Foods 2025 Conference
The 6th International Electronic Conference on Foods
28–30 October 2025 | Online
MDPI

Identification and characterization of phage-susceptible Bacillus subtilis strains isolated from Thua Nao

Asst. Prof. Dr.Ekachai Chukeatirote
Mae Fah Luang University, Thailand

INTRODUCTION & AIM

Bacillus subtilis has been reported as a predominant species used on fermented soybean foods such as Japanese Natto, Indian Kinema, Korean Cheonggukjang, and Thai Thua Nao (Gopikrishna et al., 2021). It is an essential bacterium contributing to distinct attributes of the fermented soybean products, including appearance, aroma, flavor, and texture. Thua Nao (Thai fermented soybean food), is mainly used as an important ingredient in a variety of local dishes. Several *B. subtilis* strains with desired characteristics have been used as starter cultures in Thua Nao production to improve nutritional value (Chukeatirote et al., 2025).(Chukeatirote et al., 2025 and references therein).

Bacteriophages (or phages) are widespread in nature, and usually seen as a hazard for food industry as they can harm the bacterial inoculum and thus accountable for fermentation failure and economic losses. As a result, impacts of phages on fermented foods involving bacteria are of great concerns (Verreault et al., 2011, Lavelle et al., 2018, Pujato et al., 2019). In contrast, information of the bacteriophages in the fermented soybean foods is scarce, and is limited to a few products. There is almost no information regarding the bacteriophage of *B. subtilis* in Thua Nao. In earlier work, Gewtaisong et al. (2023) isolated a BasuTN3 bacteriophage specific to *B. subtilis* strain TN3, a potential candidate for Thua Nao starter culture. The information obtained is anticipated to be useful as a reference for future study dealing with phage-sensitive and phage-resistant *Bacillus* bacterial strains.

METHOD

***Bacillus* bacterial strains** obtained from Asian fermented soybean foods: **DM1-2, FM2-3, and TN3 from Thua Nao, and ASA from Natto.**

Efficiency of plating (EOP) and adsorption studies

Characterization improvement bacteriophage sensitivity was measured by agar overlay test and calculated as a ratio of PFU between the target and reference bacteria (Khan Mirzaei and Nilsson, 2015). To isolate phage-adsorbed cells, the phage suspension (MOI 1.0) was incubated at 37°C and centrifuged. The supernatant contained unabsorbed phages, which were counted and reported as a proportion of the initially determined phage counts (Chen et al., 2016). The optimum variation pH (5–9) and temperature (4, 37, and 45°C) contributed to adsorption.

Identification of phage-sensitive bacterial strains

Phenotypic assessments included Gram-staining, spore formation, oxygen requirement, catalase activity, salt tolerance (5 and 7% NaCl), temperature tolerance (50 and 65°C), IMViC reactions, nitrate reduction, and carbohydrate fermentation. Species identification was conducted using the API-50 CHB kit (bioMerieux).

Bacterial strains were further identified using 16S rRNA sequence, by G-spin genomic DNA extraction kit (iNtRON Biotechnology, Inc.). PCR amplify 16S rRNA using primers 518F and 800R (Ghyselinck et al., 2013). Clean up the PCR products and send them to U2Bio (Thailand) for DNA sequencing. To build a phylogenetic tree, used MEGA 11 software (Tamura et al., 2021) containing *Bacillus* species sequences from the GenBank database (Sayers et al., 2020).

Antibiogram analysis

The phage-sensitive bacterial isolates were examined for their antibiogram pattern by using the disc agar diffusion technique (CLSI, 2020). In this study, antibiotic discs (Oxoid, England) used were vancomycin (30 µg), tetracycline (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), kanamycin (30 µg), ampicillin (30 µg), bacitracin (10 U), and streptomycin (10 µg).

RESULTS & DISCUSSION

The proteolytic activity was determine the potential of isolated *Bacillus* strains to be starter inoculum for soybean fermentation as the relative index value (RI values), FM2-3, TN3, and ASA shown 1.48±0.24, 1.50±0.15, and 1.20±0.06, respectively (DM1-2 was shown no clear zone in this activity).

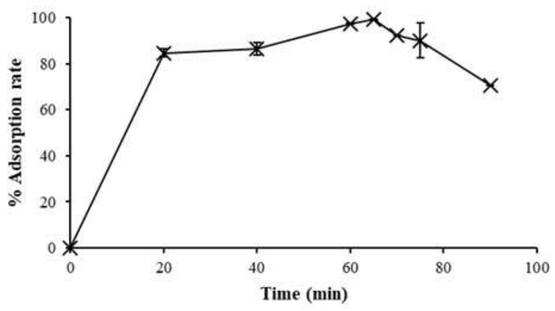

Fig. 1. Adsorption rate of the BasuTN3 phage to *B. subtilis* TN3.

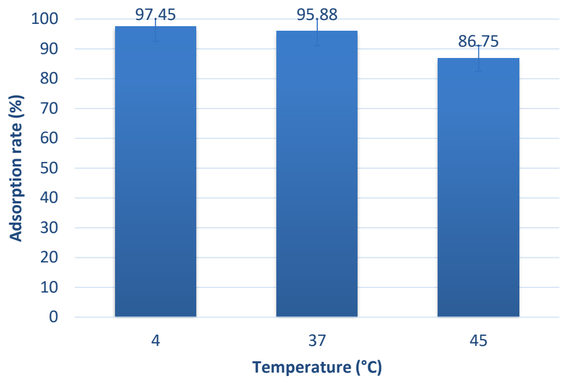

Fig. 2. Effect of temperature on the adsorption of phage BasuTN3 after 60 min.

The EOP values revealed that *B. subtilis* strain TN3 was greatest to be host than strain ASA, which The EOP values for TN3 was 1.00 and strain ASA was 0.88.

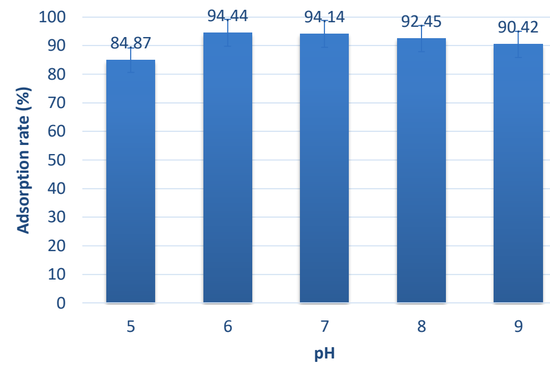

Fig. 3. Influence of pH on the adsorption of the phage BasuTN3 after 60 min.

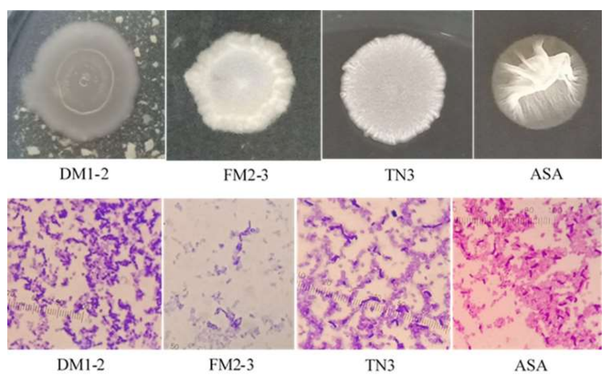

Fig. 4. Morphological features of colony (top) and cell (bottom) of *B. subtilis* strains used in the present study.

16S rRNA gene and phylogenetic analysis

The sequence information showed that their 16S rRNA genes shared more than 99% homology with various strains of *B. subtilis*, *B. velezensis*, and *B. amyloliquefaciens* (Fig.5)

Biochemical characteristics

Gram-positive, rod-shaped bacteria are present in all strains (Fig. 4), and biochemical assays indicate that they are facultative anaerobes, positive in Voges-Proskauer, starch hydrolysis, and glucose fermentation. Thau nao strain showed an ability that is similar to its biochemical profile. It has been demonstrated that ASA, FM2-3, and TN3 have a significant potential as inoculums for soybean fermentation in protease production tests.

Antimicrobial susceptibility test

All *B. subtilis* strain showed susceptibility to ampicillin, chloramphenicol, and tetracycline. Overall data obtained suggest that these strains shown ability to used in food fermentation and useful for industrial application considering from its origin and antibiogram data.

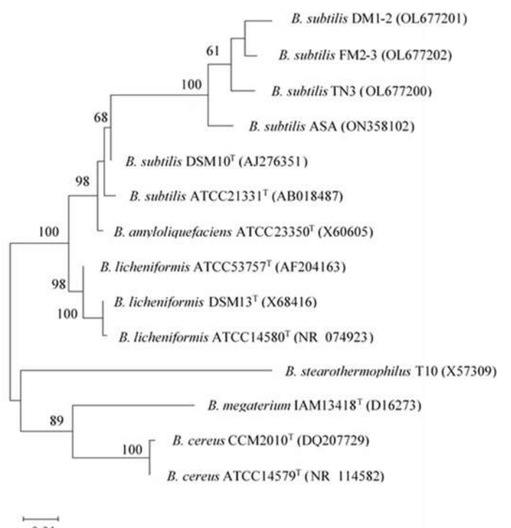

Fig. 5. Phylogenetic relationships of *Bacillus* strains ASA, DM1-2, FM2-3, and TN3 based on their 16S rRNA gene sequence similarity to the reference *Bacillus* strains.

CONCLUSION

Considering that these strains are potential for starter culture use, an occurrence of the lytic phage (e.g., BasuTN3) will be a threat for this utilization. For comparative purpose, these given reference data are expected to be useful for future studies dealing with *B. subtilis* strains (or even other closely related bacteria).

FUTURE WORK / REFERENCES