Renewal
Assessment Report

Bacillus thuringiensis ssp. aizawai strain ABTS-1857

Volume 3 – B.1 Identity

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: Germany

Version history

When	What
September 2018	

Table of contents

B Summary of the data and information

B.1 Identity of the micro-organism

B.1.1 Applicant

Applicant: Sumitomo Chemical Agro Europe S.A.S
Parc d'Affaires de Crécy
10A rue de la Voie Lactée
FR – 69370 Saint Didier au Mont d'Or

Contact Point: [redacted]

Phone: [redacted]

Fax: [redacted]

B.1.2 Producer

Confidential information see Volume 4/Confidential Volume

B.1.3 Name and species description, strain characterisation

This document is an assessment of the active substance *Bacillus thuringiensis* ssp. *aizawai* strain ABTS-1857 and the plant protection product XenTari® WG. The active substance name has been abbreviated throughout the document to BTa ABTS-1857. XenTari® DF is interchangeable with XenTari® WG.

B.1.3.1 Accession number in culture collection

Based on the DAR final addendum XenTari WG formulation contains a production culture of Bacillus thuringiensis subsp. aizawai, strain ABTS-1857. This culture has been placed in the Safe Deposit frozen storage facilities of the American Type Culture Collection (ATCC), Rockville, MD. Safe Deposit Number: SD-1372, 11 October 1990. This was converted to ATCC Patent Deposit 69074 on 7 July 1992.

B.1.3.2 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism

The scientific name and taxonomic grouping has been identified in the Draft Assessment Report and EFSA conclusion for BTa ABTS-1857

Kingdom	Bacteria
Division	Firmicutes
Class	Bacilli

Order	Bacillales
Family	Bacillaceae
Genus	*Bacillus*
Species	*thuringiensis*
Subspecies	*aizawai*
Serotype	H-7
Strain	ABTS-1857
First description	*Bacillus thuringiensis* was first described by Berliner in 1911 *Bacillus thuringiensis* subsp. *aizawai* was first described by Bonnefoi & de Barjac in 1963)

Indigenous or non-indigenous	Indigenous, wild type
Spontaneous or induced mutant*	The strain is not described as a mutant
Genetically modified according to Directive 2001/18/EC	This strain originates from a natural, indigenous wild type and is not genetically modified.

* All known differences between the modified micro-organism and the parent wild strain must be provided

Bacillus thuringiensis is naturally occurring in the environment and has been isolated from a range of habitats including soil, phylloplane, dust, plant material and insects throughout the world (Glare, O'Callaghan, 2000 DAR).

B.1.3.3 Test procedures and criteria used for identification at strain level

Test procedures and criteria used for identification at strain level has been identified in the DAR final addendum:

Bacillus thuringiensis subsp. *aizawai* (Bta), strain ABTS-1857 is used to produce ABG-6305 Technical. Bta is a Gram-positive, spore forming, rod-shaped bacterium that produces a crystalline protein inclusion, which is toxic to larvae of some Lepidopteran insects upon ingestion.
Strain identification was performed by standard biochemical and morphological testing, flagellar antigen serotyping, standard Gram-positive antibiotic sensitivity, insecticidal toxins produced, DNA analysis and plasmid profiling and description of crystalline proteins (Smith, 1990 and Benson 2005, Confidential Information)
Biochemical characteristics of *Bacillus thuringiensis* strain ABTS-1857 were determined according to methods prescribed by Bergey's Manual of Systematic Bacteriology, Vol 2 [2,19]. Characteristics of *Bacillus thuringiensis* subsp. *aizawai* were reported by researchers at the American Type Culture Collection (ATCC), Rockville, MD. Further characterisation was performed by evaluating flagellar antigen serotyping, antibiotic sensitivity pattern, characterisation of crystal proteins and plasmid profile.
For the biochemical characterization, AGB-6305 production strain was compared with 3 other closely related strains: Btk-HD-1, ATCC-SD-1275 (Used in the production of DiPel®). ABTS-26, HD-133

(an AIZ type strain from the U.S. Dept of Agriculture). ABTS-1883, HD-11 (an AIZ type strain for serotyping from the U.S. Dept of Agriculture).
Minor metabolic differences were observed when responses were compared to strain Btk-HD-1, ATCC-SD-1275, the strain used for production of DiPel®. The ABTS-1857 is phenotypically similar to Btk-HD-1. ABTS-1857 is more similar to Btk-HD-1 than *Bacillus thuringiensis* subsp. *tenebrionis* a subspecies used for control of Coleopteran larvae. The flagellar serotype of ABTS-1857 is *aizawai*, H-7. Strain ABTS-1857 was sensitive to gentamicin, kanamycin, erthyromycin, clindamycin, vancomycin, chloramphenicol and trimethoprim/sulfamethoxazole but not sensitive to penicillin, ampicillin or cephalothin.
The profile of the plasmid DNA from ABTS-1857 was compared to the profile from 2 *Bacillus thuringiensis* subsp. *aizawai* strains (ABTS-26 and ABTS-1883) and the *kurstaki* strain (Btk-HD-1). For the ABTS-1857-strain 5 plasmid bands migrated slower than the chromosomal DNA and 10 plasmid bands migrated faster than the chromosomal DNA
Crystalline protein inclusions, isolated both from sporulated flask cultures and from Technical Powders were composed primarily of one major protein approximately 135 kDa as determined by SDS-PAGE. (Smith, 1990).
The strain ABTS-1857 has been designated as an *aizawai* subspecies of *Bacillus thuringiensis* based upon the results of diagnostic tests in which it was compared to two known *aizawai* subspecies. Of the characterisation tests conducted, the responses to flagellar antibodies and to protein gel (SDS-PAGE) protoxin banding patterns were most diagnostic. Plasmid gel patterns were also shown to be similar (Smith, 1996).
The Cry toxins present in *Bacillus thuringiensis* subsp. *aizawai* (Bta), strain ABTS-1857, include Cry IAa, Cry IAb, Cry IC and Cry ID (Rowell, 2005; Confidential Information)
The DNA of *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 has been analysed by amplified fragment length polymorphism (AFLP). This technique produces a visual fingerprint of the DNA and the data obtained from over 300 strains of *Bacillus thuringiensis*, *Bacillus cereus* and *Bacillus anthracis* have been used to construct a phylogenetic tree which shows that the strains could be placed into 3 clusters, each of which contained 3 or 4 branches. The DNA of *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 has been placed into Cluster 1 Branch C (Benson, 2005; Confidential Information).

For Bacillus thuringiensis ssp. aizawai, strain ABTS-1857 new genomotyping studies are available to unequivocally identify the organisms down to strain level. These studies are presented in B.5.

B.1.3.4 Common name or alternative and superseded names and code names used during the development

This document is an assessment of the active substance *Bacillus thuringiensis* ssp. *aizawai* strain ABTS-1857 and the plant protection product XenTari® WG. The active substance name has been abbreviated throughout the document to BTa ABTS-1857. XenTari® DF is interchangeable with XenTari® WG.

B.1.3.5 Relationship to known pathogens

See B.2.5 of the CA part of this RAR.

B.1.4 Specification of the material used for manufacturing of formulated products

See volume 4.

B.1.4.1 Content of the micro-organism

Information presented in the original DAR: The active ingredient (metabolite) of the technical powder is a crystal protein which is enclosed within the organism. Therefore the organisms do not need to be viable to maintain the activity of the technical material, and methods for the enumeration of the micro-organism is not an appropriate measure of active substance. Measurements of the crystal protein content and measurements of insecticidal potency are more relevant to the active substance.

In the addendum to the DAR (2012) the weight percent of 135 kDa protein toxin from Bacillus thuringiensis subsp. aizawai, strain ABTS-1857 in 5 batches of ABG-6305 Technical Powder is identified to be 19% w/w on average (range 13.2 to 20.5 %w/w). XenTari WG formulation contains 54% w/w Technical Powder, which is equivalent to 10% w/w active toxin (135 kDa). Inerts include fermentation solids and/or solubles and residual moisture in addition to formulation additives. XenTari WG has a bio-potency of 15,000 IU/mg.

Based on the total Viable Spore Count (CFU/g) - rounded for the nominal we would indicate 5.5 x 10^{10} CFU/g and for a minimum 3 x10^{10} CFU/g and for a maximum 9 x 10^{10} CFU/g (see volume 4).

BTa ABTS-1857 is the main ingredient of the formulated product XenTari® DF (equivalent to XenTari® WG) and further information concerning the composition of the formulated product XenTari® WG) is confidential to Valent Biosciences Corp. and is presented in volume 4.

B.1.4.2 Identity and content of impurities, additives, contaminating micro-organisms

Microbial impurities

In the addendum to the DAR (2012) is described that quality controls are performed on the product to ensure the absence of potential contaminants and human pathogens. Based on these methods, the following data have been generated:

5 lots of XenTari WG formulation have been tested for E-coli, Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium perfringens, Salmonella enteriditis and Enterococcus to assess the presence of six species of potential contaminants. Results showed that only very low levels ([redacted] of [redacted]

[redacted] were present. (Copeland, 1990)

1 lot of XenTari WG formulation (Lot No. 77-805-PG) has been tested following 2 years storage at room temperature. Results showed the absence of Salmonella species and <10 cfu/g count for Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium perfringens and yeast and molds. A low count of [redacted] species ([redacted]) and other [redacted] species ([redacted] of which 98% [redacted]) were detected (Brand, 2004).

Another new study performed by Nei-Long Lyang, M.S. (2016) conducted to GLP standards is available for the XenTari DF Technical Powder and provides data to show compliance with SANCO/12116/2012 –rev. 0, September 2012 'Working Document on Microbial Contaminant Limits for Microbial Pest Control Products' (based on OECD Issue Paper on Microbial Contaminants Limits for Microbial Pest Control Products, September 03, 2014) through analysis of five batches of the applicant's candidate MPCA (see further B.5 Volume 3 for the analytical methods and Volume 4 for the 5-batch analysis).

Non-microbial impurities

β -exotoxin

In the addendum to the DAR (2012) is described that methods for the determination of β -exotoxin by HPLC-UV and by House Fly Bioassay have been developed and validated. 5 separate batches of ABG 6305 Technical Powder have been analysed and show that β -exotoxin is not detected (Lee, 1990, Jaronski, 1991).

Additional validation of the HPLC-UV method was performed prior to analysis of β -exotoxin in 3 further batches of ABG 6305 Technical Powder. β -exotoxin is not detected (<0.7ppm) (Chang, 1994).

Three separate batches of XenTari Technical slurry have been analysed for Type I and Type II β -exotoxin using the House Fly Bioassay. Results show that neither Type I or Type II β -exotoxin were detected (Benzon, 2005)

The toxicity of XenTari formulation to house fly larvae (Musca domestica) has been compared along with other Bt strains to the toxicity of β -exotoxin. Tests showed that treatments with β-exotoxin demonstrated significant toxicity whilst treatments with Bt strains showed toxicity either similar or only slightly above those effects seen in the untreated controls. (Teixeira, 2000)

A new study performed by Benzon, G.L. (2016) is available for XenTari® (Bacillus thuringiensis aizawai) and provides data to show that β-exotoxin was not detected in any of the samples of XenTari® technical slurry tested using a house fly larval feeding bioassay (see C.1.2.2 in volume 4).

Enterotoxins

The final fermentation beer (XenTari run 2672) and the final product (Lot# 108-234-PG) have been tested for the presence of enterotoxin proteins. The test method used was the TECRA Bacillus Diarrhoeal Enterotoxin Visual Immunoassay produced by Tecra International Pty Ltd. The TECRA BDE VIA system detects the 40-45 kDa protein referred to as the BDE Nonhemolytic enterotoxin or NHE. The results showed that both tests on the final fermentation beer and final XenTari product were negative (Bowman, 2004).

B.1.4.3 Analytical profile of batches

Confidential information, see volume 4.

B.1.5 References relied on

See B.6 MA for summary literature search.

OECD data point / number	Author(s)	Year	*Title* *Source (where different from Company)* *Company Report No.* *GLP or GEP status (where relevant),* *Published or not*	Data Protection Claimed Y/N	Owner
IIM 1.3.1/01	Glare, T.R., O'Callaghan, M.	2000	*Bacillus thuringiensis*, Biology, Ecology and Safety. Publ. Wiley. Not GLP; Published	N	--