gov.au

or

via mail to: The Office of the Gene Technology Regulator, MDP 54 GPO Box 9848, Canberra ACT 2601

Please note that issues regarding food safety and labelling, the use of agricultural chemicals, and marketing and trade implications **do not** fall within the scope of these evaluations as they are the responsibilities of other agencies and authorities.

Summary of the Risk Assessment and Risk Management Plan

(Consultation Version) for

Licence Application DIR 231

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application to use genetically modified (GM) yeasts for fermentation using plant-based sugars (lignocellulosic hydrolysate) to produce ethanol and xylitol. It qualifies as Dealings Involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment under the *Gene Technology Act 2000* (the Act).

The applicant, Ethanol Technologies Pty Ltd (Ethtec), proposes to use GM yeast strains from *Saccharomyces cerevisiae* and *Candida sojae* to assess their performance for the fermentation of sugars, derived from woody and grassy agricultural, forestry and municipal green waste materials, into ethanol and xylitol.

S. cerevisiae is a non-pathogenic yeast that is a model organism widely used in research and development world-wide. *Candida sojae* has not been widely used in research and is predominantly found in agricultural products, food processing environments, and plastic pollutants.

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed trial poses negligible risks to human health and safety and the environment. Licence conditions have been drafted for the proposed trial. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether to issue a licence.

The application

Project Title	Fermentation of plant material-derived sugars using genetically modified yeast for ethanol production ¹
Parent organisms	<i>Saccharomyces cerevisiae</i> and <i>Candida sojae</i>
Genetic modifications	Modifications are made to <i>S. cerevisiae</i> and <i>C. sojae</i> to enable production of ethanol and xylitol from xylose by introducing or modifying: <ul style="list-style-type: none"> in <i>S. cerevisiae</i> - genes from yeasts, anaerobic gut bacteria and a plant able to use xylose to produce ethanol in <i>C. sojae</i> - genes from yeasts and anaerobic gut bacteria able to convert xylose into ethanol and xylitol.
Principal purpose	To assess the performance of GM <i>S. cerevisiae</i> and <i>C. sojae</i> in using sugars derived from plant material (lignocellulosic sugar hydrolysate) for large-scale ethanol and xylitol fermentation.
Previous releases	There has been no previous application in Australia for these GMOs.
Proposed limits and controls	
Proposed duration	5 years

¹ The title of the project as supplied by the applicant is 'Lignocellulose hydrolysate fermentation by yeast for second generation ethanol production'.

Proposed release size	The proposed dealings will involve a maximum of 1300 litres (L) total fermentation volume per large-scale experiment, consisting of one or more individual culture vessels from 50 L up to 250 L.
Proposed location	The Hunter Pilot Biorefinery (HPB) in Muswellbrook, New South Wales
Proposed controls	<ul style="list-style-type: none"> • Work with the GMOs will be carried out within the secure HPB facility which is internally bunded and has a large sub-floor tank for collection and decontamination of any spills. • The GMOs will be grown in closed vessels. • Large scale cultures will be transferred between vessels using a closed system. • Spills can be decontaminated readily by using 70% ethanol, H₂O₂ or bleach. • Storage of viable GMOs will follow the Regulator's <i>Guidelines for the Transport, Storage and Disposal of GMOs</i>. • Staff handling the GMOs will undergo licence specific training. • Stringent manufacturing practices and quality control procedures will be followed to ensure that GM yeasts will not be released into the environment.

Risk assessment

The risk assessment process considers how the genetic modifications and proposed activities conducted with the GMO might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application (including proposed controls), relevant previous approvals and current scientific/technical knowledge. Both the short- and long-term impacts are considered.

Credible pathways to potential harm that were considered included exposure of people or other organisms to the GM yeasts, and the potential for persistence or dispersal of the GM yeasts. Potential harms associated with these pathways included allergenicity to people, and environmental harms due to the potential for the GM yeasts to spread in the environment.

The risk assessment concludes that the proposed dealings pose negligible risks to human health and safety and the environment. No specific risk treatment measures are required to manage these negligible risks.

The principal reasons for the conclusion of negligible risks are that the GM yeasts will not be used for human food or animal feed, and that the proposed limits and controls will effectively minimise exposure to and dispersal of the GM yeasts.

Risk management

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions. Draft licence conditions are detailed in Chapter 4 of the RARMP.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the licence includes limits on the location and duration of the release. Controls are included to prohibit the use of the GM yeasts in human food and animal feed, to minimise dispersal of the GM yeasts from the fermentation facility, to store GM yeasts in accordance with the Regulator's guidelines, and to destroy GM yeasts at the end of the ethanol production process.

In addition, there are several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects.

Table of contents

SUMMARY OF THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN	I
INTRODUCTION	I
THE APPLICATION	I
RISK ASSESSMENT	II
RISK MANAGEMENT	II
TABLE OF CONTENTS	IV
ABBREVIATIONS	VI
CHAPTER 1 RISK ASSESSMENT CONTEXT	1
SECTION 1 BACKGROUND	1
1.1 Interface with other regulatory schemes	2
SECTION 2 THE PROPOSED DEALINGS	2
2.1 Proposed limits of the dealings (duration, location, scale, people)	2
2.2 Proposed controls to restrict spread and persistence of the GMO in the environment	3
2.3 Details of the proposed dealings	3
SECTION 3 PARENT ORGANISM	7
3.1 Classification	7
3.2 Characteristics	7
3.3 Genome	7
3.4 Life cycle	7
3.5 Pathogenicity and toxigenicity.....	8
3.6 Reservoir	8
3.7 Environmental stability.....	9
3.8 Decontamination	9
3.9 Risk Group and containment	9
SECTION 4 THE GMO - NATURE AND EFFECT OF THE GENETIC MODIFICATION	10
4.1 Metabolic pathway for fermentation of C6 and C5 sugars	10
4.2 Development of GM yeasts	11
4.3 Expected properties of the GM yeasts	14
SECTION 5 THE RECEIVING ENVIRONMENT	15
5.1 Presence of related fungal species in the receiving environment	15
5.2 Presence of similar genes or products in the environment	15
SECTION 6 PREVIOUS AUTHORISATIONS.....	15
CHAPTER 2 RISK ASSESSMENT	16
SECTION 1 INTRODUCTION	16
SECTION 2 RISK IDENTIFICATION	17
2.1 Risk source	17
2.2 Causal pathway	18
2.3 Potential harms.....	19
2.4 Postulated risk scenarios	19
SECTION 3 UNCERTAINTY	23
SECTION 4 RISK EVALUATION	24
CHAPTER 3 RISK MANAGEMENT PLAN	26
SECTION 1 BACKGROUND.....	26
SECTION 2 RISK TREATMENT MEASURES FOR SUBSTANTIVE RISKS	26
SECTION 3 GENERAL RISK MANAGEMENT	26
3.1 Limits and controls on the release.....	26

3.2	Other risk management considerations	28
SECTION 4	ISSUES TO BE ADDRESSED FOR FUTURE RELEASES.....	30
SECTION 5	CONCLUSIONS OF THE CONSULTATION RARMP.....	30
CHAPTER 4	DRAFT LICENCE CONDITIONS	31
SECTION 1	INTERPRETATIONS AND DEFINITIONS	31
SECTION 2	GENERAL CONDITIONS AND OBLIGATIONS	32
SECTION 3	LIMITS AND CONTROL MEASURES	34
SECTION 4	REPORTING AND DOCUMENTATION.....	37
ATTACHMENT A.....		39
REFERENCES		43

Abbreviations

CO ₂	Carbon dioxide
DAFF	Department of Agriculture, Fisheries and Forestry
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
EFSA	The European Food Safety Authority
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
GRAS	Generally Recognised as Safe
GTTAC	Gene Technology Technical Advisory Committee
HGT	Horizontal gene transfer
IBC	Institutional Biosafety Committee
L	Litre
Mb	Megabase
μl	Microliter
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced form of NAD
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced form of NADP
OGTR	Office of the Gene Technology Regulator
PC2	Physical containment level 2
ppm	Part per million
PPP	Pentose phosphate pathway
RAF	Risk Assessment Framework
RARMP	Risk Assessment and Risk Management Plan
RPE	Ribose-phosphate epimerase
RPI	Ribose-phosphate isomerase
SWEET	Sugar Will Eventually be Exported Transporter
TAL	Transaldolase
the Act	The <i>Gene Technology Act 2000</i>
the Regulations	The Gene Technology Regulations 2001
the Regulator	The Gene Technology Regulator
TKL	Transketolase
TSD guidelines	Regulator's <i>Guidelines for Transport, Storage and Disposal of GMOs</i>
WHO	World Health Organization
XDH	Xylitol dehydrogenase

XI	Xylose isomerase
XK	Xylulose kinase
XR	Xylose reductase
XT	Xylose transporter

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.
2. The Act and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.
3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.
4. The *Risk Analysis Framework* (RAF) (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator ([OGTR website](#)).
5. Figure 1 shows the information that is considered, within the regulatory framework, in establishing the risk assessment context. This information is specific for each application. Risks to the health and safety of people or the environment posed by the proposed dealings are assessed within this context. Chapter 1 describes the risk assessment context for this application.

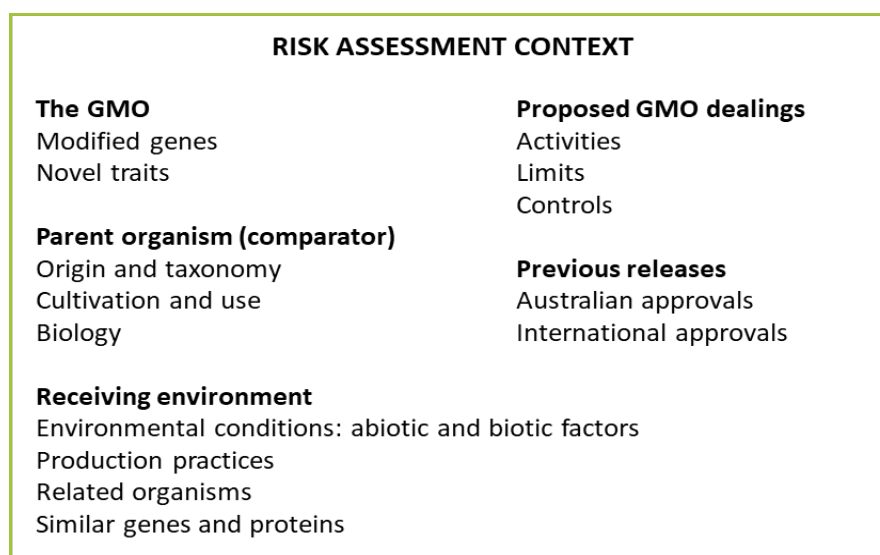


Figure 1 Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

6. In accordance with Section 50A of the Act, this application is considered to be a limited and controlled release application, as the Regulator was satisfied that it meets the criteria prescribed by the Act. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

7. Section 52 of the Act requires the Regulator to seek comment on the consultation RARMP from agencies - the Gene Technology Technical Advisory Committee, State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils and the Minister for the Environment - and from the public.

1.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority, the Therapeutic Goods Administration, the Australian Industrial Chemicals Introduction Scheme and the Department of Agriculture, Fisheries and Forestry (DAFF).

9. The DAFF regulates products imported into Australia to protect Australia from biosecurity risks. Under the *Biosecurity Act 2015*, the importation of biological material such as live GMOs requires a permit from the DAFF.

Section 2 The proposed dealings

10. Ethanol Technologies Pty Ltd (Ethtec) is seeking authorisation to carry out large scale fermentation using genetically modified (GM) *Saccharomyces cerevisiae* and *Candida sojae* (referred to as GM yeasts from hereon) for production of ethanol and xylitol. The overall aim of the project is to assess the performance of the GM yeasts in using sugars in lignocellulosic hydrolysate derived from plant material for ethanol fermentation that involves a maximum of 1300 L cultures per experiment. The experiments are designed around yeast strains capable of converting all of the sugars present in the hydrolysate into ethanol, or to produce ethanol plus xylitol (a high value sweetener used extensively in the food and beverage industry).

11. The dealings involved in the proposed fermentation process are to:

- (a) culture the GMOs in volumes exceeding 25 litres per vessel;
- (b) conduct the following experiments with the GMOs:
 - i) to assess the performance of GM *Saccharomyces cerevisiae* and *Candida sojae*; and
 - ii) to optimise the scale-up fermentation process;
- (c) use the GMOs in the course of manufacture of a thing that is not the GMOs;
- (d) dispose of the GMOs;

and the possession (including storage), supply and use of the GMOs for the purposes of, or in the course of, any of these dealings.

12. Transport of the GMOs is not proposed to be conducted under this licence.

2.1 Proposed limits of the dealings (duration, location, scale, people)

13. The ethanol production experiments are proposed to take place over a five-year period from the date of issue of the licence.

14. The ethanol production experiments will involve fermentation of GM yeast cultures up to a maximum total fermentation volume of 1300 L per experiment at the Hunter Pilot Biorefinery (HPB) in Muswellbrook, New South Wales.

15. Only trained personnel will conduct dealings with the GM yeasts.

2.2 Proposed controls to restrict spread and persistence of the GMO in the environment

16. The applicant has proposed several controls to restrict the spread and persistence of the GMO in the environment. These include:

- Work with the GMOs will be carried out within the secure HPB facility which is internally bunded and has a large sub-floor tank for collection and decontamination of any spills.
- The GMOs will be grown in closed vessels.
- Large scale cultures will be transferred between vessels using a closed system.
- Spills can be decontaminated readily by using 70% ethanol, H₂O₂ or bleach.
- Storage of viable GMOs will follow the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* (TSD Guidelines).
- Staff handling the GMOs will undergo licence specific training.
- Stringent manufacturing practices and quality control procedures will be followed to ensure that GM yeasts will not be released into the environment.

2.3 Details of the proposed dealings

2.3.1 Classification of the dealings and other regulatory schemes

17. The fermentation process involving culture of GM *S. cerevisiae* in volumes of no more than 25 L per vessel is classified as an exempt dealing (Schedule 2, Item 4 of the Regulations). When culturing volume of more than 25 L of GM *S. cerevisiae*, those dealings can be conducted under notifiable low risk dealings (NLRD) in a physical containment level 2 (PC2) large scale facility (Schedule 3, Part 2, 2.1(f) of the Regulations). However, if the facility is not certified by the OGTR, a licence is required.

18. Culture of *C. sojae* in less than 25 L per vessel, which involves a host/vector system not mentioned in paragraph 1.1(c) or Part 2 of Schedule 2, is classified as an NLRD as long as those dealings take place in a facility certified to at least PC2 ((Schedule 3, Part 2, 2.1(c) of the Regulations). Culture of GM *C. sojae* in volumes greater than 25 L per vessel is a licensable dealing regardless of whether the facility is certified as an OGTR PC2 large scale certified facility (Schedule 3, Part 3, 3.1(l) of the Regulations).

19. Work with the GM yeasts in volumes less than 25 L per vessel will be carried out in OGTR certified PC2 laboratories Cert-1449 at the University of Newcastle (UoN) and Cert-4994 inside the HPB under an NLRD authorisation. This DIR application is for a fermentation process where more than 25 L per vessel of GM yeast culture will be produced in a non-OGTR certified area of the HPB.

20. The GM yeast will be imported into Australia, and this will be covered under an exempt or an NLRD authorisation. GMOs will be imported to the University of Newcastle with an appropriate DAFF import permit and then transferred to Cert-4994 at the HPB in accordance with the TSD Guidelines. The GMOs may also be generated at the University of Newcastle Cert-1449.

2.3.2 The fermentation facility

21. The proposed dealings will be carried out at the HPB, which is located at the edge of the Muswellbrook in NSW. The HPB is surrounded by secure perimeter fencing to prevent unauthorised access. It is a locked and fully enclosed building with a roof and walls and includes two sections: one section for the processing of lignocellulosic material to produce sugar solutions, which will then be used in the second section to produce ethanol. The second section includes a small-scale facility and a 430 m² large-scale fermentation facility. The small-scale area is the PC2 laboratory covered by Cert-4994. The large-scale fermentation area, the subject of this application, has bunded flooring and includes a large sub-floor tank for containment and decontamination of accidental spills. All liquid wastes are contained within the building, with no external draining allowed.

2.3.3 Fermentation process and ethanol/xylitol recovery

22. The applicant states that the purpose of the work in the fermentation facility is to optimise fermentation conditions and identify strain types that perform optimally for ethanol or xylitol production. The applicant has proposed to use GM *S. cerevisiae* and *C. sojae* for ethanol production or use GM *C. sojae* for co-production of ethanol and xylitol. For ethanol production, the GM *S. cerevisiae* and *C. sojae* strains may be used in separate batches or mixed in a single culture.

23. The proposed fermentation process will include shorter single batch fermentations, up to 28-day fermentations for high-performing strains. The main fermentation steps to produce ethanol include:

1. Seed train in seed fermenter;
2. Large scale fermentation; and
3. Ethanol extraction.

24. A “seed train” process, i.e. a stepwise process of expanding the cell number from an initial glycerol stock up to larger volumes using vessels of increasing size, will be used to ensure there is sufficient cell mass for the large-scale fermentation. This seed train will be started in the PC2 laboratory Cert-4994 at HPB from a glycerol stock, scaling up until 5L of culture is transferred to the non-certified large-scale facility and transferred to a 50L seed fermenter, then a 100L seed fermenter. The culture is transferred into the 50L and 100L vessels using closed system fittings and compressed CO₂ to generate the pressure for the transfer. Each vessel will be agitated using a recirculation pump, and the pH of the medium will be adjusted prior to fermentation. The pH will be manually monitored during the fermentation in the 50 and 100 L vessels through a sample port, such that media samples can be taken both to measure the pH and the optical density (OD). This will allow to determine the volume of seed necessary for the largest scale fermentation in 250L vessels.

25. For large scale fermentation, the culture from the final seed fermenter (100 L) can be transferred to any one of four interlinked 250L fermenters. The maximum total fermentation volume for any large-scale fermentation experiment will be up to 1300 L (2 x 50 L, 2x100 L seed train ferments + 4 x 250 L ferments). The actual volume used within each fermenter is overestimated as all fermenters are likely to be operating at approximately 50% capacity.

26. Once fermentations are complete in the 250 L vessels, the medium is transferred to a large post-fermentation buffer tank for the ethanol recovery step. A second vessel will be run in parallel for smaller experiments which is capable of being cooled to 1°C to prevent contamination and limit ethanol losses prior to ethanol recovery. These vessels are closed to prevent GMO release and possess a recirculation line to ensure the homogeneity of the spent fermentation media. The pumps will be used to resuspend cells just prior to the ethanol recovery system.

27. Following completion of a batch, the unfiltered liquid from the fermentation buffer tank will be transferred to a heat exchanger which will take the temperature up to about 100°C for ethanol recovery (Figure 2, below). The actual boiling temperature will be dependent on the final ethanol concentration, but will be close to 100°C. The near boiling liquid (including cells/cell debris) will then be transferred to the distillation column. Ethanol will rise to the top of the column where it will be harvested (dotted red line in Figure 2). The remaining liquid (including cells/cell debris) will then be recycled back to the reboiler for a further round of heating/distillation. This recycling will continue until all the ethanol has been removed from the liquid. Cells will have undergone multiple rounds of heating (near boiling), and no viable cells will remain. The applicant has completed a laboratory-scale experiment using saturated cultures of *S. cerevisiae* and *C. sojae* which were treated at 100°C for 1 and 3 minutes, respectively and 100 µl aliquots were spotted on a plate and grown overnight. After 1 minutes heating, no viable cells remained. The process of cycling ferments through the heat exchanger and distillation column will result in much more than 1 – 3 minutes exposure to temperature closed to 100°C. In the large-scale process, the sludge remaining following ethanol

recovery will be tested for cell viability, then if confirmed to be non-viable, the waste will undergo a pH adjusting process with lime (Ca_2OH), followed by separation with bag filters to separate the liquids from solids. The solids will go to landfill, and the filtered liquids may be distilled so that the water can be re-used or used for xylitol recovery.

28. Xylitol recovery will occur following ethanol recovery. The xylitol will be isolated from the remaining cell-free liquid.

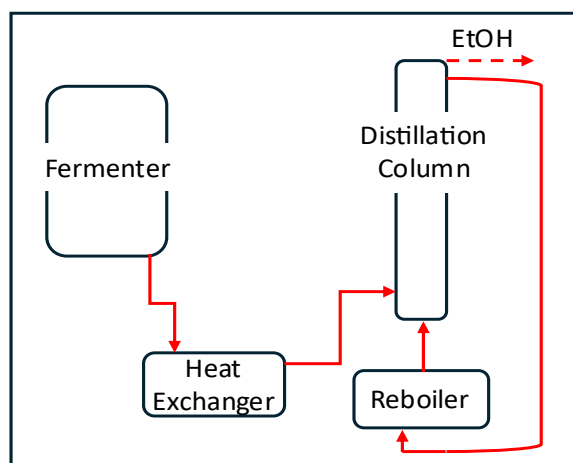


Figure 2 A schematic of the process for ethanol recovery post fermentation (source: Ethtec)

29. Ethanol may be produced by either GM *S. cerevisiae* or GM *C. sojae*. For ethanol and xylitol production, GM *C. sojae* may be used that is capable of both ethanol and xylitol production. The applicant also states that the fermentation facility may be used for ethanol and xylitol production by sequential fermentation using GM *S. cerevisiae* that is capable of ethanol production and then wild-type *C. sojae*, which is capable of xylitol production. This process involves the same large scale fermentation process as described above to convert hexose sugars in the lignocellulosic sugar hydrolysate into ethanol using GM *S. cerevisiae* in the first fermentation followed by heating to remove the ethanol, and then a second fermentation using wild-type *C. sojae* to convert the remaining xylose-rich pentose into xylitol.

2.3.4 Properties of the fermenters

30. The fermenters will form part of a closed system (this is a system for growth, processing and/or storage of large-scale cultures of GM yeast within enclosed vessels and transfer lines). The fermentation vessels are temperature controlled by a closed loop heat exchanger and have filtered (0.22 micron) compressed air supplied by a mass flow controller and a control valve for transferring the seed culture into the vessels. Medium can be transferred to each fermenter from the media batching tank or transferred between fermenters. Temperature control is achieved by a twin jacketed system on each fermenter controlled by a tank-mounted temperature sensor. Each vessel also possesses a stirrer to ensure yeast remains suspended in the fermentation medium; as well as a recirculation line with a peristaltic pump. This recirculation line contains probes to monitor both the pH and dissolved oxygen during the fermentation process. The volume in each fermenter can be controlled by a control loop between the level indicator and the media inlet feed valve.

31. The fermenters also have ports for clean in place (CIP). The CIP is achieved via a common trolley unit which can be attached to each fermenter as needed. CIP involves cleaning a vessel with a caustic solution which is then rinsed out with hot water at 80°C before being finally rinsed with peracetic acid solution and drained. The vessel can be purged with carbon dioxide and sealed until use if the vessel is to be left for a period after cleaning.

2.3.5 Training of staff

32. The applicant states that any persons covered by the licence will be notified of the licence conditions. Anyone working within the HPB facility will be required to record and sign-off on induction and training.

2.3.6 Transport and storage of the GMO

33. The applicant states that the GM yeast strains will be imported to the UoN with an appropriate DAFF import permit and/or generated at the PC2 laboratory (Cert-1449) in the UoN under an NLRD. The selected GM yeast strains will be transferred to the PC2 laboratory (Cert-4994) at the HPB for fermentation experiments in accordance with the TSD Guidelines.

34. The GM yeasts will be stored as -80°C glycerol stocks in the PC2 laboratories.

35. No transport of large-scale cultures of the GM yeast will occur under this licence. Transport of GM yeast under 25 L may occur under an NLRD. Used PPE that may be contaminated with the GM yeast will be transferred to the PC2 laboratory for disposal under the NLRD waste stream.

2.3.7 Disposal of the GMO

36. Following completion of a ferment, the unfiltered liquid containing live GM yeasts from the fermentation buffer tank is heated to about 100°C for ethanol recovery. The repeated process of cycling ferments through the heat exchanger and distillation column at 100°C is expected to effectively kill the yeast. Samples (liquid and solid) will be tested for every batch for remaining viable cells prior to disposal. If viable cells are identified, the contaminated sample will be retreated and retested until no viable cells can be detected. Any purified products will also be disposed of in accordance with waste management/Environment Protection Authority (EPA) requirements.

37. The applicant states that the HPB will store 50% hydrogen peroxide (H₂O₂) which is used for equipment cleaning and decontamination, and if required 10% solutions can be used to washdown accidental spills of *C. sojae* for effective decontamination (see Section 3.8 for further information about decontamination of the 2 parent organisms). For spills the following measures can be taken:

- *S. cerevisiae*: wash down spills with 1% H₂O₂ into subfloor tank.
- *C. sojae*: wash down spills with 10% H₂O₂ into subfloor tank.
- Following H₂O₂ wash down, cover spill site with absorbent material/paper towels and soak with 1:10 dilution of bleach. Leave for 30 minutes then clean up wet towels/material.
- Subfloor waste will be pumped into a waste blending tank prior to separation of solid and liquids for appropriate disposal. The waste blending tank will contain ports permitting sampling to determine whether any viable cells remain. If viable cells are detected additional H₂O₂/bleach can be added and mixed prior to re-testing. Disposal of contents will only occur once no viable cells can be detected.

38. As discussed above, used PPE that may be contaminated with the GM yeast will be transferred to the PC2 laboratory for disposal under the NLRD waste stream.

2.3.8 Accountability and Monitoring

39. The applicant has in place a documentation system that ensures that testing data for all ferments will be recorded and stored. All incidents will be recorded in accordance with occupational health and safety (OH&S) requirements, and the IBC informed. The OGTR will also be informed of incidents.

2.3.9 Contingency plans

40. The applicant provided measures that can be taken in case of a spill at the HPB (Section 2.3.5).

41. Samples from tanks, spill areas, and around the HPB can be taken for growth analysis on chromogenic plates and if required, by PCR and/or genome sequencing analysis to identify any GM yeasts outside the containment.

Section 3 Parent organism

42. The parent organisms are two yeast species: *Saccharomyces cerevisiae* and *Candida sojae*. The characteristics of the parent organisms provide a baseline for comparing the potential for harm from dealings with the GM yeasts. The relevant biological properties of *S. cerevisiae* and *C. sojae* will be discussed here.

3.1 Classification

43. *S. cerevisiae* and *C. sojae* share the same order, Saccharomycetales, but they split into different taxonomic families. *S. cerevisiae* belongs to the genus of *Saccharomyces* in the Saccharomycetaceae family (Suh et al., 2006). *C. sojae* belongs to the genus of *Candida* in the Debaryomycetaceae family (Mbaye et al., 2024).

3.2 Characteristics

44. *S. cerevisiae* and *C. sojae* are single-celled fungi. *S. cerevisiae* occurs in a range of natural environments (Enache-Angoulvant and Hennequin, 2005; Suh et al., 2006) and is used in baking and brewing, and as a food ingredient. *C. sojae* is not considered a highly ubiquitous organism in the natural environment and has been isolated from defatted soybean flake, insects and plastic debris (Borelli et al., 2016; Metcalf et al., 2025).

45. *S. cerevisiae* and *C. sojae* can grow both aerobically and anaerobically (Barnett and Entian, 2005; Pant et al., 2022). However, both *S. cerevisiae* and *C. sojae* shift toward higher ethanol yields under anaerobic or oxygen-limiting conditions (Pant et al., 2022; Topaloğlu et al., 2023). Optimum growth for *S. cerevisiae* occurs at 30–35°C and the maximum temperatures for growth is 37.5°C - 39.8°C (Walsh and Martin, 1977). *C. sojae* showed less growth at 38°C in liquid culture (Metcalf et al., 2025) and was unable to grow on solid medium at 37°C (information provided by the applicant).

3.3 Genome

46. The genome of *S. cerevisiae* underwent duplication millions of year ago, resulting in a haploid chromosome number of 16 (Suh et al., 2006). The 12 Mb genome encodes approximately 6000 genes (Goffeau et al., 1996).

47. *C. sojae* has a haploid genome with the size of approximately 12 Mb that encodes about 5200 genes (Borelli et al., 2016). A definitive chromosome number is not yet known.

3.4 Life cycle

48. Cells of *S. cerevisiae* exist in both haploid and diploid states (Herskowitz, 1988). Both types of cells are able to multiply by mitotic division (budding) under nutrient-replete conditions (Mitchell, 1994). Under optimum conditions, *S. cerevisiae* cells double approximately every 100 min (Herskowitz, 1988).

49. Mitotic division ceases under nutrient limitation (Mitchell, 1994). Pseudohyphal growth occurs when an essential nutrient (usually nitrogen) is limited and glucose is present. Sporulation is induced when an essential nutrient is limited in the absence of a fermentable carbon source, e.g. glucose (Mitchell, 1994). Not all cells are able to sporulate under favourable nutrient conditions. In order to sporulate, cells require a diploid a/α genome (Herskowitz, 1988). For haploid cells to reach sporulation, two cells of opposite mating type (a and α) must mate to become diploid.

50. In some cases (homothallism), a single haploid cell is able to produce daughter cells with both a and α genotype (Herskowitz, 1988). These two haploid genotypes are then able to mate and

sporulate. Laboratory strains of *S. cerevisiae* often have a defective version of the gene that determines homothallism, meaning that they are heterothallic (i.e., *ho* genotype) and do not switch mating types (Herskowitz, 1988).

51. *C. sojae* follows a typical anamorphic (asexual) yeast lifecycle characterised by single-celled growth and budding, as a species lacking a known true sexual or meiotic cycle (Abdel-Haq et al., 2022; Nakase et al., 1994). It is one of the rare *Candida* species categorised as NOMAT (no identifiable mating type/MTL locus components for α or α), meaning standard α and α mating-type genes are missing or undetectable with standard fungal models (Krassowski et al., 2019).

3.5 Pathogenicity and toxigenicity

52. *S. cerevisiae* and *C. sojae* are both considered as opportunistic pathogen of humans who have underlying conditions or predisposing factors (Chrenkova et al., 2022; Enache-Angoulvant and Hennequin, 2005; Pérez-Torrado and Querol, 2016; Seren and Birinci, 2024). Invasive infection with *S. cerevisiae* and *C. sojae* are rare and they are not on the WHO fungal priority pathogen list (WHO, 2022).

53. *S. cerevisiae* is considered a Generally Recognised As Safe (GRAS) organism. Some strains of *S. cerevisiae* are more likely to cause opportunistic disease (Pérez-Torrado and Querol, 2016). Characteristics of these strains include being able to grow at 37°C, causing infections in mice and better survival in human blood infection models.

54. *C. sojae* showed a low level of virulence compared to some known opportunistic *Candida* pathogens, such as *C. glabrata* and *C. tropicalis*, in a *Galleria mellonella* moth model of infection and antifungal resistance, implying that it presents a lower risk to public health (Metcalf et al., 2025). In a human cell tissue culture damage test, *C. sojae* showed similar levels of cell damage to the GRAS control *S. cerevisiae*, suggesting it is avirulent and most likely harmless, consistent with the *Galleria mellonella* test (information provided by the applicant).

55. *S. cerevisiae* does not typically establish true permanent colonisation in the human gut, acting instead as a transient dietary pass-through organism (Auchtung et al., 2018). *C. sojae* is an environmental or plant-associated yeast and is not recognised as a natural or stable coloniser of the healthy human gastrointestinal tract.

56. Some strains of *S. cerevisiae* produce specialised proteinaceous antimicrobial compounds known as killer toxins, but these killer toxins target other competing microorganisms and do not pose a toxic risk to human health (Billerbeck et al., 2024; Orentaite et al., 2016). *C. sojae* is primarily studied for its ability to assimilate xylose and produce xylitol, and there is no report to indicate that it produces any toxins.

57. FSANZ has approved several processing aids and food additives that have been produced using *S. cerevisiae* (FSANZ, 2021a, b). However, no food use of *C. sojae* has been approved by FSANZ.

58. Laboratory experiments with grapevine suggest that some strains of *S. cerevisiae* have the ability to invade plant tissues and affect plant growth (Gognies et al., 2001). However, *S. cerevisiae* is not generally considered a plant pathogen. *C. sojae* is a saprophytic yeast (feeds on dead or decaying organic matter) and has not been reported as a plant pathogen.

3.6 Reservoir

59. In nature, *S. cerevisiae* is commonly associated with grapes and other fruits, and may be transported by insects (Mortimer, 2000). *S. cerevisiae* is cultured for use in baking and brewing.

60. *C. sojae* is an emerging non-albicans *Candida* species whose environmental reservoirs are predominantly tied to agricultural products, food processing environments, and plastic pollutants in natural waterways (Metcalf et al., 2025; Nakase et al., 1994). It has shown the potential for

fermentation of lignocellulosic hydrolysate for the integrated production of ethanol and xylitol (Pant et al., 2022). The applicant states that *C. sojae* has not been identified in Australia to date.

3.7 Environmental stability

61. *S. cerevisiae* is an anhydrobiote (able to survive desiccation), and is able to survive desiccation for at least six months (Tapia and Koshland, 2014).

62. *C. sojae* is not an anhydrobiote and exhibits environmental stability, particularly in aquatic ecosystems and on artificial substrates, but has lower thermal tolerance that acts as a primary limiting factor (Metcalf et al., 2025). Data provided by the applicant indicates that *C. sojae* can only survive on a laboratory surface for up to 7 days.

3.8 Decontamination

63. Both *S. cerevisiae* and *C. sojae* are single celled yeast with chitinous cell wall. They belong to the families of Saccharomycetaceae and Debaryomycetaceae, respectively.

64. As stated above, *S. cerevisiae* is an anhydrobiote which is able to survive desiccation for at least six months (Tapia and Koshland, 2014). *S. cerevisiae* is effectively decontaminated by direct application of isopropyl alcohol or 0.3% peracetic acid (Korukluoglu et al., 2006). Quaternary amine compounds and aldehydes may be effective, depending on concentration and incubation time of the compounds.

65. The effect of hypochlorite (bleach) on *S. cerevisiae* depends on solution pH, concentration and incubation time (Hays et al., 1967). *S. cerevisiae* was destroyed after 30 seconds exposure to 25 parts per million (ppm) (0.0025% v/v) hypochlorite in solutions buffered at pH 5 or 8.5 (Hays et al., 1967). Growth of *S. cerevisiae* on solid medium was completely inhibited at 3000 ppm sodium hypochlorite, while viability was lost in over 80% of cells when incubated for 15 minutes in 50 ppm sodium hypochlorite solution buffered at pH 6 (Kwolek-Mirek et al., 2011). The applicant has provided data to show that household bleach diluted 1:10 can effectively kill *S. cerevisiae* and *C. sojae* after 10 min treatment.

66. The minimum inhibitory concentration of ethanol towards *S. cerevisiae* is approximately 13% (Kampf, 2018). The maximum concentration of ethanol reported to be tolerated by *S. cerevisiae* is 25%. Effective fungicidal activity against food-borne yeasts was reported following exposure to 70% ethanol for 5 minutes (Kampf, 2018). Data provided by the applicant also show that *C. sojae* can be effectively controlled by 70% ethanol.

67. The applicant has provided data on the sensitivity of *S. cerevisiae* and *C. sojae* to H₂O₂. *S. cerevisiae* is highly sensitive to 1% of H₂O₂ and treatment at this concentration for 10 minutes can achieve a complete kill. *C. sojae* is more tolerate to H₂O₂ and remains viable even following 30 minutes exposure to 5% of H₂O₂. However, it can be completely killed by a concentration at 10% with 10 minutes treatment.

3.9 Risk Group and containment

68. The Australian Standard 2243.3:2022 *Safety in Laboratories Part 3: Microbiological safety and containment* (Standards Australia New Zealand, 2022) does not provide a classification for *S. cerevisiae* and *C. sojae*. However, according to the criteria listed in the Australian Standard, *S. cerevisiae* and *C. sojae* can be classified as a Risk Group 1 organism. The Public Health Agency of Canada ([ePATHogen Risk Group Database](#)) and the [American Type Culture Collection \(ATCC\)](#) classify *S. cerevisiae* as a Risk Group 1 and Biosafety Level 1 organism, respectively (accessed September 2026). *C. sojae* has not been included in these databases but has been classified as Biosafety Level 1 and Hazard Group 1 in [BCRC Strain Collection](#) (Taiwan) and the [NCAIM Catalog](#) (Hungary), respectively (accessed September 2026). Given these, PC1 containment and work practices are considered appropriate when working with the wild type *S. cerevisiae* and *C. sojae*.

Section 4 The GMO - nature and effect of the genetic modification

69. The applicant proposes to conduct fermentation to produce ethanol from sugars in lignocellulosic hydrolysate using GM yeasts, known as the production of second generation ethanol, which uses plant waste such as agricultural waste in comparison to first generation ethanol production that is derived primarily from food crops such as corn and sugarcane. The applicant has developed a process to break down lignocellulosic material from woody and grassy agricultural, forestry and municipal wastes into lignocellulosic hydrolysate containing hexose (C6, including glucose, mannose and galactose) and pentose (C5, including xylose and arabinose) sugars by treatment with sulphuric acid, temperature and pressure. The derived lignocellulosic sugar hydrolysate is then fermented to ethanol or other high-value products (such as xylitol) using GM yeasts capable of fermenting both C6 and C5 sugars.

70. The GM yeasts will be generated by the applicant's clients from in-house or commercially available *S. cerevisiae* and *C. sojae* strains. The focus of this application is to test the performance of GM *S. cerevisiae* and *C. sojae* in using xylose for producing ethanol.

4.1 Metabolic pathway for fermentation of C6 and C5 sugars

71. The genes required for the utilisation of xylose are well characterised as most are involved in the fundamental biochemical pentose phosphate pathway (PPP) present in all yeasts (Figure 3). The PPP can fully operate under anaerobic conditions as none of the enzymatic steps in either the oxidative or non-oxidative phases require oxygen (Runquist et al., 2009b). The glyceraldehyde-3-phosphate and fructose-6-phosphate generated from the PPP can feed into glycolysis for subsequent conversion to ethanol during fermentation.

72. Once xylose has been taken up into the cell, it needs to be converted into a pentose phosphate (xylulose phosphate or ribulose phosphate) for incorporation into the PPP. There are two ways pentose phosphates can be produced:

- the first way, which is found in many yeast species, involves the sequential activity of a xylose reductase (XR) and xylitol dehydrogenase (XDH). XR is usually an NADPH-dependent enzyme, although there are some examples (e.g. from *Scheffersomyces stipitis*) that are also able to utilise NADH (dos Santos et al., 2025). XDH is a NAD⁺-dependent enzyme whose activity results in the conversion of xylitol into xylulose. Xylulose kinase (XK) activity results in the production of xylulose-phosphate which feeds into the PPP. It is thought the activity of XR results in an excess of NADP⁺ that cannot rapidly be reduced to regenerate NADPH resulting in the accumulation of xylitol.
- The second way of utilising intracellular xylose is through the activity of xylose isomerase (XI). This enzyme is not naturally present in fungi, but is often found in gut bacteria such as *Bacteroides* sp. The activity of XI is co-factor independent and so does not result in a redox imbalance in the cell or the production of xylitol, as it isomerises xylose directly into xylulose.

73. Following the formation of xylulose-phosphate by XK, the enzymes of the PPP will convert pentose phosphates into glyceraldehyde-3-phosphate and fructose-6-phosphate through the activity of transketolase (TKL) and transaldolase (TAL).

74. In media containing mixtures of hexoses and pentoses, ribulose-5-phosphate is produced through a glucose-dependent pathway, and ribose phosphate isomerase (RPI) reversibly converts it into ribose-5-phosphate. Ribose-phosphate epimerase (RPE) reversibly converts xylulose-5-phosphate into ribulose-5-phosphate.

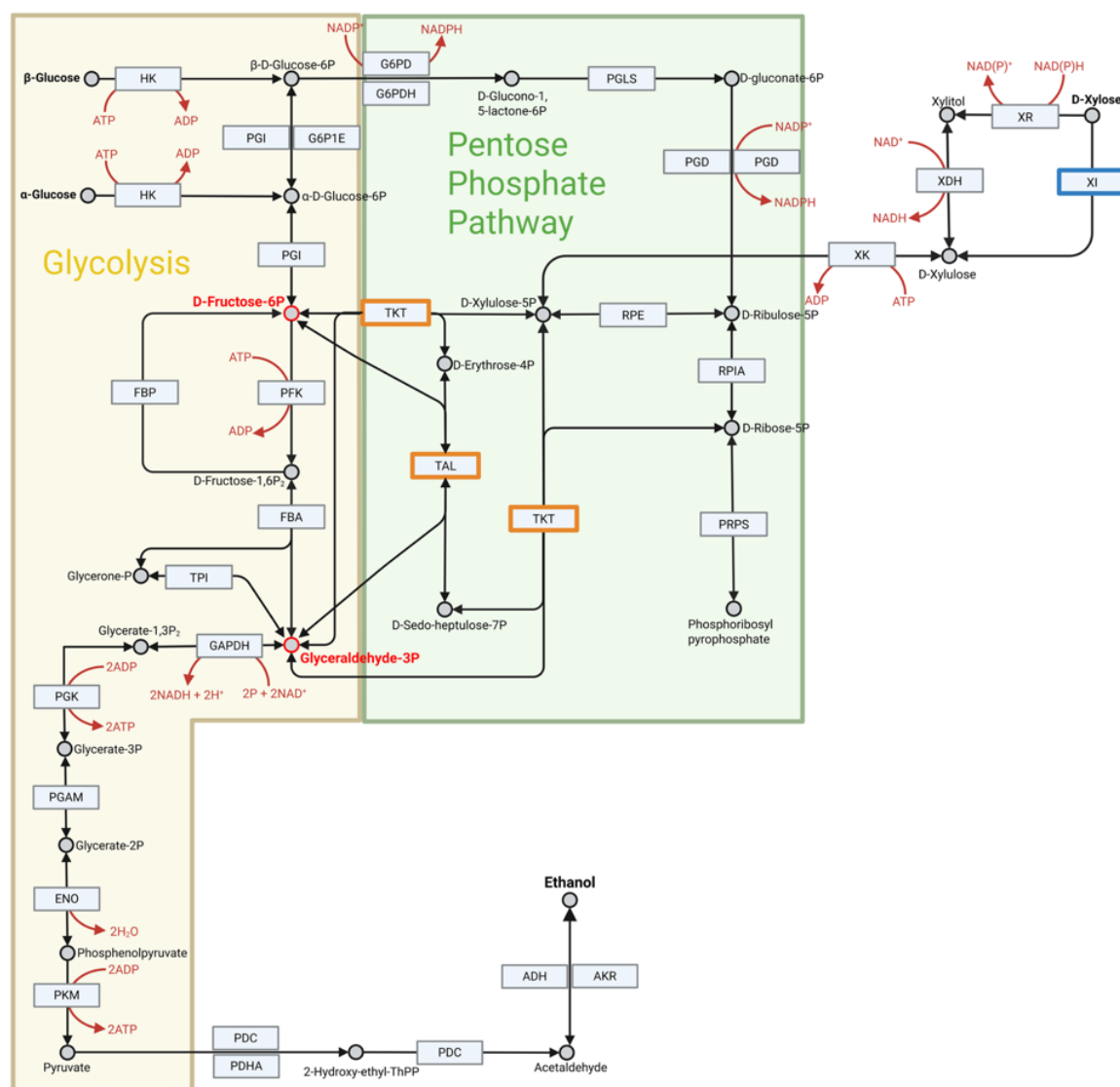


Figure 3 Metabolic pathway for fermentation of C6 and C5 sugars, including the pentose phosphate pathway, to ethanol (source: Ethtec) .

4.2 Development of GM yeasts

75. Whilst many yeast strains are capable of fermenting hexoses like glucose into ethanol, very few can also efficiently ferment pentoses like xylose into ethanol (Chandel et al., 2011). The GM yeasts will be developed by modifying their native genes in the metabolic pathways and introducing new genes from bacteria, fungi and plants for efficiently utilising and converting xylose to ethanol. Wild type *S. cerevisiae* is naturally capable of fermenting glucose into ethanol with high efficiency, but it lacks the apparatus for assimilation and utilisation of xylose. *C. sojae* is naturally able to utilise xylose, but conversion to ethanol does not occur, probably due to a redox imbalance generated from the activity of xylose reductase (XR) and xylitol dehydrogenase (XDH), and lack of XI.

4.2.1 Method of genetic modifications

76. The applicant states that genetic modifications of *S. cerevisiae* and *C. sojae* will include insertion of foreign genes that confer a new function (e.g. xylose uptake, xylose isomerase activity), site directed mutagenesis (e.g. modify binding affinity for xylose over glucose in a sugar transporter or NADH over NADPH in a xylose reductase), increased expression of a specific gene (e.g. for

transaldolase) using a stronger promoter than the native promoter, or through amplification of the genes (e.g. by exploiting natural properties such as haploinsufficiency (Deutschbauer et al., 2005; Norris et al., 2013)).

77. *C. sojae* is from the CTG clade of yeast (CTG yeast) which translate CTG (CUG) as serine rather than leucine (standard genetic code). Genes from other organisms to be expressed in CTG yeast must be modified so that there is no mis-translation of serine/leucine codons. DNA from bacterial, plant or fungal organisms such as *Bacteroides*, *Arabidopsis* and *Neocallimastigomycota* anaerobic fungi will be codon optimised and amplified using standard PCR approaches and inserted into plasmids for propagation in *E. coli*. Genes with optimised sequences may also be synthesised as **gBlocks™** gene fragments. No such modifications are required when inserting genes into *S. cerevisiae* which has a standard genetic code, but for genes from CTG yeast such as *S. stipitis*, they will need to be synthesised with appropriate codon changes.

78. For *C. sojae*, chromosomal constructs will be made using standard molecular biology approaches. Genes synthesised as **gBlocks™** will be inserted into plasmid vectors using restriction digest/ligase, **Gibson assembly** or **TEDA assembly**. Mutagenesis will either be performed using **gBlocks™** mutated gene fragments or using standard primer/PCR-based approaches. CRISPR-based approaches will also be used for gene deletion, insertion or modification using the *Candida*-specific pCT-tRNA system (Lombardi et al., 2019) or the SAT-1 flipper system (Reuss et al., 2004).

79. Genetic modifications to *S. cerevisiae* will be made using classical genetic methods including a/α haploid mating, homologous recombination, CRISPR-Cas9 gene editing and Cre/Lox system (Stepchenkova et al., 2023).

4.2.2 Genetic modifications

80. The GM yeasts to utilise xylose for the production of second generation ethanol will have the genes modified or new genes introduced as listed in Table 1. The GM yeasts may have any number or combination of the modifications.

Table 1 Modified genes required for second generation ethanol production

Genes for	Source	Function	Reference
High activity xylose transporter: XT (Pentose transporting MFS, Pentose transporting SWEET)	MFS: e.g. GXF1 from <i>Candida intermedia</i> , STP7 from <i>Arabidopsis thaliana</i> SWEET: e.g. NcSWEET1 from xylose metabolising yeast <i>Neocallimastix californiae</i>	Membrane proteins that move pentose sugars across cell boundaries	(Runquist et al., 2009a), (Rottmann et al., 2018), (Podolsky et al., 2021)
NADPH-dependent Xylose reductase: XR	xylose metabolising yeast e.g. <i>Candida sp.</i> , <i>S. stipitis</i>	Catalyses the first reduction step in microbial xylose metabolism by converting D-xylose into xylitol using a cofactor like NADPH or NADH	(Webb and Lee, 1992)

Xylitol dehydrogenase: XDH	<i>Candida sp.</i> , <i>S. stipitis</i>	Converts xylitol into D-xylulose using NAD ⁺ as a coenzyme	(Yoshiwara et al., 2023)
Xylose isomerase: XI	anaerobic commensal gut bacteria e.g. <i>Bacteroides sp.</i>	Converts D-xylose into D-xylulose	(Silva et al., 2021)
Xylulose kinase: XK	xylose metabolising yeast e.g. <i>Candida sp.</i> , <i>S. cerevisiae</i>	Converts the sugar D-xylulose into D-xylulose 5-phosphate by using energy from adenosine triphosphate (ATP)	(Johansson et al., 2001)
Ribose-phosphate epimerase: RPE	xylose metabolising yeast e.g. <i>Candida sp.</i> , <i>S. cerevisiae</i>	Catalyses the reversible conversion between D-ribulose 5-phosphate and D-xylulose 5-phosphate	(Bertels et al., 2021)
Ribose-phosphate isomerase: RPI	xylose metabolising yeast e.g. <i>Candida sp.</i> , <i>S. cerevisiae</i>	Converts D-ribose 5-phosphate and D-ribulose 5-phosphate back and forth in cellular metabolism	(Miosga and Zimmermann, 1996)
Transaldolase: TAL	xylose metabolising yeast e.g. <i>Candida sp.</i> , <i>S. cerevisiae</i>	Catalyses the reversible transfer of a three-carbon dihydroxyacetone unit between sugar phosphates	(Senac and Hahn-Hägerdal, 1991)
Transketolase: TKL	xylose metabolising yeast e.g. <i>Candida sp.</i> , <i>S. cerevisiae</i>	Sugar phosphate transfer	(Matsushika et al., 2012)

81. Promoter sequences from the host strains and other fungi associated with high levels of gene expression may be used for enhancing the expression of native or introduced genes listed in Table 1. These include GAPDH, PGI, TPI, ACT1 and TEF1 promoters (Defosse et al., 2018; Partow et al., 2010; Peng et al., 2015; Yang et al., 2015).

82. GM *S. cerevisiae* strains capable of high efficiently fermenting hexoses and pentoses into ethanol will require some/all of the following modifications:

- High activity XT
- Bacterial XI codon-optimised for expression in *S. cerevisiae*
- NAD(P)H-dependent XR with NADPH-dependent XR from another organism (e.g. codon-optimised *S. stipitis* XR)
- Insertion and amplification of xylulose production genes (codon-optimised XI, or XR and XDH)

- Amplification of XK, RPE, RPI, TKL, TAL
- Insertion of high activity promoters upstream of the xylose-utilisation genes (including PPP genes) such as the GAPDH, TPI, PGI, ACT1 and TEF1 promoters.

83. GM *C. sojae* strains capable of high efficiently fermenting hexoses and pentoses into ethanol will require some/all of the following modifications:

- High activity XT
- Bacterial XI codon-optimised for expression in *C. sojae*
- NADPH-dependent XR through mutation of native gene or replacement with NADPH-dependent XR from another organism (e.g. *S. stipitis*)
- Amplification of xylulose production genes (codon-optimised XI, or XR and XDH)
- Amplification of XK, RPE, RPI, TKL, TAL
- Insertion of high activity promoters upstream of xylose-utilisation genes (including PPP genes) such as the GAPDH, TPI, PGI, ACT1 and TEF1 promoters.

4.2.3 Sources of the introduced genes

84. The applicant states that some of the genes required for efficient ethanol production may be introduced into *S. cerevisiae* and *C. sojae*. These include genes encoding high activity XT from the plant species *Arabidopsis thaliana* and fungal species *Candida intermedia* and *Neocallimastix californiae*, genes encoding XR and XDH from the fungal species *Scheffersomyces stipites* (also known as *Pichia stipites*) and genes encoding XI from bacterial species *Bacteroides* sp. (Table 1).

85. *Arabidopsis thaliana* is a model plant widely used in plant molecular biology and not known to contain any toxins.

86. *Candida intermedia* is only considered an opportunistic human pathogen and rarely reported to cause disease.

87. *Neocallimastix californiae* resides in the rumen and faeces of herbivores, such as goats and sheep. It plays a crucial mutualistic role in helping these animals break down tough plant materials. It is not reported as a human pathogen.

88. *S. stipites* is commonly found in hardwood forests, decaying wood and inside the guts of wood-ingesting beetles and is not considered pathogenic to humans.

89. *Bacteroides* sp. are gram-negative anaerobic bacteria that form a major part of the normal human gut microbiota. They are generally considered beneficial commensal microbes inside the human gastrointestinal tract but can act as opportunistic pathogens that cause tissue damage and infections if they breach the intestinal barrier due to trauma, surgery or disease.

4.3 Expected properties of the GM yeasts

90. The applicant states that all GM yeast strains selected for fermentation of lignocellulosic sugar hydrolysate to produce ethanol at the HPB will be diploid strains. *C. sojae* strains are expected to have highly stable phenotypes as they lack mating type genes. For *S. cerevisiae* strains, genetic drift is a natural phenomenon and so resultant heterogeneity within a large population of cells fermented for an extended period is expected. The phenotypes of the GM *C. sojae* and *S. cerevisiae* strains will be assessed as part of the large-scale benchmarking fermentation experiments.

91. All of the GM yeast strains will be free of any selectable markers (e.g. containing no genes for antibiotic resistance).

Section 5 The receiving environment

92. The receiving environment forms part of the context in which the risks associated with dealings with the GMOs are assessed. Relevant information about the receiving environment includes abiotic and biotic interactions of the organism with the environment where the release would occur; production practices for the organism; presence of organisms that are sexually compatible with the GMO; and background presence of the gene(s) used in the genetic modification (OGTR, 2013).

93. The GM yeasts to be used for the fermentation process will be developed from laboratory *C. sojae* and *S. cerevisiae* strains which only grow under conducive culture conditions. In the event of a spill or leak, they are unlikely to survive in the environment and form a self-sustaining population.

5.1 Presence of related fungal species in the receiving environment

94. As discussed in Section 3.6, *S. cerevisiae* is commonly found in grapes and other fruits, which are not present in the vicinity of the HPB. *C. sojae* is commonly found in agricultural products, food processing environments, and plastic pollutants in natural waterways but not known to be in the environment in Australia. Agricultural wastes, which may host *C. sojae*, will be used in the sugar hydrolysate preparation process in the section next to the fermentation facility. However, this process involves acid and heat treatments that the wild-type *C. sojae* is unlikely to survive.

95. As discussed in Section 2.3.3, the applicant may use wild-type *C. sojae*, which naturally produces xylitol, for sequential fermentation after using GM *S. cerevisiae* to produce ethanol. This will introduce the wild type *C. sojae* into the fermentation facility. However, the GM yeast will be rendered non-viable through the heat treatment process before the second fermentation with wild-type *C. sojae* and the applicant states that the same procedure for decontamination and test for viability will be followed to ensure no viable cells remain following this second fermentation process. Hence, it is unlikely that the GM yeasts will interact with a wild-type yeast in the environment.

5.2 Presence of similar genes or products in the environment

96. The genes and their encoded proteins from the anaerobic gut bacteria, xylose metabolising yeasts and plants are ubiquitous in the environment.

Section 6 Previous authorisations

97. The GM yeasts have not been previously authorised for fermentation trials in Australia.

Chapter 2 Risk assessment

Section 1 Introduction

98. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 4). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

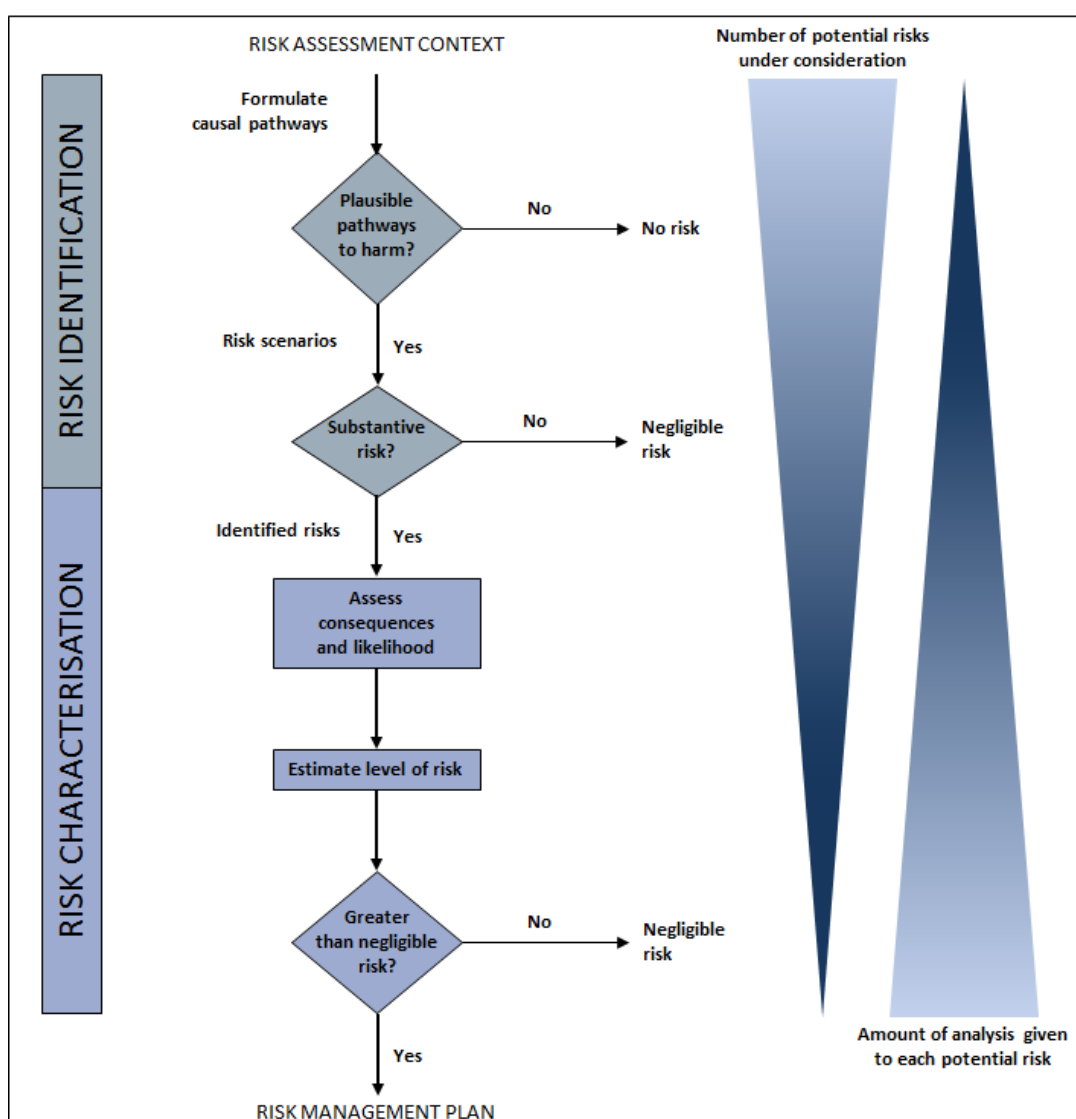


Figure 4 The risk assessment process

99. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, previous agency experience, reported international experience and consultation (OGTR, 2013).

100. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating plausible causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are risk scenarios.

101. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly

occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 4), i.e., the risk is considered no greater than negligible.

102. Risk scenarios identified as substantive risks are further characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The consequence and likelihood assessments are combined to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk identification

103. Postulated risk scenarios are comprised of three components (Figure 5):

- i. the source of potential harm (risk source)
- ii. a plausible causal linkage to potential harm (causal pathway)
- iii. potential harm to people or the environment.

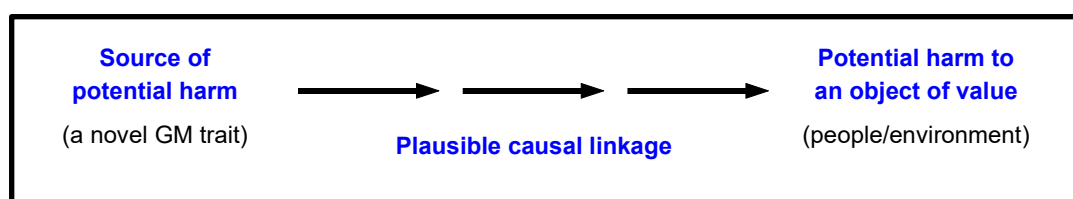


Figure 5 Components of a risk scenario

104. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors detailed in Chapter 1:

- the proposed dealings;
- the proposed limits including the extent and scale of the proposed dealings;
- the proposed controls to limit the spread and persistence of the GMO; and
- the characteristics of the parent organism(s).

2.1 Risk source

105. The sources of potential harms can be intended novel GM traits associated with one or more introduced genetic elements, or unintended effects/traits arising from the use of gene technology.

106. As discussed in Chapter 1, Section 4, the GM yeasts will be modified by modification of the native genes, introduction of new genes and enhancing gene expression using stronger promoters. These modifications are considered further as a potential source of risk.

107. The expression of native and introduced genes may be controlled by introduced regulatory sequences such as promoters. These sequences are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary DNA has no toxicity (Society of Toxicology, 2003). Hence, the introduced regulatory sequences will not be further considered as sources of potential harm for this application.

Unintended effects due to the process of genetic modification

108. Genetic modifications have the potential to cause unintended effects in several ways. These include insertional effects, where the introduced genetic elements are randomly integrated into the host's genome. These effects, such as interruptions, deletions, duplications or rearrangements of the genome, can lead to altered expression of endogenous genes as reviewed in plants (Schnell et al., 2015). Hence the potential for the processes of genetic modification to result in unintended effects will not be considered further.

109. While the principal purpose of the Act is the regulation of GMOs, licences may include conditions that impose obligations in relation to GM products, which are defined as a thing (other than a GMO) derived or produced from a GMO, if considered necessary. Examples include ethanol and xylitol produced from the fermentation of GM yeasts. The applicant has stated that there will be no commercial or other use of purified products beyond what is required for research and development purposes (e.g. assessment of purity). Therefore, risks posed during handling those products after separating them from the GM yeasts will not be considered further in this risk analysis.

110. Risks arising from cross-contamination of fermentation equipment, leading to unintended expansion of the GM yeasts while culturing other organisms, or product contamination at downstream processing stages, will not be considered further. Fermenters and processing equipment undergo cleaning processes after each use and are sterilised before initiating a new culture.

2.2 Causal pathway

111. The following factors are taken into account when postulating plausible causal pathways to potential harm:

- the proposed dealings with the GMO;
- proposed limits, including the extent and scale of the proposed dealings;
- characteristics of the parent organism;
- potential effects of introduced or deleted gene(s) on the properties of the organism;
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s);
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment;
- the receiving environment;
- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential);
- potential for gene transfer to sexually compatible organisms
- gene transfer by horizontal gene transfer (HGT); and
- unauthorised activities.

112. Although all these factors were considered, some are not included in the risk scenarios below as a plausible pathway to harm could not be identified.

113. As discussed in Chapter 1 Section 3.4, under conditions such as depletion of essential nutrients, *S. cerevisiae* resorts to formation of mating-types which produce diploid cells. These diploid cells can readily sporulate. *C. sojae* is an anamorphic (asexual) yeast species that does not form ascospores or sporulate. The culture conditions proposed by the applicant are not conducive for sporulation. Hence, the potential for spread through spore formation will not be considered further.

114. The commercially available strains proposed to be used in the application are laboratory adapted *S. cerevisiae* and *C. sojae* strains that can only grow under optimal conditions. As a result, these strains are unlikely to grow in the environment in an event of escape. Moreover, the wild-type *S. cerevisiae* is typically found in fruits like grapes, which are not found in the vicinity of the HPB. *C. sojae* is also not known to occur in the environment in Australia. *C. sojae* is an anamorphic yeast and the genetic modifications are unlikely to change this. If the GM *S. cerevisiae* and *C. sojae* strains are used in a single culture as discussed in Chapter 1, Section 2.3.3, the strains would not be able to mate. When a GM strain is used together with a wild-type strain in a single culture, the mating, if occurs, will result in cells that are similar to the GMOs. Hence, the potential of gene transfer to sexually compatible organisms or homologous recombination will not be considered further.

115. As discussed in Chapter 1 Section 3.5, *S. cerevisiae* and *C. sojae* do not produce any toxins and are not considered pathogenic to healthy humans. Hence, harms relating to toxicity and pathogenicity will not be considered further. Similarly, the potential for reversion of the GM yeasts to the parental phenotype is not a plausible pathway to harm because the parent organisms are not pathogenic or harmful (Chapter 1, Section 3). Therefore, reversion will not be considered further.

116. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities by the applicant will not be considered further.

2.3 Potential harms

117. The introduced genes encode for enzymes involved in ethanol production. Therefore, the potential harms that will be considered are:

- risks to people undertaking this dealing from exposure to the GM yeast; and
- risks to people and the environment from an unintentional release of GM yeast.

2.4 Postulated risk scenarios

118. Two risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 2 and discussed in depth in Sections 2.4.1 – 2.4.2.

119. In the context of the activities proposed by the applicant and considering both the short and long term, neither of the two risk scenarios gave rise to any substantive risks.

Table 2 Summary of risk scenarios from the proposed dealings with GM yeast

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reasons
1	GM yeasts	Fermentation of GM yeasts ↓ Exposure of people at the fermentation facility via ingestion, inhalation of aerosols or contact ↓ Expression of modified or introduced genes	Infection by GM yeasts causing acute or chronic diseases	No	<ul style="list-style-type: none"> • The limits and controls of the proposed fermentation process would minimise exposure of people to the GM yeasts. • The fermentation facility will be limited to one location which has restricted access. • Closed system equipment will limit exposure to GM yeasts. • <i>S. cerevisiae</i> and <i>C. sojae</i> are not known to cause disease in healthy individuals and are not expected to colonise the human gut
2	GM yeasts	Culture of GM yeasts ↓	Adverse effects in	No	<ul style="list-style-type: none"> • The proposed limits and controls would minimise the likelihood

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reasons
		Escape of GM yeasts into the environment via: <ul style="list-style-type: none"> Leakage from culture tanks or pipes During sample collection ↓ Presence of GM yeasts outside the fermentation facility ↓ Establishment and persistence of GM yeasts in the environment	people or animals		of dispersal of the GM yeasts. <ul style="list-style-type: none"> Restricted access and closed systems will prevent animals and non-staff getting into contact with GM yeasts. The culture tanks and pipes will be regularly inspected for any leakage. The introduced or modified genes are not expected to confer any selective advantage to the GM yeasts to establish and persist in the environment. Following each fermentation, the GM yeasts will be rendered non-viable using a validated method (e.g. heat treatment).

2.4.1 Risk scenario 1

Risk source	GM yeasts
Causal pathway	Fermentation of GM yeasts ↓ Exposure of people at the at the fermentation facility via ingestion, inhalation of aerosols or contact ↓ Expression of modified or introduced genes
Potential harm	Infection by GM yeasts causing acute or chronic disease

Risk source

120. The source of potential harm for this postulated risk scenario is the GM yeasts.

Causal Pathway

121. The GM yeasts will be cultured at the fermentation facility for ethanol production.

122. People working at the fermentation facility may be exposed to GM yeasts via direct contact with the GM yeast culture solution or by inhalation of aerosolised GM yeasts resulting in infection by the GM yeasts. This would be most likely to occur when people are working with GM yeasts, e.g., while monitoring culture conditions, or in the event of a failure of closed systems, e.g., over pressurisation resulting in bursting of a fermenter and leakage or spill during transfer of GM yeasts to a fermenter.

123. Spills or leaks during culturing, harvest or post-fermentation cleaning may lead to dermal contact of people with GM yeasts or to inhalation from aerosol exposure.

124. As mentioned in Chapter 1, fermentation will be carried out using a closed system which will have probes for monitoring culture parameters; filtered gas exchange vents, ports for inputs and sample collection, thus significantly limiting the need to open the system. The fermentation for ethanol production needs to be conducted under anaerobic conditions (Chapter 1, Section 3.2) so the fermenters do not need to be aerated, reducing the risk of aerosol generation from the aeration process. Further, transfer of fluids will be via closed transfer lines. Moreover, staff working with the GM yeast will be trained and will wear appropriate PPE, which includes face masks and gloves. All these measures will prevent people from aerosol exposure or from direct contact with GM yeast cultures.

125. Oral ingestion of the GM yeasts may lead to GM yeasts entering the human gut and locally producing ethanol. Non-GM yeasts like *S. cerevisiae* have been recognised to ferment ethanol in the human gut. In rare cases, this may cause auto-brewery syndrome, also known as gut fermentation syndrome, which is a rare condition where an overgrowth of certain microorganisms such as yeast in the gastrointestinal tract produce excessive ethanol from ingested carbohydrates, leading to symptoms of alcohol intoxication (Paramsothy et al., 2023). Treatments include anti-fungal medications, probiotics and a low carbohydrate diet. However, ingestion of the GM yeasts by staff is highly unlikely to occur as the GM yeasts will be cultured in closed systems and appropriate PPE will be worn. Also, the GM yeasts are not expected to colonise the healthy human gut and form sustainable populations, so any effect will be transient.

126. The production process will be restricted to one location and for five years from the date of issue of the licence. Access to the fermentation facility would be only given to authorised and appropriately trained people. This means that a limited number of people would be in the fermentation facility or handling the GM yeasts.

127. As mentioned in Chapter 1, no material from this fermentation process would be used for human food or animal feed. The GM yeasts will be inactivated by exposure to heat during the ethanol recovery process. This would limit the exposure of people to viable GM yeast.

128. In the event of a spill or leakage, spill kits placed adjacent to the fermenters would be used. The spill kits contain absorbent material, PPE and decontamination solution. This could be deployed quickly to soak up and kill the GM yeasts. In case of large spills, the drains lead to a large sub-floor tank for collection and chemical inactivation of any spills that occur.

129. These measures are expected to minimise exposure of people to GM yeasts during a spill or leakage.

130. Overall, the proposed limits and controls minimise the potential for exposure of GM yeasts to people working at the fermentation facility.

Potential harm

131. In the case of exposure to GM yeasts, people in contact with the GM yeasts may be infected and develop acute or chronic diseases. However, as discussed in Chapter 1, Section 3.5, both *S. cerevisiae* and *C. sojae* were only known as opportunistic pathogens of humans who have underlying conditions or predisposing factors. Intrusive infection to healthy people is rare. The genetic modifications to *S. cerevisiae* and *C. sojae* to generate the strains used in this application are unlikely to change the pathogenicity of the GM strains as the introduced or modified genes are all involved in the metabolisms of sugars. Therefore, if any staff at the fermentation facility are exposed to the GM yeasts the possibility of serious disease developing is unlikely.

Conclusion

132. Risk scenario 1 is not identified as a substantive risk due to limited potential for exposure to the GM yeasts and the infection by the GM yeast causing acute or chronic diseases is unlikely. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	GM yeasts
Causal pathway	<p>Culture of GM yeasts</p> <p>↓</p> <p>Escape of GM yeasts into the environment via:</p> <ul style="list-style-type: none"> • Leakage from fermenters or pipes • During sample collection <p>↓</p> <p>Presence of GM yeasts outside the fermentation facility</p> <p>↓</p> <p>Establishment and persistence of GM yeasts in the environment</p>
Potential harm	Adverse effects in people or animals

Risk source

133. The source of potential harm for this postulated risk scenario is the GM yeasts.

Causal Pathway

134. The GM yeasts could escape from the fermentation facility during events such as leakage from fermenters and pipelines or sample collection. The GM yeasts could establish and persist in the environment. This could result in exposure of people, other desirable organisms, or the environment.

Potential for persistence at the fermentation facility

135. The GM yeasts could persist at the fermentation facility:

- in fermenters between batches of GM yeast culture
- in equipment other than fermenters used in connection with the GM yeasts or
- in spills of culture solution suitable to grow yeast.

136. The applicant has proposed that GM yeast cultures will be grown at the site in closed systems; all equipment used in connection with the GM yeasts will be cleaned/sterilised after use and before use for a new batch; and to regularly inspect the equipment for any leakages. These controls would minimise the chances of GM yeasts dispersing and persisting in the fermentation facility.

137. As mentioned above in risk scenario 1, if there is any leak or spill, area or cultures will be decontaminated using chemical inactivation. Alternatively, for large spills, drains lead to the large sub-floor tank for chemical inactivation.

138. In addition, the GM yeasts could persist at the fermentation facility due to ineffective inactivation of GM yeast. The applicant has stated that the yeast strains can be effectively decontaminated by H₂O₂. This method of decontamination will be used in the event of a spill at the site. The GM yeasts will be rendered non-viable during the ethanol recovery process as the ferments

will be heated to 100°C and the applicant states that the ferments will be tested to ensure no viable cells remain. Clean in place (CIP) with caustic solution will be used to decontaminate the culture vessels.

Dispersal through human activity

139. Dispersal through human activity could occur if staff did not adhere to protocols or by accident. For example, GM yeast could be transported on clothing or footwear following a spill of GM yeast at the fermentation facility.

140. Access to the site would be restricted to authorised, trained staff. The site will be checked for spills of GM yeast culture solution and action will be taken to decontaminate any such spills. Transport of large volume of culture will not occur under this DIR, which would minimise the opportunity for dispersal of GM yeast.

Dispersal by animals

141. The fermentation facility has restricted access, and the fermentation process will be conducted in closed systems. This will prevent access by wild or domestic animals to the facility and being exposed to the GMOs.

Potential harm

142. The potential harms for this risk scenario are that the GM yeasts could spread and persist in the environment. This could lead to exposure of animals and people potentially causing adverse effects. The potential harms for this risk scenario for people are the same as the potential harms described in detail in Risk Scenario 1.

143. The yeast itself and proteins expressed in GM yeast are commonly present in the environment and there are no reports of toxicity in animals.

144. As discussed in Chapter 1, Section 3.5 *S. cerevisiae* and *C. sojae* are not known to be plant pathogens and the genetic modifications are not expected to change this nature.

145. The GM yeasts could spread and persist in the environment as an invasive species and out compete native yeasts. However, as the strains used for development of GMOs are laboratory strains that require a conducive environment for growth, they are not expected to survive well in the environment.

146. The fermentation facility is in an area where the natural hosts of *S. cerevisiae* do not exist. *C. sojae* is not known to be present in the environment in Australia, and, if it is present, it is not expected to survive the heat and acid treatment during sugar extraction to create the lignocellulosic hydrolysate. This will minimise the GM yeasts from finding a suitable environment to spread and persist.

Conclusion

147. Risk scenario 2 is not identified as a substantive risk due to restricted access to the fermentation facility and using closed systems that would limit potential dispersal into the environment. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

148. Uncertainty is an intrinsic part of risk analysis and is present in all aspects of risk analysis. This is discussed in detail in the Regulator's [Risk Analysis Framework](#) document.

149. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios

involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

150. For DIR 231, uncertainty is noted particularly in relation to molecular characterisation of the GMOs.

151. Additional data, including information to address these uncertainties, may be required to assess possible future applications with reduced limits and controls, such as the commercial release of these GM yeasts.

152. Chapter 3, Section 4, discusses information that may be required for future release.

Section 4 Risk evaluation

153. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

154. Factors used to determine which risks need treatment may include:

- risk criteria,
- level of risk,
- uncertainty associated with risk characterisation, and
- interactions between substantive risks.

155. Two risk scenarios were postulated whereby the proposed dealing might give risk to harm to people or the environment.

156. A risk is substantive only when the risk scenario may, because of gene technology, have some chance of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent a substantive risk and do not advance in the risk assessment process.

157. Two risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the control measures proposed by the applicant, and considering both the short and long term, neither of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 2 and include:

- fermentation will occur in closed systems.
- no GM yeast would enter human food or animal feed;
- limits on the scale and duration of the proposed release;
- people conducting dealings will wear appropriate PPE;
- waste containing GM yeast will be decontaminated before its disposal; and
- suitability of proposed controls to restrict the spread and persistence of the GM yeasts and their genetic material.

158. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM yeasts into the environment are considered negligible. The *Risk Analysis Framework* (OGTR, 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no controls are required to treat these negligible risks. Hence, the Regulator

considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment².

² As none of the proposed dealings are considered to pose a significant risk to people or the environment, Section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP.

Chapter 3 Risk management plan

Section 1 Background

159. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan addresses risks evaluated as requiring treatment and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through proposed licence conditions.

160. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

161. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: Section 64 requires the licence holder to provide access to premises to OGTR inspectors and Section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

162. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in Section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under Section 152 of the Act.

Section 2 Risk treatment measures for substantive risks

163. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed trial of GM yeast. The risk scenarios were considered in the context of the proposed release (Chapter 1, Section 2), the proposed control measures (Chapter 1, Section 2.2) and the receiving environment (Chapter 1, Section 5) and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

164. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible to low. Therefore, to maintain the risk context, draft licence conditions have been imposed to limit the number of production trial sites and duration of the trial, as well as a range of controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in the draft licence.

3.1 Limits and controls on the release

165. Section 2.1 and Section 2.2 in Chapter 1 list the limits and controls proposed by the applicant in their application. Many of these are discussed in the two risk scenarios considered in Chapter 2. The appropriateness of the limits and controls is considered further in the following sections.

3.1.1 Consideration of limits proposed by Ethtec

166. The proposed work would take place at a single location at the Ethtec's purpose-built biorefinery facility in NSW, which is owned and managed by Ethtec. The proposal is to run the fermentation and ethanol production trials for five years from the issue of licence. The fermentation experiments would be run for up to a maximum total fermentation volume of 1300 L per large-scale experiment. The limited size and duration of the trial would restrict the potential exposure of people and desirable animals to the GMOs (Risk scenario 1). Considering a possibility that some culture runs might fail, a restriction on the number of runs per annum has not been proposed in the draft licence, but a limit of the maximum combined capacity of all culture vessels used for large scale fermentation up to 1300 L has been included. Taking into consideration the limits proposed, a licence condition is included to restrict the location to the one site (Ethtec's biorefinery facility) and licence period to five years from the issue of the licence.

167. The applicant proposes that only trained and authorised staff would be permitted to deal with the GM yeasts. Standard conditions included in the licence state that only people authorised by the licence holder are covered by the licence and that the licence holder must inform all people dealing with the GM yeasts of applicable licence conditions. These measures would limit the exposure of people to potential harm from the GMOs.

3.1.2 Consideration of proposed controls to manage exposure to the GMOs

168. The biorefinery facility has restricted access to authorised people, and this is included as a draft licence condition. This condition restricts the exposure of people not authorised under the licence and animals to the GM yeasts.

169. The applicant has proposed that for all fermentation experiments staff would be required to wear long sleeve tops and full-length pants, steel capped boots with chemical resistant soles, safety glasses/goggles and appropriate gloves (latex or heavy duty depending on the task). A draft licence condition is included that requires staff to wear coveralls, P2 mask and gloves where there is possibility of exposure to the GM yeasts.

170. The applicant has stated that the GM yeasts and the fermentation products will not be used in human food or animal feed. As discussed in Chapter 2 Section 2.1, while the principal purpose of the Act is the regulation of GMOs, licences may include conditions that impose obligations in relation to GM products, which are defined as a thing (other than a GMO) derived or produced from a GMO, if considered necessary. The draft licence requires that the GM yeast material, which means any GM yeasts, whether viable or not, or any product of these yeasts, must not be used as food for humans or feed for animals. This condition would maintain the risk context by restricting the exposure of people and desirable animals to the GM yeasts by consumption.

171. The applicant has not proposed to transport GMOs under this licence but have proposed that storage of GMOs will follow the Regulator's *Guidelines for Transport, Storage and Disposal of GMOs* (TSD guidelines). Draft conditions have been included for storage of the GMOs within the fermentation facility according to the TSD guidelines.

3.1.3 Consideration of proposed controls to manage dispersal and persistence of the GMOs

172. The applicant has proposed control measures to limit the dispersal of GM yeasts during the fermentation process. The applicant proposes that fermentation will be carried out in closed fermenters to minimise the likelihood of aerosol release, direct contact and ingestion. The closed fermenters and transfer lines would be decontaminated before and after contact with the GM yeasts. These controls are considered appropriate to minimise the likelihood of dispersal of the GM yeasts and are covered in several draft licence conditions.

173. The applicant has proposed physical and behavioural control measures to limit the dispersal of GM yeasts during fermentation and ethanol recovery, by minimising aerosol formation, direct

contact and ingestion. Following each fermentation, the unfiltered liquid with GM yeasts is transferred from the fermenters directly to the heat exchanger to bring the temperature up to about 100°C for ethanol recovery, during which the GM yeasts would be rendered non-viable. The applicant will test aliquots of the ferments during this heating process to ensure that no viable cells remain. All the vessels and piping network will be inspected regularly for any leakage. These controls are considered appropriate and are included in the draft licence.

174. The methodology for inactivation of the GMOs will be tested during the initial stages of batch culture for effectiveness of killing the GM yeast. A licence condition is included that stipulates that both methods of heat and chemical inactivation be tested and validated for their effectiveness in killing the GM yeasts after the first run and these results must be provided to the Regulator before proceeding with further runs.

175. The applicant has proposed a contingency plan to deal with any inadvertent spills. As an additional back-up, for example in case of a large spill volume, the drain in the culture area could be blocked and the GM yeast culture pumped to a large sub-floor tank where it could be chemically inactivated. These steps are considered appropriate and are drafted as licence conditions.

176. A standard condition is included in the draft licence requiring the licence holder to ensure that dealings are conducted to not compromise the health and safety of people and minimise unintentional exposure to the GM yeast.

3.1.4 Summary of licence conditions to be implemented to limit and control the dealings

177. Licence conditions have been drafted to limit and control the proposed dealings, based on the above considerations. These include requirements to:

- limit the duration of the release to a maximum of five years from issue of the licence;
- limit the release to a single location in NSW: Ethtec's Hunter Pilot Biorefinery at Muswellbrook, NSW;
- limit the combined capacity of the culture vessels to 1300L;
- the fermenters be of closed systems;
- the fermentation facility must have restricted access;
- inspect the culture area for spilled media regularly during culture of the GM yeasts;
- clean areas and equipment exposed to the GM yeasts after use;
- clean any equipment used with the GM yeasts before use for any other purpose;
- store the GM yeasts in accordance with the Regulator's guidelines;
- not allow the GM yeasts to be used for human food and animal feed;
- not allow the GM yeasts to be released into the environment; and
- dispose of the GM yeasts via effective decontamination methods.

3.2 Other risk management considerations

178. All DIR licences issued by the Regulator contain conditions that relate to general risk management. These include conditions relating to:

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting requirements
- access for the purpose of monitoring for compliance.

3.2.1 Applicant suitability

179. In deciding whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under Section 58 of the Act, matters that the Regulator must take into account include:

- any relevant convictions of the applicant
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the capacity of the applicant to meet the conditions of the licence.

180. If a licence were issued, the conditions would include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

181. In addition, the applicant organisation must have access to an IBC and be an accredited organisation under the Act.

3.2.2 Contingency plans

182. The applicant has described measures to be undertaken in the event of unintentional release of the GMO into the environment. This includes accidental release during operations of the fermentation system on-site. Draft licence conditions are included in case of accidental release of the GMOs.

183. Before culturing the GM yeasts, the applicant would also be required to provide the Regulator with a method to detect the GM yeast reliably and uniquely.

3.2.3 Identification of the persons or classes of persons covered by the licence

184. If issued, the persons covered by the licence would be the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. The applicant states that the PPE used in the fermentation facility will be decontaminated and disposed of in the Cert-4994 PC2 laboratory under an NLRD authorisation. Therefore, no external service provider will be engaged in this respect.

185. Prior to culture of the GM yeast, the applicant would be required to provide a list of people and organisations that are covered by the licence, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

186. If issued, the licence would require the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the dealings
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the dealings.

3.2.5 Monitoring for compliance

187. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

188. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

189. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to the health and safety of people or the environment could result.

Section 4 Issues to be addressed for future releases

190. Additional information has been identified that may be required to assess an application for a commercial release of these GM yeasts, or to justify a reduction in limits and controls. This includes additional molecular and biochemical characterisation of the GM yeasts, and the effectiveness of the closed system in limiting the release of live GMOs into the environment.

Section 5 Conclusions of the consultation RARMP

191. The RARMP concludes that the proposed limited and controlled release of GM yeasts poses negligible risks to the health and safety of people or the environment as a result of gene technology, and that these negligible risks do not require specific risk treatment measures.

192. If a licence were issued, conditions would be imposed to limit the proposed size, location and duration of the release, and to restrict the spread and persistence of the GM yeasts and their genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.

Chapter 4 Draft licence conditions

Section 1 Interpretations and definitions

1. In this licence:

- (a) unless defined otherwise in this licence, words and phrases used in this licence have the same meaning as they do in the Act and the Gene Technology Regulations 2001;
- (b) words importing a gender include every other gender;
- (c) words in the singular number include the plural and words in the plural number include the singular;
- (d) expressions used to denote persons generally (such as “person”, “party”, “someone”, “anyone”, “no one”, “one”, “another” and “whoever”), include a body politic or corporate as well as an individual;
- (e) references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;
- (f) where a word or phrase is given a particular meaning, other grammatical forms of that word or phrase have corresponding meanings;
- (g) specific conditions prevail over general conditions to the extent of any inconsistency.

2. In this licence:

‘Act’ means the *Gene Technology Act 2000* (Commonwealth) or the corresponding State law under which this licence is issued.

‘Contingency Plan’ means a written plan detailing Decontamination measures to be taken in the event of the unintentional release of the GMOs.

‘Closed system’ means a system for growth, processing and/or storage of large-scale cultures of GMOs consisting of an enclosed vessel or vessels and transfer lines.

‘Culture Vessel’ means any of the Closed system located in the Fermentation facility which is used for the culture of the GMOs pursuant to this licence.

‘Decontaminate’ (or **‘Decontamination’**) means, as the case requires, kill the GMOs by one or more of the following methods:

- (a) chemical treatment;
- (b) autoclaving;
- (c) high-temperature incineration; or
- (d) a method approved in writing by the Regulator.

Note: ‘As the case requires’ has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate.

‘Fermentation facility’ means the purpose-built biorefinery as defined in Condition 20.

‘GM’ means genetically modified.

‘GMO’ means the genetically modified organisms that are the subject of the dealings authorised by this licence.

‘NLRD’ is a notifiable low risk dealing. Dealings conducted as an NLRD must be assessed by an institutional biosafety committee (IBC) before commencement and must comply with the requirements of the Gene Technology Regulations 2001.

‘Personal information’ has the same meaning as in the *Privacy Act 1988*. Personal information means information or an opinion about an identified individual, or an individual who is reasonably identifiable:

- (a) whether the information or opinion is true or not; and
- (b) whether the information or opinion is recorded in a material form or not.

‘Regulations’ means the Gene Technology Regulations 2001.

‘Regulator’ means the Gene Technology Regulator.

‘Sample’ means any culture volume collected from Culture Vessels for analysis as part of the protein production process.

‘Yeast Material’ means any GM yeasts, whether viable or not, or any product of these yeasts.

Section 2 General conditions and obligations

Holder of licence

3. The licence holder is Ethanol Technologies Pty Ltd.

Remaining an Accredited Organisation

4. The licence holder must, at all times, remain an accredited organisation.

Validity of licence

5. This licence remains in force until it is suspended, cancelled, or surrendered. No dealings with the GMOs are authorised during any period of suspension, or after the licence has been cancelled or surrendered.

Note: Although this licence has no expiry date, the duration of culture of the GMO is restricted in accordance with Condition 20.

Persons covered by this licence

6. The persons covered by this licence are:
 - (a) the licence holder, and any employees or agents engaged by the licence holder; and
 - (b) the project supervisor(s); and
 - (c) other persons who are, or have been, engaged or otherwise authorised by the licence holder or the project supervisor to conduct any of the dealings authorised by this licence.
7. The licence holder must keep a record of:
 - (a) all persons covered by this licence; and
 - (b) the contact details of the project supervisor(s) for the licence.
8. The licence holder must provide information related to the persons covered by the licence when requested to do so in writing by the Regulator and must provide the information within a time period stipulated by the Regulator.

Description of GMOs covered

9. The licence authorises specified dealings in respect of the GMOs identified and described in **Attachment A**.

Dealings authorised by this licence

10. The licence holder and persons covered by this licence may conduct the following dealings with the GMOs:

- (a) culture the GMOs in volumes exceeding 25 litres per vessel;
- (b) use the GMOs in the course of manufacture of a thing that is not the GMOs;
- (c) conduct the following experiments with the GMOs:
 - i) to assess the performance of GM *Saccharomyces cerevisiae* and *Candida sojae*; and
 - ii) to optimise the scale-up fermentation process;
- (d) dispose of the GMOs;

and may possess, use or store the GMO for the purposes of, or in the course of, any of these dealings.

11. This licence does not apply to dealings with the GMOs conducted as an NLRD or pursuant to another authorisation under the Act.

Conditions imposed by the Act

Note: The Act mandates the following 3 conditions.

Informing people of licence conditions (section 63)

12. The licence holder must inform any person covered by the licence, to whom a particular condition of the licence applies, of the following:

- (a) the particular condition, including any variations of it; and
- (b) the cancellation or suspension of the licence; and
- (c) the surrender of the licence.

Monitoring and audits (section 64)

13. If a person is authorised by this licence to deal with the GMOs and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Additional information to be given to the Regulator (section 65)

14. The licence holder must immediately inform the Regulator if they become aware of:

- (a) additional information about any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or
- (b) any contraventions of the licence by a person covered by the licence; or
- (c) any unintended effects of the dealings authorised by the licence.

Note 1: For the purposes of this condition:

- (a) *The licence holder is taken to have become aware of additional information if they were reckless as to whether such information existed; and*
- (b) *The licence holder is taken to have become aware of contraventions, or unintended effects, if they were reckless as to whether such contraventions had occurred, or such unintended effects existed.*

Note 2: Contraventions of the licence may occur through the action or inaction of a person.

Note 3: Additional information includes any changes to the Fermentation facility, which might increase the likelihood of unintentional exposure of people or release of the GMO into the environment.

Note 4: An example of informing immediately is contact made at the time of the incident via the OGTR free call phone number 1800 181 030 or email to OGTR.M&C@health.gov.au.

Informing the Regulator of any material changes of circumstance

15. The licence holder must immediately, by notice in writing, inform the Regulator of:

- (a) any relevant conviction of the licence holder occurring after the commencement of this licence;
- (b) any revocation or suspension after the commencement of this licence, of a licence or permit held by the licence holder under a law of the Commonwealth, a State, or a foreign country, being a law relating to the health and safety of people or the environment;
- (c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the licence holder to meet the conditions in it.

16. The licence holder must provide information related to the licence holder's ongoing suitability to hold a licence when requested to do so in writing by the Regulator and must provide the information within a time period stipulated by the Regulator.

Further conditions with respect to informing persons covered by the licence

17. If a particular condition, including any variation of it, applies to a person with respect to any dealing, the licence holder must not permit a person covered by this licence to conduct that dealing unless:

- (a) the licence holder has obtained from the person a signed and dated statement that the person:
 - i) has been informed by the licence holder of the condition and, when applicable, its variation; and
 - ii) has understood and agreed to be bound by the condition, or its variation; and
 - iii) has been trained in accordance with sub-condition 17(b) below; and
- (b) the licence holder has trained that person in a manner which enables them to conduct the dealings in accordance with the conditions of this licence.

18. The licence holder must notify all persons covered by the licence, from whom Personal information relevant to the administration and/or enforcement of the licence is collected by the licence holder, that such Personal information may be disclosed to the Regulator.

19. The licence holder must ensure that a copy of the licence is readily available to all persons covered by the licence who are conducting dealings with the GMO.

Note: The licence may be made available electronically.

Section 3 Limits and control measures

Limits on the release

The following licence conditions maintain the risk assessment context within which the application was assessed, by imposing limits on where and when the GMOs may be cultivated, and on other activities that can be undertaken.

20. Culture of the GMOs may only occur at the Fermentation facility listed below and must be completed within five years from the date of issue of the licence.

Fermentation Facility

Location	Local government area	Facility features
The Hunter Pilot Biorefinery (HPB) 24 Carramere Road, Muswellbrook NSW 2333 Australia	Muswellbrook Shire Council	i. walls, a roof and a concrete floor, ii. a concrete bunded area where the Culture Vessels will be placed within, iii. a sub floor tank.

21. The maximum combined capacity of all Culture Vessels used for large scale fermentation must not exceed 1300 litres.

Control measures

The following licence conditions restrict the spread or persistence of the GMOs and their genetic material in the environment.

GMOs must not enter food or feed

22. Yeast Material must not be used, sold or otherwise disposed of for any purpose, which would involve or result in their use as food for humans or feed for animals.

Control measures related to exposure of the GMOs

23. Staff conducting dealings with the GM yeast must wear appropriate personal protective equipment (PPE) including coveralls, a P2 mask or equivalent and gloves.

Control measures related to dispersal of the GMOs

24. Culture of GMOs may only be undertaken in:

- (a) closed Culture Vessels; or
- (b) vessels approved in writing by the Regulator.

25. Transfer of fluids must be either through closed transfer lines or a closed transfer vessel.

26. Secondary containment, such as bunding and a sub-floor tank, must be provided to retain any leakage from the closed system. It must be of sufficient capacity to contain:

- (a) the volume of fluid held in the largest single Culture Vessel, or group of vessels where interconnection could result in leakage from multiple vessels; plus
- (b) the volume of any disinfectant that might be used

with additional capacity to prevent any expected general fluid movement from breaching the secondary containment.

27. During culture of the GMOs, and until all Culture Vessels and other parts of the Closed system in contact with GMOs have been Decontaminated, any person that may come into contact with the GMOs must:

- (a) employ work practices that minimise the production of aerosols;
- (b) clearly label containers containing the GMOs;
- (c) use appropriate protective clothing as specified in Condition 23; and
- (d) Decontaminate hands before leaving the Fermentation facility.

28. While the GMOs are growing in a Culture Vessel, the Fermentation facility must be inspected by people trained to recognise spills/leaks of culture medium, and actions taken as follow:

Area	Period of inspection	Inspection frequency	Inspect for	Action
Fermentation facility	From the commencement of culture of any GMOs in a Culture Vessel until culture of any GMOs in a Culture Vessel has ceased, and the Culture Vessel and all equipment in contact with GMOs has been Decontaminated.	Daily. Inspection records must be kept and provided to the Regulator, if requested.	Spilled culture solution containing GM yeast	Implement contingency plan in accordance with Condition 36
			Failures of any components of the Closed system	Repair

29. Any large-scale culture of the GMOs must be decontaminated using a validated method, then tested for GMO viability. If viable GMOs are present, they must be destroyed. Records of methods and testing must be kept and provided to the Regulator on request.

Facility access and authority

30. While dealings with the GMOs are being conducted at the Fermentation facility, the licence holder must ensure that access to the GMOs is restricted to persons covered by this licence.

31. The licence holder must have authority, or obtain such authority in writing from the owner of the premises before GMO dealings commence, to:

- (a) maintain the Fermentation facility and associated equipment; and
- (b) admit persons to the Fermentation facility and associated areas, and to exclude persons from these areas.

Storage of the GMOs

32. Unless covered under another authorisation under the Act, the licence holder must ensure that storage of the GMOs or samples containing GMOs within the Fermentation facility follows these sub-conditions:

- (a) GMOs must be contained within a sealed, unbreakable primary container, with the outer packaging labelled to indicate at least:
 - i) that it contains GMOs; and
 - ii) the contact details for the licence holder; and
 - iii) instructions to notify the licence holder in case of loss or spill of the GMOs; and
- (b) procedures must be in place to ensure that GMOs can be accounted for and that a loss of GMOs during storage can be detected; and
- (c) access to the GMOs is restricted to authorised persons for whom Condition 17 has been met; and

Note: All stored GMOs remain the responsibility of the licence holder.

- (d) a consolidated record of all GMOs being stored under this condition is maintained and made available to the Regulator upon request.

Disposal of the GMOs

33. Unless covered under another authorisation under the Act, the licence holder must ensure that all GMOs and all waste reasonably expected to contain GMOs are Decontaminated:

- (a) prior to disposal, unless the method of disposal is also a method of Decontamination; and

- (b) before or upon suspension, cancellation or surrender of the licence, unless covered by another authorisation under the Act, or exported; and
- (c) by autoclaving, heat or chemical treatment, or any other method approved in writing by the Regulator.

34. The GMOs and equipment that comes into contact with GMOs must be decontaminated by a heat treatment or chemical inactivation that must be tested and validated for effectiveness in killing the GMOs.

35. The decontamination method and test results from the first run using methods as validated in Condition 34 must be documented and provided to the Regulator before proceeding with the next run.

Contingency plans

36. If there is a spill or an unintentional release of GMO at the Fermentation facility, the following measures must be implemented:

- (a) the GMOs must be contained to prevent further dispersal;
- (b) persons cleaning up the GMO must wear appropriate PPE as specified in Condition 23;
- (c) the exposed area must be Decontaminated with an appropriate chemical disinfectant effective against the GMOs or in case of a large spill, the effluent that goes to the large sub-floor tank must be decontaminated;
- (d) any material used to clean up the spill or PPE worn during clean-up of the spill must be Decontaminated; and
- (e) the licence holder must be notified as soon as reasonably practicable;

37. The licence holder must inform the Regulator as soon as reasonably possible:

- (a) In the event of a loss or spill of the GMO; and
- (b) In the event of the exposure of a person to the GMO.

38. Upon request from the Regulator, the licence holder must provide any records or documentation collected under a condition of this licence, within a time period stipulated by the Regulator.

Section 4 Reporting and Documentation

*Note: The following licence conditions are imposed to demonstrate compliance with other conditions and facilitate monitoring of compliance by staff of the OGTR. Notices and reports may be emailed to OGTR.M&C@health.gov.au. A summary of notification and reporting requirements is provided at **Attachment B**.*

Notifications to the Regulator

39. At least 14 days prior to commencing more than 25 L culture per vessel, or a timeframe agreed to in writing by the Regulator, the licence holder must provide the Regulator with a Compliance Management Plan, specifying:

- (a) the role and contact details for key persons responsible for the management of the fermentation process at the site;
- (b) the proposed reporting structure for the fermentation process at the site and how the reporting structure enables the licence holder to become aware of all reportable events including but not limited to Conditions 14, 15, 28 and 39;
- (c) details of how the persons covered by the licence (for that type of dealing) will be informed of licence conditions applicable to them and how they will be trained to safely conduct the dealings;
- (d) the person(s) or class of persons working with the GMO;

- (e) the expected date of first culture of the GMO; and
- (f) method of analysis of detecting the GMO in the environment. The detection method must be capable of identifying each GMO under this licence.

Note: For the purpose of finding out whether the Act has been complied with, an OGTR inspector may, if entry is at a reasonable time, enter a facility occupied by the licence holder or a person covered by the licence and exercise monitoring powers.

40. Upon request from the Regulator, the licence holder must provide any records, signed statements, written agreements or documentation collected under a condition of this licence, within a time period stipulated by the Regulator.

Attachment A

DIR No: 231

Title: Fermentation of plant material-derived sugars using genetically modified yeast for ethanol production

Organisation Details: Ethanol Technologies Pty Ltd

GMO Description

GMOs covered by this licence:

Saccharomyces cerevisiae and *Candida sojae* genetically modified by introduction or modification of only the genes listed in Table 1 below.

Common Name: Yeast

Scientific Name: *Saccharomyces cerevisiae* and *Candida sojae*

Modified traits:

Categories: Ethanol production

Description: The GMOs, modified for efficient ethanol production from sugars derived from plant material.

Introduced or modified genes are listed in Table 1.

Fungal gene promoters and terminators for enhanced expression of the introduced or modified genes.

Table 1: Genes responsible for conferring the modified traits

Genes encoding	Source	Function
High activity xylose transporter: XT (Pentose transporting MFS, Pentose transporting SWEET)	MFS: <i>e.g.</i> GXF1 from <i>Candida intermedia</i> , STP7 from <i>Arabidopsis thaliana</i> SWEET: <i>e.g.</i> NcSWEET1 from xylose metabolising yeast <i>Neocallimastix californiae</i>	Membrane proteins that move pentose sugars across cell boundaries
NADPH-dependent Xylose reductase: XR	xylose metabolising yeast <i>e.g.</i> <i>Candida sp.</i> , <i>S. stipitis</i>	Catalyses the first reduction step in microbial xylose metabolism by converting D-xylose into xylitol using a cofactor like NADPH or NADH
Xylitol dehydrogenase: XDH	<i>Candida sp.</i> , <i>S. stipitis</i>	Converts xylitol into D-xylulose using NAD ⁺ as a coenzyme
Xylose isomerase: XI	anaerobic commensal gut bacteria <i>e.g.</i> <i>Bacteroides sp.</i>	Converts D-xylose into D-xylulose
Xylulose kinase: XK	xylose metabolising yeast <i>e.g.</i> <i>Candida sp.</i> , <i>S. cerevisiae</i>	Converts the sugar D-xylulose into D-xylulose 5-phosphate by using energy from adenosine triphosphate (ATP)

Ribose-phosphate epimerase: RPE	xylose metabolising yeast <i>e.g. Candida sp., S. cerevisiae</i>	Catalyses the reversible conversion between D-ribulose 5-phosphate and D-xylulose 5-phosphate
Ribose-phosphate isomerase: RPI	xylose metabolising yeast <i>e.g. Candida sp., S. cerevisiae</i>	Converts D-ribose 5-phosphate and D-ribulose 5-phosphate back and forth in cellular metabolism
Transaldolase: TAL	xylose metabolising yeast <i>e.g. Candida sp., S. cerevisiae</i>	Catalyses the reversible transfer of a three-carbon dihydroxyacetone unit between sugar phosphates
Transketolase: TKL	xylose metabolising yeast <i>e.g. Candida sp., S. cerevisiae</i>	Sugar phosphate transfer

Attachment B – Summary of reporting requirement*

Prior to the commencement of the protein production process	Condition	Timeframe for reporting
<p>A written Compliance Management Plan for the Fermentation facility:</p> <ul style="list-style-type: none"> (a) the role and contact details for key persons responsible for the management of the protein production process at the site; (b) the proposed reporting structure for the protein production process at the site and how the reporting structure enables the licence holder to become aware of all reportable events including but not limited to Conditions 15, 16 and 39; (c) details of how the persons covered by the licence (for that type of dealing) will be informed of licence conditions applicable to them and how they will be trained to safely conduct the dealings; (d) the person(s) or class of persons working the GMO; (e) the expected date of first culture of the GMO (f) method of analysis of detecting the GMO in the environment. 	39	At least 14 days prior to commencing work with the GMOs, or a timeframe agreed to in writing by the Regulator
Information to be provided at any time during the protein production process	Condition	Timeframe for reporting
Any additional information related to the health and safety of people and the environment associated with the dealing covered by the licence, or any unintended effect of the dealing authorised by the licence	14(a),(c)	Immediately
Information related to any contravention of the licence by a person covered by the licence	14(b)	Immediately
Any relevant conviction of the licence holder	15(a)	Immediately
Any revocation or suspension of a licence or permit held by the licence holder under a law of the Commonwealth, a State or a foreign country, being a law relating to the health and safety of people or the environment	15(b)	Immediately
Any event or circumstances that would impact the licence holder capacity to meet the licence conditions	15(c)	Immediately
Spill or failure identified during weekly inspections	28	As soon as reasonably possible

Any loss or spill of the GMO, or exposure of a person or animal to the GMO	37	As soon as reasonably possible
Information to be provided on request by the Regulator	Condition	Timeframe for reporting
Information related to the persons covered by the licence	8	Within a timeframe stipulated by the Regulator
Information related to the licence holder's ongoing suitability to hold a licence	16	Within a timeframe stipulated by the Regulator
Copies of signed and dated statements and training records	17	Within a timeframe stipulated by the Regulator
The decontamination method and test results must be documented and provided to the Regulator on request.	35	Test results from the first run must be documented and provided to the Regulator before proceeding with the next run
Any records or documentation collected under a condition of this licence	40	Within a timeframe stipulated by the Regulator

*Notifications and documents to be sent to OGTR.M&C@health.gov.

References

- Abdel-Haq, N., Asmar, B.I., Ang, J.Y., Natarajan, G., Fairfax, M., and Salimnia, H. (2022). Central line-associated blood stream infection (CLABSI) due to *Candida sojae* in an infant with short bowel syndrome: The first human case report. *IDCases* 29, e01559.
- Auchtung, T.A., Fofanova, T.Y., Stewart, C.J., Nash, A.K., Wong, M.C., Gesell, J.R., Auchtung, J.M., *et al.* (2018). Investigating Colonization of the Healthy Adult Gastrointestinal Tract by Fungi. *mSphere* 3.
- Barnett, J.A., and Entian, K.D. (2005). A history of research on yeasts 9: regulation of sugar metabolism. *Yeast* 22, 835-894.
- Bertels, L.K., Fernandez Murillo, L., and Heinisch, J.J. (2021). The Pentose Phosphate Pathway in Yeasts-More Than a Poor Cousin of Glycolysis. *Biomolecules* 11.
- Billerbeck, S., Walker, R.S.K., and Pretorius, I.S. (2024). Killer yeasts: expanding frontiers in the age of synthetic biology. *Trends Biotechnol* 42, 1081-1096.
- Borelli, G., Jose, J., Teixeira, P.J., Dos Santos, L.V., and Pereira, G.A. (2016). De Novo Assembly of *Candida sojae* and *Candida boidinii* Genomes, Unexplored Xylose-Consuming Yeasts with Potential for Renewable Biochemical Production. *Genome Announc* 4.
- Chandel, A., Chandrasekhar, G., Konakalla, R., Ravinder, R., and Ravindra, P. (2011). Bioconversion of pentose sugars into ethanol: A review and future directions. *Biotechnology and Molecular Biology Review* 6, 8-20.
- Chrenkova, V., Vadkertiova, R., Vlachova, K., Babjuk, M., Lischke, R., Bebrova, E., and Hubacek, P. (2022). *Candida sojae*: First report of a human infection. *J Mycol Med* 32, 101309.
- Defosse, T.A., Courdavault, V., Coste, A.T., Clastre, M., de Bernonville, T.D., Godon, C., Vandeputte, P., *et al.* (2018). A standardized toolkit for genetic engineering of CTG clade yeasts. *J Microbiol Methods* 144, 152-156.
- Deutschbauer, A.M., Jaramillo, D.F., Proctor, M., Kumm, J., Hillenmeyer, M.E., Davis, R.W., Nislow, C., *et al.* (2005). Mechanisms of haploinsufficiency revealed by genome-wide profiling in yeast. *Genetics* 169, 1915-1925.
- dos Santos, L.V., Neitzel, T., Lima, C.S., de Carvalho, L.M., de Lima, T.B., Ienczak, J.L., Correa, T.L.R., *et al.* (2025). Engineering cellular redox homeostasis to optimize ethanol production in xylose-fermenting *Saccharomyces cerevisiae* strains. *Microbiol Res* 290, 127955.
- Enache-Angoulvant, A., and Hennequin, C. (2005). Invasive *Saccharomyces* infection: a comprehensive review. *Clinical Infectious Diseases* 41, 1559-1568.
- FSANZ (2021a). Approval report – Application A1207 Rebaudioside M as a Steviol Glycoside from *Saccharomyces cerevisiae*. (Food Standards Australia New Zealand, Canberra, Australia).
- FSANZ (2021b). Approval report – Application A1210 Maltogenic alpha-amylase enzyme from GM *Saccharomyces cerevisiae*. (Food Standards Australia New Zealand, Canberra, Australia).
- Goffeau, A., Barrell, B.G., Bussey, H., Davis, R.W., Dujon, B., Feldmann, H., Galibert, F., *et al.* (1996). Life with 6000 Genes. *Science* 274, 546-567.

- Gognies, S., Belarbi, A., and Ait Barka, E. (2001). *Saccharomyces cerevisiae*, a potential pathogen towards grapevine, *Vitis vinifera*. FEMS Microbiology Ecology 37, 143-150.
- Hays, H., Elliker, P.R., and Sandine, W.E. (1967). Microbial destruction by low concentrations of hypochlorite and iodophor germicides in alkaline and acidified water. Applied Microbiology 15, 575-581.
- Herskowitz, I. (1988). Life cycle of the budding yeast *Saccharomyces cerevisiae*. Microbiological Reviews 52, 536-553.
- Johansson, B., Christensson, C., Hobley, T., and Hahn-Hagerdal, B. (2001). Xylulokinase overexpression in two strains of *Saccharomyces cerevisiae* also expressing xylose reductase and xylitol dehydrogenase and its effect on fermentation of xylose and lignocellulosic hydrolysate. Appl Environ Microbiol 67, 4249-4255.
- Kampf, G. (2018). Ethanol. In Antiseptic Stewardship: Biocide Resistance and Clinical Implications, G. Kampf, ed. (Cham: Springer International Publishing), pp. 9-35.
- Korukluoglu, M., Sahan, Y., and Yigit, A. (2006). The fungicidal efficacy of various commercial disinfectants used in the food industry. Annals of Microbiology 56, 325-330.
- Krassowski, T., Kominek, J., Shen, X.X., Opulente, D.A., Zhou, X., Rokas, A., Hittinger, C.T., *et al.* (2019). Multiple Reinventions of Mating-type Switching during Budding Yeast Evolution. Curr Biol 29, 2555-2562 e2558.
- Kwolek-Mirek, M., Bartosz, G., and Spickett, C.M. (2011). Sensitivity of antioxidant-deficient yeast to hypochlorite and chlorite. Yeast 28, 595-609.
- Lombardi, L., Oliveira-Pacheco, J., and Butler, G. (2019). Plasmid-Based CRISPR-Cas9 Gene Editing in Multiple Candida Species. mSphere 4.
- Matsushika, A., Goshima, T., Fujii, T., Inoue, H., Sawayama, S., and Yano, S. (2012). Characterization of non-oxidative transaldolase and transketolase enzymes in the pentose phosphate pathway with regard to xylose utilization by recombinant *Saccharomyces cerevisiae*. Enzyme Microb Technol 51, 16-25.
- Mbaye, B., Wasfy, R.M., Alou, M.T., Borentain, P., Gerolami, R., Dufour, J.C., and Million, M. (2024). A catalog of ethanol-producing microbes in humans. Future Microbiol 19, 697-714.
- Metcalf, R., Akinbobola, A., Woodford, L., and Quilliam, R.S. (2025). Thermotolerance, virulence, and drug resistance of human pathogenic Candida species colonising plastic pollution in aquatic ecosystems. Environ Sci Pollut Res Int 32, 14993-15005.
- Miosga, T., and Zimmermann, F.K. (1996). Cloning and characterization of the first two genes of the non-oxidative part of the *Saccharomyces cerevisiae* pentose-phosphate pathway. Curr Genet 30, 404-409.
- Mitchell, A.P. (1994). Control of meiotic gene expression in *Saccharomyces cerevisiae*. Microbiological Reviews 58, 56-70.
- Mortimer, R.K. (2000). Evolution and variation of the yeast (*Saccharomyces*) genome. Genome Research 10, 403-409.

- Nakase, T., Suzuki, M., Takashima, M., Miyakawa, Y., Kagaya, K., Fukazawa, Y., and Komagata, K. (1994). *Candida sojae*, a new species of yeast isolated from an extraction process of water-soluble substances of defatted soybean flakes. *The Journal of General and Applied Microbiology* 40, 161-169.
- Norris, M., Lovell, S., and Delneri, D. (2013). Characterization and prediction of haploinsufficiency using systems-level gene properties in yeast. *G3 (Bethesda)* 3, 1965-1977.
- OGTR (2013). Risk Analysis Framework 2013, 4th edn (Canberra: Office of the Gene Technology Regulator).
- Orentaite, I., Poranen, M.M., Oksanen, H.M., Daugelavicius, R., and Bamford, D.H. (2016). K2 killer toxin-induced physiological changes in the yeast *Saccharomyces cerevisiae*. *FEMS Yeast Res* 16, fow003.
- Pant, S., Ritika, Prakash, A., and Kuila, A. (2022). Integrated production of ethanol and xylitol from *Brassica juncea* using *Candida sojae* JCM 1644. *Bioresource Technology* 351, 126903.
- Paramsothy, J., Gutlapalli, S.D., Ganipineni, V.D.P., Okorie, I.J., Ugwendum, D., Piccione, G., Ducey, J., *et al.* (2023). Understanding Auto-Brewery Syndrome in 2023: A Clinical and Comprehensive Review of a Rare Medical Condition. *Cureus* 15, e37678.
- Partow, S., Siewers, V., Bjørn, S., Nielsen, J., and Maury, J. (2010). Characterization of different promoters for designing a new expression vector in *Saccharomyces cerevisiae*. *Yeast* 27, 955-964.
- Peng, B., Williams, T.C., Henry, M., Nielsen, L.K., and Vickers, C.E. (2015). Controlling heterologous gene expression in yeast cell factories on different carbon substrates and across the diauxic shift: a comparison of yeast promoter activities. *Microb Cell Fact* 14, 91.
- Pérez-Torrado, R., and Querol, A. (2016). Opportunistic strains of *Saccharomyces cerevisiae*: A potential risk sold in food products. *Frontiers in Microbiology* 6:1522, 1-5.
- Podolsky, I.A., Seppala, S., Xu, H., Jin, Y.S., and O'Malley, M.A. (2021). A SWEET surprise: Anaerobic fungal sugar transporters and chimeras enhance sugar uptake in yeast. *Metab Eng* 66, 137-147.
- Reuss, O., Vik, A., Kolter, R., and Morschhäuser, J. (2004). The SAT1 flipper, an optimized tool for gene disruption in *Candida albicans*. *Gene* 341, 119-127.
- Rottmann, T., Klebl, F., Schneider, S., Kischka, D., Ruscher, D., Sauer, N., and Stadler, R. (2018). Sugar Transporter STP7 Specificity for l-Arabinose and d-Xylose Contrasts with the Typical Hexose Transporters STP8 and STP12. *Plant Physiol* 176, 2330-2350.
- Runquist, D., Fonseca, C., Rådström, P., Spencer-Martins, I., and Hahn-Hägerdal, B. (2009a). Expression of the Gxf1 transporter from *Candida intermedia* improves fermentation performance in recombinant xylose-utilizing *Saccharomyces cerevisiae*. *Appl Microbiol Biotechnol* 82, 123-130.
- Runquist, D., Hahn-Hägerdal, B., and Bettiga, M. (2009b). Increased expression of the oxidative pentose phosphate pathway and gluconeogenesis in anaerobically growing xylose-utilizing *Saccharomyces cerevisiae*. *Microb Cell Fact* 8, 49.
- Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N., Pearson, C., *et al.* (2015). A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. *Transgenic Research* 24, 1-17.

- Senac, T., and Hahn-Hägerdal, B. (1991). Effects of increased transaldolase activity on D-xylulose and D-glucose metabolism in *Saccharomyces cerevisiae* cell extracts. *Appl Environ Microbiol* 57, 1701-1706.
- Seren, C., and Birinci, A. (2024). First report of *Candida sojae* sepsis in a preterm infant; A new fungal threat in neonatal care. *Curr Med Mycol* 10.
- Silva, P.C., Ceja-Navarro, J.A., Azevedo, F., Karaoz, U., Brodie, E.L., and Johansson, B. (2021). A novel D-xylose isomerase from the gut of the wood feeding beetle *Odontotaenius disjunctus* efficiently expressed in *Saccharomyces cerevisiae*. *Sci Rep* 11, 4766.
- Society of Toxicology (2003). Society of Toxicology position paper: The safety of genetically modified foods produced through biotechnology. *Toxicological Sciences* 71, 2-8.
- Standards Australia New Zealand (2022). The Australian Standard 2243.3:2022 *Safety in Laboratories Part 3: Microbiological safety and containment*.
- Stepchenkova, E.I., Zadorsky, S.P., Shumega, A.R., and Aksenova, A.Y. (2023). Practical Approaches for the Yeast *Saccharomyces cerevisiae* Genome Modification. *Int J Mol Sci* 24.
- Suh, S.-O., Blackwell, M., Kurtzman, C.P., and Lachance, M.-A. (2006). Phylogenetics of *Saccharomycetales*, the ascomycete yeasts. *Mycologia* 98, 1006-1017.
- Tapia, H., and Koshland, Douglas E. (2014). Trehalose is a versatile and long-lived chaperone for desiccation tolerance. *Current Biology* 24, 2758-2766.
- Topaloglu, A., Esen, O., Turanli-Yildiz, B., Arslan, M., and Cakar, Z.P. (2023). From *Saccharomyces cerevisiae* to Ethanol: Unlocking the Power of Evolutionary Engineering in Metabolic Engineering Applications. *J Fungi (Basel)* 9.
- Walsh, R.M., and Martin, P.A. (1977). Growth of *Saccharomyces cerevisiae* and *Saccharomyces uvarum* in a temperature gradient incubator. *Journal of the Institute of Brewing* 83, 169-172.
- Webb, S.R., and Lee, H. (1992). Characterization of xylose reductase from the yeast *Pichia stipitis*: Evidence for functional thiol and histidyl groups. *Microbiology* 138, 1857-1863.
- WHO (2022). WHO fungal priority pathogens list to guide research, development and public health action (World Health Organization).
- Yang, C., Hu, S., Zhu, S., Wang, D., Gao, X., and Hong, J. (2015). Characterizing yeast promoters used in *Kluyveromyces marxianus*. *World J Microbiol Biotechnol* 31, 1641-1646.
- Yoshiwara, K., Watanabe, S., and Watanabe, Y. (2023). Molecular evolutionary insight of structural zinc atom in yeast xylitol dehydrogenases and its application in bioethanol production by lignocellulosic biomass. *Sci Rep* 13, 1920.