

BASELINE DOSSIER

Bacillus subtilis QST 713

Microbial pest control agent against plant pathogenic fungi and bacteria

Dossier according to OECD guidance for industry data submissions for microbial pest control products and their microbial pest control agents – August 2006

Summary documentation, Tier II

Annex IIM, Section 4

Point IIM 6: Metabolism and residue studies

Date: July 2013

Applicant

Bayer CropScience AG



M-535397-01-2

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Introduction

This document summarizes all data submitted for the initial evaluation of *Bacillus subtilis* QST 713 as an active substance under Directive 91/414. Data provided in the initial dossier and in subsequent additional submissions are listed chronologically under the respective data point according to the OECD dossier guidance (2006).

This document is further named as “**Baseline Dossier**” since it presents all data previously submitted.

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IIM 6 Metabolism and residues studies on the microbial pest control agent**IIM 6.1 Rationale for waiver of residue data based on information showing that MPCAs is not hazardous to mammals, i.e. lack of potential for a known mammalian toxin and negative result from the acute oral toxicity test**

Studies were submitted in Annex II, Section 3, Points IIM 5.3.2 – 5.3.3 on acute oral and inhalatory toxicity in rats. The results showed the absence of any clinical signs and therefore it is concluded that *B. subtilis* poses no risk to mammals, and can be classified as non-toxic. Since *B. subtilis* QST713 is not infectious to mammals and does not produce metabolites toxic to other organisms, deposits of this microorganism after application of the plant protection product will not impose any health risk for consumers. Consequently no residues data are required.

IIM 6.2 Rationale for waiver based on a substantiated estimation that MPCAs is unlikely to occur on treated food/feed stuffs in concentrations considerably higher than under natural conditions

Report: KIIM 6.2/01; Priest, F. G.; 1993; M-484952-01-1
Title: Systematics and ecology of Bacillus
Report No.: M-484952-01-1
Document No.: M-484952-01-1
Guideline(s): not specified
Guideline deviation(s): not specified
GLP/GEP: no

Based on reported characteristics of *B. subtilis* species, as uniformly found in the scientific literature, under consideration of the envisaged application and relevant properties of strain QST713, we can conclude that populations on treated food/feed stuffs may be higher only directly after application, and will decrease significantly until the harvest of the treated crop. This refers also to Point IIM 6.3, which presents the data on the possibility of multiplication of the strain of interest on food stuffs and feeding stuffs.

It has to be considered that *B. subtilis* is a non-pathogenic, ubiquitous micro-organism, prevalent in the micro-flora of different environmental compartments and media, including the leaf surface and foodstuff (Priest 1993).

IIM 6.3 Persistence and likelihood of multiplication in or on crops, feedingstuffs or foodstuffs**EU-Dossier: Doc M-III, Point 6.1**

Report: KIIM 6.3/01; Campbell, R.; 1989; M-488048-01-1
Title: Biocontrol on leaf surface
Report No.: M-488048-01-1
Document No.: M-488048-01-1
Guideline(s): not specified
Guideline deviation(s): not specified
GLP/GEP: no

Report: KIIM 6.3/02; Zimmerman, S. B.; Schwartz, C. D.; Monaghan, R. L.; Pleak, B. A.; Weissberger, B.; Gilfillan, E. C.; Mochaels, S.; Hernandez, S. A.; Currie, S. A.; Tejera, E.; Stapley, E. O.; 1997; M-528163-01-1
Title: Final decision document: TSCA section 5 (H) (4) exemption for Bacillus subtilis
Report No.: M-528163-01-1
Document No.: M-528163-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: no

Report: KIIM 6.3/03; [REDACTED]; 2000; M-497595-01-1
Title: Longevity study of Serenade (QST713) on pepper leaf surface in greenhouse conditions
Report No.: M-497595-01-1
Document No.: M-497595-01-1
Guideline(s): not specified
Guideline deviation(s): not specified
GLP/GEP: no

Report: KIIM 6.3/04; Priest, F. G.; 1993; M-484952-01-1
Title: Systematics and ecology of Bacillus
Report No.: M-484952-01-1
Document No.: M-484952-01-1
Guideline(s): not specified
Guideline deviation(s): not specified
GLP/GEP: no

Report: KIIM 6.3/05; Sholberg, P. L.; Machi, A.; Bechara, S.; 1995; M-528182-01-1
Title: Biocontrol of postharvest diseases of apple using Bacillus sp. isolated from stored apples
Report No.: M-528182-01-1
Document No.: M-528182-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: no

Strain QST 713 of *B. subtilis* is intended to be applied on the foliage. Regarding the intended fields of use residues of *B. subtilis* on leaf surfaces are associated with the establishment of colonization of the plant phenomenon of this contact biofungicide and bactericide. Colonization of treated foliage provides a protective layer, and basically is involved in the mode of action of *B. subtilis* against pathogen attack (see Fig. 6.3-01 and see Section 1, Point 2.3.2).

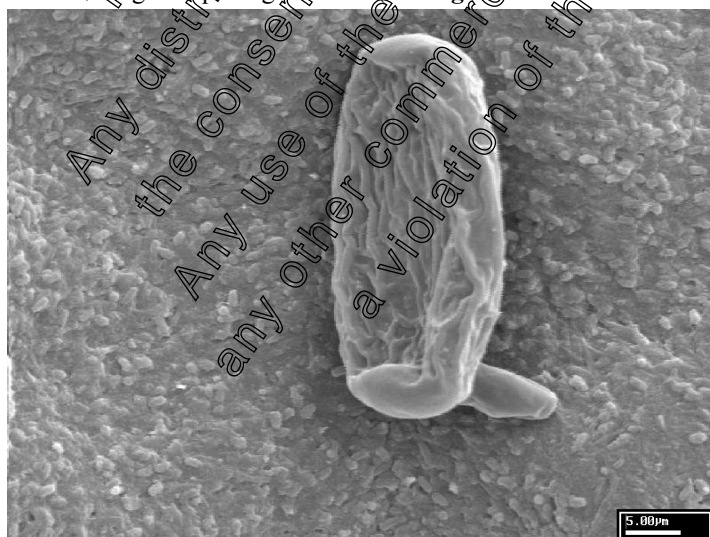


Fig. 6.3-01: Grape powdery mildew spore on a dense “carpet” of *B. subtilis* cells on squash

Campbell (1989) addresses to the problems involved in foliar application of biocontrol agents, including adhesion of preparations to the mainly hydrophobic leaf surface and the usually unfavourable environmental conditions restricting microbial growth (and explaining the generally

slight occurrence of growing saprophytic bacteria on the leaf surface and the low covering percentage (<1%) of micro-organisms on the leaf surface of temperate plants). In addition, *B. subtilis* cells will stop growth after depletion of organic matter supply (EPA 1997), e.g. the fungal pathogen.

Therefore, the protective layer of *B. subtilis* cells colonizing the leaf surface will not maintain stability or persistence for long in this stressed micro-habitat.

The applicant conducted a study on longevity of QST 713 strain of *B. subtilis* on pepper leaf surface under greenhouse conditions, applied at a rate of 6.7 kg/ha formulated product (█ & █ 2000): an initial increase in colony forming units (CFU) was followed by a sharp decline in CFU-counts by day 5 to low levels of surviving cells persisting for ~ 3 weeks. (Fig. 6.1-02).

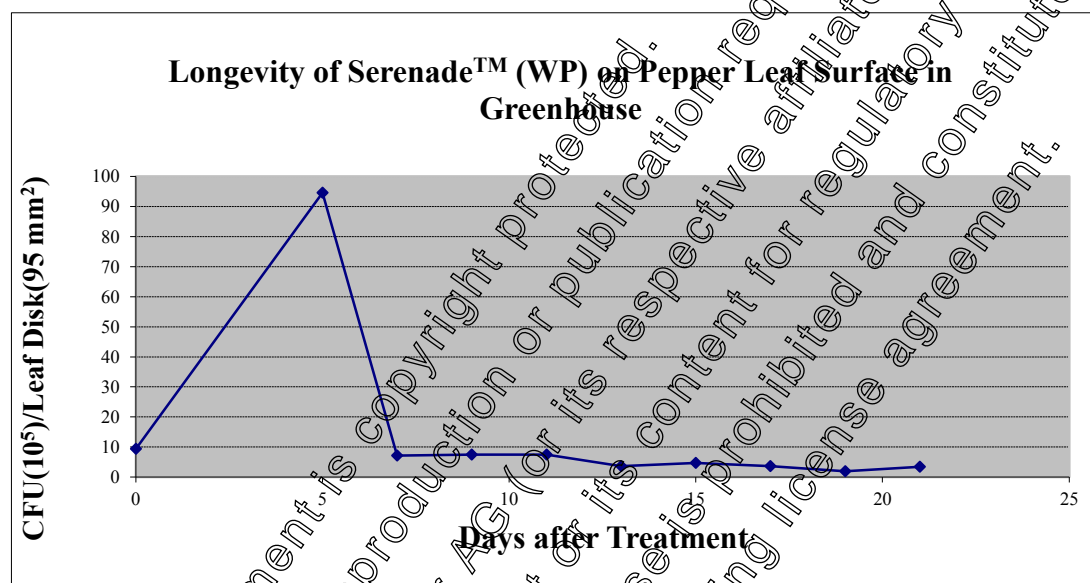


Fig. 6.1-02: Time course of CFU-counts of *B. subtilis* strain QST 713 recovered from pepper leaf surface

To assess the survival of this strain in the field and in summertime, especially with an assumed negative effect of increased UV radiation, further studies under ambient field conditions are planned.

However, the unfavourable environmental conditions prevailing on the leaf surface will not impede the efficacy of QST 713 strain of *B. subtilis* since the preparation will be added several times at appropriate intervals and in sufficient amounts so that long-term survival or long-term colonization of the foliage is not necessitated – in fact for the induction of systemic resistance in the plant, presenting one mode of action (please refer to Annex IIM, Section 1, Point 2.3.2), an initial challenge by a non-pathogenic species may elicit the resistance response (Campbell, 1989).

B. subtilis may form endospores under nutrient shortage and environmental stress. These endospores might possibly be dissipated via wind and water to other environmental compartments (Priest, 1993), where they may contribute to the existent population of *B. subtilis*, e.g.:

Sholberg et al (1995) recovered 25 bacterial isolates from stored apples for screening of biocontrol activity and identified among 30 Bacilli isolates as *B. subtilis* which acted effectively against molds.

With regard to *B. subtilis* cells remaining on harvested fruit the chance of proliferation during processing of raw products (grapes, apples to vine, juice respectively) is not relevant since a) in vine fermentation conditions are unfavourable and b) in juice production microbial contaminants are heat killed (at a processing temperature of ~ 90°C), while conditions do not favour endospore formation.

In this context it has to be considered that *B. subtilis* is a non-pathogenic, ubiquitous micro-organism, prevalent in the micro-flora of different environmental compartments and media, including the leaf surface and foodstuff (Priest, 1993).

IIM 6.4 Further information required**IIM 6.4.1 Non-viable residues**

Report: KIIM 6.4.1/01; Zimmerman, S. B.; Schwartz, C. D.; Monaghan, R. L.; Pleak, B. A.; Weissberger, B.; Gilfillan, E. C.; Mochaels, S.; Hernandez, S. A.; Currie, S. A.; Tejera, E.; Stapley, E. O.; 1997; M-528163-01-1

Title: Final decision document: TSCA section 5 (H) (4) exemption for Bacillus subtilis

Report No.: M-528163-01-1

Document No.: M-528163-01-1

Guideline(s): --

Guideline deviation(s): --

GLP/GEP: **no**

Report: KIIM 6.4.1/02; [REDACTED], D.; 2000; M-528847-01-1

Title: Absence of pharmaceuticals in 713

Report No.: M-528847-01-1

Document No.: M-528847-01-1

Guideline(s): not specified

Guideline deviation(s): not specified

GLP/GEP: **no**

This refers to Annex II, Section 1, Point IIM 2.6. *B. subtilis* does not produce significant quantities of extracellular enzymes or toxins and is generally considered to have a low degree of virulence (EPA 1997). The results of the submitted toxicological studies on rodents demonstrate that QST 713 strain of *B. subtilis* does evidently not produce toxins (please refer to Annex II, Section 3, Point IIM 5.3.2 – 5.3.3).

In conclusion, non-viable residues originating from *B. subtilis* QST 713 are not considered to pose a risk to human health and the environment.

Included under 1st Additional Submission. Amendment not included in monograph

Secondary metabolites of strain QST 713 of *B. subtilis* have been analyzed and determined to be in the known class of lipopeptides ([REDACTED], 2001, submitted in Doc JII). The complete chemical analysis of the strain QST 713 proved that 60 compounds are produced that are not well known in the literature. Based on literature search, it can be stated that the strain QST 713 does not produce metabolites that are used in human medicine ([REDACTED], 2000). Please refer to Annex II, Section 1, Point IIM 2.6.

Included under 2nd Additional Submission

Bacillus subtilis produces several different secondary metabolites. Detailed information on secondary metabolites formed by the strain QST 713 of *B. subtilis* has been submitted to all Member States in October 2001 for the evaluation for the Annex I inclusion ([REDACTED], 2001). Please refer to Annex II, Section 1, Point IIM 2.6.

Included under 3rd Additional Submission

Additional information was submitted in response to the EU Evaluation to provide further information on toxins produced by *B. subtilis* QST 713 (date of submission: June 2002). Please refer to Annex II, Section 1, Point IIM 2.6.

IIM 6.4.2 Viable residues

Report: KIIM 6.4.2/01; Sietske de Boer, A.; Diderichsen, B.; 1991; M-486912-01-1
Title: On the safety of Bacillus subtilis and B. amyloliquefaciens: a review
Report No.: M-486912-01-1
Document No.: M-486912-01-1
Guideline(s): not applicable
Guideline deviation(s): not applicable
GLP/GEP: no

Report: KIIM 6.4.2/02; Zimmerman, S. B.; Schwartz, C. D.; Monaghan, K. L.; Peak, B.; Weissberger, B.; Gilfillan, E. C.; Mochaels, G.; Hernandez, A.; Currie, S. A.; Tejera, E.; Stapley, E. O.; 1997; M-528163-01-1
Title: Final decision document: TSCA section 4(H) (4) exemption for Bacillus subtilis
Report No.: M-528163-01-1
Document No.: M-528163-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: no

Report: KIIM 6.4.2/03; Priest, F. G.; 1993; M-484952-01-1
Title: Systematics and ecology of Bacillus
Report No.: M-484952-01-1
Document No.: M-484952-01-1
Guideline(s): not specified
Guideline deviation(s): not specified
GLP/GEP: no

Report: KIIM 6.4.2/04; [REDACTED], J.; 2001; M-487847-01-1
Title: Screenade WP residue on wine grapes.
Report No.: 00147
Document No.: M-487847-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: no

EU-Dossier: Doc M-IIB, Point 6.2

Due to the fact that the active ingredient is a viable micro-organism of ubiquitous occurrence and predominance in the soil-microflora the term *residue* is not applicable to this preparation.

Specifically, no residue metabolism can be stated, since a micro-organism does not follow first order kinetics.

Residue studies were regarded as being dispensable for the above given and following additional reasons:

- With regard to its natural global distribution and non-pathogenic character *B. subtilis* cells left on the surface of treated areas or plant products do not imply health or environmental impacts (Boer & Diderichsen 1991; EPA 1997).
- *B. subtilis* has been used for enzyme production on a large industrial scale, and is even used for food production without having caused health or environmental hazards or damages (e.g. Priest, 1993).
- *B. subtilis* has no special attachment ability to plants or plant products, i.e. there is no compatibility comparable to host-pathogen interactions (EPA 1997).

- Colonization of the leaf surface by *B. subtilis* contributes largely to the protective effect against bacterial and fungal pathogens and according to the recommended frequent applications this protective layer may be renewed almost weekly.
- A plant product (fruit) carrying a layer built up of *B. subtilis* can easily be washed with water prior to consumption or juice production.

Included under 3rd Additional Submission

A relevant study which demonstrates the decline of *Bacillus subtilis* strain QST 713 on grapes after application of Serenade WP (■■■■■ ■■■■, 2001) was submitted in June 2007 and is cited in the Addendum 1 to the Monograph (date of issue: 04.12.2002). According to this study, *Bacillus subtilis* strain QST 713 declines on grapes after application of 10 kg Serenade WP/ha within four weeks after the last application to values below 7.1×10^3 CFU/berries.

On the ECCO Working Group Evaluation Meeting on 26.03.2003 it was stated that no data are required for the time being as MRLs are not considered necessary.

IIM 6.5 Summary of residue behaviour and overall evaluation

EU-Dossier: Doc M-IIB, Point 6.3

Primarily the low health and environmental risk potential of *B. subtilis* and its ubiquitous distribution indicate that residual *B. subtilis* cells may present only a low risk potential.

Secondly, the unfavourable environmental conditions prevailing on the leaf surface and the dependence of *B. subtilis* on organic matter supply are restricting its growth, as shown in the submitted study report (please refer to Point IIM 6.3). In addition, in processing of raw products no growth or sporulation of *B. subtilis* is expected to occur.

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