Bacillus amyloliquefaciens 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: September 2015

Applicant

Bayer CropScience AG

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Introduction

The company Bayer CropScience AG is submitting a dossier for the re-approval of the microorganism *Bacillus amyloliquefaciens* QST 713 as an active substance under regulation (EC) 1107/2009, previously designated as *Bacillus subtilis* QST 713. Due to most current information on taxonomy, *B. subtilis* QST 713 is classified as a member of *B. amyloliquefaciens* group. As a consequence, the active substance is now named as *B. amyloliquefaciens* subsp. *plantarum* QST 713, hereinafter named as *B. amyloliquefaciens* QST 713.

The initial evaluation of *Bacillus subtilis* QST 713 was performed under Directive 91/414. Data provided in the initial dossier and in subsequent additional submissions according to the OECD dossier guidance (2006) are submitted as a "Baseline Dossier", separately.

Here we submit all new data and information basing on previous literature searches and studies.

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IIM 6 Metabolism and residues studies on the microbial pest control agent

Report: KIIM 6/01 – [redacted] (2015), literature review on bacillus amyloliquefaciens QST713:
Residues in or on treated products, food and feed. Owner Bayer CropScience AG.
Unpublished
Report No. 6791109-A2-06-01
M-535709-01-1

Please refer to the baseline dossier for the background data. In the current dossier several new literature reports resulting from the literature search conducted in 2015 are submitted ([redacted], 2015).

IIM 6.1 Rationale for waiver of residue data based on information showing that MPCA is not hazardous to mammals, i.e. lack of potential for a known mammalian toxin and negative result from the acute oral toxicity test

Please refer to the baseline dossier.

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

Please refer to the baseline dossier.

IIM 6.3 Persistence and likelihood of multiplication in or on crops, feedingstuffs or foodstuffs

B. amyloliquefaciens QST 713 is intended to be applied onto the foliage. Regarding the intended fields of use residues of *B. amyloliquefaciens* on leaf surfaces are associated with the establishment of colonization, the prime 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.

For details please refer to the baseline dossier.

A literature search was conducted on the DIMDI database provided by the German Institute of Medical Documentation and comprised of searches in MEDLINE, BIOSIS, CAB Abstracts and SCISEARCH databases. Search strategy aimed to find all references that are of relevance regarding reports of residues on food and feed, proliferation and persistence in the field as well as consumer risk referring to the species *Bacillus amyloliquefaciens* and *Bacillus subtilis.* For more details on the literature search, please refer to [redacted] (2015), submitted in Point IIM 6.

Several reports were found to provide supporting information on the behaviour of *B. amyloliquefaciens* or *B. subtilis* after treatment. [redacted] el at. (2011) evaluated the efficacy of the biocontrol agent *B. amyloliquefaciens* PPCB004 in papaya. A set of fruits at 25-30% yellow was subjected to the postharvest treatment with PPCB004 (100 µL, 1×10^9 CFU/mL). At completion of the treatment, fruits were packed and stored for 14 d at 10°C and at 80% RH. After cold storage, fruits were allowed to ripen at 25°C. After storage and ripening fruits were washed in Ringer's solution and the washing was filtered through 0.22 µm filters. Subsequently filters were transferred to Ringer´s solution, and bacteria and fungi and yeast were grown on agar plates. Survival of *B. amyloliquefaciens* was expressed in CFU, based on colony morphology. The PPCB004 population showed first an increase in population after ripening at 25°C. However a similar increase was observed for fungi and yeast present after the treatment, when the conditions allow their growth. Another study describes the postharvest treatment in wounds of citrus fruits with *Pichia guilliermondii* BCC 5389 and *B. subtilis* ABS-S14 ([redacted] et al., 2013). While *P. guilliermondii* colonized the fruit wounds rapidly within 6 days at 25°C, *B. subtilis* increased only marginally during first 3 days, and then the population stabilized for the remaining incubation period (9 days in total). Similar postharvest treatment with *B. subtilis* from Avogreen was investigated by [redacted] et al., (2007): litchi fruit cv. McLean's Red was harvested at commercial maturity, dipped in *B. subtilis* solution at 1×10^8 CFU/mL, dried, packed, and cold stored at 2°C and 90% RH for 18 days, and subsequently at 14°C and 75% RH for 2 days, to simulate market-shelf conditions. Different packaging/treatment operations were tested: *B. subtilis* and prochloraz, packed in low

density polyethylene (LDPE) or polypropylene (PP). Effective recovery of *B. subtilis* was observed in the *B. subtilis* + PP combination, while it failed to survive in *B. subtilis* + LDPE combination. These studies clearly indicate that the colonization capacities and population growth is strain and condition dependent, and change even for one strain, when conditions vary.

It can be summarized that there exists a possibility of survival and even growth of microorganism population on treated crops, but no risk is anticipated due to the fact that *B. amyloliquefaciens* is a non-pathogenic, ubiquitous microorganism, prevalent in the microflora of different environmental compartments and media. This was further demonstrated in numerous studies with the strain *B. amyloliquefaciens* QST713 (formerly classified as *B. subtilis* QST713) which demonstrated absence of toxicity, pathogenicity, and infectivity. Even if an initial growth of *B. amyloliquefaciens* is observed, it is meaningless, since the population will stabilize or decrease, due to the nutrient competition, antagonism and environmental conditions.

Report: KIIM 6.3/06 – [redacted], M.S., [redacted], [redacted] (2011). Effect of biocontrol agent *Bacillus amyloliquefaciens* and 1-methyl cyclopropene on the control of postharvest diseases and maintenance of fruit quality, published report
Crop Prot, 30, 173-178
M-518664-01-1

Abstract: Efficacy of biocontrol agent *Bacillus amyloliquefaciens* PPCB004 was evaluated on the control of anthracnose and phomopsis rot in 'Solo' papaya pre-treated with 1-methyl cyclopropene (100 mL) (1- MCP) during storage. This treatment was compared to the untreated control, commercial treatment (washing in chlorinated water), standalone 1-MCP and PPCB004 treatment. Although fruit pre-treated with 1-MCP delayed the ripening (100% yellow) after cold storage by 9e10 d, it showed higher incidence and severity of anthracnose and phomopsis rot than the fruit subjected to commercial treatment. Application of PPCB004 after 1-MCP pre-treatment (1-MCP + PPCB004) reduced the anthracnose and phomopsis incidence and severity after cold storage (10°C, 85% RH for 14 d) and ripening at 25°C. The 1-MCP + PPCB004 treatment helped to retain the fruit firmness, overall quality and uniform yellow skin (100%) and flesh colour after ripening. The PPCB004 was effectively recovered from standalone PPCB004 and 1-MCP + PPCB004 treated fruit after cold storage and ripening. The PPCB004 population showed an increase by 1 log units after ripening in 1-MCP + PPCB004 treated fruit. After ripening the recovery of PPCB004 population was higher (0.7 log units) in 1-MCP + PPCB004. The total recovery of fungal population on the fruit surface after ripening was lower in 1-MCP + PPCB004 and standalone PPCB004 treated fruit. It can be concluded that application of *B. amyloliquefaciens* PPCB004 with 1-MCP pretreated papaya (at 25-30% skin yellow stage) can significantly reduce disease incidence associated with 1-MCP treatment. This treatment has the potential for commercial application in the 'organic' papaya industry.

Report: KIIM 6.3/07 – [redacted], [redacted], [redacted], (2013) Biocontrol of citrus green mould and postharvest quality parameters, published report
Int Food Research Journal, 20, 3381-3396
M-518930-01-1

Abstract: The potential for using *Pichia guilliermondii* BCC 5389 or *Bacillus subtilis* ABS-S14 by themselves or in combination for the control *Penicillium digitatum* in citrus, and their effects on postharvest quality of fruit was investigated. The percentage of disease with the combined antagonists was completely inhibited. Rapid colonization of *P. guilliermondii* was observed in the wounds during the first day to 6 days at 25°C, whereas *B. subtilis*, increased marginally over 3 days. The populations then stabilized for the remaining incubation period. The percentage of spore germination of *P. digitatum* incubated with all treatments was inhibited by 100%. At concentrations of the combined antagonists of 1x10^8 CFU/mL, the incidence of green mould was reduced to 0% compared with the pathogen control itself (92.93%) after 5 days of incubation at 25°C. The combination did not impair any of the quality parameters of fruit following incubation at 25°C for 7 days.

Report: KIIM 6.3/08 – [redacted], [redacted], [redacted] (2007) Effect of a biocontrol agent (*Bacillus subtilis*) and modified atmosphere packaging on postharvest decay control and quality retention of litchi during storage, published report
Phytoparasitica, 35, 507-518
M-530513-01-1

Abstract: The efficacy of biological control and two types of modified atmosphere packaging (MAP) alone and in combinations was evaluated under cold storage as well as simulated market-shelf conditions to control decay and pericarp browning on litchi cv. 'McLean's Red'. Fruits were dipped for 2 min at 15°C in *Bacillus subtilis* or prochloraz separately, packed in MAP [low density polyethylene (LDPE) or polypropylene (PP)], heat sealed and stored at 2°C and 90% r.h. for 18 days followed by 2 days at 14°C and 75% r.h. to simulate market-shelf conditions. A commercially adopted sulfur dioxide treatment was included as a comparative control. Fruits treated with *B. subtilis* + PP or prochloraz + PP and stand-alone PP treatment did not show decay or browning at 2°C. Decay and browning were controlled significantly after 2 days at 14°C in *B. subtilis* + PP or prochloraz + PP treatments. However, the prochloraz + PP affected the natural pinkish-red color of the pericarp and gave higher h° (hue angle) values. The stand-alone PP treatment (~14% O_2, ~5% CO_2) showed 11.3% decay due mainly to *Alternaria alternata* and *Cladosporium* spp. at 14°C. The effectiveness of the MAP was improved at 14°C when *B. subtilis* was combined with PP, controlling decay and pericarp browning and retaining the fruit color and quality. *B. subtilis* survived in PP at 2° and 14°C, but not in LDPE. Stand-alone LDPE (~3% O_2, ~10% CO_2) and combination treatments *B. subtilis* + LDPE or prochloraz + LDPE failed to control decay and pericarp browning. Higher yeast populations were observed in LDPE or *B. subtilis* + LDPE at both 2° and 14°C.*Candida, Cryptococcus* and *Zygosaccharomyces* spp. were the predominant yeasts in all LDPE treatments.

IIM 6.4 Further information required

IIM 6.4.1 Non-viable residues

B. subtilis does not produce significant quantities of extracellular enzymes or toxins and is generally considered to have a low degree of virulence.

Please refer to the baseline dossier Annex II, Section 1, Point IIM 2.6 and Section 6, Point IIM 6.4.1.

IIM 6.4.2 Viable residues

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.
Please refer to the baseline dossier Annex II, Section 6, Point IIM 6.4. 2.

IIM 6.5 Summary of residue behaviour and overall evaluation

Primarily the low health and environmental risk potential of *B. amyloliquefaciens*, and its ubiquitous distribution indicate that residual cells of *B. amyloliquefaciens* QST713 may present only a low risk potential.

Secondly, the unfavourable environmental conditions prevailing on the leaf surface and the dependence of *B. amyloliquefaciens* on organic matter supply are restricting its growth.