**Review Article**

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**Significance of bacteriophages in fermented soybeans: A review**

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**Abstract:** Bacteriophages are ubiquitous and have been reported to have been found in many food products. Their presence is important as they have the ability to interact with their bacterial host in food matrices. Fermented soybean products, one of the most widely consumed ethnic foods among Asian people, are prepared naturally and include Japanese Natto, Indian Kinema, Korean Chongkukjang and Thai Thua Nao. This review highlights bacteriophages which have been isolated from fermented soybean products and also includes an overview of their diversity, occurrence as well as their significance.

**Keywords:** bacteriophage; biocontrol; fermented soybean; influence.

**Introduction**

Fermented foods are defined as those which have been processed by microbial activity. In general, fermented food products can be classified on the basis of the raw materials used (i.e. beverages, dairy, fish or meat or fruit and vegetable products) (1). Well known fermented foods which have been popularly consumed include fermented dairy products and fermented sausages. Others may be considered ‘indigenous’ and consumed only by particular consumer groups; these include fermented soybeans and fish sauce. According to the definition, fermented foods must be produced by microbes (as well as their enzymes), it is therefore not surprising that fermented food products are a reliable reservoir for microbiota, including bacteriophages (especially for those products produced by the action of bacteria).

Bacteriophages or phages, are viruses which can infect bacteria (Figure 1A). They are ubiquitous and may be the most abundant entities on Earth (2). Once denied, it is now widely accepted that phages have played a major role in the regulation of bacterial communities in every ecosystem (3, 4). Phages can interact with their host by either the lytic pathway or lysogenic mode. Lytic phages are known to disrupt bacterial metabolism and result in cell lysis. In contrast, lysogenic phages (also known as temperate phages) can integrate their DNA into the host genome, replicate together, and thus establish a long-term relationship with their bacterial host (Figure 1B) (5). Due to their simple structure and host specificity, bacteriophages have played a pivotal role in the field of molecular biology and biotechnology. Many biological concepts can be resolved by the use of bacteriophages, for example the Hershey–Chase experiment (6). More recently, bacteriophages have captured interest among the scientific community specifically for phage therapy, phage display systems, vaccine delivery vehicles and even for diagnostic use (7).

Phages have also contributed considerably to the food industry. Many phages have been isolated and studied from various food products. Table 1 gives examples of fermented food products in which bacteriophages were isolated and studied. Based on different food substrates, the abundance of phages has been demonstrated in a wide range of fermented food products. Most bacteriophages commonly isolated and studied to date are specific to lactic acid bacteria (LAB) (23). It should be noted however, that other bacteriophages infecting other bacterial groups are also present and remain to be studied, for example the bacteriophage groups present in fermented foods not associated with LAB. In general, the presence of phages appears to be detrimental to bacterial starter cultures used and often results in food production failure (24). On the other hand, phage infecting bacterial pathogens can be considered beneficial, as many have...
Table 1: Fermented food products and their associated bacteriophages.

<table>
<thead>
<tr>
<th>Fermented foods</th>
<th>Bacterial host</th>
<th>Phage effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy-based</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Cheese</td>
<td>LAB</td>
<td>Lytic activity to LAB strains and commercial starter culture</td>
<td>8 - 11</td>
</tr>
<tr>
<td>- Yoghurt</td>
<td>S. thermophilus\n L. delbrueckii</td>
<td>Lytic activity and temperate phage</td>
<td>12 - 14</td>
</tr>
<tr>
<td>Vegetable-based</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Fermenting cucumber</td>
<td>LAB</td>
<td>Lytic phage against commercial starter culture</td>
<td>15 - 17</td>
</tr>
<tr>
<td>- Kimchi</td>
<td>LAB</td>
<td>Lytic activity to LAB population</td>
<td>18, 19</td>
</tr>
<tr>
<td>- Sauekraut</td>
<td>LAB</td>
<td>Lytic activity to starter culture</td>
<td>20</td>
</tr>
<tr>
<td>Animal-based</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Fermented sausages</td>
<td>LAB</td>
<td>Lytic activity to starter culture</td>
<td>21</td>
</tr>
<tr>
<td>- Nham (fermented pork)</td>
<td>Weissella cibaria</td>
<td>Lytic activity</td>
<td>22</td>
</tr>
</tbody>
</table>

Notes: LAB = Lactic acid bacteria; S. = Streptococcus; L. = Lactobacillus.

Figure 1: Bacteriophage and its life cycle. (A) The structure of a tailed bacteriophage; (B) The phage life cycle: lytic and lysogenic cycle.
reported the ability to control foodborne pathogenic bacteria (25, 26). Fermented soybeans (FSB) are another type of fermented food for which soybeans are used as the raw material. Interestingly, several studies have shown that the predominant bacterial group of this product is *Bacillus* species, and not LAB (1, 27). With only a few studies having been performed to date regarding phages infecting the *Bacillus* species in fermented soybean products, the aim of this review was to increase awareness of phage occurrence, and its potential role and significance during the soybean fermentation process.

### Fermented soybeans (FSB)

Soybeans [*Glycine max* (L.) Merrill] belong to the family Leguminosae and are one of the most important crops worldwide (28). Soybean seeds are protein-rich and contain many beneficial nutrients, however in order for human consumption, the soybeans must be cooked to destroy anti-nutritional factors such as trypsin inhibitor and hemaglutinins (lectins) (29). The soybeans can then be safely consumed or further processed into various products. The fermentation of soybeans is a process which results in the formation of novel foods products (fermented soybeans) with benefits including prolonging the shelf life.

There are many kinds of FSB products including Japanese Natto, Korean Chongkukjang, Indian Kinema and Thai Thua Nao (30). Figure 2 shows representative images of Thai Thua Nao products. (A - B) Fresh Thua Nao products. (C - D) Dried Thua Nao products.

The majority of FSB products are traditionally produced, using naturally occurring microbes. Natto is one of the best-known examples and is prepared
using a pure starter culture of *B. subtilis* strain *natto*, for commercial purposes (31). The *Bacillus* species are predominant in FSB products and thus considered to play a key role during fermentation (38, 39). Other bacterial strains have also been detected, although their presence is often considered a ‘contaminant’ as they do not appear to play a role in the fermentation process (27).

### Historical study of bacteriophages in relation to FSB products

Since their discovery by Twort (40) and d’Herelle (41), bacteriophages have been isolated and studied by many and are of great interest due to their simple structure, useful not only for fundamental biological studies but also for their potential use in biotechnological applications (42, 43). In terms of food-related bacteriophages, their study can be traced back to 1946, where they were used for staphylococci typing (44, 45).

The bacteriophages related to FSB products were initially isolated from Natto; where the presence of phages resulted in the abnormal production of Natto whereby the viscous polymer polyglutamic acid (PGA) was absent (46). Natto products affected by phages, were then considered ‘spoiled’ and lead to a tremendous loss to the Natto manufacturing business. Since 1967, many bacteriophages infecting Natto bacterial starter cultures have been isolated and characterized, and include the work of Yoshimoto and Hongo (47), Yoshimoto et al. (48), Fujii et al. (49, 50), Yamamoto (51), Tsutsumi et al. (52), Nagai and Yamasaki (53) and Umene et al. (54). In addition, further research relating to the influence of phages on PGA degradation have been performed and include the work of Hara et al. (55), Hongo and Yoshimoto (56-58), Nagai and Itoh (59), Kimura and Itoh (60), Kimura (61), Fujimoto et al. (62) and Ozaki et al. (63). It should be noted however, that although most references relating to the isolation and characterization of Natto phages (between 1967 and 1986) were written in Japanese, there are two excellent English reviews related to Natto bacteriophages (64, 65). Based on these studies, a report describing guidelines to improve the fermentation procedure of Natto was proposed (66).

Subsequent research on FSB-associated phages has been performed on Korean Cheonggukjang (or *Jang*). The first record detailing phage isolation from Korean soybean paste came from the work of Lee in 1978 (67). Lee reported that this phage could infect *B. subtilis* var. 816, and thus result in fermentation failure. However, there has been no further work on this topic until 2011 by Korean scientists and to date, there have been only seven research papers on this topic (between 2011 and 2018). In contrast, the bacteriophage findings have been related to *Bacillus*, normal flora strains in Cheonggukjang products. This was expected, as no specific inoculum is required for Cheonggukjang fermentation. Cheonggukjang and other FSB products (except Natto) are typically produced in households using naturally occurring bacterial cultures. Much work has focused on phages infecting *B. cereus* and aim to employ them as biocontrol agents (32, 68 - 71). The bacteriophages specific to *B. subtilis* have been found (72) and confirmed to play a detrimental role in the quality of resulting Cheonggukjang products (73). For other FSB products, there are currently no reports describing phages. Figure 4 shows a timeline of the major studies associated with phages isolated from FSB products.

### Classification and occurrence of fermented soybean bacteriophages: an overview

One of the distinct features of Natto is the presence of a mucous slime substance known as polyglutamic acid (PGA) (74). Notably, Natto affected by phages fail to produce PGA and it was this observation that initiated a focus on the presence of phages in FSB products. In 1967, Fujii et al. reported on the isolation of a phage known as PN-1 infecting the *B. subtilis (natto)* starter cultural strain from an abnormal Natto product (reduced or no PGA present). From there, many Japanese groups found further evidence of Natto phages from such products, as well as
Natto factories and have studied their characteristics (64, 65).

During this period, isolated Natto phages were screened and classified by a serological method. Work by several research groups resulted in the classification of Natto phages into between 2 and 4 groups (46, 48). There has been no further studies describing the relationship of these phages at this time. The phages were also named differently. It was not until 2009, after a detailed study of the Natto bacteriophages was performed by Nagai and Yamasaki, that a reclassification occurred. The Natto phages were analysed using a DNA hybridization technique, and their morphology uncovered, as well as other physicochemical analyses. Based on this data, many Natto phages previously isolated were then grouped into two types: Group 1 (represented by the JNDMP isolate) and Group 2 (represented by the ONPA isolate) (53). Key features of the two Natto phage groups are summarised in Table 3. In addition, based on the phage PM1 genome, a specific primer set was developed, designed for the selective amplification of a 0.53kb DNA region of the 1.1kb *Eco*RI fragment of the PM1 DNA (54). The existence of this amplified product, was distinct and strongly associated with the PM1 and PM1-related phages, and proved useful for the detection and distribution of FSB phages. It is expected that this finding may be useful in the FSB production pipeline (such as in the Natto’s factory) to prevent and control phage infection (54).

At present, bacteriophage nomenclature and classification are governed by the International Committee on Taxonomy of Viruses (ICTV). Many criteria have been proposed for phage classification, including nucleic acid nature, particle structure and nucleotide or amino acid sequences (75), although it should be noted that viral morphology observed by electron microscopy

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**Table 3:** Key characteristics of the two Natto phage groups (adapted from 64, 65).

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (JNDMP)</th>
<th>Group 2 (ONPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head (diameter, nm)</td>
<td>60</td>
<td>89</td>
</tr>
<tr>
<td>Tail (width x length, nm)</td>
<td>7 x 200</td>
<td>9 x 200</td>
</tr>
<tr>
<td>Sheath (width, nm)</td>
<td>Absent</td>
<td>23</td>
</tr>
<tr>
<td>Genomic size (kb)</td>
<td>42</td>
<td>91</td>
</tr>
<tr>
<td>Latent time (min)</td>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>Burst size</td>
<td>46</td>
<td>72</td>
</tr>
<tr>
<td>Heat stability (°C)</td>
<td>53</td>
<td>63</td>
</tr>
<tr>
<td>Mg²⁺ requirement</td>
<td>Required</td>
<td>Not required</td>
</tr>
</tbody>
</table>

Host range
- *B. subtilis (natto)*    +  +
- *B. subtilis Marburg*    -  -
- *B. cereus*              -  -
- *B. brevis*              -  -
- *B. megaterium*          -  -
remains important (75). Based on this aspect, phages are either tailed, polyhedral, filamentous or pleomorphic. Of these structures, tailed phages (Order Caudovirales) constitute the largest, most widespread group of bacterial viruses (ca. 96%) and can be classified into three families: Myoviridae (with contractile tail), Siphoviridae (with long, non-contractile tail), and Podoviridae (with extremely short, non-contractile tail) (75). A diagram showing the morphological structures of these three tailed phage families is presented in Figure 5. All phages isolated from the FSB products to date have been tailed phages, grouped into Myoviridae or Siphoviridae. For the two Natto phage groups, Group 1 is Siphoviridae, and Group 2 is Myoviridae (Figure 5 and Table 4).

Other studies of FSB phages have come from Cheonggukjang products. In 2011, the phages infecting B. subtilis and B. cereus have been isolated from Korean Cheonggukjang (33, 72). Since then, further work regarding this topic has been extended and most studies have focused on phages infecting B. cereus, aiming to use these phages as a biocontrol agent for controlling foodborne pathogenic bacteria (33, 68, 71). Recently, Ghosh et al. (73) has shown that the phages infecting B. subtilis are abundant in Cheonggukjang products and may cause detrimental effect to the fermentation process, as well as the products quality. The majority of phages isolated from Cheonggukjang products are Myoviridae and only the phages reported by Oh et al. (71) are Siphoviridae (Table 4).

Significance of phages in soybean fermentation and the FSB products

Phages are abundant, found in both natural and man-made environments. This includes the fermentation of soybeans which provides a unique niche for the Bacillus-phages. As mentioned above, many phages related to the Bacillus bacterial group can be isolated during the soybean fermentation process, where a particular group of Bacillus species is present. The occurrence of phages is usually considered detrimental as they have the potential to cause failure (or delay) in food fermentation, ultimately leading to poor quality products. However, phages found naturally in food can also represent an alternative means to combat foodborne pathogenic bacteria—a major concern within the food industry. Similarly, significance of the phages found during soybean fermentation and within FSB products can be viewed both positively and negatively, and this will be discussed below.

Degradation risk to FSB products

From the beginning, phages within FSB products have been isolated from failed soybean fermentations or from poor quality FSB products. In 1967, Fujii et al. reported the first isolation of bacteriophages from abnormal Natto (i.e. due to the absence of the viscous biopolymer). These abnormal Natto products were considered 'spoiled' and thus discarded, a major problem for the Natto production industry. During 1960 - 1980, several Japanese researchers used abnormal Natto as a major source for the isolation and screening of phages (46, 48, 49). It was shown that these isolated phages could infect B. subtilis strain natto, an inoculum used for Natto preparation. The Natto production process relies on the metabolic capabilities of the B. subtilis starter culture, which could then be delayed, or even worse disrupted, resulting in poor quality Natto products.

One of the best-known examples of the phage effect on Natto products is the degradation of γ-polyglutamic acid (γ-PGA). PGA is a water-soluble, biodegradable substance comprising of D- and L-glutamic acids polymerized via γ-glutamyl bonds (77). In general, PGA is produced predominantly by bacteria belonging to Bacillus species, such as B. licheniformis, B. subtilis, B. megaterium, B. pumilis, B. mojavensis and B. amyloliquefaciens. It is worth mentioning that at least one Gram-negative bacterium, Fusobacterium nucleatum, and some archaea are also able to produce the PGA (78). PGA biosynthesis is a multi-step reaction occurring via a cytoplasmic membrane-bound enzyme complex (the CapABC proteins) using L-glutamic
Acid as a substrate (77). Physiological functions of PGA in these bacteria have been proposed; potentially as a barrier to attack via phages, or perhaps as a virulence factor (77). For FSB products, some PGA-producing *Bacillus* strains have been isolated from Natto, Doenjang and Kinema (54-63, 79-81). It should be noted however, that the ability to produce PGA is strain-specific, for example *B. subtilis* strain *natto* can produce PGA, whereas *B. subtilis* Marburg cannot (64). The presence of PGA is one of the key characteristics of Natto generating its sticky appearance.

Although there is no reported range for optimum PGA quantities, it is generally acknowledged that the higher the PGA content, the higher the products quality (especially for Natto products) (59, 64, 65).

PGA degradation in Natto was initially described by Fujii et al. (46), followed by isolation of the phage culprit. It has been shown that *B. subtilis* strain *natto* infected with the phage was not able to accumulate PGA and resulted in 'spoiled' Natto. Further research revealed that this phage could produce extracellular PGA depolymerase enzyme.

### Table 4: Detailed characteristics of FSB-related phages.

<table>
<thead>
<tr>
<th>FSB-related phages</th>
<th>Host</th>
<th>Family</th>
<th>Genome size</th>
<th>Morphology</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Natto</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JNDMP</td>
<td>BS</td>
<td>S</td>
<td>42 kb</td>
<td>Hexagonal head (diameter, 60 nm) Non-contractile tail (7 x 200 nm)</td>
<td>53</td>
</tr>
<tr>
<td>ONPA</td>
<td>BS</td>
<td>M</td>
<td>91 kb</td>
<td>Hexagonal head (diameter, 89 nm) Contractile tail (9 x 200 nm) with a sheath (23 nm)</td>
<td>53</td>
</tr>
<tr>
<td>ϕNIT1</td>
<td>BS</td>
<td>M</td>
<td>155,631 bp</td>
<td>Isometric head (100nm in diameter) Contractile tail (250 nm long)</td>
<td>63</td>
</tr>
<tr>
<td>PM1</td>
<td>BS</td>
<td>S</td>
<td>50 kb</td>
<td>A long, non-contracted tail virus (similar to JNDMP)</td>
<td>54</td>
</tr>
<tr>
<td>BN100</td>
<td>BS</td>
<td>S</td>
<td>42 kb</td>
<td>Hexagonal head (60 by 67 nm) Non-contractile tail (7 x 200 nm) (similar to JNDMP)</td>
<td>59</td>
</tr>
<tr>
<td>PBND8</td>
<td>BS</td>
<td>M</td>
<td>ND</td>
<td>Hexagonal head (diameter, 39 nm) Contractile tail</td>
<td>52</td>
</tr>
<tr>
<td><strong>Cheonggujkjang</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>816 phage</td>
<td>BS</td>
<td>ND</td>
<td>ND</td>
<td>Hexagonal head (diameter, 160 - 240 nm)</td>
<td>67</td>
</tr>
<tr>
<td>JBP901</td>
<td>BC</td>
<td>M</td>
<td>159, 492 bp</td>
<td>Hexagonal head (diameter, 95 nm) Contractile tail (170 nm long)</td>
<td>33, 69</td>
</tr>
<tr>
<td>BCP1-1</td>
<td>BC</td>
<td>M</td>
<td>150 kb</td>
<td>Icosahedral head (diameter, 95 nm) Contractile tail (220 nm long)</td>
<td>68</td>
</tr>
<tr>
<td>BCP8-2</td>
<td>BC</td>
<td>M</td>
<td>159,071 bp</td>
<td>Similar to BCP1-1, with 210 nm long contractile tail</td>
<td>68, 70</td>
</tr>
<tr>
<td>Bp-K2</td>
<td>BS</td>
<td>M</td>
<td>21 kb</td>
<td>Icosahedral head (50 by 80 nm) Contractile tail (85 - 90 nm long) with a basal plate</td>
<td>72</td>
</tr>
<tr>
<td>BSP18</td>
<td>BS</td>
<td>M</td>
<td>ND</td>
<td>Isometric head (90 nm in diameter) Contractile tail (200 nm long)</td>
<td>73</td>
</tr>
<tr>
<td>CAU series</td>
<td>BC</td>
<td>S</td>
<td>ND</td>
<td>Hexagonal head (100 nm in diameter) Non-contractile tail (197 nm long, 11 nm wide)</td>
<td>71</td>
</tr>
<tr>
<td><strong>Soy sauce</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ϕD10</td>
<td>TH</td>
<td>S</td>
<td>ND</td>
<td>Hexagonal head (55 nm in diameter) Non-contractile tail (200 nm long, 9 nm wide)</td>
<td>76</td>
</tr>
</tbody>
</table>

Notes: BS = *Bacillus subtilis*; BC = *B. cereus*; TH = Tetragenococcus halophila; S = Siphoviridae; M = Myoviridae; ND = No data.
which digested the PGA into di- and tri-γ-glutamate, and hence a reduction in viscosity (57, 58). Subsequently, Kimura and Itoh reported another PGA-degrading enzyme, the so-called ‘PghP’, from the phage ΦNIT1 (60). The PghP (γ-PGA hydrolase of phage) is a monomeric enzyme of 22.9 kDa able to digest the PGA to tri-, tetra-, and penta-γ-glutamate, suggesting that the PghP enzyme catalyzes the PGA differently from the PGA depolymerase of Hongo et al. (58). Unfortunately, Hongo’s enzyme has not been purified or studied further. The PghP work also showed that the phage ΦNIT1 has an apparent advantage over a phage that did not produce PghP when infecting the PGA-forming Bacillus cells (60). This finding supports the possible role of PGA as a host barrier, preventing the host cells from phage infection. Additionally, the PghP has been crystallized to determine its 3D-structure, in which the enzyme was found to belong to space group P3_21 or P3_21(62).

Apart from the phage’s effect on PGA hydrolysis, a study of the phage ΦNIT1 genome revealed a putative levanase gene (orf477) encoding a possible levansucrase (63). Levan, a fructan polysaccharide that has also been found in FSB products as another biopolymer (82-84). Based on the comparative genomic analysis, the existence of the pghP and levP (orf477) was found in several Natto phages (including the ΦNIT1) (63). The role of the PghP enzyme produced by the ΦNIT1 phage as a strategy to infect the PGA-forming host cells was confirmed in this study (63). LevP was expected to degrade fructan, however, this was not clear from the phage growth and infection, and thus requires further investigation (63).

As a result, phage contamination poses a serious threat to the Natto production process, as sensitive B. subtilis host cells were lysed when encountering the phages. It has been hypothesized that the phages were brought into the factory and contaminated the subsequent fermentation process via the raw soybeans or dust in the air (64). Further investigations revealed that apart from abnormal Natto, the phages were also found in the factory and in the effluent (48, 49). It should also be noted that the phages were often isolated from the old factories where the factory walls were made of clay (an old style of Japanese building). As expected, the phages were found abundantly in the fermentation room where the bacterial starter cultures were inoculated (49). With this knowledge, a guideline to improve the Natto fermentation process was compiled and proposed by Nakajima (66) and Takiguchi et al. (89). Critical regulations to prevent phage contamination included i) the strict separation of the entrance and exit; ii) implementation of correct hand washing; iii) the immediate removal of abnormal Natto; iv) the use of sterilized water; v) UV installation; vi) the use of stainless steel equipment (no wooden tools); vii) regular cleaning of the factory; and viii) educating the workers about hygiene practices. Since the implication of these rules and regulations, phage detection has decreased enormously and in fact is rarely detected in the modern Natto factories (64).

Information regarding the effect of bacteriophages on other FSB products however, is limited. Apart from Natto, the only FSB phages known to date were isolated from Korean Cheonggukjang. The first report of phage presence and its effect on Korean soybean paste can be traced back to 1978 (67), although information related to this topic is minimal. Lee (67) reported a phage which could infect B. subtilis var. 816, a starter culture in manufacturing the soybean paste. This bacterial host-phage infection caused an occasional failure in soybean fermentation, subsequently leading to poor quality products (67). It was not until 2011, that Kim et al. (72) further described this event in which the phage Bp-K2 isolated from Chungkookjang exhibited lytic activity on various strains of B. subtilis. However, only the phages characteristics were determined with no report of its effect on soybean fermentation. Detailed information relating to this scope has recently been published in 2018, describing a similar phenomenon which was also found in Korean FSB products. Ghosh et al. (73) revealed that there was a high prevalence of phages infecting B. subtilis in Cheonggukjang. Phages in contact with bacterial host cells stall bacterial growth and yield products of poor quality (73).

Beneficial role as biocontrol agents

Among the naturally produced FSB products, currently Natto is the only product for which a pure starter culture of B. subtilis strain Natto is used in the production process (31). Due to Natto’s popularity among Japanese consumers, the production process of Natto has been strictly controlled to guarantee the products quality and safety. Therefore, the microbial contamination of Natto product is considered to be ‘minimal’. However, unlike Natto, other FSB products are conventionally produced by artisanal techniques. These FSB products can be considered ‘indigenous’ and are produced locally from knowledge passed from generation to generation. More importantly, their fermentation process occurs naturally by mixed microbial cultures, although many studies confirm that Bacillus species are predominant (27). As a result, these FSB production processes (except Natto) can
be regarded as ‘non-sterile’. Microbial contamination, especially from pathogens can be expected and once developed generates a serious issue for consumers.

There are no specific regulations regarding the microbiological safety of FSB products, and varies from country to country. For example, in Thailand, there is only a guideline of ‘Community Product Standards’ for Thua Nao products, which does not include enough detail for microbiological analyses (85). Nevertheless, to ensure public safety, the FDA of Thailand has established guidelines related to microbiological contaminates of foods and related food products (86) in accordance with other international standards such as Codex and ICMSF (87, 88). Noted specifications to indicate acceptable quality of foods includes: i) total aerobic count of less than 4 log CFU/g; ii) Enterobacteriaceae count of less than 100 CFU/g; iii) Escherichia, Listeria, Vibrio, and Staphylococcus count of less than 20 CFU/g; and iv) pathogenic Bacillus including B. cereus count of less than 3 log CFU/g (88).

Of these FSB products (except Natto), there have been many reports describing events of microbial contamination. These include an occurrence of the Enterobacteriaceae members in Kinema (34), Escherichia coli, and B. cereus in Thua Nao (90), and B. cereus in Korean soybean paste (71). In these cases, the FSB-isolated phages can be acknowledged as ‘beneficial’ against such foodborne pathogenic bacteria. In terms of food safety, the presence of these allied phages is useful and once studied could be used as alternative means to eliminate these pathogens. To date, many research groups have focused on phages which exhibit lytic activity against B. cereus from FSB products. This is not surprising, considering that Bacillus species are widely found in these FSB foods. In particular, many FSB phages infecting B. cereus have been isolated from Korean Cheonggukjang (33, 68 - 71). These FSB phages specific to B. cereus exhibit strong lytic activity and are therefore a promising tool to tackle B. cereus contamination. Although most of the present studies have focused on the FSB phages of B. cereus, there are other contaminated bacterial strains which include E. coli, Salmonella and Staphylococcus (35, 90). Further work screening and isolating phages against these foodborne pathogenic bacteria is therefore of great importance when considering the impact on food safety.

Concluding remarks

Phages are versatile and can be found in all ecosystems where their bacterial hosts are present. In this review, we have focused on the occurrence of the phages relating to FSB products. The majority of phages isolated to date are associated with the Bacillus species. Although FSB phages are a viable risk factor to the bacterial starter cultures used in FSB preparations, they may offer a promising tool in combating foodborne pathogenic bacteria. Up until recently, FSB phages have only been isolated and studied from Natto and Cheonggukjang. It would be of great interest if FSB phages were isolated and studied from other FSB products. Further studies related to phage-host interactions are challenging, but important when considering that the starter culture could be selected or developed for optimal FSB production. In terms of food safety issues, some FSB phages against the foodborne pathogenic bacteria can be applied potentially as biocontrol agents.

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