FORM NO2N

Application for approval to

IMPORT INTO CONTAINMENT ANY NEW ORGANISM THAT IS NOT GENETICALLY MODIFIED

under section 40 of the Hazardous Substances and New Organisms Act 1996

Application Title: Importation of extremophilic microorganisms from geothermal sites for research purposes
Applicant Organisation: Institute of Geological & Nuclear Sciences

ERMA Office use only	
Application Code: ☐☐☐☐☐☐☐☐	Formally received:____/____/____
ERMA NZ Contact: _____	Initial Fee Paid: $
Application Status:	

Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

IMPORTANT

1. An associated User Guide is available for this form. You should read the User Guide before completing this form. If you need further guidance in completing this form please contact ERMA New Zealand.
2. This application form covers importation into containment of any new organism that is not genetically modified, under section 40 of the Act.
3. If you are making an application to import into containment a **genetically modified organism** you should complete **Form NO2G,** instead of this form (Form NO2N).
4. This form, together with form NO2G, replaces all previous versions of Form 2. Older versions should not now be used. You should periodically check with ERMA New Zealand or on the ERMA New Zealand web site for new versions of this form.
5. You can talk to an Applications Advisor at ERMA New Zealand who can help you scope and prepare your application. We need all relevant information early on in the application process. Quality information up front will speed up the process and help reduce costs.
6. This application form may be used to seek approvals for importing more than one new (non-genetically modified) organism into containment where the organisms are of a similar nature.
7. Any extra material that does not fit in the application form must be clearly labelled, cross-referenced, and included as appendices to the application form.
8. Commercially sensitive information must be collated in a separate appendix. You need to justify why you consider the material commercially sensitive, and make sure it is clearly labelled as such.
9. Applicants must sign the form and enclose the correct application fee (plus GST). The initial application fee can be found in our published Schedule of Fees and Charges. Please check with ERMA New Zealand staff or the ERMA New Zealand website for the latest schedule of fees. We are unable to process applications that do not contain the correct initial application fee.
10. Unless otherwise indicated, all sections of this form must be completed for the application to be progressed.
11. Please provide an electronic version of the completed application form, as well as sending a signed hard copy.

You can get more information by contacting us. One of our staff members will be able to help you.

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Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

Section One – Applicant Details

1.1 Name and postal address in New Zealand of the organisation or individual making the application:

Name > Institute of Geological & Nuclear Sciences

Postal Address > Private Bag 2000, Taupo, NZ.

Physical Address > Institute of Geological and Nuclear Sciences
114 Karetoto Road, SH1
Wairakei, New Zealand

Phone > 07-374-8211

Fax > 07-374-8199

E-mail >

1.2 If application is made by an organisation, provide name and contact details of a key contact person at that organisation

This person should have sufficient knowledge to respond to queries and have the authority to make decisions that relate to processing of the application.

Name > Matthew Stott

Position > Scientist - Microbiologist

Address > Institute of Geological and Nuclear Sciences
114 Karetoto Road, SH1
Wairakei, New Zealand

Phone > 07-376-0132

Fax > 07-374-8199

E-mail > m.stott@gns.cri.nz

1.3 If the applicant is an organisation or individual situated overseas, provide name and contact details of the agent authorised to transact the applicant's affairs in relation to the application

This person should have sufficient knowledge to respond to queries and have the authority to make decisions that relate to processing of the application.

Name > N/A

Position >

Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

Address >

Phone >

Fax >

E-mail >

Section Two – Purpose of the Application

This form is to be used for an application to import into containment any new organism that is not genetically modified. For an application to import into containment **a genetically modified organism**, use **Form NO2G**.

2.1 Give a short summary statement of the purpose of this application to be used on ERMA New Zealand's public register. (Maximum of 255 characters).

Briefly describe the organism(s) to be imported into containment, and the purpose(s) for which you wish to import the organism(s).

To import for research purposes including taxonomy, ecology, biodiversity and biotechnology studies, cultures of non-pathogenic extremophilic microorganisms.

2.2 Provide a short description of the background and aims of the project suitable for lay readers.

Describe the purpose of the importation and rationale for the overall project these organisms are to be used in so that people not directly connected with the research can understand why these organisms are required.

The Extremophile Research Group (ERG) at the Institute of Geological & Nuclear Sciences (GNS) is undertaking research based around the enrichment and isolation of novel New Zealand indigenous extremophilic microorganisms from their natural environments. Extremophilic organisms live in environments not normally inhabitable by the majority of life, such as hot springs, highly salty brine water, deep underground oil reserves, acidic or alkaline waters or deep-sea hydrothermal vents. We hope that by studying these unique environments and the microorganisms that inhabit them, we will learn more about how these cells are able to survive in these harsh conditions and how they interact with the food chain and surrounding geochemical/mineral environments. Understanding the ecology of these systems has implications for understanding the origins of life and the possibilities of life on other planets. This research will assist in defining New Zealands native biota and in doing so opens up possibilities of developing potentially new beneficial technologies such as bioremediation of pollutants, medicine and drug discovery, and more efficient industrial processes.

In order to undertake such research, isolated strains must be compared with other, taxonomically defined strains to accurately describe their physiological and molecular characteristics. We wish to import individual strains of microorganisms into containment from recognised microbial culture collections (such as the German Collection of Microorganisms and Cell Culture (DSMZ - http://www.dsmz.de/species/strains.htm), the American Type Culture Collection (ATCC - http://www.atcc.org/Home.cfm), National Collection of Industrial and Marine Bacteria (NCIMB - http://www.ncimb.com/index.php) or the Japanese Collection of Microorganisms (JCM - http://www.jcm.riken.jp/)), to use as reference strains for molecular and

physiological comparison with isolates enriched from New Zealand extreme environments. However, as the internationally accepted list of microbial species is constantly changing due to reclassifications and continual species discoveries, we request that the risk assessment and permits for importation of the microbial types be assessed as classes or types of extremophilic microorganisms rather than importation assessment be made on the basis of individual microbial species. No microbial species containing harmful or pathogenic species have been considered.

Section Three – Information on the Organism(s) to be imported

If the application is for importation of more than one organism, this section must be completed separately for each organism. If there are commercial reasons for not providing full information here, alternative approaches must be discussed with and agreed by ERMA New Zealand.

3.1 Give the unequivocal identification of the organism(s) to be imported

These names will be on the public register and should clearly identify the organisms. Please provide details of the following:

Latin binomial, including full taxonomic authority:
Common name(s), if any:
Type of organism (eg bacterium, virus, fungus, plant, animal, animal cell):
Taxonomic class, order and family:
Strain(s) if relevant:
Other information, including presence of any inseparable or associated organisms:

We would like the Authority to give consideration to a risk assessment of classes or metabolic types of extremophilic microorganisms for importation into containment. The classes of microorganisms we would like considered are all PC1 rated (risk 1) microbes that, because of their metabolic requirements, are unlikely to be pathogenic to plants, animals or humans. We suggest that such classes could include thermophiles, acidophiles, barophiles, alkaliphiles, methanotrophs, methanogens, nitrifiers and halophiles[1]. Because of their uncharacteristic metabolic requirements, these extremophiles live outside the bounds of so-called normal life. They therefore pose very low risk to the community or environment and when maintained within a MAF-registered containment facility, there is little chance of a microbial discharge into the environment.

In the table below we have listed a comprehensive list of “extremophilic” microbial genera we wish to be considered for importation into containment as part of this application. Relevent taxanomic information has also been included. In addition, a table containing a list of current species included in each genus, physiological information and risk classification have been included in Appendix 1.

[1] In this application the microbial classes described above have been collectively grouped as extremophiles (see definition in glossary, section 6.4). However, strictly speaking, methanotrophs, methanogens and nitrifiers are not classically grouped as “extremophiles” (see Schlegel and Jannasch, 1999 for classical definition). None-the-less, these three metabolic types still growth obligately in conditions outside the bounds of what is considered “normal-life”.

TABLE 3.1. List of non-pathogenic, non-infectious "extremophilic" microbial genera, including taxonomic and risk classifications to be considered for imporation into containment

Description	Domain	Phylum	Genera	Risk classification
Thermoproteales	*Archaea*	*Crenarchaeota*	***THERMOPROTEUS, PYROBACULUM, THERMOCLADIUM, THERMOFILUM, CALDIVIRGA, VULCANISAETA***	1
Caldisphaerales	*Archaea*	*Crenarchaeota*	***CALDISPHAERA***	1
Cenarchaeales	*Archaea*	*Crenarchaeota*	***CENARCHAEUM***	1
Desulfurococcales	*Archaea*	*Crenarchaeota*	***DESULFUROCOCCUS, ACIDILOBUS, AEROPYRUM, IGNICOCCUS, STAPHYLOTHERMUS, STETTERIA, SULFOPHOBOCOCCUS, THERMODISCUS, THERMOSPHAERA, PYRODICTIUM, PYROLOBUS, HYPERTHERMUS***	1
Sulfolobales	*Archaea*	*Crenarchaeota*	***SULFOLOBUS, ACIDIANUS, METALLOSPHAERA, SULFUROCOCCUS, SULFURISPHAERA, STYGIOLOBUS***	1
Methanogens	*Archaea*	*Euryarchaeota*	***METHANOBACTERIUM, METHANOBREVIBACTER, METHANOCALCULUS, METHANOCALDOCOCCUS, METHANOCOCCOIDES, METHANOCOCCUS, METHANOCORPUSCULUM, METHANOCULLEUS, METHANOFOLLIS, METHANOGENIUM, METHANOHALOBIUM, METHANOHALOPHILUS, METHANOLACINIA, METHANOLOBUS, METHANOMETHYLOVORANS, METHANOMICROBIUM, METHANOMICROCOCCUS, METHANOSPHAERA, METHANOSPIRILLUM, METHANOPLANUS, METHANOTHERMOBACTER, METHANOPYRUS, METHANOTHERMOCOCCUS, METHANOTHERMUS, METHANOSAETA, METHANOTHRIX, METHANOTORRIS, METHANOSALSUM, METHANOSARCINA***	1
Halobacteria	*Archaea*	*Euryarchaeota*	***HALOBACTERIUM, HALOARCULA, HALOBACULUM, HALOCOCCUS, HALOFERAX, HALOGEOMETRICUM, HALORHABDUS, HALORUBRUM, HALOTERRIGENA, NATRIALBA, NATRINEMA, NATRONOBACTERIUM, NATRONOCOCCUS, NATRONOMONAS, NATRONORUBRUM, HALOMICROBIUM, HALOQUADRATUM, HALOSIMPLEX, NATRONOLIMNOBIUS***	1
Thermoplasmata	*Archaea*	*Euryarchaeota*	***FERROPLASMA, PICROPHILUS, THERMOPLASMA***	1
Thermococci	*Archaea*	*Euryarchaeota*	***THERMOCOCCUS, PALAEOCOCCUS, PYROCOCCUS***	1

Archaeoglobi	*Archaea*	*Euryarchaeota*	***ARCHAEOGLOBUS, FERROGLOBUS***	1
Nanoarchaeota	*Archaea*	unclassified	***NANOARCHAEUM***	1
Aquificales	*Bacteria*	*Aquificae*	***AQUIFEX, HYDROGENOBACTER, THERMOCRINIS, DESULFUROBACTERIUM, HYDROGENIVIRGA, HYDROGENOBACULUM, HYDROGENOTHERMUS, PERSEPHONELLA, SULFURIHYDROGENIBIUM***	1
Methanotrophs	*Bacteria*	*Alphaproteobacteria*	***METHYLOCYSTIS, METHYLOSINUS, METHYLOCELLA, METHYLOCAPSA***	1
Methanotrophs	*Bacteria*	*Gammaproteobacteria*	***METHYLOCOCCUS, METHYLOMICROBIUM, METHYLOHALOBIUS, METHYLOBACTER, METHYLOCALDUM, METHYLOSPHAREA, METHYLOSARCINA METHYLOTHERMUS***	1
Nitrifiers	*Bacteria*	*Gammaproteobacteria*	***NITROSOCOCCUS***	1
Nitrifiers	*Bacteria*	*Betaproteobacteria*	***NITROSOMONAS, NITROSOSPIRA LEPTOSPIRILLUM***	1
Nitrifiers	*Bacteria*	*Alphaproteobacteria*	***NITROBACTER***	1
Acidobacteria	*Bacteria*	*Acidobacteria*	***ACIDOBACTERIUM, GEOTHRIX, HOLOPHAGA***	1
Deinococcus-Thermus	*Bacteria*	*Deinococcus-Thermus*	***DEINOCOCCUS, THERMUS***	1
Thermotogales	*Bacteria*	*Thermotogales*	***FERVIDOBACTERIUM, GEOTOGA, MARINITOGA, PETROTOGA, THERMOPALLIUM, THERMOSIPHO, THERMOTOGA***	1
Thermodesulfobacteria	*Bacteria*	*Thermodesulfobacteria*	***GEOTHERMOBACTERIUM, THERMODESULFATATOR, THERMODESULFOBACTERIUM***	1
Acidophiles	*Bacteria*	*Proteobacteria*	***ACIDITHIOBACILLUS, ACIDIMICROBIUM, FERROMICROBIUM, THIOMONAS***	1
Bacillales	*Bacteria*	*Fermicutes*	***GEOBACILLUS, ANOXYBACILLUS SULFOBACILLUS***	1

Appendix 1 contains relavent physiological information, risk classifications and a list of species currently included in each genus.

3.2 Characteristics of the organism(s) to be imported

Provide information on the biology, ecology and the main features or essential characteristics of each organism(s) to be imported. For example, note production of spores/seeds/pollen, conditions for growth and reproduction. Also provide information on affinities of the organism(s) with other organism(s) in New Zealand. This information should be relevant to the identification of the risks of the organism (section 5).

All extremophilic microorganisms known to date (examples of extremophilic genera are listed in section 3.1 and individual species of listed genera (Appendix 1)) have been catagorised with Risk classifications consistant with AS/NZ Standard 2243:3:2002. All listed microbial genera and species are classified as Risk Group 1: "*Risk group 1 (low individual and community risk);* a microorganism or microbial culture that is unlikely to cause human, plant or animal disease" (AS/NZ Standard 2243:3:2002). All risk group classifications have been referenced from the various microbial databases listed above in Section 2.2. The risk classifications of these microbial databases and the AS/NZ Standard 2243:3:2002 risk classification definitions are all based on WHO standards.

The growth requirements of all microbial genera listed are best described as extreme. They require elevated temperatures, pressures, highly acidic or basic solutions, highly salty environments (Schlegel and Jannasch, 1999) or specialised energy sources (methanogens, methanotrophs, nitrifiers; Whitman *et al.*, 1999; Bowman, 2000; Bock and Wagner, 2001) or a combination of the above for normal growth. In general, the microorganisms that tolerate and grow under the most extreme conditions are obligately adapted to their particular environment and fail to grow at lower intensities of the same environmental factor. Such an organism has acquired the ability to grow in one extreme environment at the expense of its ability to grow in others (Schlegel and Jannasch, 1999). It therefore follows that if extremophilic microorganisms obligately require non-normal conditions for growth, in most cases because of their specific (mostly chemolithotrophic) growth requirements, then these microorganisms will lack the ability to infect organisms that operate under "normal conditions" and will be therefore highly unlikely to be pathogenic to flora, fauna and humans. It should also be noted that no member of the Domain *Archaea* (over half of the example genera included in this application) have ever been identified as a pathogen for humans or plants and animals (Reeve, 1999). It should also be noted that methanogens, all of which are *Archaea* are normal inhabitants of cattle rumen, gastrointestinal tracts and sewage (Whitman *et al.*, 1999), but do not cause disease (all have a risk classification of 1) and methanotrophs have never been isolated from humans, plants or animals (Bowman, 2000) and therefore should not be considered capable of infection or growth in human, plant or animal hosts.

Of the extremophilic genera listed above, only members of the *Geobacillus*, *Sulfobacillus* and *Anoxybacillus* genera are capable of endospore production (see individual species references in Apendix I). However, due to their requirements for moderately thermophilic temperatures and chemosynthetic growth requirements, all are classified as Risk Group 1[2]. While endospores formed by prokaryotic Gram-positive microorganisms such as *Geobacillus* or *Anoxybacillus* are highly chemical and desiccation resistant, the generation of endospores (unlike spores from *Actinomycetes*, fungi and algae or cysts from Protozoa) is not part of the *normal* cell cycle and only form when conditions become non-conducive to growth (Madigan *et al*., 1997). Aseptic laboratory techniques, trained staff and routine subculturing negate the exposure and formation of endospore-inducing conditions.

[2] a number of species within the *Geobacillus* and *Anoxybacillus* genera are already included on the ERMA list of microorganisms authorised for importation into containment.

Section Four – The Proposed Containment System and its Effectiveness

4.1 Describe the proposed containment system (physical and operational) and the ability of the organism(s) to escape from this system. The adequacy of the containment regime is a principal consideration so you need to provide comprehensive information on the containment system. Containment facilities must be registered by MAF, and you should provide documentary evidence of this. Refer to relevant containment manuals as appropriate. Please also ensure that ERMA New Zealand has an up-to-date copy of the containment manual relating to this facility. Identify possible pathways of escape of the organism(s) from containment, including through lapses of security or sabotage. Describe the biological features of the organism(s) that might affect its ability to escape from containment.

The ERG containment facility has been registered as a PC1 type laboratory with MAF according to the MAF Biosecurity Authority Standard 154.03.02. An inspection of the containment facilities has been conducted by a MAF officer (Mike Aitkenhead, Mike.Aitkenhead@maf.govt.nz).

All proposed work using the soil/sediment and fluid samples described in this application will be conducted in the aforementioned containment facility according the protocols described in the ERG Containment and Quarantine Manual (a copy of this manual is held with ERMA from the ERG's previous NO20 application, application NOC04013, 18 October, 2004). While specifics for the maintenance of a containment facility are described in detail in the manual, the overarching premises for containment regime are as follows;

- Only authorised, trained personnel will be permitted to conduct research within the containment facility. All staff with be trained in the correct and safe handling of microbial cultures
- All trained personnel are (or will be) required to ensure that work practices comply with the requirements of the Biosecurity and HSNO Acts and any additional conditions stipulated by ERMA
- All personnel are required to understand and implement contingency plans in the unlikely situation where containment is compromised
- All microbiological material, regardless of whether sourced from within New Zealand or classified as a "new organism" (see HSNO Act 1996) will be destroyed (using the appropriate methods described in the ERG Containment and Quarantine Manual (and AS/NZ Standard 2243:3:2002) before leaving the containment facility
- A register of all HSNO-restricted microbial cultures will be maintained in the ERG Containment and Quarantine Manual
- Transfer of HSNO-restricted microbial cultures to other containment facilities will only be conducted with the written approval of MAF and according to the transport container requirements of the IATA Dangerous Goods Regulations

PATHWAYS OF ESCAPE for microorganisms

The laboratory will be run in accordance with the principles of AS/NZS 2243.3:2002 "Safety in Laboratories: Microbiological aspects and containment facilities", as required by MAF Standards 154.03.02 and 152.04.03F and the HSNO act (1998). If the procedures described in the Standard Work Practices (Waste Disposal and Treatment) are followed (Section 6.3, ERG Containment Manual), the likelihood of this occurring is remote. Not-withstanding, the following areas have been identified as *possible* points of escape;

- *During transit between containment facilities*. Correct IATA packaging will prevent the escape of the microbial culture beyond the secondary packaging (see IATA packing instruction No: 650)
- *Accidental release of viable material into storm water without sterilisation*. Drain traps have been installed such that if release is suspected, the contaminated waters can be correctly treated with a chemical disinfectant.
- *Sabotage, lapse of security or fire*. Up-to-date safety equipment, and in-house and contracted security at the containment facility means that any accidental release of the facility by sabotage, lapse of security or fire is unlikely and will be minimised.

As mentioned is Section 3.2, a small number of Gram-positive endospore-forming *Bacteria* have been included in this importation into containment application. Endospores are chemical and desiccation resistant. However, endospore generating conditions are not part of the *normal* reproduction cycle and only occur when conditions are non-conducive, for example, where an energy source is exhausted. Growth of these cells will always be in closed containers that can be autoclaved and therefore the risk of aerosol generation is minimal if endospore stimulating conditions were to occur. Where a vessel containing spore-forming microorganisms (or any microbial cultures for that matter) is broken, the spill would be chemically treated according to

AS/NZS 2243.3:2002 "Safety in Laboratories: Microbiological aspects and containment facilities" (and as described in the ERG Quarantine and Containment Manual).

In the advent of escape from the containment facilities, there is a negligible probability that the imported microbial strains would be able to proliferate and out-compete indigenous microflora due to their specific growth requirements. In addition, due to their type Risk 1 profile, no microorganisms within these samples are likely to pose any threat to human flora or fauna.

Section Five - Identification and Assessment of Risks, Costs, and Benefits
This section must include information on the beneficial and adverse effects referred to in the HSNO Act. It is easier to regard risks and costs as being adverse (or negative) and benefits as being positive. You should consider costs and benefits with respect to both non-monetary and monetary (dollar) terms and also consider the distribution of this incidence. Provide a brief description of where the information in the application has been sourced from, e.g. from in-house research, independent research, technical literature, community or other consultation.

5.1 Ability of organism(s) to establish a self-sustaining population.

Discuss the ability of the organism(s) to establish an undesirable self-sustaining population, should an escape from containment occur, and the ease with which such a population could be eradicated. You should consider the ability of the organism(s) to survive and reproduce if it did escape from containment.>

As outlined in Section 4, in the event of escape from the containment facilities, it is highly improbable that any of the listed microbial strains will be able to proliferate and outcompete indigenous microflora due to their specific growth requirements, primarily temperature, but also their need for specific energy sources and terminal electron acceptors. Only small populations of microorganisms will be used in routine laboratory work (≤1L at maximum densities of 10^9 microorganisms/ml). At these levels, there are no predicted environmental perturbations because the numbers in the environment are usually much greater[3].

The primary route of escape from the facility, *if* containment protocols *and* back-up safeguards failed would be via wastewater drains to a sump. Conditions within the sump are non-conducive to growth due to the lack of elevated temperatures and energy sources/nutrients. Any indigenous microbial populations within the sump would out-compete the escaped microflora (or microflora generated from endospores). Likewise, spillage or transport and release of the thermophilic microbial populations outside the containment facility would require the cultures to be transported and released *specifically* to a geothermally heated environment in large volumes. The microorganisms would then need to be in large enough population to out-compete an established population[3] and to survive the specific environment. The likelihood of this occurring is essentially non-existent.

In the theorectical case where a microbial culture was transported from the containment facility to a geothermal feature that identically mimicked the ideal geochemical and geophysical conditions required for growth AND a large volume of restricted microorganisms were then transported to this site and released, then the establishment of a self-sustaining population within a geothermal environment would be difficult to eradicate. However, in this case, it would be highly probable that a similar, if not identical indigenous microorganism would also be present.

[3] ESR Ltd GMC99004: In this approved application it states. "Such small amounts of organism being used in individual experiments….means that there would be no predicted environmental perturbation as the number already in the environment are much greater." Thus, GMC99004 is referenced as a precedent of the argument and for its documentation.

5.2 Identify all potential adverse effects of the organism(s). Identify potential adverse effects associated with the organism(s) and with any inseparable organisms, both within containment, and outside of containment (should an escape occur). Consider effects on the environment, and human health and safety (e.g. of workers in the containment facility), and any ethical and cultural effects. It is important to think about the source of the risk, i.e. the way in which the risk is created (the exposure pathway), and then the consequences of exposure. Adverse effects should be identified for the following categories:

A. Potential adverse effects on the environment, in particular on ecosystems and their constituent parts (e.g. adverse effects on: life supporting capacity of air, water, soil and ecosystems; native and valued introduced flora and fauna; natural habitats and the intrinsic value of ecosystems; New Zealand's inherent genetic diversity; animal or plant health)

No potential adverse effects on the environment have been identified within containment or if the microbial cultures were to escape containment. It is a common premise in environmental microbiology that the environment dictates the type of microbial population. Therefore in the event of escape, it is highly likely that a similar or identical microbial species will already inhabit the environment. Most non-pathogenic microorganisms are at the genus level pandemic. Members of a single genus can be found in geographically distant locations: at either pole, for example (Fenchel and Finlay, 2004). If a niche for a microbe occurs in a location, it is almost certain to be filled already. If escape of a microbial species from the laboratory were ever to occur, then no perceived adverse effects on the environment can be envisaged.

All microorganisms will be purchased from reputable microbial database libraries (DSMZ, ATCC or JCM) and are guaranteed to be single strains. No Virus or other organisms are included making up the delivery.

B. Potential adverse effects on public health (including occupational exposure)

Risk to public health (including ERG personnel) will be negligible as the microorganisms listed are all Risk Type 1, are not human pathogens and not transmissible.

C. Potential adverse effects 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). For example, you should consider whether the organism(s) would have an effect on specific native flora or fauna if they escaped from containment.

No evidence of any adverse effects on the relationships of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu or valued flora and fauna and other taonga have been identified. As outlined in section 5.2A, in the unlikely event of escape into the environment, no adverse effect would be noted. The risks are no greater than outlined above.

D. Other potential adverse effects (such as New Zealand's international obligations, social or economic adverse effects, ethical issues)

We can conceive of no way in which the escape of these microorganisms could have any adverse effects on NZ's international obligations, nor any negative social or economic effects or ethical issues.

5.3 Provide an assessment of the adverse effects identified in Sections 5.2. The assessment should include the nature, likelihood or probability of occurrence, and magnitude of each adverse effect (i.e. **the risk**), and the value (in monetary or non-monetary terms) of a

particular adverse effect (i.e. **the cost**). The uncertainty bounds of the information contained in the assessment should also be discussed.
Adverse effects should be assessed in relationship to:

A. Potential adverse effects on the environment, in particular on ecosystems and their constituent parts (e.g. adverse effects on: life supporting capacity of air, water, soil and ecosystems; native and valued introduced flora and fauna; natural habitats and the intrinsic value of ecosystems; New Zealand's inherent genetic diversity; animal or plant health)

The risk of escape and establishment of a self-propagating microbial population are minimal to non-existent and therefore the value associated with this is negligible.

B. Potential adverse effects on public health (including occupational exposure)

N/A

C. Potential adverse effects 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). For example, you should consider whether the organism(s) would have an effect on specific native flora or fauna if they escaped from containment. If consultation with Maori has been undertaken, provide details of the process used and the outcome.

N/A

D. Other potential adverse effects (such as New Zealand's international obligations, social or economic adverse effects, ethical issues)

N/A

5.4 Identification of beneficial effects (benefits)

Identify and describe monetary and non-monetary benefits associated with importing the organism(s) into containment. Outline and discuss the purpose(s) for the importation and the potential use of the organism(s). Focus on the immediate benefits, as well as longer-term benefits. For example, "increase in scientific knowledge", "increased production of agricultural produce". Substantiate claims by reference to sources of information. Specify whether the benefits identified are environmental, public health or economic benefits; and/or are specific benefits to Maori.

We hope that by studying New Zealand's extreme environments and the microorganisms that inhabit them we will learn more about how these cells are able to survive in these harsh conditions and how they interact within food chain and surrounding geochemical/mineral environments. To conduct a thorough scientific investigation, isolated microbial cells must be compared to phylogenetically and taxonomically similar described microbial strains. Therefore, we believe by importing these microorganisms into containment we will firstly, and most importantly, increase scientific knowledge. Understanding the ecology of these extreme ecosystems has implications for understanding the origins of life, life on other planets and the similarities between extreme microbial survival and pathogen evasion techniques. Furthermore, the discovery of new microorganisms opens up possibilities of developing potentially new beneficial technologies such as bioremediation of pollutants, generation of environmentally sustainable energies, medicine and drug discovery and efficient industrial processes. It is proposed that these samples will be collected with international collaborators; therefore the study of these microorganisms will bolster New Zealand's international science reputation.

5.5 Provide an assessment of the benefits identified in Section 5.4.

Estimate the likelihood that the benefits will be realised, the magnitude of benefits associated with importing the organism(s) into containment, and any uncertainties associated with this assessment. You should also indicate who would receive the benefits and the expected time-course of delivery of the benefits.

While there will be direct benefits from importing these proposed microorganisms into containment outside increasing scientific knowledge, there is no doubt that in the medium term, novel microorganisms from New Zealand's extreme environments have potential to generate medical and environmental biotechnologies. Likewise, there is potential for the development of environmentally sustainable technologies such as eco-friendly fuels or pollutant bioremediation.

A list of scientific publications by members of the research team at GNS have been provided in the reference list.

5.6 Overall evaluation of risks, costs, and benefits

This overall evaluation is the main task of the Authority. The Authority has to decide whether the beneficial effects of having the organism in containment outweigh the adverse effects of the organism and any associated inseparable organisms. The Authority must also be satisfied that the organism can be safely contained. You may wish to express a view on the relative importance of the different risks, costs and benefits and how they should be brought together in making a decision.

The risks associated with importing into containment and cultivating these microorganisms are minimal to non-existent. The characteristics of each imported extremophilic microbial genus is clearly known prior to importation, all microbes are Risk Group 1-type microorganisms and all will be held in a MAF approved containment facility operated by competent and microbiologically-trained staff. If a microbial strain were to escape containment, the likelihood of one of these microbial strains establishing a self-propagating community outside containment is minimal due limited number of conducive environments in which individual microbial strains could proliferate. In addition, there is minimal risk to public health for a containment failure or occupational exposure, due to the microorganism's lack of pathogenesis and inability to grow in all but extreme conditions.

Conversely, the immediate benefits of importing extremophilic microorganisms into containment will include the characterisation of unknown NZ extremophilic isolates, it will assist in establishing a sound scientific knowledge base of the microbial ecology in NZ's geothermal and hotspring environments and it will increase staff skills and scientific knowledge base. In doing so we, may gain some insights to the origins of life, perhaps life on other planets and the similarities between extreme microbial survival and pathogen evasion techniques. Furthermore, the discovery of new microorganisms may open up possibilities of developing potentially new beneficial technologies such as bioremediation of pollutants, generation of environmentally sustainable energies, medicine and drug discovery and efficient industrial processes. The benefits for New Zealand and science if any of these technologies were realised could potentially be huge.

In order to conduct this science efficiently and to maintain the relevance of our work, it is critical that we have access to all "extreme" microbial genera as new species are described. To do this, we request that the Authority assess the microorganisms we wish to import by classes or metabolic types of extremophilic microorganisms as opposed to the standard method of species. All current extremophilic microbial genera we wish to import are listed in Table 3.1. All of these organisms are risk classification 1 and are not transmittable and do not cause disease in humans, flora or fauna.

Section Six – Additional Information

6.1 Do any of the organism(s) need approvals under any other New Zealand legislation or are affected by international obligations?

For example, indicate whether the organism is subject to other New Zealand legislation, e.g. the Biosecurity Act 1993, or Animal Welfare Act 1999; or if the organism(s) are listed in CITES, then approval is required from both the importing and exporting countries.

No approvals are required for the Animal Welfare Act 1999 or CITES. The Biosecurity Act 1993 requires that a containment facility be established according to the directions dictated by MAF Standards 154.03.02 for the importation of HSNO restricted microorganisms (ERMA). The Wairakei laboratory has a MAF containment facility permit number of 846 (see section 4.1 for details). The Biosecurity Act also requires environmental samples containing microorganisms to be imported under permit from MAF. This will be done following any ERMA decision relating to this application.

6.2 Have any of the new organism(s) in this application previously been considered in New Zealand or elsewhere?

For example, has the organism(s) been previously considered for import (e.g. under the Plants Act)?

A number of extremophilic microbial species previously considered and accepted and are listed on the ERMA permitted species list[4]. However, this application concerns the importation of all species within listed genera. The applicants have not applied previously to import any microbial species.

[4] Some genera have been reclassified. For example, many of the moderately thermophilic members of the *Bacillus* genus have been reclassified into the *Geobacillus* and *Anoxybacillus* genera.

6.3 Is there any additional information that you consider relevant to this application that has not already been included?

No

6.4 Provide a glossary of scientific and technical terms used in the application.

- **Extremophilic microorganisms** require 'extreme' physio-chemical conditions for optimal growth. These conditions are usually outside those that permit the majority of life to exist;
    - acidophiles (acidity pH <3.0)
    - thermophiles (Temperatures >60˚C)
    - hyperthermophiles (Temperatures >80˚C)
    - methanogens (microorganisms that generate methane and are strict anaerobes)
    - methanotrophs (microorganisms that consume methane and are generally aerobic)
    - nitrifiers (microorganisms that oxidise ammonium or nitrite to nitrate and are generally aerobic).
    - barophiles (requirement of a atmospheric pressure greater than 1atm for growth)
    - halophiles (requirement for NaCl, optimal growth at higher salinity than seawater)
- Metabolic descriptions
    - **Autotroph**; a microorganism that obtains its carbon from carbon dioxide (ie not an organic compound)
    - **Heterotroph**; a microorganism that obtains its carbon from an organic compound

- **Chemolithotroph**; a microorganism that grows (gains energy) by the oxidation of inorganic compounds. Generally chemolithotrophic microorganisms are autotrophic
- **Chemoorganotroph**; a microorganism that grows (gains energy) by the oxidation of organic compounds. Generally chemoorganotrophic microorganisms are heterotrophic
- **Terminal electron acceptor;** a terminal electron acceptor (or oxidant) is required to gain energy from the oxidation of an energy source. In the case of aerobic organisms (like ourselves) oxygen is the terminal electron acceptor. In the case of anaerobic organisms componds such as nitrate, sulfate and carbon dioxide are terminal electron acceptors.
- **Anaerobe**; a microorganism that grows in the absence of free oxygen, usually using an inorganic compound in place of oxygen as the terminal electron acceptor
- **Aerobe**; a microorganism that grows in the presence of atmospheric oxygen (as the terminal electron acceptor).
- **Microaerobe**; a microorganism that grows in the presence of reduced concentrations of atmospheric oxygen, but is damaged by normal oxygen concentrations

- A **new microorganism** is (see HSNO Act 1998);
  - An organism belonging to a species that was not present in New Zealand immediately before 29 July 1998;
  - An organism belonging to a species , subspecies, infrasubspecies, variety, strain, or cultivar prescribed as a risk species, where that organism not present in New Zealand at the time of promulgation of the relevant regulation;
  - A genetically modified organism
- **NZ EEZ**; New Zealand Exclusive Economic Zone
- **Microbial classifications;**
  - **Prokaryote;** single cellular organisms encompassing the Domains *Bacteria* and *Archaea*. Prokaryotic DNA is not enclosed by a membrane (nucleus).
  - **Eukaryote;** Cells that contain a membrane-enclosed nucleus. The cells are generally larger than prokaryotes. Many eukaryotes are multicellular organisms (plants and animals). Eukaryotes encompass protista, algae, fungi, plants and animals
  - ***Archaea*;** a prokaryotic cell distinct from *Bacteria*. Archaeal cells lack a muramic acid in their cell wall structure, have ether-linked membrane lipids and are insensitive to many antibiotics. *Archaea* tend to be the dominant microbial type in extreme environments.
  - ***Bacteria***; All prokaryotes that are not of the domain *Archaea.* Bacterial cells contain muramic acid in their cell wall structure, have ether-linked membrane lipids and are sensitive to many antibiotics.
  - **Gram positive/Gram negative;** all prokaryotic cells can be classified by a cell wall stain known as a Gram stain. Gram-positive cells are characterised by a single plasma membrane and a thick peptidoglycan envelope. Gram negative cells have a double membrane and a thin peptidoglycan layer between these.
  - **Algae;** Photosynthetic unicellular eukaryotes
  - **Fungi;** Non-photosynthetic unicellular or multicellular eukaryotes with rigid cell walls
  - **Acintomyces;** Gram-positive aerobic bacteria that form branching filaments or hyphae and asexual spores
  - **Protozoa;** Unicellular eukaryotes that lack a cell wall
- **WHO**; World Health Organisation

REFERENCES within text

- ERMA applications; GMC99004, NOC99021 & NOC99013
- AS/NZS 2243.3:2002 "Safety in Laboratories: Microbiological aspects and containment facilities"
- MAF Standards 154.03.02 and 152.04.03F
- HSNO act (1998).
- The German Collection of Microorganisms and Cell Cultures (DSMZ); www.dsmz.de
- The Ammerican Type Culture Collection (ATCC); http://www.atcc.org/Home.cfm
- The Japanese Collection of microorganisms (JCM); http://www.jcm.riken.jp/
- IATA *Dangerous Goods Regulations*
- ERG Containment and Quarantine Manual

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6.5 List of appendices. List any appendices included with this application. Any information that is commercially sensitive, or additional material included with the application (such as details of consultations, referenced articles) should be contained in appendices. The main application should refer to the relevant appendices but be able to be read as a stand-alone document.

See over page

Appendix 1. Current list of extremophilic prokaryotic microbial genera and species. Also included are the optimal growth temperatures, culture collection references, journal reference and risk classifications for the *type strain* for each genera.

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
Acidianus	*infernus* (Type species)	*Archaea*	1	Sulfolobales	88˚C	DSM 3191	Segerer *et al*., 1986
	ambivalens		1				
	brierleyi		1				
Acidilobus	*aceticus* (Type species)	*Archaea*	1	Desulfurococcales	80˚C	DSM 11585	Prokofeva *et al*., 2000
Acidimicrobium	*Ferrooxidans* (type strain)	*Bacteria*	1	Acidophiles	45°C	DSM 10331	Clark and Norris, 1996
Acidobacterium	*capsulatum* (Type species)	*Bacteria*	1	Acidobacteria	30˚C	DSM 11244	Kishimoto *et al*., 1991
Acidithiobacillus	*ferrooxidans*	*Bacteria*	1	Acidophiles	30˚C	DSM 14882	Kelly and Wood, 2000
	caldus		1		45°C		
	thiooxidans		1		26°C		
Aeropyrum	*pernix* (Type species)	*Archaea*	1	Desulfurococcales	90˚C	DSM 11879	Sako *et al*., 1996
	camini		1				
Anoxybacillus	*pushchinoensis*	*Bacteria*	1	Bacillales	37-66°C	DSM 12423^{T}	Pikuta *et al.*, 2003
	gonensis		1				
	flavothermus		1				
	ayderensis		1				
	kestanbolensis		1				
Aquifex	*pyrophilus* (Type species)	*Bacteria*	1	Aquificales	85˚C	DSM 6858	Huber *et al*., 1992
Archaeoglobus	*fulgidus* (Type species)	*Archaea*	1	Archaeoglobi	85˚C	DSM 4304	Stetter, 1988
	profundus		1				
	veneficus		1				
Caldisphaera	*lagunensis*(Type species)	*Archaea*	1	Caldisphaerales	75˚C	DSM 15908	Itoh *et al.*, 2003
Caldivirga	*maquilingensis* (Type species)	*Arhaea*	1	Thermoproteales	83˚C	DSM 13496	Itoh *et al.*, 1999
Cenarchaeum	*symbiosum*	*Archaea*	-	Cenarchaeales	10˚C	N/A	Preston *et al.*, 1996
Deinococcus	*radiodurans* (Type species)	*Bacteria*	1	Deinococcus-Thermus	30˚C	DSM 20539	Brooks and Murray, 1981
	erythromyxa		1				
	geothermalis		1				
	grandis		1				
	indicus		1				
	murrayi		1				
	proteolyticus		1				

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
	radiophilus		1				
	radiopugnans		1				
Desulfurobacterium	*thermolithotrophum* (Type species)	*Bacteria*	1	Aquificales	70˚C	DSM 11699	L'Haridon *et al*., 1998
Desulfurococcus	*mucosus* (Type species)	*Archaea*	1	Desulfurococcales	85˚C	DSM 2162	Zillig *et al.*, 1982
	amylolyticus		1				
	mobilis		1				
Ferrimicrobium	*acidophilus*	*Bacteria*	1			N/A	Johnson and Roberto, 1997
Ferroglobus	*placidus* (Type species)	*Archaea*	1	Archaeoglobi	85˚C	DSM 10642	Hafenbradl *et al*., 1996
Ferroplasma	*acidiphilum* (Type species)	*Archaea*	1	Thermoplasmata	35˚C	DSM 12658	Golyshina *et al*., 2000
Fervidobacterium	*nodosum* (Type species)	*Bacteria*	1	Thermotogales	70˚C	DSM 5306	Patel *et al*., 1985
	gondwanense		1				
	islandicum		1				
	pennivorans		1				
Geobacillus	*stearothermophilus* (Type species)	*Bacteria*	1	Bacillales	65°C	DSM 22^T	Nazina *et al*., 2001
	uzenensis		1				
	subterraneus		1				
	thermoleovorans		1				
	thermocatenulatus		1				
	kaustophilus		1				
	thermoglucosidasius		1				
	thermodenitrificans		1				
Geothermobacterium	*ferrireducens* (Type species)	*Bacteria*	1	Geothermobacterium	85˚C	JCM 12379	Kashefi *et al*., 2002
Geothrix	*fermentans* (Type species)	*Bacteria*	1	Acidobacteria	35˚C	DSM 14018	Coates *et al*., 1999
Geotoga	*patraea* (Type species)	*Bacteria*	1	Thermotogales	55°C	ATCC 51226	Davey *et al*., 1993
	subterranea		1				
Haloarcula	*vallismortis* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 3756	Torreblanca *et al*., 1986
	argentinensis		1				
	hispanica		1				
	japonica		1				
	marismortui		1				
	quadrata		1				
Halobacterium	*salinarum* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 3754	Buchanan and Gibbons, 1974
	cutirubrum		1				
	halobium		1				
Halobaculum	*gomorrense*	*Archaea*	1	Halobacteria	35˚C	DSM 9297	Oren *et al.*, 1995

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
Halococcus	*morrhuae* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 1307	Buchanan and Gibbons, 1974
	dombrowskii		1				
	saccharolyticus		1				
	salifodinae		1				
Haloferax	*volcanii* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 3757	Torreblanca *et al.*, 1986
	alexandrinus		1				
	denitrificans		1				
	gibbonsii		1				
	lucentense		1				
	mediterranei		1				
	sulfurifontis		1				
Halogeometricum	*borinquense* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 11551	Montalvo-Rodriguez *et al.,* (1998)
Halomicrobium	*mukohataei* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 12286	Oren *et al.*, 2002
Haloquadratum	*walsbyi*	*Archaea*	1	Halobacteria	37˚C	N/A	Bolhuis *et al*., 2004
Halorhabdus	*utahensis* (Type species)	*Archaea*	1	Halobacteria	30˚C	DSM 12940	Waino *et al*., 2000
Halorubrum	*saccharovorum* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 1137	McGenity and Grant, 1995
	alkaliphilum		1				
	coriense		1				
	distributum		1				
	lacusprofundi		1				
	sodomense		1				
	tebenquichense		1				
	terrestre		1				
	tibetense		1				
	trapanicum		1				
	vacuolatum		1				
	xinjiangense		1				
Halosimplex	*carlsbadense* (Type species)	*Archaea*	1	Halobacteria	40˚C	ATCC BAA-75	Vreeland *et al*., 2002
Haloterrigena	*turkmenica* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 5511	Ventosa *et al.*, 1999
	thermotolerans		1				
Holophaga	*foetida* (Type species)	*Bacteria*	1	Acidobacteria	30˚C	DSM 6591	Liesack *et al*., 1994
Hydrogenivirga	*caldilitoris* (Type species)	*Bacteria*	1	Aquificales	75˚C	DSM 16510	Nakagawa *et al*., 2004
Hydrogenobacter	*thermophilus* (Type species)	*Bacteria*	1	Aquificales	70˚C	DSM 6534	Kawasumi *et al*., 1984
	hydrogenophilus (Basonym: *Calderobacterium hydrogenophilum*)		1				

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
	subterraneus		1				
Hydrogenobaculum	*acidophilum* (Type species)	*Bacteria*	1	Aquificales	65˚C	DSM 11251	Stohr *et al*., 2001
Hydrogenothermus	*marinus* (Type species)	*Bacteria*	1	Aquificales	65˚C	DSM 12046	Stohr *et al*., 2001
Hyperthermus	*butylicus* (Type species)	*Archaea*	1	Desulfurococcales	99˚C	DSM 5456	Zillig *et al*., 1991
Ignicoccus	*islandicus* (Type species)	*Archaea*	1	Desulfurococcales	90˚C	DSM 13165	Huber *et al*., 2000
	pacificus		1				
Leptospirillum	*ferrooxidans* (Type species)	*Bacteria*	1	Nitifiers	30°C	DSM 2705	Hippe, 2000
	ferriphilum		1				
Marinitoga	*camini* (Type species)	*Bacteria*	1	Thermotogales	55˚C	DSM 13578	Wery *et al*., 2001
	piezophila		1				
Metallosphaera	*sedula* (Type species)	*Archaea*	1	Sulfolobales	65˚C	DSM 5348	Huber *et al.*, 1989
	hakonensis		1				
	prunae		1				
Methanomicrobium	*mobile* (Type species)	*Archaea*	1	Methanogens	37˚C	DSM 1539	Balch *et al.*, 1979
Methanobacterium	*formicicum* (Type species)	*Archaea*	1	Methanogens	37˚C	DSM 1535	Kluyver and van Niel, 1936
	aarhusense		1				
	alcaliphilum		1				
	beijingense		1				
	bryantii		1				
	congolense		1				
	defluvii		1				
	espanolae		1				
	ivanovii		1				
	oryzae		1				
	palustre		1				
	subterraneum		1				
	thermoaggregans		1				
	thermoalcaliphilum		1				
	thermoformicicum		1				
	uliginosum		1				
Methanobrevibacter	*ruminantium* (Type species)	*Archaea*	1	Methanogens	37˚C	DSM 1093	Balch *et al*., 1979
	acididurans		1				
	arboriphilicus		1				
	curvatus		1				
	cuticularis		1				

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
	filiformis		1				
	gottschalkii		1				
	oralis		1				
	smithii		1				
	thaueri		1				
	woesei		1				
	wolinii		1				
Methanocalculus	*halotolerans* (Type species)	*Archaea*	1	Methanogens	37˚C	DSM 14092	Ollivier *et al*., (1998)
	chunghsingensis		1				
	pumilus		1				
	taiwanensis		1				
Methanocalculus	*jannaschii* (Type species)	*Archaea*	1	Methanogens	80-85°C	DSM 2661	Jones *et al.,* 1983
	fervens		1				
	infernus		1				
	vulcanius		1				
	indicus		1				
Methanococcoides	*methylutens* (Type species)	*Archaea*	1	Methanogens	30˚C	DSM 2657	Sowers and Ferry, 1983
	burtonii		1				
Methanococcus	*vannielii* (Type species)	*Archaea*	1	Methanogens	30-40˚C	DSM 1224	Mah and Kuhn, 1984
	deltae		1				
	maripaludis		1				
	voltae		1				
Methanocorpusculum	*parvum* (Type species)	*Archaea*	1	Methanogens	37˚C	DSM 3823	Xun *et al*., 1989
	aggregans		1				
	bavaricum		1				
	labreanum		1				
	sinense		1				
Methanoculleus	*bourgensis* (Type species)	*Archaea*	1	Methanogens	37˚C	DSM 3045	Maestrojuan *et al*., 1990
	chikugoensis		1				
	marisnigri		1				
	oldenburgensis		1				
	olentangyi		1				
	palmolei		1				
	submarinus		1				
	thermophilus		1				

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
Methanofollis	*tationis* (Type species)	*Archaea*	1	Methanogens	37˚C	DSM 2702	Zellner *et al.*, 1999
	aquaemaris		1				
	liminatans		1				
Methanogenium	*cariaci* (Type species)	*Archaea*	1	Methanogens	20-25˚C	DSM 1497	Maestrojuan *et al*., 1990
	frigidum		1				
	frittonii		1				
	marinum		1				
	organophilum		1				
Methanohalobium	*evestigatum* (Type species)	*Archaea*	1	Methanogens	50˚C	DSM 3721	Zhilina and Zavarzin, 1987
Methanohalophilus	*mahii* (Type species)	*Archaea*	1	Methanogens	37˚C	DSM 5219	Paterek and Smith, 1988
	halophilus		1				
	oregonensis		1				
	portucalensis		1				
	zhilinae		1				
Methanolacinia	*paynteri* (Type species)	*Archaea*	1	Methanogens	37˚C	DSM 2545	Zellner *et al.*, 1989
Methanolobus	*tindarius* (Type species)	*Archaea*	1	Methanogens	25˚C	DSM 2278	Konig and stetter, 1982
	bombayensis		1				
	oregonensis		1				
	siciliae		1				
	taylorii		1				
	vulcani		1				
Methanomethylovorans	*hollandica* (Type species)	*Archaea*	1	Methanogens	37˚C	DSM 15978	Lomans *et al.*, 1999
Methanomicrococcus	*blatticola* (Type species)	*Archaea*	1	Methanogens	35˚C	DSM 13328	Sprenger *et al.*, 2000
Methanoplanus	*limicola* (Type species)	*Archaea*	1	Methanogens	30-35˚C	DSM 2279	Wildgruber *et al*., 1982
	endosymbiosus		1				
	petrolearius		1				
Methanopyrus	*kandleri* (Type species)	*Archaea*	1	Methanogens	98°C	DSM 6324	Kurr *et al.,* 1991
Methanosaeta	*Concilii* (Type species	*Archaea*	1	Methanogens	35˚C	DSM 3671	Patel and Sprott, 1990
	thermoacetophila		1				
Methanosalsum	*zhilinae* (Type species	*Archaea*	1	Methanogens	45˚C	DSM 4017	Boone and Baker, 2001
Methanosarcina	*barkeri* (Type species)	*Archaea*	1	Methanogens	30-37˚C	DSM 800	Mah and Kuhn, 1984
	acetivorans		1				
	baltica		1				
	frisia		1				
	lacustris		1				

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
	mazei		1				
	methanica		1				
	semesiae		1				
	siciliae		1				
	thermophila		1				
	vacuolata		1				
Methanosphaera	*stadtmanae* (Type species)	*Archaea*	1	Methanogens	37˚C	DSM 3091	Miller and Wolin, 1985
	cuniculi		1				
Methanospirillum	*hungatii* (Type species; synonym: *hungatei*)	*Archaea*	1	Methanogens	37˚C	DSM 864	Ferry *et al*., 1974
Methanothermobacter	*thermautotrophicus* (Type species)	*Archaea*	1	Methanogens	65˚C	DSM 1053	Wasserfallen *et al*., 2000
	defluvii		1				
	marburgensis		1				
	thermoflexus		1				
	thermophilus		1				
	wolfei		1				
Methanothermococcus	*thermolithotrophicus* (Type species)	*Archaea*	1	Methanogens	65°C	DSM 2095	Whitman, 2001
	okinawensis		1				
Methanothermus	*fervidus* (Type species)	*Archaea*	1	Methanogens	83˚C	DSM 2088	Stetter *et al.*, 1981
	sociabilis		1				
Methanothrix	*Soehngenii* (Type species)	*Archaea*	1	Methanogens	35˚C	DSM 2139	Huser *et al.*, 1982
	thermoacetophila (synonym: *Methanosaeta thermoacetophila*)		1				
	thermophila		1				
Methanotorris	*Igneus* (Type species)	*Archaea*	1	Methanogens	85˚C	DSM 5666	Whitman, 2001
	formicicus		1				
Methylobacter	*luteus* (Type species)	*Bacteria*	1	Methanotrophs	30˚C	ATCC 49878	Bowman *et al*., 1995
	marinus		1				
	psychrophilus		1				
	whittenburyi		1				
Methylocaldum	*szegediense* (Type species)	*Bacteria*	1	Methanotrophs	37-62˚C	NCIMB 13486	Bodrossy *et al.*, 1997
	gracile		1			NCIMB 11912	
	tepidum		1				
Methylocapsa	*acidiphila* (Type species)	*Bacteria*	1	Methanotrophs	20-24˚C	DSM 13967	Dedysh *et al*., 2002
Methylocella	*palustris*(Type species)	*Bacteria*	1	Methanotrophs	20˚C	ATCC 700799	Dedysh *et al*., 2000

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
	silvestris		1				
	tundrae		1		20˚C		
Methylococcus	*capsulatus* (Type species)	*Bacteria*	1	Methanotrophs	37-45˚C	ATCC 33009	Bowman *et al.*, 1993
	bovis		1				
	chroococcus		1				
	mobilis		1				
	thermophilus		1				
	vinelandii (synonym: *Methylobacter whittenburyi*)		1				
Methylocystis	*parvus* (Type species)	*Bacteria*	1	Methanotrophs	25°C	ATCC 35066	Bowman *et al.*, 1993
	echinoids						
Methylohalobius	*crimeensis*	*Bacteria*	1	Methanotrophs	30˚C	ATCC BAA967	Heyer *et al.*, 2005
Methylomicrobium	*agile* (Type species)	*Bacteria*	1	Methanotrophs	30˚C	ATCC 35068	Bowman *et al.*, 1995
	album		1				
	buryatense						
	pelagicum		1				
Methylosarcina	*fibrata* (Type species)	*Bacteria*	1	Methanotrophs	30˚C	DSM 13736	Wise *et al.*, 2001
	quisquiliarum		1				
	lucus						
Methylosinus	*trichosporium* (Type species)	*Bacteria*	1	Methanotrophs	25°C	ATCC 49243	Bowman *et al.*, 1993
Methylosinus	*sporium*	*Bacteria*	1	Methanotrophs	30˚C	ATCC 35069	
Methylospharea	*hansonii* (Type species)	*Bacteria*	1	Methanotrophs	10-13˚C	ACAM 549	Bowman *et al*., 1998
Methylothermus	*thermales*	*Bacteria*	1	Methanotrophs	55°C	VKM B-2345	Tsubota *et al., 2005*
Nanoarchaeum	*equitans*	*Archaea*	1	Nanoarchaeota	70-98˚C	N/A	Huber *et al*., 2002
Natrialba	*asiatica* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 12278	Kamekura and Dyall-Smith, 1995
	aegyptia		1				
	chahannaoensis		1				
	hulunbeirensis		1				
	magadii		1				
	taiwanensis		1				
Natrinema	*pellirubrum* (Type species)	*Archaea*	1	Halobacteria	37˚C	JCM 10476	Xin *et al*., 2000
	pallidum		1				
	versiforme		1				
Natronobacterium	*gregoryi* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 3393	Tindall *et al*., 1984
Natronococcus	*occultus* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 3396	Tindall *et al*., 1984

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
	amylolyticus		1				
Natronolimnobius	*baerhuensis*	*Archaea*	1	Halobacteria	37-45˚C	JCM 12253	Itoh *et al*., 2004
	innermongolicus					JCM 12255	
Natronomonas	*pharaonis* (Type species)	*Archaea*	1	Halobacteria	37˚C	DSM 2160	Kamekura *et al.*, 1997
Natronorubrum	*bangense* (Type species)	*Archaea*	1	Halobacteria	37˚C	JCM 10635	Xu *et al.*, 1999
	sp.		1				
	tibetense		1				
Nitrobacter	*winogradskyi* (Type species)	*Bacteria*	1	Nitifiers	28˚C	DSM 10237	Buchanan and Gibbons, 1974
	alkalicus		1				
	hamburgensis		1				
	vulgaris		1				
Nitrosococcus	*nitrosus* (Type species)	*Bacteria*	1	Nitifiers	N/A	N/A	Buchanan and Gibbons, 1974
	oceani	*Bacteria*	1		26˚C	ATCC 19707	Watson, 1971
Nitrosomonas	*europaea* (Type species)	*Bacteria*	1	Nitifiers	26˚C	ATCC 19718	Buchanan and Gibbons, 1974
	cryotolerans		1				
	aestuarii		1				
	communis		1				
	eutropha		1				
	halophila		1				
	marina		1				
	nitrosa		1				
	oligotropha		1				
	ureae		1				
Nitrosospira	*multiformis* (Type species)	*Bacteria*	1	Nitifiers	26-30˚C	ATCC 25196	Watson and Mandel, 1971
	tenuis		1				
Palaeococcus	*ferrophilus* (Type species)	*Archaea*	1	Thermococci	80-83˚C	DSM 13482	Takai *et al.*, 2000
Persephonella	*marina* (Type species)	*Bacteria*	1	Aquificales	70˚C	DSM 14350	Gotz *et al.*, 2002
	guaymasensis		1				
	hydrogeniphila		1				
Petrotoga	*miotherma* (Type species)	*Bacteria*	1	Thermotogales	55˚C	DSM 10691	Davey *et al.*, 1993
	mexicana		1				
	mobilis		1				
	olearia		1				
	sibirica		1				
Picrophilus	*oshimae* (Type species)	*Archaea*	1	Thermoplasmata	55-60˚C	DSM 9789	Schleper *et al*., 1996

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
	torridus		1				
Pyrobaculum	*islandicum* (Type species)	*Archaea*	1	Thermoproteales	95-100˚C	DSM 4184	Huber *et al.*, 1987
	aerophilum		1				
	arsenaticum		1				
	oguniense		1				
	organotrophum		1				
Pyrococcus	*furiosus* (Type species)	*Archaea*	1	Thermococci	97-100˚C	DSM 3638	Fiala and Stetter, 1986
	glycovorans		1				
	horikoshii		1				
	woesei		1				
Pyrodictium	*occultum* (Type species)	*Archaea*	1	Desulfurococcales	85-105˚C	DSM 2709	Stetter *et al*., 1983
	abyssi		1				
	brockii		1				
Pyrolobus	*fumarii* (Type species)	*Archaea*	1	Desulfurococcales	103˚C	DSM 11204	Blochl *et al*., 1997
Staphylothermus	*marinus* (Type species)	*Archaea*	1	Desulfurococcales	85-90˚C	DSM 3639	Fiala *et al.*, 1986
	hellenicus		1				
Stetteria	*hydrogenophila* (Type species)	*Archaea*	1	Desulfurococcales	93˚C	DSM 11227	Jochimsen *et al*., 1997
Stygiolobus	*azoricus* (Type species)	*Archaea*	1	Sulfolobales	80˚C	DSM 6296	Segerer *et al*., 1991
Sulfobacillus	*thermosulfidooxidans* (Type species)	*Bacteria*	1	Bacillales	50°C	DSM 9293	Golovacheva and Karavaiko, 1991
	acidophilus						
	disulfidooxidans						
Sulfolobus	*acidocaldarius* (Type species)	*Archaea*	1	Sulfolobales	70˚C	DSM 639	Brock *et al.*, 1972
	metallicus		1				
	shibatae		1				
	solfataricus		1				
	tokodaii		1				
	yangmingensis		1				
Sulfophobococcus	*zilligii* (Type species)	*Archaea*	1	Desulfurococcales	90˚C	DSM 11193	Hensel *et al*., 1997
Sulfurihydrogenibium	*subterraneum* (Type species)	*Bacteria*	1	Aquificales	62˚C	DSM 15120	Takai *et al.*, 2003
	azorense		1				
	yellowstonensis		1				
Sulfurisphaera	*ohwakuensis* (Type species)	*Archaea*	1	Sulfolobales	75˚C	DSM 12421	Kurosawa *et al.*, 1998
Sulfurococcus	*mirabilis* (Type species)	*Archaea*	1	Sulfolobales	70-75˚C	INMI AT-59	Prokofeva *et al.,* 2000
	yellowstonensis		1				
Thermocladium	*modestius* (Type species)	*Archaea*	1	Thermoproteales	75˚C	JCM 10088-	Itoh *et al*., 1998

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
Thermococcus	*celer* (Type species)	*Archaea*	1	Thermococci	88˚C	10090 DSM 2476	Zillig *et al*., 1983
	acidaminovorans		1				
	aegaeus		1				
	aggregans		1				
	alcaliphilus		1				
	atlanticus		1				
	barophilus		1				
	chitonophagus		1				
	fumicolans		1				
	gammatolerans		1				
	gorgonarius		1				
	guaymasensis		1				
	hydrothermalis		1				
	litoralis		1				
	pacificus		1				
	peptonophilus		1				
	profundus		1				
	sibiricus		1				
	siculi		1				
	stetteri		1				
	waiotapuensis		1				
	zilligii		1				
Thermocrinis	*ruber* (Type species)	*Bacteria*	1	Aquificales	80˚C	DSM 12173	Huber *et al*., 1998
	albus		1				
Thermodesulfatator	*indicus* (Type species)	*Bacteria*	1	Geothermobacterium	70˚C	DSM 15286	Moussard *et al*., 2004
Thermodesulfobacterium	*commune* (Type species)	*Bacteria*	1	Geothermobacterium	70˚C	DSM 2178	Jeanthon *et al.*, 2002
	hveragerdense		1				
	hydrogeniphilum		1				
	mobile		1				
Thermodiscus	*maritimus* (Type species)	*Archaea*	1	Desulfurococcales	90˚C	JCM 11597	Stetter, 2001
Thermofilum	*pendens* (type species)	*Archaea*	1	Thermoproteales	88˚C	DSM 2475	Zillig *et al*., 1983
	librum		1				
Thermopallium	*natronophilum*	*Bacteria*	1	Thermotogales	70˚C	N/A	Wiegel, 1998
Thermoplasma	*acidophilum* (Type species)	*Archaea*	1	Thermoplasmata	55-60˚C	DSM 1728	Darland *et al*., 1970

Microbial genus and species name		Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
	volcanium		1				
Thermoproteus	*tenax* (Type species)	*Archaea*	1	Thermoproteales	85˚C	DSM 2078	Zillig *et al.*, 1981
	neutrophilus		1				
	uzoniensis		1				
Thermosipho	*africanus* (Type species)	*Bacteria*	1	Thermotogales	75˚C	DSM 5309	Huber *et al.*, 1989
	atlanticus		1				
	geolei		1				
	japonicus		1				
	melanesiensis		1				
Thermosphaera	*aggregans* (Type species)	*Archaea*	1	Desulfurococcales	85˚C	DSM 11486	Huber *et al.*, 1998
Thermotoga	*maritima* (Type species)	*Bacteria*	1	Thermotogales	80˚C	DSM 3109	Huber *et al.*, 1986
	elfii		1				
	hypogea		1				
	lettingae		1				
	naphthophila		1				
	neapolitana		1				
	petrophila		1				
	subterranea		1				
	thermarum		1				
Thermus	*aquaticus* (Type species)	*Bacteria*	1	Deinococcus-Thermus	70˚C	DSM 625	Brock and Freeze, 1969
	antanikianii		1				
	brockianus		1				
	filiformis		1				
	igniterrae		1				
	oshimai		1				
	profundus (synonym: *Thermococcus profundus*)		1				
	scotoductus		1				
	thermophilus		1				
Thiomonas	*intermedia (Type species)*	*Bacteria*	1	Acidophiles		DSM 5495	Moreira and Amils, 1997
	perometabolis						
	cuprina						
	perometabolis						
	thermosulfata						
Vulcanisaeta	*distribute (Type species)*	*Archaea*	1	Thermoproteales	90˚C	DSM 14429	Itoh *et al.*, 2002

Microbial genus and species name	Domain	Risk classification*	Description	Optimal growth temp.	Culture collection reference	reference
souniana		1				

* *Risk classification according to AS/NZ Standard 2243:3:2002.*

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GNS researchers publication record

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Section Seven – Application Summary

Summarise the application in clear, simple language that can be understood by the general public. Include a description of the organism(s) to be imported into containment, and any risks and benefits associated with their importation. This summary will be used to provide information for those people and agencies who will be notified of the application (e.g Ministry of Agriculture and Forestry, Department of Conservation, Crown Research Institutes) and for members of the public who request information. Do not include any commercially sensitive information in this summary.

This application is for the importation of groups of non-pathogenic, non-transmissable extremophilic microbial genera isolated from geothermal and hydrothermal sites from International microbial libraries for research purposes including ecology, biodiversity and biotechnology studies.

In order to undertake such research, isolated strains must be compared with other like strains to accurately describe their physiological and molecular characteristics. We wish to import individual extremophilic strains of microorganisms into containment from recognised microbial databases (such as the German Collection of Microorganisms and Cell Culture (DSMZ), the American Type Culture Collection (ATCC), National Collection of Industrial and Marine Bacteria (NCIMB) or the Japanese Collection of Microorganisms (JCM) to use as reference strains for molecular and physiological comparison with isolates enriched from New Zealand extreme environments. However, as the internationally accepted list of microbial

species is constantly changing due to reclassifications and continual species discoveries, we request that the risk assessment and permits for importation be made on the basis of a generic extremophile application. We suggest that the microbial physiologies classified as extremophiles include thermophiles, acidophiles, alkaliphiles, barophiles, halophiles, methanotrophs, methanogens and nitrifiers.

According to this definition, the microbial genera of *current* extremophiles include *Acidianus, Acidilobus, Acidimicrobium, Acidobacterium, Acidithiobacillus, Aeropyrum, Anoxybacillus, Aquifex, Archaeoglobus, Caldisphaera, Caldivirga, Cenarchaeum, Deinococcus, Desulfurobacterium, Desulfurococcus, Ferrimicrobium, Ferroglobus, Ferroplasma, Fervidobacterium, Geobacillus, Geothermobacterium, Geothrix, Geotoga, Haloarcula, Halobacterium, Halobaculum, Halococcus, Haloferax, Halogeometricum, Halomicrobium, Haloquadratum, Halorhabdus, Halorubrum, Halosimplex, Haloterrigena, Holophaga, Hydrogenivirga, Hydrogenobacter, Hydrogenobaculum, Hydrogenothermus, Hyperthermus, Ignicoccus, Leptospirillum, Marinitoga, Metallosphaera, Methanomicrobium, Methanobacterium, Methanobrevibacter, Methanocalculus, Methanocalculus, Methanococcoides, Methanococcus, Methanocorpusculum, Methanoculleus, Methanofollis, Methanogenium, Methanohalobium, Methanohalophilus, Methanolacinia, Methanolobus, Methanomethylovorans, Methanomicrococcus, Methanoplanus, Methanopyrus, Methanosaeta, Methanosalsum, Methanosarcina, Methanosphaera, Methanospirillum, Methanothermobacter, Methanothermococcus, Methanothermus, Methanothrix, Methanotorris, Methylobacter, Methylocaldum, Methylocapsa, Methylocella, Methylococcus, Methylocystis, Methylohalobius, Methylomicrobium, Methylosarcina, Methylosinus, Methylosinus, Methylospharea, Methylothermus, Nanoarchaeum, Natrialba, Natrinema, Natronobacterium, Natronococcus, Natronolimnobius, Natronomonas, Natronorubrum, Nitrobacter, Nitrosococcus, Nitrosomonas, Nitrosospira, Palaeococcus, Persephonella, Petrotoga, Picrophilus, Pyrobaculum, Pyrococcus, Pyrodictium, Pyrolobus, Staphylothermus, Stetteria, Stygiolobus, Sulfobacillus, Sulfolobus, Sulfophobococcus, Sulfurihydrogenibium, Sulfurisphaera, Sulfurococcus, Thermocladium, Thermococcus, Thermocrinis, Thermodesulfatator, Thermodesulfobacterium, Thermodiscus, Thermofilum, Thermopallium, Thermoplasma, Thermoproteus, Thermosipho, Thermosphaera, Thermotoga, Thermus, Thiomonas* and *Vulcanisaeta*.

The risks associated with importing into containment and cultivating these microorganisms are minimal to non-existent. The characteristics of each imported microbial genus is clearly known prior to importation, all microbes are Risk Group 1-type microorganisms and all will be held in a MAF approved containment facility operated by competent and microbiologically-trained staff. The importation into containment of extremophilic microorganisms will immediately benefit NZ through the characterisation of novel NZ extremophilic isolates, it will assist in establishing a sound scientific knowledge base of the microbial ecology in NZ's geothermal and hotspring environments and will increase staff skills and scientific knowledge base. In doing so we, may gain some insights to the origins of life, perhaps life on other planets and the similarities between extreme microbial survival and pathogen evasion techniques. Furthermore, the discovery of new microorganisms may open up possibilities of developing potentially new beneficial technologies such as bioremediation of pollutants, generation of environmentally sustainable energies, medicine and drug discovery and efficient industrial processes. The benefits for New Zealand and science if any of these technologies were realised could potentially be huge.

Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

Checklist

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 additional references attached (*only selected references*)	yes
Cheque for initial fee (incl. GST) enclosed	Yes
If "yes", state amount:	$1125
Direct credit made to ERMA bank account:	No
If "yes" give date of direct credit …/…/… and amount deposited:	$…….
Application signed and dated	Yes
Electronic copy of application e-mailed to ERMA New Zealand	Yes

*NA – not applicable

Signed: **Date:**