

FACT SHEET

APPLICATION FOR APPROVAL TO RELEASE SINGLE CELL PROTEIN (SCP), LIQUID FERTILIZER AND SOLID FERTILIZER, FROM THE FERMENTATION PRODUCTION OF L-METHIONINE USING *E.coli* KCCM11252P AND *E.coli* KCCM11340P FROM *E.coli* K12, FOR SUPPLY OR OFFER TO SUPPLY FOR SALE/PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/15

The objective of the Biosafety Act is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act, the National Biosafety Board (NBB) is currently assessing an application for approval submitted by **CJ Bio Malaysia Sdn. Bhd.** to release Single Cell Protein (SCP), Liquid Fertilizer and Solid Fertilizers, from the fermentation production of L-Methionine using *E.coli* KCCM11252P and *E.coli* KCCM11340P from *E.coli* K12.

1. What is this application for?

CJ Bio Malaysia Sdn. Bhd. has established the fermentative production of L-Methionine by using several microorganisms. Origins of these microorganisms are *E.coli* K12 W3110. Some of them have been made by random mutation which is non-living modified organisms (LMO) and two of them (*E.coli* KCCM 11252P, *E.coli* KCCM 11340P) have been made by genetic modification.

This application is for the release of fertilizers co-produced with L-Methionine by using these two genetically modified microorganisms from *E.coli* K12 W3110 by CJ Bio Malaysia Sdn. Bhd. These are Single Cell Protein (SCP), Liquid Fertilizer and Solid Fertilizer.

2. What is the purpose of release?

These microorganisms are used for the fermentative production of L-methionine, a feed additive. After the fermentation, the microorganisms are separated and dried. The resultant SCP has a large amount of nitrogen and can be used as a good organic nitrogen source in the fertilizer market. The liquid is treated further to obtain L-Methionine and Ammonium Sulfate. The final liquid also has a respectable amount of nitrogen, and can be sold as fertilizer. We call this Liquid Fertilizer, but in Malaysia, the usage is very limited, so it is dried and sold as Solid Fertilizer.

3. How has the LMO been modified?

The two microorganisms (*E.coli* KCCM 11252P, *E.coli* KCCM 11340P) have been made by genetic modification from *E.coli* K12 W3110 strains in CJ Research Institute of Biotechnology located in Seoul, South Korea. *E.coli* K12 is one of the most well recognised and safe microorganism which has been approved for the production of pharmaceuticals and feed additives in the world. Each of the two microorganisms has one foreign gene from microorganisms which have already been approved as safe. There is no antibiotic resistant marker gene, and no transferable mobile element in the final microorganism and the final microorganisms express no genetic instabilities. These microorganisms are separated, dried and killed to become SCP. Hence there are no remaining viable cells in the SCP. There are also no viable cells in the Liquid Fertilizer and Solid Fertilizer because the microorganisms are separated from the liquid.

4. Characteristics of the LMO

(a) Details of the parent organism

The parent organism is the wild type of *Escherichia coli* K12 W3110. There is no known precedent for the crossing events of this strain with other species. This strain cannot survive in the environment or any animal body. Hence, *E.coli* K12 has a long history of safe use. Its derivatives are currently used in a large number of industrial applications, including the production of specialty chemicals (e.g., L-aspartic, inosinic, and adenylic acids), amino acids (L-threonine, L-tryptophane, L-Valine, and L-isoleucine) and human drugs such as insulin and somatostatin. Furthermore, *E.coli* K12 is also used for the production of some chemicals such as enzymes. In general, *E.coli* K12 is one of the most extensively studied bacteria, and has been used in genetic studies in laboratories worldwide.

(b) Details of the donor organism

KCCM11252P from *E.coli* K12 W3110 is used for the production of O-acetylhomoserine, the precursor of L-Methionine. In this strain, one gene from *E.coli* H155 has been introduced, which is the sucrose utilizing gene. This gene has already been approved for safety in other amino acid production such as valine and isoleucine in Europe.

KCCM 11340P from *E.coli* K12 W3110 is used for the production of conversion enzyme which is used for the conversion of O-acetylhomoserine to L-Methionine. The conversion enzyme named O-acetylhomoserine sulfhydrylase is coded by a gene from *Corynebacterium glutamicum*. *Corynebacterium glutamicum* is a GRAS stain widely used for MSG production and other amino acid production.

(c) Description of the trait(s) and characteristic which have been introduced or modified

LMO	Genus, Species and Common name of parent organism (recipient)	Genus, Species and Common name of donor organism	Modified trait
<i>E.Coli KCCM 11252P</i>	- Genus: <i>Escherichia</i> - Species: <i>E.Coli</i> - Common name: <i>Escherichia Coli K12</i>	- Genus: <i>Escherichia</i> - Species: <i>E.Coli</i> - Common name: <i>Escherichia Coli K12 & Escherichia Coli H155</i>	i) sucrose utilisation ii) production of O-Acetylhomoserine
<i>E.Coli KCCM 11340P</i>	- Genus: <i>Escherichia</i> - Species: <i>E.Coli</i> - Common name: <i>Escherichia Coli K12</i>	- Genus: <i>Corynebacterium</i> - Species: <i>C.glutamicum</i> - Common name: <i>Corynebacterium glutamicum</i>	i) production of O-Acetylhomoserine Sulfhydrylase enzyme

5. Modification method

(a) Characterization of the modification

We use well-published genetic modification methods in Deleting and Inserting the genes. After these modifications, the LMOs have no transferring or replicating genetic materials, and also no antibiotic resistant genes.

(b) Safety of the expressed protein

Newly expressed protein from the production strain is sucrose utilizing protein and O-acetylhomoserine sulfhydrylase protein. These two proteins are widely present in the wild-type microorganisms and already regarded and confirmed as safe by acute toxicity analysis.

6. Assessment of risks to human health

(a) Nutritional data

(b) Toxicology

(c) Allergenicity

E.coli K12 has a long history of safe use. Its derivatives are currently used in a large number of industrial applications and human drugs. After the genetic modification, the two microorganisms have no transferring or replicating genetic materials, and no antibiotic marker genes. As a result, these two microorganisms show long-term genetic stability. Therefore, there is no risk of genetic transfer to other organisms.

7. Assessment of risks to the environment

There are no viable cells in the SCP, Liquid Fertilizer and Solid Fertilizer. Also, it was confirmed that there is no phenotype change between the parent organism, *E.coli* K12 and the two microorganisms except for Intended change. Hence, these two microorganisms showed the same characteristics with their parent organism. *E.coli* K12 does not produce spores and will not likely survive for long periods in soil, water, or air. *E.coli* K12 has lost the ability to colonize in animal gut. The ability of *E.coli* to survive under environmental conditions is thus very limited. *E.coli* K12 has no known survival mechanism in the environment, such as the ability to produce spores. *E.coli* K12 is a non-pathogenic and non-toxic group of *E.coli* strains, verified by numerous studies.

8. What is the emergency response plan?

(a) First aid measures

There are no reports of adverse consequences of use, but, we have established general first aid measures as follows:-

- (1) Inhalation – Immediately relocate to a fresh air environment. Rinse mouth with water. If not breathing, give artificial respiration. If breathing becomes difficult, give oxygen and seek medical attention.
- (2) Skin Contact – Wash with soap and copious amounts of water. If irritation persists, seek medical attention.
- (3) Eye Contact – Immediately flush eyes with copious amounts of water for at least 15 minutes. Ensure adequate flushing by separating eyelids with fingers. If contact lenses are being worn, remove lenses and continue rinsing. Seek medical attention.
- (4) Ingestion – Rinse mouth with water and seek medical attention.

(b) Accidental release measures

There are no viable cells in the SCP, Liquid Fertilizer and Solid Fertilizer. Also, it was confirmed that there is no phenotype change between the parent organism, *E.coli* K12 and the two microorganisms except for Intended change. Hence, these two microorganisms showed the same characteristics with their parent organism. *E.coli* K12 has no known survival mechanism in the environment. *E.coli* K12 is a non-pathogenic and non-toxic group of *E.coli* strains, verified by numerous studies. Also, there are no transferring or replicating genetic materials, and no antibiotic marker genes in the two microorganisms. Hence DNA from these two microorganisms cannot cause any ecological effects. Considering all these information, even though there is an unintentional

release to the environment, the possibility of any hazard to plant, and animal health, as well as the environment will be negligible.

(c) Handling and storage

- (1) Handling – Follow the practice in good housekeeping and personal hygiene.
- (2) Storage – Store in closed container in a dry area. Avoid direct sunlight and high temperature.

(d) Disposal considerations

There are no specific instructions or recommendations for waste disposal and treatment because there are no viable cells in SCP, Liquid Fertilizer and Solid Fertilizer. Dispose as you would dispose with a non-hazardous material in accordance with all applicable national, state and local regulations.

9. How can I comment on this application?

Any member of the public may submit their comments or queries on publicly notified information about the application. Before submission of comments or queries, the person should review the information provided. Your comments and queries on any possible impacts/risks to the health and safety of the people and the environment that may be posed by the proposed release are appreciated. The submission of the comments or queries should be prepared carefully as it will be given the same scrutiny as the application by the NBB. The submission of comments and clarifications of queries should contribute to the NBB's assessment. Even if the submission is not science-based, and focuses on cultural or other values, it should still be developed in the form of a well-founded argument.

Please note that the consultation period closes on 14 February 2014 and written submissions are required by that date. Submissions must be addressed to:

The Director General,
Department of Biosafety
Ministry of Natural Resources and Environment
Level 1, Podium 2, Wisma Sumber Asli
No. 25, Persiaran Perdana, Precinct 4
62574 Putrajaya, MALAYSIA
Email: biosafety@nre.gov.my
Fax: 03-88904935

Please indicate your full name, address and contact details in your submission.