Application title:

Importation of plant bacteria and fungi for the development of diagnostic methods & research

Applicant organisation:

Plant Health & Environment Laboratory, Investigation & Diagnostic Centre – MAF Biosecurity New Zealand

Please provide a brief summary of the purpose of the application (255 characters or less, including spaces)

To import and hold exotic plant pathogenic and saprophytic bacteria and fungi in containment, in order to develop diagnostic methods and for laboratory-based research purposes.

PLEASE CONTACT ERMA NEW ZEALAND BEFORE SUBMITTING YOUR APPLICATION

Please clearly identify any confidential information and attach as a separate appendix.

Please check and complete the following before submitting your application:

All sections completed	Yes
Appendices enclosed	Yes
Confidential information identified and enclosed separately	NA
Copies of references attached	NA
Application signed and dated	Yes
Electronic copy of application e-mailed to ERMA New Zealand	Yes

Signed: **Date:**

Section One – Applicant details

Name and details of the organisation making the application:	
Name:	Plant Health & Environment Laboratory, Investigation & Diagnostic Centre – MAF Biosecurity New Zealand
Postal Address:	PO Box 2095, Auckland 1140
Physical Address:	231 Morrin Road, St Johns
Phone:	09 909 3015
Fax:	09 909 5739
Email:	Not applicable
Name and details of the key contact person *(if different from above)***:**	
Name:	
Postal Address:	Plant Health & Environment Laboratory Manager
Physical Address:	As above
Phone:	
Fax:	
Email:	
Name and details of a contact person in New Zealand, if the applicant is overseas:	
Name:	Not applicable
Postal Address:	
Physical Address:	
Phone:	
Fax:	
Email:	

Note: The key contact person should have sufficient knowledge of the application to respond to queries from ERMA New Zealand staff.

Section 2: Purpose of the application

Lay summary of the application (approximately 200 words)

Note: This summary should include a description of the organism(s), the purpose of the application or what you want to do with the organisms(s).

Use simple non-technical language

New Zealand has relatively few of the bacteria and fungi that are associated with plants overseas. If introduced, diseases caused by such micro-organisms would lead to huge economic losses in New Zealand's crops and might have unforeseen effects on native biota. To reduce the risks of introducing such diseases, imported plants must be sourced from disease-free areas or be inspected, tested and/or treated for the diseases of concern. New Zealand also has surveillance programmes to enable the early detection and potential eradication of introduced diseases. For import, export and surveillance programmes, robust methods are required for the detection of plant bacteria and fungi associated with plants, and other materials crossing the border that could harbour risk organisms.

The purpose of this application is to seek approval to import and hold plant pathogenic and saprophytic bacteria and fungi in containment to facilitate the development and implementation of testing procedures for exotic diseases, thereby ensuring New Zealand’s biosecurity. More specifically, access to these micro-organisms will improve New Zealand’s ability to identify exotic plant diseases by providing controls to ensure correct test performance, and enable the development of new diagnostic tests.

Describe the background and aims of the project

Note: This section is intended to put the organism(s) in perspective of the wider project(s) that they will be used in. You may use more technical language but please make sure that any technical words are included in the Glossary.

The Plant Health & Environment Laboratory (PHEL) supports the Ministry of Agriculture and Forestry (MAF) Biosecurity New Zealand's activities in the identification of new pests and diseases affecting plants and the environment. PHEL is responsible for the identification and/or validation of all suspected exotic, new and emerging pests and diseases of plants (including horticultural species, arable crops, forest species and species of amenity and environmental value) and arthropod pests affecting the environment and human health.

PHEL covers all aspects of plant entomology (including insects, mites, molluscs and spiders), nematology, and pathology (including bacteria, fungi, phytoplasmas, viruses and viroids) and has specific skills and experience including:

- investigation and control of exotic plant pests and diseases affecting the economy, environment and human health;
- accreditation to ISO 17025 "General requirements for the competence of testing and calibration laboratories";
- specialist facilities including containment laboratories and glasshouses;
- specialist reference collections;
- technical advice on plant pests and diseases;
- technical audit of accredited laboratories; and
- world-class diagnostic testing capabilities.

Because of its physical, and until recently economic, isolation, New Zealand has relatively few of the plant pathogens that plague many crops overseas. If introduced, such pathogens (e.g. *Xylella fastidiosa*, the causal agent of Pierce's disease) would cause huge economic losses in New Zealand's crops and might have unforeseen effects on native biota (discussed further in Section 5 page 18). To reduce the risks of introducing such pathogens, MAF Biosecurity New Zealand has introduced a series of measures such as sourcing material from pathogen-free areas or accredited offshore facilities, and pre- and post-export inspection, testing and treatment. MAF Biosecurity New Zealand also uses surveillance programmes to enable the early detection and potential eradication of organisms that have passed the border. For import, export and surveillance programmes, robust testing procedures are required for the detection of plant bacteria and fungi.

The purpose of this application is to seek permission for PHEL to import and hold reference isolates of plant pathogenic and saprophytic bacteria and fungi in containment to facilitate the development and implementation of testing procedures for the identification and detection of exotic plant diseases (as permitted under Section 39 (1) (g) of the HSNO Act), thereby ensuring New Zealand's biosecurity. More specifically, access to such reference material (the micro-organisms) will significantly improve New Zealand's ability to identify plant pathogens in quarantine or surveillance samples by:

1. avoiding "false negatives" during diagnostic testing by providing confirmation of test performance; and
2. developing new diagnostic tests and/or validating the performance of published methods.

The types of experiments that will be done include, but are not restricted to, research into diagnostics (e.g. morphological identifications, and biochemical, serological and molecular tests), plant biology and pathology (e.g. *in planta* pathogenicity testing and biochemical studies), systematics and taxonomy. Fungi of the orders *Uredinales* (rusts) and *Ustilaginales* (smuts) are specifically excluded from this application for the purposes of *in planta* pathogenicity testing, but are included for other purposes such as diagnostic research.

Isolates will generally be imported from either recognised culture collections or experts in the field (including laboratories) and where possible, will be identified to species prior to importation. In some instances isolates may require testing and examination in New Zealand to resolve their taxonomic identity. Bacteria and fungi that are new organisms will also be isolated from the New Zealand environment, e.g. during incursions, and may be held for subsequent research.

Isolates may be imported as pure cultures or growing *in planta* (e.g. infected seeds, cuttings, bulbs and other plant material) or on a substrate (e.g. soil, water, artificial media, dead or living plant material, or dead animal material [e.g. hair, feathers and other keratinaceous material, dried skins, dairy products and animal dung])

Appropriate controls will be taken to ensure viable bacterial and fungal material, including spores, are contained and do not leave the containment facility through any outlets including exhaust system, air filters and water discharge. All sub-culturing and the initial stages of DNA extraction will be done in a Class II biological safety cabinet (BSC).

While some organisms to be imported are capable of causing diseases in plants, with the containment standards and conditions provided at PHEL the likelihood of these organisms escaping from containment and infecting a susceptible plant is essentially nil.

The ability to diagnose the presence of exotic diseases at the border and during incursions in New Zealand, and to rule out exotic diseases in negative cases with suspicious symptoms, is essential to New Zealand's economy. Rapid diagnosis during incursions could mean the difference of the disease being confined to one or two production sites, rather than hundreds.

Section Three – Identification of the organism(s) to be imported

Complete this section separately for **each new organism** to be imported.

Identification of the organism to be imported

Latin binomial, including full taxonomic authority:	Where possible, all organisms will be identified to species level prior to importation.
Common name(s), if any:	Not relevant.
Type of organism (eg bacterium, virus, fungus, plant, animal, animal cell):	Bacterium, Chromista, Fungi and Protozoa
Taxonomic class, order and family:	1. Kingdom Fungi, including those in the Phyla Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota, and the artificial taxonomic group Anamorphic fungi (Kirk *et al*., 2001) 2. Kingdom Chromista, including those in the Phyla Oomycota, Hyphochytriomycota and Labyrinthulomycota (Kirk *et al*., 2001) 3. Kingdom Protozoa, including those in the Phyla Plasmodiophoromycota (Kirk *et al*., 2001) 4. Kingdom Bacteria: including those in the Phyla Acidobacteria, Actinobacteria, Aquificae, Bacteroidetes, Chlamydiae, Chlorobi, Chloroflexi, Chrysiogenetes, Cyanobacteria, Deferribacteres, Deinococcus-Thermus, Dictyoglomi, Fibrobacteres, Firmicutes (this includes the Class Mollicutes, although subsequent classifications have placed this class in the Phylum Tenericutes), Fusobacteria, Nitrospirae, Planctomycetes, Proteobacteria, Spirochaetes, Thermodesulfobacteria, Thermomicrobia, Thermotogae and Verrucomicrobia (Garitty *et al*.,2001). **Note:** Fungal taxonomy and nomenclature at all levels has been and continues to be in a state of flux (Guarro *et al*., 1999; Crous, 2005). Therefore in this application we have elected to apply for organism groups that would be, at the moment, higher than the taxonomic level of class. We note that this is a broad group of bacteria fungi but emphasise that it is narrowed by the purpose

	statement which excludes human and animal pathogens. There has also been a long history of the manipulation of these organisms in the lab which has enable the accumulation of an extensive body of best practise knowledge.
Strain(s) if relevant:	Not relevant.
Other information, including presence of any inseparable or associated organisms and any related organisms present in New Zealand:	We are not aware of any inseparable associated or related micro-organisms to the bacteria and fungi in this application. However we accept that there may be such organisms but the standard microbiological practises in our lab are sufficient to contain such organisms.

Section Four – The proposed containment system

Describe the containment facility and the proposed containment system (physical and operational)

Question	Answer
Which MAF/ERMA Standard is this containment facility approved under?	The organisms to which this application refers will be imported into a containment facility which operates according to the *MAF Biosecurity New Zealand and ERMA New Zealand Standard, Facilities for Microorganisms and Cell Culture: 2007* (the Standard).
What physical containment level (AS/NZS 2243: 2002) is this containment facility registered to (where relevant)?	All work using these organisms will be done in a PC2 registered laboratory (as defined by the AS/NZS 2243.3: 2002 Standard "Safety in Laboratories, Part 3 Microbiology") operated within a containment facility. The facility is registered by MAF (ref. number 4209) and the containment manual is attached in Appendix 1.
What other physical measures do you propose to use to contain this organism?	To reduce the risk of escape of viable airborne micro-organisms or propagules, the movement of potentially contaminated air is controlled by conducting all sub-culturing and the initial stages of DNA extraction in a Class II BSC. This provides environmental, personnel, and product protection. A centrifuge with either sealed rotors or safety cups is used when large volumes or high concentrations of infectious materials are used. BSCs are decontaminated after use according to the AS/NZS 2243.3: 2002 Standard. Laboratory staff are required to remove their coats and gloves, and wash their hands and fingernails as the final step in safe microbiological practices. The BSCs at PHEL are subject to an annual certification check. PHEL's PC2 laboratory in Tamaki is mechanically ventilated where a directional air-flow into the laboratory is maintained by extracting room air. Re-circulated air is not released into areas outside the Facility unless vented through a High Efficiency Particulate Air (HEPA) filter. All external windows are sealed and closed. Specific physical measures we propose to use to contain the micro-organisms are outlined below.

	Storage Once imported the cultures of bacteria or fungi will be maintained in a sealed receptacle (e.g. Petri dish, culture bottle, vial etc.) which will be opened only to remove material as required to do experiments. In some instances (e.g. where it is not possible to obtain further samples), cultures may be maintained by growing in/on liquid or solid media, or may be transferred to the International Collection of Microorganisms from Plants (ICMP) curated by Landcare Research. The ICMP is co-located with PHEL in Tamaki. **Access** Access to the PC2 laboratory at PHEL is restricted to trained personnel. Maintenance and service personnel, and visitors are only permitted entry provided if accompanied by trained personnel. A biological hazard symbol specifying restricted access is displayed near the entrance of the facility. Visits are recorded in a visitors' log book for security purposes, and all personnel are required to adhere to access procedures. Entrance to the facility is locked when not in active use. Facility doors are closed while work is in progress. Laboratory coats are removed when leaving the laboratory and cleaned by autoclaving at least once a month. Refer to section 4.1 of the containment manual CM-07 "PC2 Containment of Exotic Organisms and GMO's and PC3 Rearing of Insects" (the Containment Manual). **Treatment and Disposal of Biological Waste** All micro-organisms and biological waste are disposed of and/or treated according to the guidelines in the AS/NZS 2243.3: 2002 Standard which is by autoclaving and/or chemical sterilisation and/or incinerating (refer to section 4.5.3 of the Containment Manual). **Exposure of Plants to Micro-organisms** Plants will only be exposed to the imported bacteria or fungi if contained within a BSC or contained growth chamber. Plants will not be infected with fungi belonging to the order *Uredinales* (rusts) or *Ustilaginales* (smuts).

What procedural or operational measures do you propose to use to contain this organism?	Specific procedural or operational measures we propose to use to contain these micro-organisms are discussed below. **Training Programme** The micro-organisms will be handled only by staff with experience and training in bacteriology and/or mycology. The general requirements for handling of micro-organisms are covered in the Standard (also refer to section 3.4 of the Containment Manual). **Transfer (Import and Export) of Micro-organisms between Facilities** All material will generally be imported from either recognised culture collections (including scientific companies) or experts in the relevant field (including laboratories) and where possible, will be identified to species level prior to importation. Isolates may be imported as pure cultures or growing *in planta* (e.g. infected seeds, cuttings, bulbs and other plant material) or on a substrate (e.g. dead or living plant material, soil, water or artificial media). Each imported species will be clearly labelled and packaged so that containment may not be breached accidentally during transit. The packaging and transportation of these micro-organisms from overseas and for transfers between remotely located (i.e. not located in the same building) facilities of equivalent containment, will be in accordance with Packaging Instruction No. 650 of the International Air Transport Association (IATA) Dangerous Goods Regulations (refer to section 8.8.4 of the Standard). No viable bacteria or fungi will be removed from the facility unless approved by a MAF inspector to be transported to an appropriate containment facility e.g. Landcare Research's ICMP facility. A register will be kept of all samples of reference material imported, to include the number of importations, their identity, origin (source, country) and fate. **Contingency Plans** In the event of failure of the containment of these micro-organisms (e.g. following a spill,

	through personal contamination, fire, sabotage or theft), there is a small but finite risk that some plant pathogens may be released and cause disease in native and valued introduced plants, and could thus also affect taonga (see section 5). Section 4.3 of the Containment Manual has specific procedures that are followed if containment of such types were to be breached. This involves notifying the MAF Supervisor of the laboratory immediately or at least within 24 hours of noticing breach of containment. Spills management is also covered in section 5 of the AS/NZS 2243.3: 2002 Standard. The Incursion Response Team (part of the Investigation & Diagnostic Centre Directorate [MAF Biosecurity New Zealand], co-located with PHEL) would also be notified and would decide on a course of action depending on their assessment of the risk. Security measures (e.g. locked access, security alarms) applying to the building and facility in which these micro-organisms are housed are designed to minimise the chance of sabotage and the earthquake risk is considered small due to the location in Auckland and solid construction of the building. Therefore, the likelihood of breaching containment is considered negligible.
Any other information relevant to the containment of the organism.	No other information relevant to the containment of the organism.

Describe the characteristics of the organism to be imported that may influence its ability; to escape from containment, to form a self sustaining population, or to cause adverse effects. *Refer to sample applications for guidance on how to answer these questions.*

Question	Answer *attach copies of the references used in an appendix*
What are the characteristics of the organism that may prevent/enable it to escape from containment? *eg size, spore production, infectivity, seed/pollen characteristics etc.*	Characteristics of the micro-organisms that may prevent/enable it to escape from containment are outlined below. **Fungi** Fungi are decomposers of organic matter and a major cause of plant disease. They exist primarily as filamentous hyphae, producing spores sexually and asexually. Their life modes varies from saprobic, endophytic, mutualistic, opportunistic pathogenic, to obligate pathogenic. Fungal spores are commonly c. 10 to 100 micrometres in diameter. Fungi can be transmitted by various means, i.e. as actively dispersed spores, or passively as spores carried by wind, water splash, and through the movement of animals; by the growing of infected seeds, cuttings, bulbs, and other plant material and on contaminated tools and equipment. Most plant pathogenic fungi infect several plant species, though some of them are restricted to a single plant. Some infect a restricted number of plants within a single plant family while others may infect many plants in different families. **Chromista** Chromists were once classified as fungi and many of them cause plant diseases. They exist primarily as filamentous hyphae, producing spores sexually and asexually. Their life modes vary from saprobic, opportunistic pathogenic, to obligate pathogenic. They can be transmitted by various means such as the passive dispersal of spores in water or by the wind, and by animals; on infected seeds, cuttings, bulbs, and other plant material; on contaminated tools and equipment. Some plant pathogenic chromists infect several plant species, others like the downy mildews are host specific. **Protozoa** Some protozoa were once classified as fungi and cause plant diseases. They exist primarily as single cells, reproducing sexually and asexually. They can be transmitted by various means, such as active dispersal of spores, or passively by water and animals; growing infected plant material; carried over by contaminated

	tools and equipment. Most plant pathogenic protozoa infect several plant species, a few have a very restricted host range. **Bacteria** Many plant diseases are caused by bacteria. They exist primarily as single cells, mainly reproducing asexually. They can be transmitted by various means, i.e. passively in water droplets, windborne aerosols, by insects, infected seeds, cuttings, bulbs, and other plant material; and on contaminated tools and equipment. The most damaging plant pathogenic bacteria tend to be specialised and have a restricted host range.
How could this organism escape from containment? *ie what are the possible pathways for escape?* *How does the proposed containment regime address these pathways?*	Theoretically escape from containment could occur by: • release of viable air- or water-borne micro-organisms or propagules; or • release of contaminated equipment or clothing To address these risks: • all work is done in a PC2 registered laboratory; • access to the facility is restricted to trained personnel; • the laboratory is mechanically ventilated where a directional air-flow is maintained by extracting room air. Re-circulated air is not released outside unless vented through a HEPA filter. All external windows are sealed and closed; • any sub-culturing and the initial stages of DNA extraction is done in a Class II BSC. Cabinets are decontaminated after use according to the AS/NZS 2243.3: 2002 Standard; • staff are required to remove their coats and gloves, and wash their hands and fingernails after working with these organisms; • all cultures of imported micro-organisms are stored in sealed receptacles (e.g. petri dish, culture bottle, vial etc.); • all micro-organisms and biological waste are disposed of and/or treated according to the guidelines in the AS/NZS 2243.3: 2002 Standard; and • no viable bacteria or fungi will be removed from the laboratory unless approved by a MAF inspector to be transported to an appropriate containment facility. All organisms moved into/out of the laboratory to a remotely located facility will be packaged and transported from the laboratory in accordance with Packaging Instruction No. 650 of the IATA) Dangerous Goods Regulations (refer to section 8.8.4 of the Standard).

If it were to escape, could this organism establish a population outside of containment in New Zealand? *ie what conditions are required for growth and reproduction? And are those conditions present in New Zealand? What factors might prevent this from occurring?*	Establishment of a self-sustaining population and ease of eradication of a particular micro-organism would depend on its pathogenicity and presence of suitable host plants and vectors in New Zealand. For example, the fungus *Fusarium circinatum* (Pine pitch canker) infects *Pinus* spp. (pine) and *Pseudotsuga menziesii* (Douglas-fir) which are important forestry plants of New Zealand (Crop Protection Compendium, 2005). It can infect vegetative and reproductive tissues of susceptible hosts at all ages, from seedlings through to mature trees and can be spread by water splash, wind-borne inoculum, infected plant material, and contaminated tools and equipment. The known insect vectors of this fungus overseas are not established in New Zealand. It can survive in soil for 6 months and in wood for over 12 months. Its teleomorph, *Gibberella circinata*, has never been observed in nature and has only been produced in culture by crossing complementary mating types (Liberato *et al*., 2006). Currently there is no effective treatment for the disease in mature trees (MAF Biosecurity New Zealand, 2007). Considering the mode of dispersal, this fungus would have a relatively high risk of establishment in New Zealand if containment were breached in areas where *Pinus* spp. were grown. Although its dissemination could be assisted by wind, there are no susceptible hosts proximal to PHEL therefore it is unlikely that the fungus would become established if containment were breached in the Auckland suburb in which PHEL is located. In contrast, a smut fungus such as *Tilletia horrida* (Rice kernel smut) is an obligate pathogen and infects only two plants, *Oryza sativa* and *Oryza rufipogon*, which are not commercially cultivated and rarely planted in New Zealand due to the climatic and economic constraints. The disease is seed and soil-borne (Shivas, 2006). The fungus can be dispersed by wind, contaminated tools and equipment. Considering the rarity of the host organisms, this fungus has a low risk of establishment in New Zealand if containment were breached in any location. The micro-organisms will be held in a high level of containment at all times (generally a PC2 laboratory, although a BSC in PC1 may suffice for organisms such as saprophytes). Most plant pathogens are specific to a relatively small number of plant species, and require specific environmental conditions for transmission and for infection of a plant. These factors significantly reduce the possibility of escape and establishment of a self-sustaining population in the environment.

	In the unlikely event of an incident or accident that may lead to an escape, the PHEL PC2 laboratory has a micro-organism (all organisms within the scope of this application are micro-organisms) release contingency plan (refer to section 4.3 of the Containment Manual). This involves notifying the MAF Supervisor of the laboratory as soon as possible and at least within 24 hours of noticing breach of containment. Procedures to manage spillage of any micro-organisms within and outside the facility and for fire, personal decontamination, theft and sabotage are also addressed in this containment manual. MAF Biosecurity New Zealand's Incursion Response Team would also be notified and would decide on a course of action depending on their assessment of the risk.
If a population did establish could it be eradicated? How? Would it be noticed immediately? How would such a population be identified?	Eradication of such species if containment were breached would depend on the micro-organism causing significant detectable symptoms and the availability of a specific and sensitive laboratory test for detection of an infection. The latter requirement is, of course, one of the main reasons for this application.
Additional information	No additional information.

Section Five – Identification and assessment of effects

Identify and assess the effects of the organism. Look primarily at the effects if the organism remains in containment, but also consider what might happen if the organism were to escape. If the organism were to escape think about what additional things would need to occur for these effects to be realised.

What are the beneficial effects of the organism(s) and the application? *These benefits must be relevant to the purpose and scope of the application*
Access to reference material of bacteria and fungi will significantly improve New Zealand's ability to identify exotic plant pathogens in quarantine or surveillance samples thereby preventing their entry or assisting their control by: 1. avoiding "false negatives" during diagnostic testing by providing confirmation of test performance; and 2. developing new diagnostic tests and/or validating the performance of published methods. Additional benefits include: • Improved import and export testing abilities; • Improved surveillance programmes; • Opportunity to increase scientific knowledge and expertise of researchers and diagnosticians; • Protection and assurance of the unique disease-free status of the New Zealand plant-based industries; • Protection of native flora from exotic plant diseases; • Protection of the economy from a severe exotic disease outbreak and consequently leading to a reduction in exports and more expensive imports of plants and plant products; and • Rapid diagnosis of suspected exotic plant diseases without having to be dependent on overseas laboratories.
What adverse effects could this organism have on the environment? *For all stages of the life cycle*
Potential adverse effects on the environment, in particular on ecosystems and their constituent parts were only identified in this application if a micro-organism escaped containment. These potential adverse effects were: • disease and/or loss of native and valued introduced flora; and • deterioration of ecosystems if the disease-causing agent could not be eradicated from the environment and caused loss of genetic diversity and health of our native and valued introduced flora. If containment were breached, it is unlikely that the micro-organisms would become established in New Zealand since most plant pathogens require specific environmental conditions for transmission and for infection of a plant. Many of the specific vectors such as insects are also not present in New Zealand. Furthermore, many plant pathogens (especially bacteria and to a lesser extent fungi) are specific to a small number of plant species which often have a restricted distribution in New Zealand. An organism would also be required to be present in sufficient numbers to cause an infection in a susceptible host plant and for the infection to be self-sustaining within the host plant species. However, there is a possibility that some micro-organisms in this application would find

suitable conditions in New Zealand and would be capable of establishment and causing harm to the environment. For example, the fungus *Puccinia psidii* (Guava rust) infects many fruit and timber trees of the family Myrtaceae (e.g. eucalyptus, rata and pohutukawa) and Heteropyxidaceae which are common in New Zealand (Liberato *et al.*, 2007b). It is transmitted long distances as windborne aeciospores, in rain splash, infected plant material, and contaminated tools and equipment. There are many host plants present in New Zealand for this fungus to establish although the climate may not be sufficiently warm in the winter in many parts of New Zealand for this tropical fungus to become established. Note that the use of fungi in the orders *Uredinales* (rusts) and *Ustilaginales* (smuts), e.g. *Puccinia psidii*, to infect plants is outside the scope of this application.

In contrast, a smut fungus such as *Tilletia horrida* (Rice kernel smut) is an obligate pathogen and infects only two plants, *Oryza sativa* and *O. rufipogon*, which are not commercially cultivated and rarely planted in New Zealand due to the climatic and economic constraints. The disease is seed and soil-borne (Shivas, 2006). The fungus can be dispersed by wind, contaminated tools and equipment. Considering the rarity of the host organisms, this fungus has a low risk of establishment in New Zealand if containment were breached and is therefore unlikely to cause harm to the environment.

What adverse effects could this organism have on public health? *For all stages of the life cycle*

Allergic or toxic reactions to some species covered in this application were identified as a potential adverse effect on human health. Species of fungus in genera such as *Aspergillus*, *Fusarium*, *Penicillium*, *Pythium* and *Thielaviopsis* are known to cause allergic or toxic reactions in susceptible people. In this case, laboratory workers are at most risk as they are more likely to come into direct contact with such organisms. Measures to prevent contact with such micro-organisms are a requirement of working in the containment laboratory, e.g. wearing protection clothing such as eye wear, face masks and laboratory coats. In addition, all sub-culturing and the initial stages of DNA extraction is done in a Class II BSC, this ensures personnel protection.

If in the unlikely event an allergy-producing fungi escaped containment to cause an adverse effect on public health it would first have to find suitable environmental conditions to survive and establish. It would have also to be present in amounts sufficient enough to cause harm to humans. The risk posed to humans from these micro-organisms is considered negligible.

What adverse effects could this organism have on the relationship of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna and other taonga (taking into account the principles of the Treaty of Waitangi)?

The potential adverse effects to the environment and economy caused by the escape of organisms from containment have been addressed elsewhere in this section. If an adverse effect were to occur to native and/or valued species through an escape from containment, there would be likely flow-on effects to the mauri (life essence) of those species and the role of Māori as kaitiaki (guardians/stewards) in the maintenance and management of mauri. However, as described earlier, the nature of containment and likelihood of suitable environment conditions are such that the risk of escape and subsequent infection are essentially nil.

Consultation with local Māori groups (namely Ngati Paoa Whanau Trust Board, Ngai Tai

Umupuia Te Waka Totara Trust, and Ngati Whatua o Orakei Corporate Ltd) has taken place during March and April 2008 by letter and telephone (see Appendix 3). No issues were raised during consultation.

Are there any other potential adverse effects (including effects on New Zealand's international obligations, society and community or the market economy)?
Potential adverse effects (such as New Zealand's international obligations, social or economic adverse effects, ethical issues) were only identified in this application if an micro-organism escaped containment. These were: • decreased yield and quality of production from plant-based industries if a new disease became established, potentially leading to reduced domestic and export sales; and • loss of access to export markets if a new disease were to become established or suspected but could not be confirmed/ruled-out by testing. The economic impacts vary depending on the micro-organisms and the industry affected. For example, the escape of *Fusarium circinatum* could devastate New Zealand plantation forests which primarily consist of *Pinus radiata*, i.e. a susceptible host to *Fusarium circinatum*. There are however, no commercial plantations of *Pinus radiata* near PHEL therefore the risk to *Pinus radiata* is considered negligible. In contrast, although some species may be considered harmful they are unlikely to cause harm to the New Zealand economy if they escaped containment. For example, the bacterium *Xylella fastidiosa* (Pierce's disease) can multiply on over 100 plant species from many families. Although no disease symptoms are produced in many hosts, it has caused losses in economically important plants such as alfalfa, citrus, grape, oak and stone fruit. Some individual strains are able to infect different hosts, but others do not (Liberato *et al*., 2007a). It is xylem-limited and transmitted by leafhopper vectors (Hemiptera: Cicadellidae), infected plant material (Purcell & Hopkins, 1996), and contaminated tools and equipment. Given the wide host range, this bacterium might have a high risk of establishment in New Zealand if containment were breached. However, because it requires the presence of an insect vector to inject it into the host xylem for it to become established, it is low risk because none of the known vectors are present in New Zealand. In addition, the containment laboratory has a contingency plan in place for breaches of containment (see section 4 of this application) and a team of exotic disease investigators that, in the unlikely event of an escape, would minimise the adverse effect of such an escape and further reduce the probability of the organisms establishing a self-sustaining population.
Are there any ethical considerations associated with the organism(s) to be imported or the proposed research?
We do not consider that there are any ethical considerations associated with the micro-organisms to be imported for the proposed research.

Section Six – Additional information

Additional Information	**Y/N**	**If yes, explain**
Do any of the organism(s) need approvals under any other New Zealand legislation?	Y	Many of the micro-organisms included in this application have been declared as "unwanted organisms' under the Biosecurity Act 1993. Multiplication of such micro-organisms requires approval by a Chief Technical Officer (Section 53 (2) of the Biosecurity Act). PHEL has approval to do such work (Appendix 2) unless the organism is a 'high-impact pest' in which case specific approval from a Chief Technical Officer must be obtained.
Does New Zealand have any international obligations relating to (any of) the organism(s)?	N	New Zealand does not have any international obligations relating to any of the micro-organism(s) within the scope of this application.
Have any of the new organism(s) in this application previously been considered in New Zealand or elsewhere? What was the outcome?	Y	Landcare Research has approval to import pure strains of bacteria and fungi from plants and soil into the ICMP. The ERMA New Zealand register record for this approval is NOC99023. The current application is to allow the PHEL to import pure isolates of plant pathogenic and saprophytic bacteria and fungi for the primary purpose of development of detection methods for exotic pests and diseases. The New Zealand Forest Research Institute Ltd also has approval to import micro-organisms into containment to be used as reference cultures in the diagnosis and identification of fungi; to develop new diagnostic tools; to perform pathogenicity tests in containment; and to carry out taxonomic research. The ERMA New Zealand register for this approval is NOC00003.
Is there any additional information that you consider relevant to this application that has not already been included?	N	There is no further information that PHEL considers relevant to this application.

Provide a glossary of scientific and technical terms used in the application:
Chromista: a eukaryotic group which may be treated as a separate kingdom or included in the Kingdom Protista. The groups include all algae whose chloroplasts contain chlorophylls a and c, as well as closely related colourless forms. There are three different groups: Heterokonts or stramenopiles (brown algae, diatoms, water moulds, etc); Haptophytes; and Cryptomonads. **Obligate pathogen**: a pathogen which requires its host for multiplication and which can not multiply *in vitro* in the absence of its host, e.g. all powdery mildews, phytoplasmas, viroids and viruses. **Phytoplasma**: a genus of bacteria characterised by their lack of a cell wall, a pleiomorphic or filamentous shape, normally with a diameter less than 1 micrometer, and compartively small genomes (compared to other bacteria). Members of the genus cannot be cultured *in vitro* and are obligate plant pathogens, usually transmitted by planthoppers or leafhoppers. **Teleomorph**: the sexual reproductive stage of fungi in the phyla Ascomycota and Basidiomycota, typically a fruiting body (cf. **anamorph**, the asexual reproductive stage). **Viroid**: plant pathogens that have a small (a few hundred bases), circular, single-stranded RNA genome, without the protein coat that is typical for viruses. Approximately 30 species have been identified and they are usually transmitted by seed or pollen.
List of appendices:
Appendix 1: Plant Health & Environment Laboratory Standard Operating Procedure CM-07 “PC2 Containment of Exotic Organisms and GMO’s and PC3 Rearing of Insects”. Appendix 2: Permission to propagate or multiply unwanted organisms or pests. Appendix 3: Correspondence during consultation with Ngati Paoa Whanau Trust Board, Ngai Tai Umupuia Te Waka Totara Trust, and Ngati Whatua o Orakei Corporate Ltd.
List of references:
Crop Protection Compendium 2005 Edition. *Gibberella circinata* (pitch canker). CAB International, Wallingford, UK. Crous, P.W. 2005. Plant pathology is lost without taxonomy. Outlooks on Pest Management 16: 119-123. Garrity GM, Boone DR, Castenholz RW (2001) Bergey's Manual of Systematic Bacteriology. Volume One: The Archaea and the Deeply Branching and Phototrophic Bacteria. 2nd edition. Springer. Guarro, J, Gené, J, & Stchigek, AM (1999) Developments in fungal taxonomy. Clinical Microbiology Reviews 12: 454-500. Kirk PM, Cannon PF, David JC, Stalpers JA (2001) Ainsworth & Bisby’s Dictionary of the Fungi. 9th edition. CABI Bioscience. Liberato JR, Matheson C & Summerell (2006) Pitch canker disease of pine (*Fusarium circinatum*) Pest and Diseases Image Library. http://www.padil.gov.au. Updated on 21/09/2006 12:01:02 PM.

Liberato JR, Queiroz-Voltan, RB, Matsuoka K, Laranjeira FF & Miles AK (2007a) Citrus variegated chlorosis (*Xylella fastidiosa*) Pest and Diseases Image Library. http://www.padil.gov.au. Updated on 11/01/2007 10:56:18 PM.

Liberato JR, Silveira SF, Junghans DT, Rocabado, JA, Aparecido CC & Shivas RG (2007b). Eucalyptus rust (*Puccinia psidii*) Pest and Diseases Image Library. Updated on 21/02/2007 3:12:51 PM. Available online: http://www.padil.gov.au

MAF Biosecurity New Zealand (2007) http://www.biosecurity.govt.nz/imports/forest/pine-pitch-canker-interception. Accessed on 3/12/07.

Purcell AH, Hopkins DL (1996). Fastidious xylem-limited bacterial plant pathogens. Annual Review of Phytopathology 34:131-151.

Shivas RG (2006) Rice kernel smut (*Tilletia horrida*) Pest and Diseases Image Library. Updated on 20/09/2006 9:39:04 AM. Available online: http://www.padil.gov.au.