

## First report of *Bacillus pumilus* causing trunk bulges of rubber tree (*Hevea brasiliensis*) in Malaysia

### ABSTRACT

The rubber industry is projected to increase to RM53 billion or USD1.3 billion of gross national income by 2020 in Malaysia (Malaysian Rubber Board 2009). Introductions of superclones with high yield potential such as RRIM 3001 are among the efforts to improve latex yield as well as timber yields. Superclone RRIM 3001, mostly cultivated by smallholders, yields about 3 tons of natural rubber latex per hectare per year. During September 2017, trunk bulging occurrences were observed in rubber trees (*Hevea brasiliensis*) RRIM 3001 superclone in Serdang, Selangor, Malaysia. These bulges appeared in different sizes and were similar to tumor-like bacteriosis on the whole trunk. Other symptoms were also observed, including depressed canker wounds with different sizes at the tapping zone and large bleeding lesions on the trunk and branches of the rubber trees. Symptomatic bark tissue of various rubber trees was collected at 1.52 m by using a sterile knife. The infected tissues were cut approximately at 0.5 × 0.5 cm, surface sterilized in 10% sodium hypochlorite, and washed two times in sterilized water. The tissues were mashed using pestle and mortar, shaken for 15 min in sterilized water, streaked onto nutrient agar (NA) medium, and incubated at 30°C for 24 h. From 15 putative strains, three potential strains (SM1, SM2, and SM3) were chosen for further characterization. Bacterial colonies were gram-positive, motile, endospore formers, catalase-positive and oxidase-negative, and produced a whitish pigment on NA medium. Polymerase chain reaction (PCR) amplification with 16S rRNA and Bsub-specific primers (Wattiau et al. 2001) was performed, and the products were sequenced. BLASTn analysis of both genes revealed that all strains were 99% identical to *Bacillus pumilus* strains M3 (GenBank accession no. MF461325) and PS23 (GenBank accession no. KP89557). The nucleotide sequences were later deposited in GenBank (accession nos. MH401100 to MH401102 for 16 rRNA gene and MH428001 to MH428003 for Bsub-specific gene). Phylogenetic analysis of Bsub-specific gene sequences showed all three strains were closely related to *B. pumilus* AGERI-PB1 reference strain in GenBank database with 99% similarity (accession no. LC385524). A pathogenicity test was carried out on 3-month-old superclone RRIM 3001 seedlings by inoculating 200 µl of 10<sup>8</sup> CFU/ml bacterial suspensions of all three strains to the upper part of the stem using a sterilized syringe. Parafilm was used to cover the inoculation sites. Six replicates were used for each treatment. Control seedlings were inoculated with sterilized water. Within 21 to 28 days postinoculation, rubber seedlings inoculated with *B. pumilus* strains produced symptoms such as necrosis on the leaves, cankers, exhibiting ooze, and tumor-like bacteriosis as observed naturally in the field. Control seedlings remained asymptomatic. Reisolation of the bacterium from the symptomatic tissues confirmed the presence of *B. pumilus* based on phenotypic characteristics and molecular characterization. To the best of our knowledge, this is the first official report of *B. pumilus* causing trunk bulges on rubber trees in Malaysia. Recent reports revealed that *B. pumilus* is pathogenic by causing disease to various type of plants including muskmelon (Song et al. 2018), ginger (Peng et al. 2013), and Scots pine (Kovaleva et al. 2015).

**Keyword:** *Bacillus pumilus*; Trunk bulges; Rubber tree; *Hevea brasiliensis*; Malaysia