025/2014

This is to certify that I, Marco Antônio Rochadel, Official Public Translator, designated and installed in Office according to The Official Gazette of June 23, 1982, page 5428, have received and translated, to the best of my knowledge and belief, a document with the following contents:

Ministério da Ciência e Tecnologia

GOVERNO FEDERAL BRASIL PAÍS RICO É PAÍS SEM POBREZA

Ministry of Science and Technology – MCT
National Biosafety Technical Commission – CTNBio
Office of the Executive Secretary

comissão técnica nacional CTNBio de biossegurança

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Technical Opinion no. 3775/2013

Proceedings:	01200.001052/2013-32
Applicant:	Solazyme Brasil Óleos Renováveis e Bioprodutos Ltda.
CQB:	328/11
Proton:	8398/2013
Address:	Rua Pierre Simon de Leplace, 751, quadra A, lote 8, galpão 7, Technopark Campinas SP; CEP 13063-320.
Matter:	Request for opinion on commercial release of Class 1 biological risk genetically modified microorganism.
Meeting:	166th Regular Meeting held on October 17, 2013.
Previous Extract:	Number 3535/2013, published in the Federal Official Gazette nº 63, of April 03, 2013.
Decision:	GRANTED.

Summary: CTNBio, following examination of the request for Technical Opinion related to biosafety of the biologic risk Class I genetically modified microorganism *Prototheca moriformis* for commercial release and production of triglycerides and bio products, marketing and any other activities related to this genetically modified microorganism and progenies derived therefrom, decided for the **GRANTING** of the request on the terms of this Technical Opinion.

Prototheca moriformis is a unicellular microorganism with no chlorophyll, necessarily heterotrophic, featuring asexual reproduction without producing spores, commonly known as microalga. CTNBio states that, pursuant to paragraph 5 of Article 38 of the National Biosafety Technical Commission Internal Regulations, the secrecy requested by Solazyme Brasil Óleos Renováveis e Bioprodutos was granted for pages 223 to 345 and 356 to 413.

In the light of competences granted by Law nº 11105/05 and regulated by Decree nº 5591/2005, the Commission considered that the experimental protocols and other biosafety measures proposed comply with CTNBio rules and applicable legislation aimed at securing biosafety of the environment, agriculture, and human and animal health.

TECHNICAL GROUNDS

Prototheca moriformis is a unicellular microorganism that does not possess chlorophyll, necessarily heterotrophic, featuring asexual reproduction and failing to produce spores, commonly known as microalga. *Prototheca moriformis* is naturally found in almost all habitats, including water and soils, naturally producing oil and has never been related to adverse effects to the environment. This species is not mentioned in infectious agents lists published by the Ministry of Health or in the list of plagues requiring quarantine, published by the Ministry of Agriculture and Supply, an is classified as a risk Class I organism. When cultivated in heterotrophic way, this organism produces large quantity of neutral lipids (triacylglycerol, or TAG), typical of vegetable oils and useful in a wide range of commercial applications, and may use glucose, fructose, etc. as a source of nutrition, although not saccharose.

1. Genes introduced, origin organisms and mode of action

Lineage S2014 resulted from a genetic modification of a non-GM lineage of *Prototheca moriformis*, named S1331 by the Applicant, by insertion of two genes: the sucrose invertase SUC2 of yeast *Saccharomyces cerevisiae*, and gene FATB2 that codes for a thioesterase, derived from the herbal plant *Cuphea wrightii*. The application for commercial release submits

a large explanation of the genes used to produce GMO, origin organisms and modes of action. Detailed descriptions of the genic construct used and of the transformation process were also provided in the application.

Considering all information given in the request for commercial release and the scientific literature currently available, we may reach the conclusion that the GMO analyzed may be classified as NB-1.

2. Molecular characterization of the GMO

The precise location and the nature of the genetic transformation event on S2014 lineage (number of copies of each gene and regulating elements present in the event) were determined using the Southern blot, according to experiments presented, yet the data may not be publicly disseminated given the confidentiality request made by Applicant.

Expression of transgenes in lineages S1331 and S2014 was assessed by Western blotting to detect the quantity of recombinant protein that was present in a given moment (steady-state). Expression of proteins SUC2 and FATB2 was detected in lineage S2014 within acceptable levels. As expected, none of these two proteins were detected in the feral lineage.

Considering the host of information supplied in the request for commercial release and the scientific literature currently available, we may conclude that the *Prototheca moriformis* transformation process that generated lineage S2014 failed to generate adverse effects in the event under examination.

3. Allergenicity and toxicity of the GMO and proteins

The Applicant performed studies establishing clearly that the GMO under examination does not present toxins or metabolites that may cause adverse effects on cutaneous sensibility or acute toxicity and pathogenicity. Studies presented in the application show beyond any doubt that the lineages of *Prototheca moriformis*, original and genetically transformed, do not cause harmful effects to animals and humans through the more common routes of exposure:

ingestion and skin contact. This is based on negative results appointed by sensibility tests of dermal sensibility and acute toxicity and pathogenicity performed with rats and guinea pigs. Results of both tests indicate that the genetic change in lineage S2014 fails to make such lineage allergenic, toxic or pathogenic.

Besides, an extensive *in silico* assessment performed in the world allergenic data bank, namely the Structural Database for Allergenic Proteins (SDAP: http://www.fermi.utmb.edu/SDAP), on the allergenic potential of both recombinant proteins expressed in the transgenic lineage S2014, as well as possible allergens that may be present in the parental lineage proteome, evidenced that the protein coded by thioesterase failed to display identity with any allergene present in the data bank. Only protein SUC2 (sucrose invertase), used in the food industry for centuries, resulted in a positive association as part of its *in silico* analysis; however, we may consider this result as irrelevant, since the same enzyme has a long history of safe commercial use in the food industry since 1947, when it was used to produce inverted sugar for different products, including artificial honey, confectionery, liqueurs, ice creams and treacle (Underkofler, 1957).

Applicants additionally have shown through studies that the GMO under examination and its derivatives have no different risk on the non-transgenic isogenic lineage for the quality of water and soil, in case of accidental release into the environment. No negative effects were detected on the physical and chemical characteristics of soil and water used in the experiments that may be attributed to the genetic modification performed in the GM S2014 lineage or its derivatives. It was determined that, even after a non-intentional release of lineages S1331 and S2014 in a water environment, even disregarding the dilution effect, the water could be used for human consumption after treatment, and in irrigation, sport fishing and secondary contact recreation.

Taken into account all information given in the application for commercial release, and the scientific literature currently available, we may reach the conclusion that the *Prototheca*

moriformis transformation process that generated the S2014 lineage failed to turn the GMO either allergenic or toxic.

4. Application mentioned

The process described for the use of the GMO under examination foresees production of the organism and later processing for obtaining derivatives (oils and bioproduct) in a contention regime. *Prototheca moriformis* is not a spore-forming organism and has no special adaptations that could favor long term persistency in the environment. The organism is easily inactivated by heat and chemical products, and applicants have clearly shown that the industrial process to be undergone by the GMO under analysis to extract the byproducts mentioned (high temperature and pressure) are fully efficient to inactivate the GMO and destroy its DNA. Derived products (oil and bioproduct) fail to contain the GMO and are DNA free, and its commercial use, as foreseen by the application for commercial release, will be in the oleochemical industry, and not as food and rations for humans and animals.

5. Conclusion

Considering the reasons listed above and the host of information included in the proceedings, I am for, except better judgment, the commercial release of the genetically modified microorganism *Prototheca moriformis*, lineage S2014, aimed at production of triglyceride oils and bioproduct.

Dr. Flavio Finardi Filho

President of CTNBio

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In Witness Whereof, I have hereunto set my hand and seal in this City of Brasília, Federal District, Brazil, this Wednesday, January 22, 2014.

Fees according to

Official Gazette of 04/15/2011 Marco Antônio Rochadel

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