

# Gut microbiome of black soldier fly larvae for efficient use and purification of organic waste: An environmentally friendly development concept

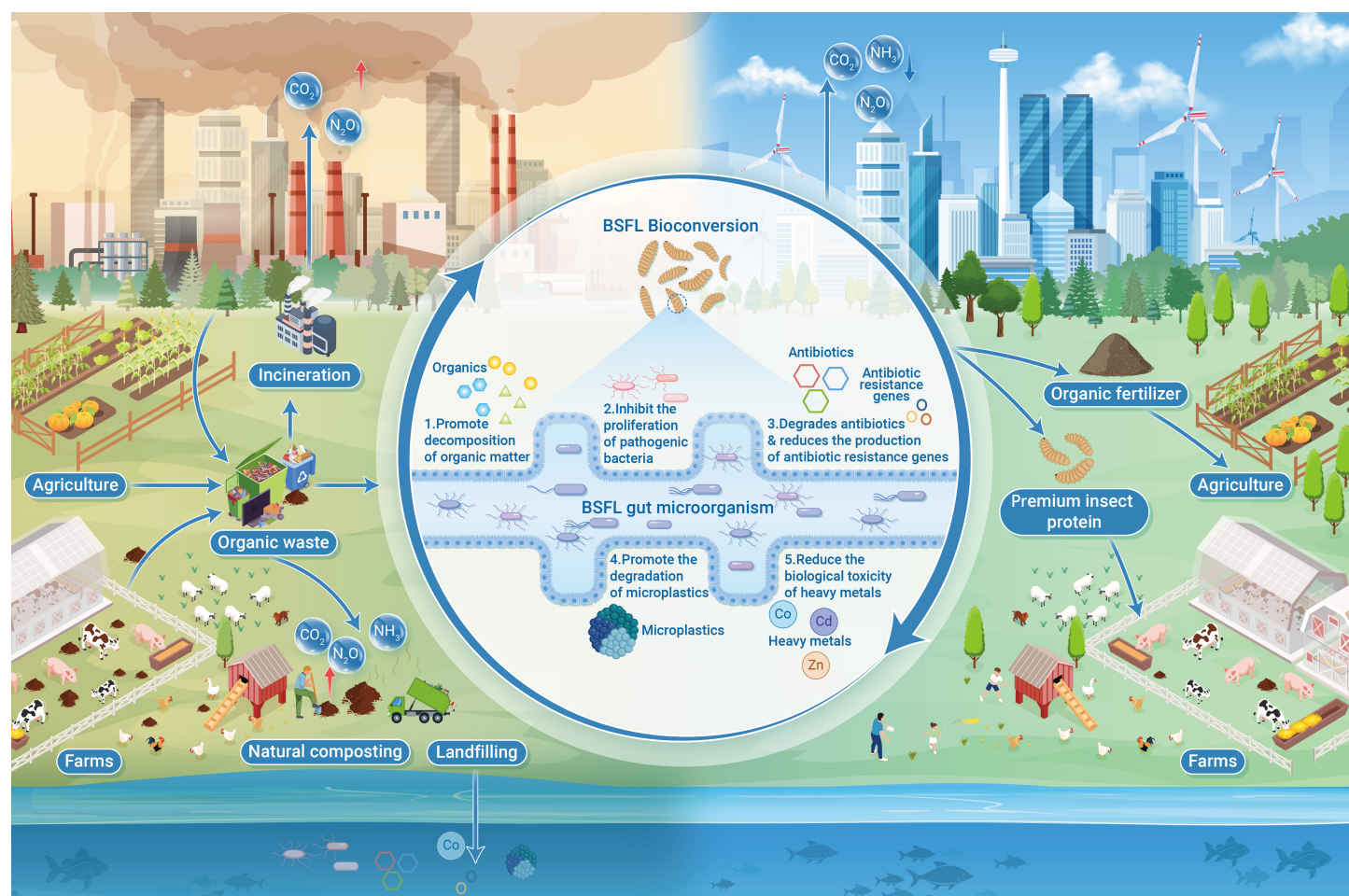
Shiqi Zheng,<sup>1,5</sup> Rui Li,<sup>2,5</sup> Yanhua Huang,<sup>1,5</sup> Menglin Yang,<sup>1</sup> Wuhao Chen,<sup>1</sup> Shuangyan Mo,<sup>1</sup> Renli Qi,<sup>4</sup> Wei Wang,<sup>1</sup> Dan Wan,<sup>2,\*</sup> Yulong Yin,<sup>2,3,\*</sup> and Jie Peng<sup>1,\*</sup>

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## GRAPHICAL ABSTRACT



## PUBLIC SUMMARY

- Black soldier fly larvae (BSFL) drive the efficient operation of sustainable crop-livestock systems.
- The gut microbiota of BSFL assist the host in accelerating the degradation and resource utilization of organic matter.
- BSFL gut microbiota can inhibit the proliferation of pathogenic bacteria through a variety of mechanisms.
- BSFL gut microbiota promote the degradation of antibiotics, heavy metals, and microplastics in the substrate.
- BSFL gut microbiota serve as a probiotic resource with diverse applications.

# Gut microbiome of black soldier fly larvae for efficient use and purification of organic waste: An environmentally friendly development concept

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The growing global population and increasing human demands have led to a rise in organic waste volumes. These wastes contain potential risk substances like pathogenic bacteria (PB), antibiotics (ABs), heavy metals (HMs), and microplastics (MPs), which can threaten public health through direct contact or food chain transmission. Black soldier fly larvae (BSFL) have emerged as a promising solution for organic waste management and crop-livestock integration. They efficiently reduce organic matter, produce high-quality proteins and fats, generate green biofertilizers, and mitigate harmful substances and greenhouse gas emissions. However, the specific mechanisms by which BSFL transform organic matter, produce organic fertilizers, and reduce harmful substances remain unclear, significantly limiting the further development and application of BSFL bioconversion technology. In recent years, numerous studies have revealed that BSFL gut microbiota possess functions such as promoting the degradation of substrate nutrients, regulating host nutrient synthesis and metabolism, directly or indirectly inhibiting the proliferation of PB, biodegrading or cometabolizing ABs, mitigating the mobility of HMs, and biodegrading MPs. Therefore, exploring and developing BSFL gut microbiota could be of great significance for optimizing the efficiency of BSFL in organic waste transformation, effectively reducing potential risk substances in organic waste, and promoting their wide applications in biomedicine and energy sectors. This review systematically summarizes the composition, function, and environmental impacts of BSFL gut microbiota, focusing on their roles in organic waste transformation and reducing PB, ABs, HMs, and MPs. We believe that in-depth exploration in this field will significantly advance the application of BSFL in integrated crop-livestock systems, which is essential for achieving humanity's sustainable development goals.

## INTRODUCTION

According to the United Nations, the world's population has increased from 2.4 billion in 1950 to 8.2 billion in 2024 and is projected to grow to 9 billion by 2050.<sup>1</sup> However, this dramatic population growth has also led to a significant rise in organic waste, including food waste (e.g., kitchen waste, household food waste), and agro-waste (e.g., crop residues, livestock manure, meat and poultry processing waste, vegetable and fruit waste).<sup>2-4</sup> The United Nations Environment Programme's 2024 report indicates that global annual food waste now exceeds 1.05 billion tonnes.<sup>5</sup> Meanwhile, in China, the discharge of livestock and poultry manure alone amounts to as high as 3.8 billion tonnes (Ministry of Agriculture and Rural Affairs of the People's Republic of China). Food waste contains significant amounts of unused proteins, oils, fats, carbohydrates, and other compounds, and when piled together, these materials can release substantial quantities of malodorous gases, such as ammonia and hydrogen sulfide. Additionally, the high concentration of pollutant liquids that leach from these piles can cause eutrophication of nearby water bodies and contaminate surrounding land through infiltration.<sup>6-9</sup> Furthermore, livestock manures harbor numerous pathogens, including *Salmonella*, *Escherichia coli*, *Bacillus anthracis*, *Shigella*, *Echinococcus granulosus*, and

*Toxoplasma gondii*. Improper treatment of these wastes increases the risk of soil and water contamination by these pathogens.<sup>10,11</sup> The presence of ABs residues and excessive levels of HMs in organic waste such as livestock manure poses significant risks to human health. ABs are widely used in livestock and poultry production due to their effectiveness in controlling diseases and promoting recovery.<sup>12</sup> However, the excessive use of ABs in livestock and poultry can result in the occurrence of antibiotic-resistance genes (ARGs), leading to the emergence of multidrug-resistant bacteria in the environment.<sup>10,13</sup> HMs in animal feed are often not fully absorbed and tend to accumulate in feces and urine. When manure containing HMs is not adequately treated, it can severely harm ecosystems.<sup>14-17</sup> Therefore, finding effective methods to transform and purify the residual nutrients and toxic substances in organic waste is an urgent issue that needs to be addressed.

Microorganisms are crucial participants in natural life activities and material cycles, playing an indispensable role in animal immune responses, growth and development, as well as the decomposition of animal and plant remains and excreta.<sup>18,19</sup> Beyond these functions, numerous studies have identified microorganisms capable of degrading pollutants such as ABs, pesticides, and microplastics (MPs). These findings provide valuable insights into the potential use of microorganisms or microbial enzymes for environmental pollutant remediation.<sup>20-23</sup> For instance, Yoshida et al.<sup>24</sup> discovered that *Ideonella sakaiensis* 201-F6 can degrade polyethylene terephthalate, a widely used plastic material, into harmless terephthalic acid and ethylene glycol. This is achieved through the secretion of PET hydrolase and MHET hydrolase, which exhibit high activity at low temperatures. The strain effectively degraded polyethylene terephthalate films within six weeks. Similarly, Zhang et al.<sup>25</sup> identified a strain of *Desulfotomaculum* capable of degrading phenanthrene, a recalcitrant organic pollutant, under anaerobic conditions through carboxylation, benzene ring reduction, and ring-opening reactions. This discovery offers new perspectives for the elimination of phenanthrene in anaerobic environments such as lakes and rivers. Therefore, the development of microorganisms or microbial enzymes with pollutant-degrading capabilities holds significant promise for ecological and environmental restoration. Insects, being the most species-rich and biomass-rich organisms in the animal kingdom, also possess complex and functionally diverse gut microbial communities.<sup>26,27</sup> The gut microbiota of these insects exhibit complex and diverse functions, including aiding *Termites* in digesting lignocellulose<sup>28,29</sup> and assisting species such as *Tenebrio molitor*, *Zophobas atratus*, *Galleria mellonella*, and *Cockroaches* in degrading plastics.<sup>30-33</sup> However, for black soldier fly larvae (BSFL), which are among the most promising insects for organic waste bioconversion, the functions of their gut microbiota and their potential for development remain unclear.<sup>34,35</sup>

BSFL are rich in protein and fat, and possess significant nutritional value due to their high content of chitin, fatty acids (including lauric acid, palmitic acid, and myristic acid), and antimicrobial peptides (AMPs). These attributes make them a viable protein source for livestock and aquaculture feeds.<sup>36-42</sup> The decomposition and transformation of organic waste by BSFL primarily rely on the biting and chewing activities of their digestive organs, as well as

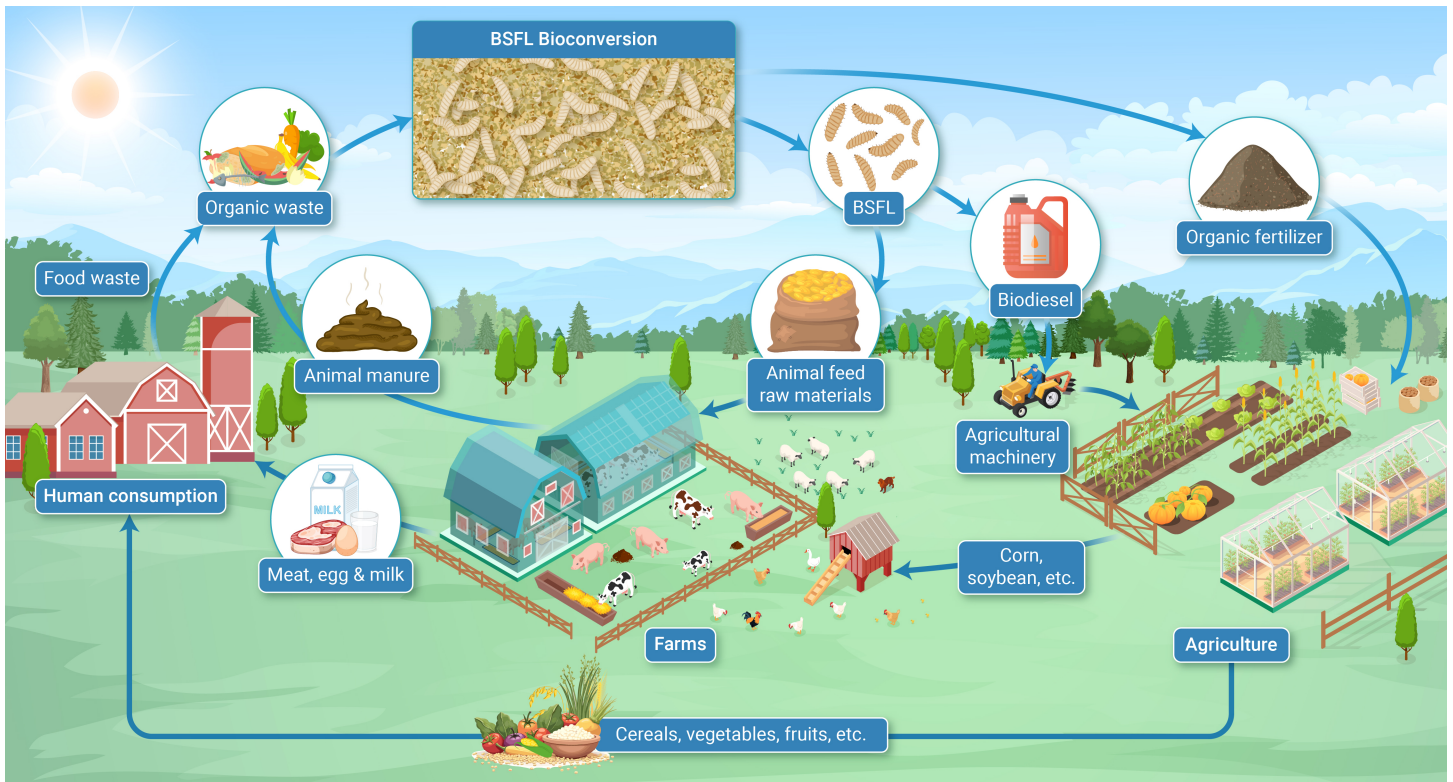


Figure 1. Role of BSFL in crop-livestock integration.

the secretion of various digestive enzymes.<sup>43</sup> The salivary glands of BSFL, which secrete mucoproteins, play a crucial role in lubricating food, while the esophagus, characterized by well-developed muscle layers and a cuticular lining, is responsible for compressing and grinding.<sup>43,44</sup> Additionally, the BSFL gut is rich in a variety of digestive enzymes, including trypsin-like protease, leucine arylamidase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\alpha$ -mannosidase, and  $\alpha$ -fucosidase. These enzymes facilitate the more efficient digestion and decomposition of organic waste by BSFL.<sup>44</sup> However, the specific functions of gut microbiota in the life activities of BSFL remain unclear. The BSFL gut is anatomically divided into the foregut, midgut, and hindgut. The midgut, which is the most developed section and harbors the most abundant microbial communities, can be further subdivided into the anterior midgut, medial midgut, and posterior midgut.<sup>45</sup> The luminal pH varies significantly across these regions: the anterior midgut is weakly acidic (pH~6) with a microbial count of  $1.14 \times 10^7$ – $2.76 \times 10^7$  CFUs/segment; the medial midgut is strongly acidic (pH~2) with a microbial count of  $6.20 \times 10^6$ – $9.20 \times 10^6$  CFUs/segment; and the posterior midgut is alkaline (pH~8.5) with a microbial count of  $6.80 \times 10^7$ – $1.50 \times 10^8$  CFUs/segment.<sup>43,45–47</sup> The distinct distribution of microbial abundance in these midgut segments is likely closely associated with the different physicochemical conditions of each gut region. With continuous research, it has become evident that the gut microbiota of BSFL may play a crucial role in organic matter decomposition, inhibition of pathogenic bacteria (PB) reproduction, and pollutant management.<sup>45,48,49</sup> Recent research has shown that the gut microbes of BSFL can significantly influence host growth and development, immune regulation, nutrient synthesis, and food digestion.<sup>50–57</sup> These microbes also negatively impact the proliferation of pathogens in the substrate, while positively affecting the degradation of ABs, ARGs, MPs, and plasticizers, as well as the reduction of HMs biotoxicity.<sup>58–67</sup> Therefore, this review aimed to systematically summarize the role of BSFL in crop-livestock integration, detailing the composition, structure, and function of their gut microorganisms and their environmental impacts. In particular, this review examined how these microorganisms efficiently utilize and convert organic waste and eliminate pathogens, ABs, HMs, and MPs from organic materials. The review also explored the possible ways to better promote the application of black soldier flies in the recycling of organic waste resources.

### BSFL PLAYS A CRUCIAL ROLE IN CROP-LIVESTOCK INTEGRATION

BSFL contributes significantly to integrating crop cultivation and animal husbandry, forming efficient, eco-friendly crop-livestock integration that support sustainable agricultural development. They effectively recycle organic waste such as food waste, livestock manure, and biological sludge, facilitating resource recycling.<sup>34,68,69</sup> Additionally, the natural antimicrobial compounds in BSFL enhance livestock disease resistance, reducing production costs.<sup>35,42,70</sup> Furthermore, BSFL frass serves as a high-quality organic fertilizer, improving soil structure and enhancing crop yield and quality (Figure 1).<sup>71–73</sup>

### BSFL improves the efficiency of organic waste utilisation

As a saprophagous insect, BSFL can rapidly decompose organic waste, including livestock manure, animal feed, food waste, and others, within a short timeframe.<sup>74–77</sup> Numerous studies have demonstrated that introducing an appropriate density of BSFL can reduce livestock manure (excluding cow manure) by approximately 52.44%, achieving a bioconversion rate of 11.68% (calculated as BSFL body weight gain divided by total organic waste added, based on dry matter content).<sup>78–83</sup> For food waste, BSFL can reduce organic dry matter by approximately 68.64%, with a bioconversion rate of 30.91%.<sup>74,79,84</sup> For animal feed, BSFL can decrease organic dry matter by up to approximately 76.25%, accompanied by a bioconversion rate of 16.90%.<sup>74,84</sup> For low-nutrient organic wastes such as cow manure and sludge, BSFL typically reduces organic dry matter by approximately 39.08%, achieving a bioconversion rate of approximately 2.24%.<sup>74,79,83,84</sup>

However, when cow manure is combined with food waste, slaughterhouse waste, or industrial by-products in specific ratios and subsequently treated with BSFL, substrate reduction rate of 47.2% to 84.9% and bioconversion rates of 15.5% to 19.8% can be achieved.<sup>84</sup> Beyond these findings, this review provides a systematic and comprehensive summary of the effects of diverse organic substrates on BSFL growth performance, nutritional composition, bioconversion rate, substrate reduction rate, etc (Table 1). Compared to organic waste such as food waste and agricultural residues, cow manure contains lower levels of amino acids, proteins, and non-fiber carbohydrates, while exhibiting higher concentrations of cellulose and lignin.<sup>84,85</sup> A deficiency of readily degradable proteins and carbohydrates in the diet of BSFL can significantly inhibit their growth, development, and substrate degradation.<sup>85,86</sup>



Table 1. Comprehensive effects of various organic substrates on growth performance, nutritional composition, and bioconversion efficiency in BSFL

| Main Substrate Type | Other Substrates and Proportions                              | Treatment Duration | Larval Age | Rearing Density               | Fat (%)          | Lauric Acid (%)<br>(as a proportion of total fatty acids) | Protein (%)      | Chitin (%) | Substrate Reduction Rate (%)            | Bioconversion Rate (%) | Protein Conversion Rate (%) | Reference |
|---------------------|---------------------------------------------------------------|--------------------|------------|-------------------------------|------------------|-----------------------------------------------------------|------------------|------------|-----------------------------------------|------------------------|-----------------------------|-----------|
| Chicken manure      | 100% Chicken manure                                           | 13 d               | 6 d        | –                             | 6.98%            | 33.70%                                                    | 46.22%           | –          | 53.33%                                  | 15.07%                 | –                           | 78        |
|                     | 100% Chicken manure                                           | 10 d               | 5 d        | 1500 individuals/kg           | 16.48%           | 27.20%                                                    | 37.07%           | –          | 64.13%                                  | 42.66%                 | –                           | 79        |
|                     | 100% Chicken manure                                           | –                  | –          | –                             | 36.18% (Prepupa) | –                                                         | 41.71% (Prepupa) | –          | –                                       | –                      | –                           | 238       |
|                     | 20-100% Chicken manure + 0-80% Soybean meal                   | 13d                | 3 d        | 2500 individuals/kg           | 15.00-22.00%     | 8.79-22.41%                                               | 40-50%           | –          | –                                       | –                      | –                           | 239       |
|                     | 100% Chicken manure                                           | 15 d               | 3 d        | –                             | 19.94%           | –                                                         | 53.46%           | –          | –                                       | 5.70%                  | –                           | 80        |
|                     | 100% Chicken manure + 0-20 mg/kg sulfadiazine                 | 12 d               | 7 d        | 900 individuals/kg            | 27.66-27.91%     | –                                                         | 38.75%-41.81%    | –          | –                                       | –                      | –                           | 72        |
|                     | 85-100% Chicken manure + 0-15% Biochar                        | 10 d               | 4 d        | 1000 individuals/kg           | –                | –                                                         | 60.88-68.88%     | –          | 43.61-59.75%                            | 5.21-8.31%             | –                           | 240       |
|                     | 100% Chicken manure                                           | 8 d                | 2 d        | 40 g/kg                       | –                | –                                                         | –                | –          | –                                       | 15.31%                 | –                           | 81        |
|                     | 15% Rice bran + 85% Chicken manure                            | 8 d                | 2 d        | 40g/kg                        | –                | –                                                         | –                | –          | –                                       | 19.74%                 | –                           |           |
| Pig manure          | 100% Pig manure                                               | 10 d               | 5 d        | 1500 individuals/kg           | 2.23%            | 4.13%                                                     | 52.74%           | –          | 49.60%                                  | 6.73%                  | –                           | 79        |
|                     | 92-100% Pig manure + 2-8% Urple potato peel powder            | 16 d               | 4 d        | –                             | 13.35-22.00%     | –                                                         | 33.10-56.44%     | –          | 34.65-43.08%                            | –                      | –                           | 241       |
|                     | 100% Pig manure                                               | –                  | –          | –                             | 36.52% (Prepupa) | –                                                         | 42.83% (Prepupa) | –          | –                                       | –                      | –                           | 238       |
|                     | Pig manure (60% moisture content)                             | 10 d               | 8 d        | 500 g/kg                      | 6.05%            | –                                                         | 42.59%           | –          | –                                       | 5.61%                  | –                           |           |
|                     | Pig manure (65% moisture content)                             | 10 d               | 8 d        | 500 g/kg                      | 6.34%            | –                                                         | 45.36%           | –          | –                                       | 6.59%                  | –                           |           |
|                     | Pig manure (70% moisture content)                             | 10 d               | 8 d        | 500 g/kg                      | 7.53%            | –                                                         | 44.91%           | –          | –                                       | 8.63%                  | –                           | 82        |
|                     | Pig manure (75% moisture content)                             | 10 d               | 8 d        | 500 g/kg                      | 8.90%            | –                                                         | 46.60%           | –          | –                                       | 9.57%                  | –                           |           |
|                     | 80% Pig manure + 20% Pineapple waste + <i>Bacillus cereus</i> | 13 d               | 6 d        | 500 individuals/kg            | –                | –                                                         | –                | –          | 42.28%                                  | 18.91%                 | –                           | 242       |
|                     | 100% Pig manure                                               | 12 d               | 10 d       | 1 individuals/cm <sup>3</sup> | –                | –                                                         | –                | –          | 42.70%                                  | 0.92%                  | –                           | 83        |
|                     | 55% Pig manure + 45% Wet distiller grains                     | 12 d               | 10 d       | 1 individuals/cm <sup>3</sup> | –                | –                                                         | –                | –          | 67.11%                                  | 10.05%                 | –                           |           |
|                     | 100% Pig manure solid                                         | 8 d                | 8 d        | 2500 individuals/kg           | –                | –                                                         | –                | –          | 7.60% (The substrate consumption index) | –                      | –                           | 243       |
| Cow dung            | 15% Rice bran + 85%Pig manure                                 | 8 d                | 2 d        | 40 g/kg                       | –                | –                                                         | –                | –          | –                                       | 19.25%                 | –                           | 81        |
|                     | 100% Cow dung                                                 | 10 d               | 5 d        | 1500 individuals/kg           | 1.50%            | 0.98%                                                     | 44.84%           | –          | 52.21%                                  | 3.75%                  | –                           | 79        |
|                     | 100% Cow dung                                                 | –                  | –          | –                             | 35.73% (Prepupa) | –                                                         | 41.25% (Prepupa) | –          | –                                       | –                      | –                           | 238       |
|                     | 100% Cow dung                                                 | 12 d               | 10 d       | 1 individuals/cm <sup>3</sup> | –                | –                                                         | –                | –          | 43.85%                                  | 1.20%                  | –                           | 83        |



Table 1. (Continued)

| Main Substrate Type | Other Substrates and Proportions                                       | Treatment Duration | Larval Age | Rearing Density                 | Fat (%)               | Lauric Acid (%)<br>(as a proportion of total fatty acids) | Protein (%)      | Chitin (%)      | Substrate Reduction Rate (%)            | Bioconversion Rate (%) | Protein Conversion Rate (%) | Reference |
|---------------------|------------------------------------------------------------------------|--------------------|------------|---------------------------------|-----------------------|-----------------------------------------------------------|------------------|-----------------|-----------------------------------------|------------------------|-----------------------------|-----------|
| Cow dung            | 55% Cow dung + 45% Wet distiller grains                                | 12 d               | 10 d       | 1 individuals/–cm <sup>3</sup>  | –                     | –                                                         | –                | –               | 73.41%                                  | 10.54%                 | –                           | 83        |
|                     | 100% Cow dung                                                          | 9 d                | 12–14 d    | –                               | –                     | –                                                         | 36.20%           | –               | 12.70%                                  | 3.80%                  | –                           | 84        |
|                     | 100% Dairy manure                                                      | 24 d               | 6 d        | 1000 individuals/–kg            | –                     | –                                                         | –                | –               | –                                       | 29.57%                 | 6.99%                       | 244       |
|                     | 60% Dairy manure + 40% Chicken manure                                  | 20 d               | 6 d        | 1000 individuals/–kg            | –                     | –                                                         | –                | –               | –                                       | 28.45%                 | 7.45%                       |           |
| Food waste          | 100% Restaurant waste (Contained potatoes, rice, pasta and vegetables) | 18 d               | 6–8 d      | 1666 individuals/kg             | 38.60% (Prepupa)      | –                                                         | 40.70% (Prepupa) | 6.70% (Prepupa) | –                                       | –                      | –                           | 245       |
|                     | 100% Feedstuffs food waste                                             | –                  | –          | –                               | 37.24% (Prepupa)      | –                                                         | 43.69% (Prepupa) | –               | –                                       | –                      | –                           | 238       |
|                     | 100% Food waste                                                        | 10 d               | 5 d        | 1500 individuals/kg             | 42.13%                | 31.30%                                                    | 30.93%           | –               | 89.53%                                  | 59.40%                 | –                           | 79        |
|                     | 100% Canteen waste                                                     | 20 d               | 3 d        | –                               | 44.35%                | –                                                         | 26.11%           | –               | –                                       | –                      | –                           | 246       |
|                     | 100% Canteen waste                                                     | 40 d               | 3 d        | –                               | 47.15%                | –                                                         | 24.94%           | –               | –                                       | –                      | –                           |           |
|                     | 100% Canteen waste                                                     | –                  | 10 d       | 0.6 individuals/cm <sup>2</sup> | 0.6 s/cm <sup>2</sup> | –                                                         | –                | –               | 55.30%                                  | 13.90%                 | 58.70%                      | 74        |
|                     | 100% Kitchen waste                                                     | 10 d               | 5 d        | 1500 individuals/kg             | 37.49%                | 31.10%                                                    | 33.71%           | –               | 84.46%                                  | 52.07%                 | –                           | 79        |
|                     | 100% Canteen waste                                                     | 9 d                | 12–14 d    | –                               | –                     | –                                                         | 36.10%           | –               | 37.90%                                  | 15.30%                 | –                           | 84        |
|                     | 100% Vegetable waste (A mixture of carrots, peas, salsify and celery)  | 15 d               | 6–8 d      | 1666 individuals/kg             | 37.10% (Prepupa)      | –                                                         | 40.10% (Prepupa) | 5.70% (Prepupa) | –                                       | –                      | –                           | 245       |
|                     | 100% Vegetable waste                                                   | 10 d               | 5 d        | 1500 individuals/kg             | 10.64%                | 31.10%                                                    | 48.32%           | –               | 94.86%                                  | 22.10%                 | –                           | 79        |
| Olive pulp          | 100% Vegetable canteen waste                                           | 9 d                | 12–14 d    | –                               | –                     | –                                                         | 24.50%           | –               | 58.40%                                  | 22.70%                 | –                           | 84        |
|                     | 100% Olive pulp                                                        | 8 d                | 8 d        | 2500 individuals/–kg            | –                     | –                                                         | –                | –               | 8.50% (The substrate consumption index) | –                      | –                           | 243       |
| Poultry feed        | 100% Chicken feed                                                      | 12 d               | 6–8 d      | 1666 individuals/kg             | 33.60% (Prepupa)      | –                                                         | 38.80% (Prepupa) | 6.20% (Prepupa) | –                                       | –                      | –                           | 245       |
|                     | 100% Poultry feed                                                      | –                  | 10 d       | 0.6 individuals/cm <sup>2</sup> | –                     | –                                                         | –                | –               | 84.80%                                  | 12.80%                 | 80.40%                      | 74        |
|                     | 100% Poultry feed                                                      | 9 d                | 12–14 d    | –                               | –                     | –                                                         | 36.30%           | –               | 67.70%                                  | 21.00%                 | –                           | 84        |
| Biogas digestate    | 100% Biogas digestate                                                  | 15 d               | 6–8 d      | 1666 individuals/kg             | 21.80% (Prepupa)      | –                                                         | 37.70% (Prepupa) | 5.60% (Prepupa) | –                                       | –                      | –                           | 245       |
| Sludge              | Primary sludge                                                         | –                  | 10 d       | 0.6 individuals/cm <sup>2</sup> | –                     | –                                                         | –                | –               | 63.30%                                  | 2.30%                  | 15.00%                      | 74        |

Table 1. (Continued)

| Main Substrate Type          | Other Substrates and Proportions                          | Treatment Duration | Larval Age | Rearing Density                 | Fat (%) | Lauric Acid (%)<br>(as a proportion of total fatty acids) | Protein (%) | Chitin (%) | Substrate Reduction Rate (%) | Bioconversion Rate (%) | Protein Conversion Rate (%) | Reference |
|------------------------------|-----------------------------------------------------------|--------------------|------------|---------------------------------|---------|-----------------------------------------------------------|-------------|------------|------------------------------|------------------------|-----------------------------|-----------|
| Sludge                       | Undigested sludge                                         | -                  | 10 d       | 0.6 individuals/cm <sup>2</sup> | -       | -                                                         | -           | -          | 49.20%                       | 2.20%                  | 7.80%                       | 74        |
|                              | Digested sludge                                           | -                  | 10 d       | 0.6 individuals/cm <sup>2</sup> | -       | -                                                         | -           | -          | 13.20%                       | 0.20%                  | 1.90%                       |           |
| Mill by-products             | 100% Bran                                                 | 8 d                | 9 d        | -                               | 35.46%  | 51.71%                                                    | 49.98%      | -          | -                            | -                      | -                           |           |
|                              | 75% Bran + 25% Wei safflower seed meal                    | 8 d                | 9 d        | -                               | 38.77%  | 37.97%                                                    | 49.14%      | -          | -                            | -                      | -                           |           |
|                              | 50% Bran + 50% Wei safflower seed meal                    | 8 d                | 9 d        | -                               | 39.08%  | 30.84%                                                    | 51.87%      | -          | -                            | -                      | -                           | 247       |
|                              | 25% Bran + 75% Wei safflower seed meal                    | 8 d                | 9 d        | -                               | 39.61%  | 28.17%                                                    | 52.64%      | -          | -                            | -                      | -                           |           |
|                              | 100% Wei safflower seed meal                              | 8 d                | 9 d        | -                               | 39.02%  | 24.41%                                                    | 53.32%      | -          | -                            | -                      | -                           |           |
|                              | 100% Bran                                                 | 20 d               | 3 d        | -                               | 14.15%  | -                                                         | 42.42%      | -          | -                            | -                      | -                           |           |
|                              | 100% Bran                                                 | 40 d               | 3 d        | -                               | 39.44%  | -                                                         | 49.79%      | -          | -                            | -                      | -                           |           |
|                              | 50% Bran + 50% Food waste                                 | 20 d               | 3 d        | -                               | 39.44%  | -                                                         | 30.79%      | -          | -                            | -                      | -                           | 246       |
|                              | 50% Bran + 50% Food waste                                 | 40 d               | 3 d        | -                               | 39.04%  | -                                                         | 33.78%      | -          | -                            | -                      | -                           |           |
|                              | 100% Mill by-products                                     | 9 d                | 12-14 d    | -                               | -       | -                                                         | 42.10%      | -          | 56.40%                       | 14.90%                 | -                           | 84        |
| Coconut endosperm            | 100% Coconut endosperm                                    | 10-11 d            | 7 d        | -                               | -       | -                                                         | 30.60%      | -          | 62.50%                       | -                      | -                           |           |
|                              | 75% Coconut endosperm + 25% Soybean curd residue          | 10-11 d            | 7 d        | -                               | -       | -                                                         | 39.10%      | -          | 57.60%                       | -                      | -                           |           |
|                              | 50% Coconut endosperm + 50% Soybean curd residue          | 10-11 d            | 7 d        | -                               | -       | -                                                         | 48.40%      | -          | 59.50%                       | -                      | -                           | 248       |
|                              | 25% Coconut endosperm + 75% Soybean curd residue          | 10-11 d            | 7 d        | -                               | -       | -                                                         | 52.60%      | -          | 58.50%                       | -                      | -                           |           |
|                              | 100% Soybean curd residue                                 | 10-11 d            | 7 d        | -                               | -       | -                                                         | 57.7%       | -          | 54.50%                       | -                      | -                           |           |
|                              | 50% Wheat bran + 30% Alfalfa meal + 20% Yellow corn flour | 7 d                | 14 d       | 7500 individuals/kg             | 25.20%  | 31.90%                                                    | 43.42%      | -          | -                            | -                      | -                           | 249       |
| Desiccated coconut           | 100% Desiccated coconut                                   | 7 d                | 14 d       | 7500 individuals/kg             | 47.30%  | 41.84%                                                    | 29.70%      | -          | -                            | -                      | -                           | 249       |
| Tofu waste                   | 100% Tofu waste                                           | 20 d               | egg        | 0.4 g/kg                        | 39.41%  | -                                                         | 38.59%      | -          | -                            | -                      | -                           |           |
|                              | 75% Tofu waste + 25% Chicken manure                       | 20 d               | egg        | 0.4 g/kg                        | 34.09%  | -                                                         | 42.98%      | -          | -                            | -                      | -                           |           |
|                              | 50% Tofu waste + 50% Chicken manure                       | 20 d               | egg        | 0.4 g/kg                        | 19.94%  | -                                                         | 41.69%      | -          | -                            | -                      | -                           | 250       |
|                              | 25% Tofu waste + 75% Chicken manure                       | 20 d               | egg        | 0.4 g/kg                        | 15.37%  | -                                                         | 26.34%      | -          | -                            | -                      | -                           |           |
| Human faeces                 | 100% Human faeces (1)                                     | 9 d                | 12-14 d    | -                               | -       | -                                                         | 26.70%      | -          | 39.10%                       | 22.70%                 | -                           | 84        |
|                              | 100% Human faeces (2)                                     | 9 d                | 12-14 d    | -                               | -       | -                                                         | 27.10%      | -          | 48.60%                       | 18.80%                 | -                           |           |
| Poultry slaughterhouse waste | 100% Poultry slaughterhouse waste                         | 9 d                | 12-14 d    | -                               | -       | -                                                         | 31.50%      | -          | 30.70%                       | 13.40%                 | -                           | 84        |

Table 1. (Continued)

| Main Substrate Type                                     | Other Substrates and Proportions                                          | Treatment Duration | Larval Rearing Age | Larval Rearing Density | Fat (%) | Lauric Acid (%) (as a proportion of total fatty acids) | Protein (%) | Chitin (%) | Substrate Reduction Rate (%) | Bioconversion Rate (%) | Protein Conversion Rate (%) | Reference |
|---------------------------------------------------------|---------------------------------------------------------------------------|--------------------|--------------------|------------------------|---------|--------------------------------------------------------|-------------|------------|------------------------------|------------------------|-----------------------------|-----------|
| A mixture of more than three types of organic materials | 23% Bran + 50% Food waste + 27% Human faeces                              | 9 d                | 12-14 d            | -                      | -       | -                                                      | 25.20%      | -          | 64.10%                       | 31.80%                 | -                           | 84        |
|                                                         | 37% Bran + 21% Food waste + 42% Cow dung                                  | 9 d                | 12-14 d            | -                      | -       | -                                                      | 33.90%      | -          | 51.10%                       | 20.90%                 | -                           |           |
|                                                         | 51% Bran + 14% Human faeces (1) + 35% Cow dung                            | 9 d                | 12-14 d            | -                      | -       | -                                                      | 38.70%      | -          | 45.30%                       | 16.40%                 | -                           |           |
|                                                         | 51% Bran + 14% Human faeces (2) + 35% Cow dung                            | -                  | -                  | -                      | -       | -                                                      | 39.00%      | -          | 49.20%                       | 14.50%                 | -                           |           |
|                                                         | 60% Bran + 20% Food waste + 20% Poultry slaughterhouse waste              | 9 d                | 12-14 d            | -                      | -       | -                                                      | 36.90%      | -          | 58.30%                       | 22.90%                 | -                           |           |
|                                                         | 33% Bran + 33% Food waste + 34% Poultry slaughterhouse waste              | 9 d                | 12-14 d            | -                      | -       | -                                                      | 28.60%      | -          | 65.20%                       | 30.90%                 | -                           |           |
|                                                         | 65% Bran + 22% Poultry slaughterhouse waste                               | 9 d                | 12-14 d            | -                      | -       | -                                                      | 38.10%      | -          | 56.60%                       | 19.80%                 | -                           |           |
|                                                         | + 13% Cow dung                                                            | -                  | -                  | -                      | -       | -                                                      | -           | -          | -                            | -                      | -                           |           |
|                                                         | 40% Pears + 45% Banana + 5% Tomatoes + 10% Various leafy green vegetables | -                  | 6 d                | -                      | 30.80%  | 61.87%                                                 | 39.88%      | 22.97%     | 60.00%                       | 8.00%                  | -                           | 251       |

Note: The substrate consumption index = (total substrate added – remaining substrate)/total substrate added; Bioconversion Rate (dry matter) = (final weight of BSFL – initial weight of BSFL)/(total substrate added – remaining substrate)\*100; Substrate Reduction Rate = (total substrate added – remaining substrate)/total substrate added\*100; Protein Conversion Rate = increase in BSFL protein content/protein content of substrate\*100.

However, the introduction of organic waste with high non-fiber carbohydrates and proteins into cattle manure can optimize the C/N ratio and potentially enhance the rapid growth of BSFL and substrate reduction by improving the activity of both BSFL gut microbiota and substrate microorganisms.<sup>83,84</sup>

Crop straw, characterized by high fiber content and low protein and fat levels, are difficult for BSFL to decompose and digest. Manurung et al.<sup>87</sup> attempted to feed BSFL with straw, but after 39 days of digestion, the reduction rate of straw was only 10.85%. Therefore, crop residues are rarely treated with BSFL bioconversion technology. However, recent studies have found that by optimizing BSFL bioconversion technology, the degradation efficiency and bioavailability of crop residues (corn and rice straw) can be significantly improved.<sup>88,89</sup> For example, alkaline peroxide pre-treatment of straw to alter its physicochemical structure and properties can increase the fiber degradation rate by approximately 12.7%.<sup>89</sup> Combining BSFL bioconversion with microbial fermentation can enhance the degradation rates of cellulose, hemicellulose, and lignin in corn straw by 68.28%, 81.43%, and 99.95%, respectively, compared to BSFL controls without microbial fermentation.<sup>88</sup> In addition to crop residues, mechanical crushing of grass clippings before BSFL treatment can increase bioconversion rates by 25%–44%.<sup>85</sup>

Furthermore, compared to untreated controls, pre-treatment with BSFL can significantly increase the concentrations of total phosphorus and available phosphorus in compost, elevating them from 7.2 g/kg and 3.3 g/kg to 44.2 g/kg and 5.5 g/kg, respectively.<sup>90</sup> In comparison to the natural composting of a mixture of wheat bran and wheat middlings, BSFL bioconversion can significantly reduce CO<sub>2</sub> emissions by 65.71% to 80.52%.<sup>91</sup> BSFL also effectively decreases NH<sub>3</sub> emissions from a mixture of cow manure and distillery waste by 21.39%, indicating that the treatment of 1 ton of this mixture can

lower NH<sub>3</sub> emissions by 6.65 kg.<sup>92</sup> Overall, BSFL bioconversion effectively transforms nutrients in organic waste into its own nutrients while converting organic matter into safe and efficient organic fertilizers. This process significantly minimizes the waste of organic resources and promotes the recycling and utilization of organic materials.

#### BSFL can serve as high-quality animal feed

BSFL exhibit exceptional protein and lipid deposition capabilities. Meta-analysis of current research indicates that BSFL reared on diverse substrates contain an average protein content of approximately 40.33% and lipid content of 27.67% on a dry matter basis. Notably, lauric acid constitutes 30.23% of total fatty acids in BSFL biomass (Table 1). As a medium-chain fatty acid with demonstrated antimicrobial properties, lauric acid enhances intestinal barrier integrity and modulates gut microbiota in livestock, offering significant potential for improving animal health.<sup>93-95</sup> Furthermore, BSFL-derived chitin and chitosan, accounting for 8.5% of larval biomass, serve as sustainable adsorbents for heavy metals and organic pollutants while exerting broad-spectrum antimicrobial activity against PB.<sup>35,96-99</sup> Numerous studies have demonstrated that BSFL-derived chitin and chitosan can effectively adsorb HMs such as zinc (Zn), cadmium (Cd), and nickel<sup>98,100</sup> and inhibit the proliferation of pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Ralstonia solanacearum*.<sup>101,102</sup> Additionally, BSFL-derived antimicrobials can serve as substitutes for ABs for treating livestock and poultry diseases, thereby promoting the healthy growth of these animals.<sup>42,70</sup> Furthermore, BSFL proteins have been reported as innovative biodegradable bioplastics for agricultural applications.<sup>103</sup>

In recent years, a substantial body of research has emerged highlighting the potential of BSFL to partially or even completely replace conventional feed



ingredients such as soybean meal and fishmeal in animal production.<sup>104-107</sup> In animal feed, soybean meal typically contains a crude protein level of about 44%, crude fat of about 6%, and crude fiber of about 7% (GB/T 19541-2017).<sup>108-110</sup> Fish meal contains about 60% protein, 8% crude fat, and 0.8% crude fiber (GB/T 19164-2021).<sup>108-110</sup> In contrast, BSFL contain a crude protein level of 41% (similar to soybean meal), crude fat of about 29% (higher than both soybean meal and fish meal), and crude fiber of about 7% (similar to soybean meal).<sup>108-111</sup> This indicates a high degree of similarity in nutritional composition between BSFL and both soybean meal and fish meal. Moreover, BSFL contain high levels of lauric acid, AMPs, chitin, and its derivative chitosan, all of which have significant antimicrobial and antibacterial properties.<sup>42,70,93-95,101,102</sup> These characteristics mean that when BSFL are used as a substitute for soybean meal and fish meal, they not only do not negatively impact the growth and development of livestock and aquaculture animals, but may even be superior to soybean meal and fish meal feeds. In poultry production, substituting approximately 10% of soybean meal or corn protein with BSFL in poultry feed can significantly enhance the daily weight gain and feed intake of broilers and ducks while reducing their mortality rates.<sup>106,107</sup> In pigs, substituting 4% to 25% of soybean meal-derived protein can improve the intestinal immune barrier and promote body weight gain.<sup>104,105</sup> Substituting 50% fishmeal-derived protein with BSFL in fish feed can significantly improve the growth rate in aquaculture.<sup>112</sup> Furthermore, several studies have shown that fully substituting soybean meal, corn, or fish meal-derived protein with BSFL in the pig, poultry, or aquatic diets does not adversely affect production performance.<sup>105,112,113</sup> Similarly, substituting soybean oil with BSFL in poultry or fish feed has no significant impact on the growth performance and health of these animals.<sup>113-115</sup> Additionally, BSFL-derived oils and fats can be converted into biodiesel, thus addressing the growing demand for fossil fuels.<sup>116-120</sup> These findings illustrate that BSFL-derived proteins and fats can effectively replace conventional plant- and animal-derived feed ingredients in livestock and aquaculture feeds. They can also be utilized to produce various value-added products, thereby enhancing their commercial value.

### BSFL efficiently produces organic fertilisers

BSFL bioconversion is a comprehensive concept that is not limited to waste treatment or fertilizer production. It also includes the transformation of organic waste into a wider range of valuable products, such as protein feed, fatty acids, chitin, AMPs, and so on. In contrast, BSFL biocomposting primarily refers to the process of using BSFL to transform organic waste into stable and safe organic fertilizers. Several studies have demonstrated that BSFL biocomposting accelerates ammonia metabolism, reduces the carbon-to-nitrogen ratio, and promotes compost humification, yielding high-quality organic fertilizer for agriculture.<sup>72,121</sup> Research indicates that kitchen waste can be repurposed for agricultural production after approximately nine weeks of BSFL decomposition and natural fermentation.<sup>71</sup> However, organic wastes such as faeces and brewery spent grains could be safely used after about 5 weeks of similar treatment without affecting soil quality.<sup>121,122</sup> Composting time for BSFL could be further reduced by mixing various types of organic wastes. For instance, combining brewery spent grains with potato peels at a ratio of approximately 9:1 for a 14-day BSFL biocomposting can produce a non-toxic product suitable for agricultural use.<sup>73</sup> Additionally, mixing kitchen waste with sawdust at a ratio of 6.5:0.5 for a 9-day BSFL biocomposting can significantly enhance fertilizer humification.<sup>123</sup> However, there is currently no unified standard for assessing the maturity of BSFL compost products. Most existing studies primarily use the seed germination index (greater than 100%), C/N ratio (below 25), and electrical conductivity (less than 4 mS cm<sup>-1</sup>) to determine the maturity of the fertilizer.<sup>124-126</sup> Fertilizers derived from BSFL biocomposting are classified as biofertilizers, which must meet relevant industry standards if intended for market sale. In China, insect frass-based biofertilizers (such as earthworm castings) must comply with the requirements outlined in the People's Republic of China Agricultural Industry Standard NY525-2021 for agricultural use. These requirements include a moisture content of no more than 30%, a pH value ranging from 5.5 to 8.5, arsenic levels not exceeding 15 mg/kg, mercury levels not exceeding 2 mg/kg, lead levels not exceeding 50 mg/kg, chromium levels not exceeding 150 mg/kg, total *Escherichia coli* not exceeding 100 CFU/g, and a seed germination rate (using cucumber or radish seeds) of at least 70%. The European Union Fertil-

ising Products Regulation (EU) 2019/1009 specifies that organic fertilizers must meet certain criteria before use. These criteria include Cd levels not exceeding 1.5 mg/kg, hexavalent chromium levels not exceeding 2 mg/kg, mercury levels not exceeding 1 mg/kg, nickel levels not exceeding 50 mg/kg, lead levels not exceeding 120 mg/kg, arsenic levels not exceeding 40 mg/kg, copper levels not exceeding 300 mg/kg, Zn levels not exceeding 800 mg/kg, total *Escherichia coli* not exceeding 1000 CFU/g, and the absence of *Salmonella* in a 25 g sample of solid or a 25 ml sample of liquid organic fertilizer.

Compared to conventional compost, BSFL-treated organic manure is richer in N, P, and K elements and exhibits a higher rate of organic nitrogen mineralization, which enhances plant nutrient uptake and utilization.<sup>73,123,127</sup> Furthermore, harmful components such as PB, ABs, MPs, and HMs are significantly reduced in BSFL-treated organic fertilizer, greatly improving the safety of the compost for use. This is particularly important for promoting the sustainable development of agriculture.<sup>64,65,128,129</sup>

### BSFL GUT MICROBIOLOGICAL COMPOSITION

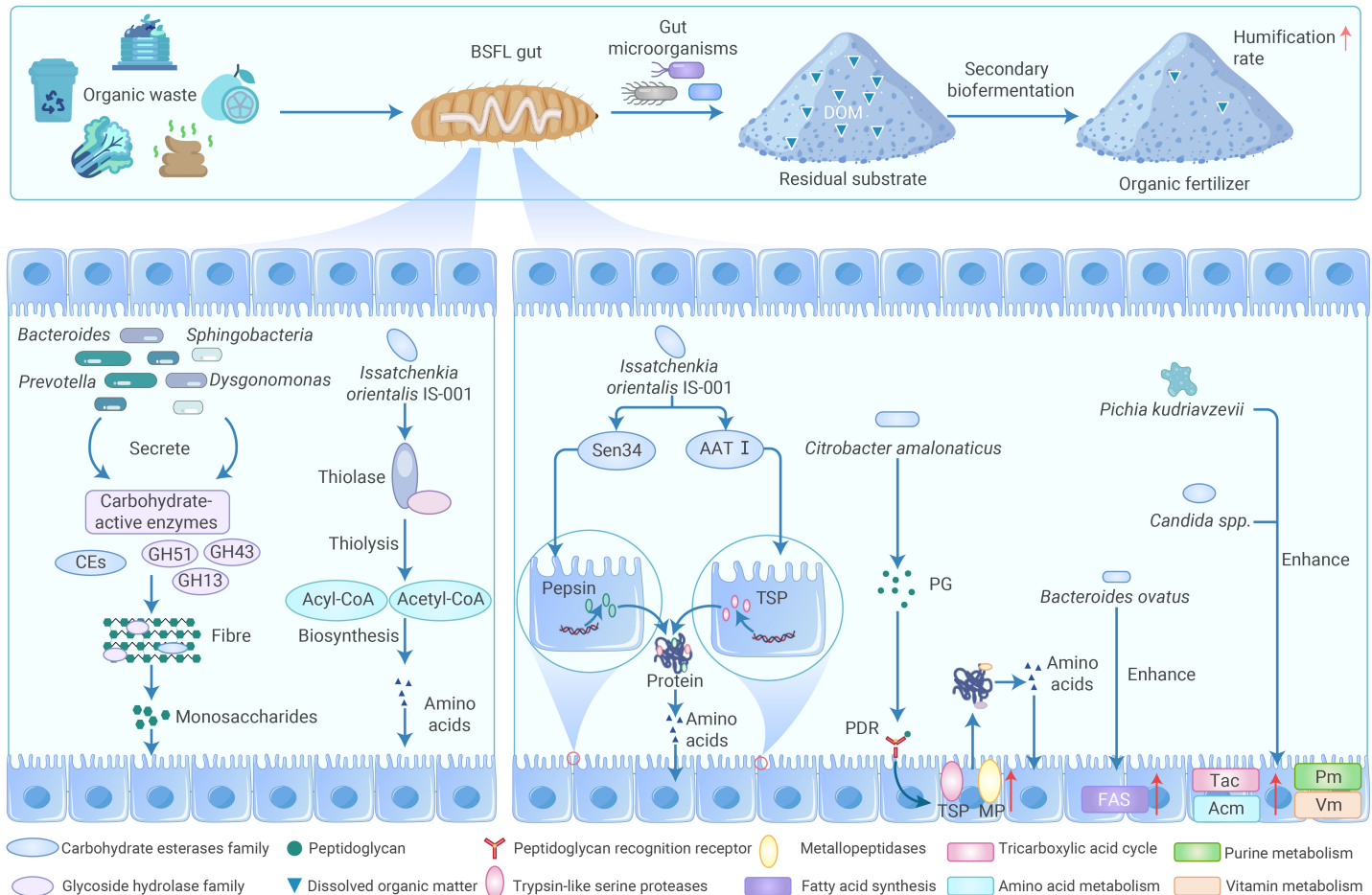
The insect gut harbors a diverse microbial community comprising bacteria (accounting for 98% of the total microbes), fungi, viruses, archaea, and protozoa. These microorganisms play various roles, including regulating host immunity, facilitating nutrient digestion and absorption, and protecting against PB.<sup>54,130</sup> In BSFL, the intestinal bacterial composition shifts in response to substrate variations. For instance, in lignocellulose-rich environments, the abundance of *Bacteroidetes*, *Actinobacteria*, and *Pseudomonas* increases progressively with the growth and development of BSFL. Among these phyla, *Actinobacteria* emerges as the predominant, followed by *Bacteroidetes*.<sup>54</sup> Conversely, in protein- and lipid-rich substrates, *Firmicutes* are the most abundant, followed by *Proteobacteria*, *Actinobacteria*, and *Bacteroidota*.<sup>52</sup> Although the gut microbiota composition of BSFL fluctuates with growth stages and substrate types, several studies reported the presence of a core group of bacteria in the BSFL gut microbiota. At the phylum level, the predominant groups include *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria*, while the main representatives at the genus level are *Prevotella*, *Bacillus*, *Enterococcus*, and *Pseudomonas*.<sup>45,131-136</sup> Additionally, the intestinal flora of BSFL varies significantly with species composition and abundance across different intestinal segments. For instance, the midgut is considerably longer than both the foregut and hindgut in BSFL, thus harboring a richer microbial flora and a more complex digestive capacity.<sup>45</sup> The core microbial community of this section primarily consists of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*.<sup>34</sup> In addition to these core flora, BSFL can recruit microorganisms from their environment, enhancing their resilience under various conditions and promoting their adaptability to different substrates. Research indicates that approximately 34.3% of bacteria from food waste can colonize the BSFL gut, with exogenous flora colonization increasing over time.<sup>58</sup> Another study revealed that when fed lignocellulose-rich substrates, BSFL can recruit *Corynebacterium*, *Brevibacterium*, *Dysgonomonas*, *Prevotella*, and *Miniimonas* to their gut, facilitating lignin and cellulose degradation.<sup>54</sup> In conclusion, the ability of BSFL to thrive in diverse environments is attributed to the synergistic interactions between their native gut and external microbes, which collectively enhance the functional diversity of BSFL.

### EFFECTS OF BSFL GUT MICROBES ON ORGANIC MATTER DEGRADATION AND RESOURCE UTILIZATION

BSFL are insects that are rich in proteins, fats, and various functional nutrients, including chitin, chitosan, fatty acids, and AMPs.<sup>35,36,42,137</sup> Although the role of BSFL gut microbes in host nutrient synthesis and metabolism remained unclear for a long time, the recent advancements in gut microbial metagenomics and metabolomics have revealed that BSFL gut microbes contribute to organic matter degradation and nutrient digestion and absorption in various ways (Figure 2).<sup>55,56,138</sup>

### BSFL gut microbes promote organic matter degradation and utilization

In the composting process involving BSFL, the gut microorganisms of BSFL play a crucial role by directly facilitating the degradation of various organic compounds, including cellulosic carbohydrates, nitrogenous



**Figure 2. Influence of BSFL gut microbes on host intestinal nutrient digestion and absorption** *Dysgonomonas*, *Prevotella*, *Bacteroides*, *Sphingobacteria*, and *Issatchenkia orientalis* IS-001 directly promote fiber catabolism and the production of amino acids in the gut of BSFL by secreting various digestive enzymes, including carbohydrate esterases, glycoside hydrolases, and thiolases. *Issatchenkia orientalis* IS-001 and *Citrobacter amalonaticus* stimulate the expression of host pepsin, trypsin-like serine proteases and metalloproteases, to promote the degradation of proteins and peptides. Additionally, *Bacteroides ovatus*, *Pichia kudriavzevii*, and *Candida* spp. improve the growth and development of the host by regulating the metabolism of various amino acids, vitamins, the tricarboxylic acid cycle, and purine, among other processes. Furthermore, the action of BSFL gut microbes significantly increases the rate of DOM decomposition during secondary fermentation and accelerates the humification rate.

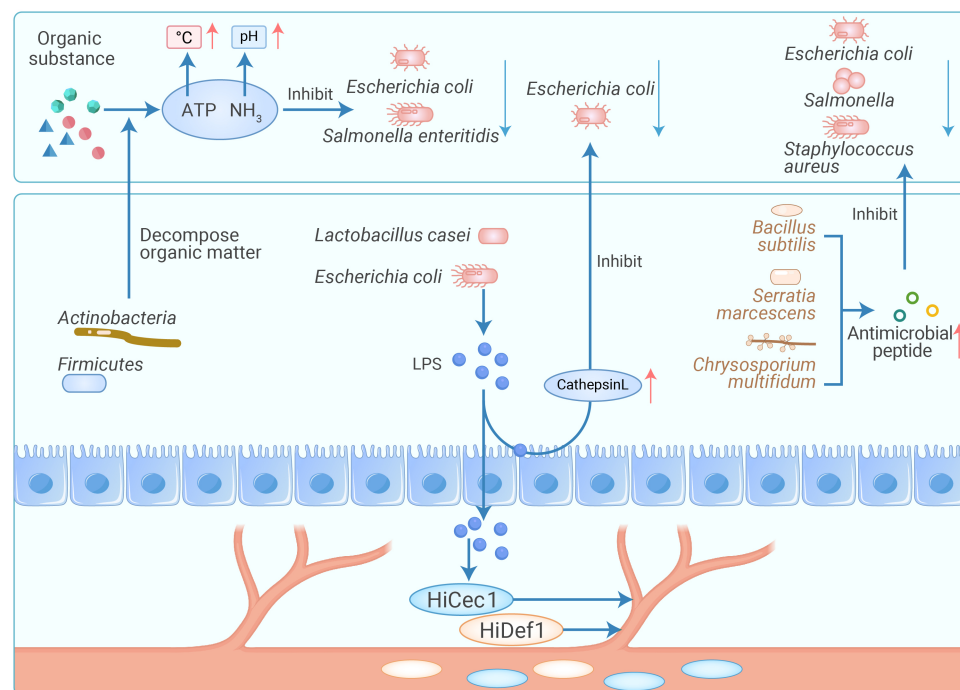
substances such as proteins, and lipids. BSFL degradation of carbohydrates is largely attributed to *Dysgonomonas*, *Prevotella*, *Bacteroides*, and *Sphingobacteria* under the phylum *Bacteroidetes*.<sup>53,54</sup> These microorganisms possess Polysaccharide Utilization Loci encoding various cellulases (depending on the type of carbohydrate), which facilitate cellulose breakdown.<sup>139</sup> Metagenomic and metatranscriptomic studies have revealed that the gut microbiota of BSFL is enriched with a diverse array of carbohydrate-active enzymes, including glycoside hydrolase family 51, glycoside hydrolase family 43, and glycoside hydrolase family 13, as well as the carbohydrate esterases.<sup>53,54</sup> These glycoside hydrolase family enzymes play a crucial role in the catalytic hydrolysis of glycosidic bonds. For instance, members of the glycoside hydrolase family 43, including  $\beta$ -D-xylosidases,  $\alpha$ -L-arabinofuranosidases, endo- $\alpha$ -L-arabinosidases, and  $\beta$ -1,3-galactosidases, degrade hemicellulose glycosidic bonds, particularly those found in xylan and pectin.<sup>140,141</sup> Enzymes from the glycoside hydrolase family 51 have distinct N-terminal ( $\beta$ / $\alpha$ )-barrel (TIM-barrel) and C-terminal  $\beta$ -sandwich fold, allowing selective hydrolysis of the glycosidic bonds of L-arabinofuranosides within hemicellulose side chains, such as arabinoxylan, arabinogalactan, and L-arabinan.<sup>141,142</sup> In the glycoside hydrolase family 13, sucrose phosphorylase catalyzes the phosphorylation of sucrose, producing  $\alpha$ -glucose 1-phosphate and fructose.<sup>143</sup> Additionally, the carbohydrate esterases family, comprising enzymes like acetyl xylan esterase, feruloyl esterase, and glucuronidase, de-esterifies complex polysaccharides, complementing the activities of glycosidases to ensure more efficient hydrolysis of these structures.<sup>144</sup>

The gut microbiota of BSFL also plays a significant role in promoting protein digestion and absorption. For example, Yu<sup>56</sup> found that the

C5L36\_0A08380 protein encoded by *Issatchenkia orientalis* IS-001 catalyzes the reversible sulfur cleavage of 3-ketoacyl-CoA into acyl-CoA and acetyl-CoA, showing a strong positive correlation with the host's amino acid metabolism. In the absence of gut microbiota, the BSFL protein digestibility significantly decreases by approximately 73%.<sup>56</sup> Furthermore, refeeding BSFL with *Candida tropicalis* and *Pichia kudriavzevii*, a strain isolated from the BSFL gut, significantly enhances histidine metabolism, aminoacyl-tRNA biosynthesis, glycerophospholipid metabolism, and the tricarboxylic acid cycle, accelerating BSFL growth.<sup>145</sup> Similarly, *Candida* spp. species isolated from the BSFL gut can enhance host growth through various metabolic pathways, including purine, vitamin B6, tyrosine, histidine, phenylalanine, and tryptophan metabolism pathways.<sup>146</sup> In addition, recent studies have shown that aseptic treatment significantly reduces the synthesis and accumulation of lauric, palmitic, myristic, and oleic acids in BSFL. However, when *Bacteroides ovatus* was introduced to aseptic BSFL, the host's fatty acid synthesis genes, *HiACP* and *HiFAS*, were significantly activated, markedly increasing the synthesis and accumulation of lauric, palmitic, myristic, and oleic acids in the host.<sup>138</sup>

#### BSFL gut microbes enhance host digestive enzyme activity

In addition to directly enzymatically degrade organic matter, BSFL gut microbes can modulate the activity of digestive enzymes in the host gastrointestinal tract. Research has shown that *Issatchenkia orientalis* IS-001 from the BSFL gut affects the bioactivity of host pepsin and trypsin-like serine proteases by regulating the expression of tRNA splicing endonuclease subunit *Sen34* and the pyridoxal phosphate (PLP)-dependent aspartate



**Figure 3. Effect of BSFL gut microorganisms on PB in substrates** In the pre-composting stage of organic waste, BSFL gut microorganisms inhibit the proliferation of PB by increasing the amount of heat and ammonia released from the digestion and decomposition of organic matter, which changes the temperature and pH of the heap. *Serratia marcescens*, *Bacillus subtilis*, and *Chrysosporium multifidum* inhibited the growth of *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* in the compost pile by directly secreting antimicrobial substances. Furthermore, the activities of antimicrobial compounds, including HiCec1, HiDef1, and cathepsin L, were significantly enhanced in BSFL by the stimulation of *Lactobacillus casei* and *Escherichia coli*, which further suppressed the proliferation of PB.

lead to a decline in the activity of various biological enzymes, thereby limiting their proliferation. During the early stages of substrate inoculation with BSFL, certain gut microorganisms derived from BSFL frass can rapidly adapt to the substrate environment and proliferate to become the dominant microbial community. These dominant bacteria (e.g., *Actinobacteria* and *Firmicutes*) generate significant heat through decomposition processes.<sup>58</sup> Specifi-

aminotransferase (AAT) superfamily (folding type I) proteins, thereby facilitating gut protein digestion in the host. Moreover, *Issatchenkia orientalis* IS-001 also influences host intestinal branched-chain amino acid metabolism and absorption by regulating the expression of aldehyde dehydrogenase, spermidine synthase, and (S)-3-amino-2-methylalanine transaminase genes.<sup>56</sup> *Citrobacter amalonaticus* is also an important regulator of BSFL intestinal protein digestive enzymes. Its cell wall component, peptidoglycan, enhances the activity of the trypsin-like serine proteases and metalloproteinases expression, promoting the degradation of intestinal proteins and peptides.<sup>55</sup>

### BSFL gut microbes accelerate substrate humification

Dissolved organic matter (DOM) is a general term for organic matter with a particle size of less than 0.45  $\mu\text{m}$  that is soluble in water, and changes in its structure can reflect the changes in organic matter during composting, indicating compost stability and maturity.<sup>147,148</sup> In BSFL composting, BSFL increases the degree of organic matter fragmentation through degradation and exports gut microorganisms to the compost pile.<sup>58</sup> These microorganisms promote DOM decomposition in the compost by secreting digestive enzymes such as catalase, lipase, protease, sucrose, urease, and cellulase. This process generates oxygen-functional groups (e.g., hydroxyl, carboxyl, and carbonyl groups), aromatic compounds, and stabilized humic substances, enhancing residue stabilization and humification of organic waste.<sup>149</sup> Moreover, BSFL treatment of compost piles results in the formation of new microbial communities that can further promote DOM decomposition during secondary natural composting. This reduces total nitrogen and ammonium nitrogen concentrations while increasing nitrate nitrogen concentrations and the production of humus-like and aromatic compounds, yielding quality land-applied biofertilizers.<sup>71</sup> However, how BSFL and their gut microorganisms enhance the enzymatic activity in the compost pile remains unclear.

### BSFL GUT MICROBES INHIBIT PROLIFERATION OF PB

PB such as *Salmonella*, *Escherichia coli* and *Campylobacter* are commonly found in animal faeces, and can survive in fecal forms (both solid and liquid) from 3 to 21 months.<sup>150-152</sup> Previous studies demonstrated that BSFL biocomposting could mitigate the risk of spreading PB to the environment in various ways (Figure 3).<sup>153-155</sup>

### BSFL gut microbes can alter the substrate microenvironment to inhibit PB

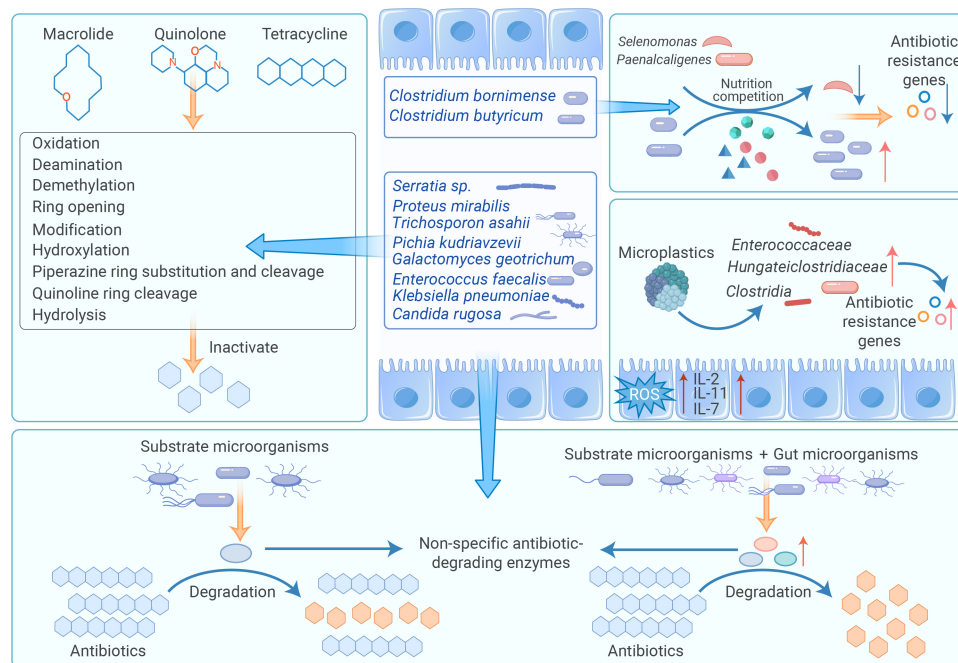
For microorganisms, temperatures outside the optimal survival range can

ally, *Actinobacteria* secrete enzyme systems that promote polysaccharide decomposition and release substantial heat.<sup>156-158</sup> Moreover, *Bacillus* within the *Firmicutes* are also key decomposers of substrate nutrients. They secrete various digestive enzymes, including cellulases, proteases, and lipases, which accelerate nutrient decomposition and enhance heat release.<sup>159,160</sup> Under the mediation of BSFL, the substrate temperature can rise to a maximum of 49.9°C, remaining as high as 43.6°C by the 10th day of composting.<sup>58</sup> Such sustained high temperatures may inhibit the enzymatic activity of mesophilic bacteria, such as *Escherichia coli* (proliferation inhibited above 41.6°C) and *Salmonella* (proliferation inhibited above 42°C), and can even disrupt their cellular structures, thereby suppressing the proliferation of PB.<sup>161-163</sup> Furthermore, changes in substrate pH may significantly contribute to the reduction of PB. Studies have shown that the pH of the substrate increases to 7–8 during BSFL-mediated bioconversion,<sup>164-166</sup> which inhibits the proliferation of *Escherichia coli* O157:H7 and *Salmonella enteritidis*.<sup>155</sup> Conversely, when the substrate pH is low (5–6), PB such as *Morganella* become significantly enriched.<sup>165</sup>

### BSFL gut microbes directly inhibit the proliferation of some PB

In complex microbial environments, BSFL gut microbes gradually form a microbial community adapted to the environment, including some probiotics that inhibit the proliferation of PB. For example, *Serratia marcescens* and *Bacillus subtilis*, which inhibit the proliferation of PB, are found in the intestines of BSFL that have been raised in pathogen-rich swine feces. *Bacillus subtilis* has an inhibitory effect on *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*, while *Serratia marcescens* can inhibit *Escherichia coli* and *Staphylococcus aureus*.<sup>59</sup> Previous studies found that *Bacillus* species can secrete various lipopeptides, including surfactin, iturin, fengycin, bacillomycin, and polymyxins, which can inhibit the proliferation of bacteria, fungi, and viruses.<sup>167</sup> These lipopeptides can rapidly bind the bacterial bilayer in the biofilm and adsorb the negatively charged phospholipids such as phosphatidylglycerol and phosphatidylcholine. This alters the local lipid organization of the biofilm and causes pore formation, thereby inhibiting microbial growth.<sup>168,169</sup> *Serratia marcescens* produces various secondary metabolites exhibiting antimicrobial, antifungal, and insecticidal activities. One such metabolite, prodigiosin, can bind the cell membranes of *Staphylococcus aureus* and disrupt its biofilm structure, although the specific mechanism of action remains unclear.<sup>170</sup> Fungi also play an important role in maintaining BSFL gut health. *Chrysosporium multifidum*, isolated from the intestines of BSFL fed on chicken manure, can significantly inhibit the proliferation of





**Figure 4. Effect of BSFL gut microorganisms on ABs and ARGs** The BSFL gut microbiota, including *Enterococcus faecalis*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Serratia* spp., *Trichosporon asahii*, *Pichia kudriavzevii*, *Candida rugosa*, and *Galactomyces geotrichum*, can inactivate ABs through various mechanisms such as hydroxylation, piperazine ring substitution and cleavage, quinoline ring cleavage, hydrolysis, oxidation, deamination, demethylation, ring opening, and modification. Moreover, BSFL gut microbiota may promote AB degradation by increasing the production of non-specific antibiotic-degrading enzymes. The BSFL gut microbiota *Clostridium butyricum* and *Clostridium bornimense* can inhibit the proliferation of antibiotic-resistant bacteria such as *Selenomonas* and *Paenalcigenes* through competition for nutrients, thereby reducing the generation of ARGs. Toxic substances such as MPs can disrupt the gut homeostasis of BSFL, promoting the proliferation of antibiotic-resistant bacteria (e.g., *Enterococcaceae*, *Hungateiclostridiaceae*, and *Clostridia*), leading to an increase in ARG levels within BSFL.

demonstrated that *Serratia* sp., *Trichosporon asahii*, *Pichia kudriavzevii*, *Candida rugosa*, *Galactomyces geotrichum*, and *Serratia marcescens* isolated from the BSFL gut

*Staphylococcus aureus*, mainly through the secretion of 5,6-dihydro-4-methoxy-6-(1-oxopentyl)-2H-pyran-2-one (4) (an  $\alpha$ -pyranone derivative).<sup>60</sup>

#### BSFL gut microbes promote host secretion of AMPs

AMPs are small molecular weight, thermally stable, alkaline polypeptides with rapid bactericidal action.<sup>171</sup> BSFL are considered one of the most abundant insect species producing AMPs, with a genome encoding approximately 50 different AMPs.<sup>132,172</sup> Among them, AMPs such as cecropins, defensins, dipterocins, and attacins have been shown to be potent antimicrobial agents that inhibit the proliferation of PB, such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.<sup>173-177</sup> Most AMPs are classified as acquired immune factors, and their production is influenced by external stimuli.<sup>178</sup> During BSFL-mediated composting, various environmental microorganisms colonize the gut of the BSFL, some of which may trigger immune responses, leading to the expression of antimicrobial peptide genes.<sup>58,59,179-181</sup> For instance, *Lactobacillus casei* could enhance the activities of cecropin 1 (HiCec1) and defensin 1 (HiDef1) in the haemolymph of BSFL (Lee et al., 2020). Furthermore, certain pathogenic strains of *Escherichia coli* reportedly stimulate the expression of intestinal cathepsin L in BSFL, which subsequently inhibits their proliferation.<sup>182</sup>

#### BSFL GUT MICROBES ENHANCE ABs DEGRADATION AND SUPPRESS THE PRODUCTION OF ARGs

Misuse of ABs increases residue concentration in animal manure, increasing the risk of resistant bacteria occurring in the environment.<sup>183,184</sup> It has been recently demonstrated that the gut microbes of BSFL not only possess significant biotransformation capabilities but can also enhance the degradation of ABs and inhibit the occurrence of ARGs (Figure 4).<sup>63,64,185,186</sup>

#### BSFL gut microbes accelerate ABs degradation

Mei et al.<sup>63</sup> found that in the presence of BSFL gut microbiota, the concentrations of tiamulin and enrofloxacin in feces decreased by 82.9% and 80.9%, respectively, within 12 days, which is 52.3% and 79.8% higher than the degradation rates observed in natural composting. *Enterococcus faecalis* and *Proteus mirabilis* isolated from the BSFL gut also significantly degraded more than 54.4% and 57.2% of tylosin and enrofloxacin, respectively, under in vitro conditions. Yang et al.<sup>64</sup> reported that ciprofloxacin was significantly degraded by 84.22% within 12 days, which is 49.85% higher than the degradation rate in natural composting. *Klebsiella pneumoniae* and *Proteus mirabilis* isolated from the BSFL gut microbiota degraded more than 98.22% and 97.83% of ciprofloxacin, respectively, under in vitro conditions. Cai et al.<sup>186</sup>

degraded 50.0%, 48.2%, 28.9%, 44.2%, 39.2%, and 28.5% of tetracycline, respectively. Luo et al.<sup>187</sup> found that lincomycin was degraded by 99.4% within 12 days, which is approximately 60.0% higher than the degradation rate in natural composting. In addition to this, BSFL also demonstrated significant degradation capabilities for ABs such as Macrolides, Quinolones, and Amino-glycosides (Table 2).

The mechanisms by which microorganisms reduce antibiotics mainly include co-metabolism, biodegradation, biosorption, and bioaccumulation.<sup>188</sup> Co-metabolism refers to the non-specific metabolic degradation of non-essential compounds (such as antibiotics) by microorganisms while they metabolize substrates required for growth.<sup>188</sup> For example, supplementing microbial growth substrates with organic substrates (e.g., glucose, sodium acetate, glycerol) can significantly enhance microbial activity, induce the upregulation of non-specific enzymes (e.g., ammonia monooxygenase, glutathione metabolic enzymes, peroxidases), and facilitate the metabolic breakdown of antibiotics.<sup>189-193</sup> Biodegradation primarily relies on the metabolic functions of microorganisms, including cellular phagocytosis and the secretion of various degrading enzymes (e.g., cytochrome P450, peroxidases, serine hydrolases) to promote antibiotic degradation.<sup>194</sup> Biosorption and bioaccumulation depend on the extracellular polymeric substances produced by microorganisms.<sup>195</sup> Extracellular polymeric substances is a complex composed of polysaccharides, proteins (including enzymes), nucleic acids, lipids, and humic substances, containing various functional groups such as methyl, amino, and phosphate groups. These functional groups can provide numerous binding sites for antibiotics and extracellular enzymes, reducing the mobility of antibiotics while promoting the digestion and degradation of antibiotics by extracellular enzymes.<sup>195-197</sup> During the process of antibiotic degradation by BSFL, the gut microbiota may assist in antibiotic degradation through co-metabolism and biodegradation (Figure 4).<sup>188</sup> In the process of organic waste transformation, the addition of BSFL can significantly enhance the activity of digestive enzymes or non-specific antibiotic hydrolases (e.g., carbohydrate hydrolases) in the substrate microorganisms, thereby promoting the degradation of organic matter.<sup>198</sup> The increased activity of these enzymes may be the main reason for antibiotic degradation in the substrate, a phenomenon that has also been partially confirmed in the study by Pei et al.<sup>199</sup> In addition, biodegradation may also be an important cause of antibiotic degradation during BSFL-mediated bioconversion. Numerous studies have confirmed that the BSFL gut harbors functional microorganisms (e.g., *Klebsiella pneumoniae*, *Proteus mirabilis*, *Serratia* sp.) capable of directly degrading antibiotics. These microorganisms may degrade antibiotics through various mechanisms, including hydroxylation, piperazine ring substi-



Table 2. Comprehensive analysis of antibiotic degradation efficiency, resistance gene dynamics, and functional gut microbial roles in BSFL-mediated organic waste treatment

| Antibiotic Type | Name            | Substrate    | Inoculation Concentration | Initial Antibiotic Concentration | Treatment Duration | Degradation Rate                              | Half-life                                               | Changes in ARGs and Detection Sites                                                                                                                                                                                                                                                                                                                                   | Changes in MGEs and Detection Sites | Functional Microbes in the Gut                                                          | Extracellular Antibiotic Degradation Capability of Gut Microbiota | References |
|-----------------|-----------------|--------------|---------------------------|----------------------------------|--------------------|-----------------------------------------------|---------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------------|------------|
| Tetracyclines   | Oxytetracycline | Soya meal    | 3 d                       | 1000 mg/kg                       | 8 d                | 77.50% (54.31% higher than the control group) | 4.18 days (compared to 17.53 days in the control group) | -                                                                                                                                                                                                                                                                                                                                                                     | -                                   | Enterococcus, Psychrobacter, Providencia, Myroides, Enterobacteriaceae, Lactobacillales | -                                                                 | 252        |
|                 |                 |              |                           |                                  |                    | 82.73%, 77.56%, and 69.27%, respectively      | -                                                       | Tetracycline resistance genes in the gut accounted for 67.00% of the total ARGs. The relative abundance of ARGs and MGEs in the gut increased from Day 1 to Day 3 and then decreased from Day 3 to Day 6. The relative abundance of tetA (48) in the gut decreased by 3.52%, tetB (48) decreased by 2.93%, tetB (60) increased by 2.76%, and tetX increased by 3.29%. | -                                   | Enterococcus, Lgnatzschineria, Bordetella, Providencia, Proteus                         | -                                                                 | 185        |
|                 | Tetracycline    | Soybean meal | 3 d                       | 434.4 mg/kg, 3042.3 mg/kg        | 7 d                | 65.90% and 63.30%, respectively               | -                                                       | -                                                                                                                                                                                                                                                                                                                                                                     | -                                   | Enterococcus, Lgnatzschineria, Providencia, Morganella, Paenaltcaligenes, Proteus       | -                                                                 | 253        |
|                 |                 |              |                           |                                  |                    | 32.21% (22.29% higher than the control group) | -                                                       | -                                                                                                                                                                                                                                                                                                                                                                     | -                                   | Enterococcus, Vagococcus, Providencia, Paenaltcaligenes                                 | -                                                                 | 199        |

Table 2. (Continued)

| Antibiotic Type | Name              | Substrate                        | Inoculation Concentration | Initial Antibiotic Concentration | Treatment Duration | Degradation Rate                             | Degradation Half-life                                                                                                        | Changes in ARGs and Detection Sites                                                                                                                                                                                               | Changes in MGEs and Detection Sites | Functional Microbes in the Gut                                                                                                                                                                                                      | Extracellular Antibiotic Degradation Capability of Gut Microbiota                                                                                                                                                                                                                                                                             | References |
|-----------------|-------------------|----------------------------------|---------------------------|----------------------------------|--------------------|----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Tetracyclines   | Tetracycline      | Wheat bran                       | 8 d                       | 20 mg/kg, 40 mg/kg, 80 mg/kg     | 12 d               | 95.90%, 96.90%, and 95.50%, respectively     | 90 h (211 h less than the control), 85 h (196 h less than the control), and 86 h (134 h less than the control), respectively | The absolute abundance of <i>tetA</i> , <i>tetC</i> , <i>tetG</i> , <i>tetM</i> , <i>tetO</i> , <i>tetQ</i> , <i>tetT</i> , <i>tetW</i> , and <i>tetX</i> genes in the gut increased before Day 4 and then decreased after Day 4. |                                     | <i>Serratia</i> sp. (BSFL-1),<br><i>Trichosporon asahii</i> (BSFL-2),<br><i>Pichia kudriavzevii</i> (BSFL-3),<br><i>Candida rugosa</i> (BSFL-3),<br><i>Galactomyces geotrichum</i> (BSFL-4),<br><i>Serratia marcescens</i> (BSFL-5) | In the substrate containing 40 mg/kg of tetracycline, BSFL-1 and BSFL-2 were able to degrade approximately 50.00%, with a half-life of 94 hours. BSFL-3, BSFL-4, BSFL-5, and BSFL-6 degraded approximately 28.90%, 44.20%, 39.20%, and 28.50%, respectively, with half-lives of 215 hours, 137 hours, 146 hours, and 177 hours, respectively. |            |
|                 |                   |                                  |                           |                                  |                    |                                              |                                                                                                                              |                                                                                                                                                                                                                                   |                                     |                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                               |            |
|                 | Chlortetracycline | 50% Wheat bran + 50% wheat flour | 6 d                       | 100 mg/kg                        | 12 d               | 78.00% (4.90% higher than the control group) | -                                                                                                                            | -                                                                                                                                                                                                                                 | -                                   | <i>Pseudomonas</i> ,<br><i>Providencia</i> ,<br><i>Dysgonomonas</i> ,<br><i>Morganella</i>                                                                                                                                          | -                                                                                                                                                                                                                                                                                                                                             | 254        |

Table 2. (Continued)

| Antibiotic Type  | Name                             | Substrate  | Inoculation Concentration | Initial Antibiotic Concentration | Treatment Duration                                            | Degradation Rate | Half-life | Changes in ARGs and Detection Sites                                                                                          | Changes in MGEs and Detection Sites                                                                                                | Functional Microbes in the Gut                                                                                                                                                  | Extracellular Antibiotic Degradation Capability of Gut Microbiota                                                                                                                                                                                                                                                                                                                                                      | References |
|------------------|----------------------------------|------------|---------------------------|----------------------------------|---------------------------------------------------------------|------------------|-----------|------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Tetracyclines    | Oxytetracycline                  | -          | -                         | -                                | -                                                             | -                | -         | -                                                                                                                            | -                                                                                                                                  | <i>Klebsiella pneumoniae</i> (A1), <i>Enterococcus faecalis</i> (B2), <i>Paenibacillus polymyxa</i> (F2), <i>Bacillus anthracis</i> (M2), <i>Lysinibacillus macrolides</i> (Y2) | At drug concentrations of 25, 50, 100, 200, and 400 mg/L, A1 degraded 76.41%, 69.28%, 70.22%, 70.52%, and 70.43%, respectively. B2 degraded 0.81%, 74.24%, 73.79%, 74.89%, and 76.38%, respectively. F2 degraded 79.36%, 70.86%, 70.72%, 70.60%, and 69.59%, respectively. M2 degraded 79.06%, 70.15%, 69.25%, 68.19%, and 70.43%, respectively. Y2 degraded 79.23%, 70.27%, 68.72%, 67.96%, and 68.93%, respectively. | 255        |
|                  |                                  |            |                           |                                  |                                                               |                  |           |                                                                                                                              |                                                                                                                                    |                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                        |            |
|                  |                                  |            |                           |                                  |                                                               |                  |           |                                                                                                                              |                                                                                                                                    |                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                        |            |
| $\beta$ -lactams | Penicillin G sodium, Amoxicillin | Pig manure | 6 d                       | -                                | 12 days of BSFL bioconversion + 16 days of natural composting | 13.70%           | -         | The absolute abundance of total ARGs in the substrate decreased by 93.20%, while the relative abundance decreased by 37.80%. | The relative abundance of plasmids, and insertion sequences in the substrate decreased by 5.50%, 41.30%, and 47.80%, respectively. | -                                                                                                                                                                               | -                                                                                                                                                                                                                                                                                                                                                                                                                      | 203        |
|                  |                                  |            |                           |                                  |                                                               |                  |           |                                                                                                                              |                                                                                                                                    |                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                        |            |

Table 2. (Continued)

| Antibiotic Type | Name                                      | Substrate                       | Inoculation Concentration | Initial Antibiotic Concentration | Treatment Duration                                            | Degradation Rate                                          | Half-life | Changes in ARGs and Detection Sites                                                                                          | Changes in MGEs and Detection Sites                                                                                             | Functional Microbes in the Gut                                                                                            | Extracellular Antibiotic Degradation Capability of Gut Microbiota                                                                                                  | References |
|-----------------|-------------------------------------------|---------------------------------|---------------------------|----------------------------------|---------------------------------------------------------------|-----------------------------------------------------------|-----------|------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Quinolones      | Norfloxacin, Ciprofloxacin, Enrofloxacin) | Pig manure                      | 6 d                       | -                                | 12 days of BSFL bioconversion + 16 days of natural composting | 24.10%                                                    | -         | The absolute abundance of total ARGs in the substrate decreased by 93.20%, while the relative abundance decreased by 37.80%. | The relative abundance of plasmids, integrons, and insertion sequences in the substrate decreased by 5.50%, 41.30%, and 47.80%. | -                                                                                                                         | -                                                                                                                                                                  | 203        |
|                 | Ciprofloxacin                             | Artificial diet, Chicken manure | 8 d                       | 50 mg/kg                         | 12 d                                                          | Artificial diet group 85.48%, Chicken manure group 84.22% | -         | -                                                                                                                            | -                                                                                                                               | <i>Klebsiella pneumoniae</i> (BSFLG-CIP1), <i>Trichosporon asahii</i> (BSFLG-CIP2), <i>Proteus mirabilis</i> (BSFLG-CIP5) | In the substrate containing 50 mg/kg ciprofloxacin, BSFLG-CIP1 was able to degrade 98.22% of the drug, BSFLG-CIP5 degraded 97.83%, and BSFLG-CIP2 degraded 16.66%. | 64         |
| Chloramphenicol | Chloramphenicol, I, Thiamphenicol         | Pig manure                      | 6 d                       | -                                | 12 days of BSFL bioconversion + 16 days of natural composting | 100.00%                                                   | -         | The absolute abundance of total ARGs in the substrate decreased by 93.20%, while the relative abundance decreased by 37.80%. | The relative abundance of plasmids, integrons, and insertion sequences in the substrate decreased by 5.50%, 41.30%, and 47.80%. | -                                                                                                                         | -                                                                                                                                                                  | 203        |



Table 2. (Continued)

| Antibiotic Type | Name                                             | Substrate                        | Inoculation Concentration | Initial Antibiotic Concentration   | Treatment Duration                                            | Degradation Rate                               | Half-life | Changes in ARGs and Detection Sites                                                                                                                                                                                                                                                   | Changes in MGEs and Detection Sites                                                                                             | Functional Microbes in the Gut                                           | Extracellular Antibiotic Degradation Capability of Gut Microbiota                                       | References |
|-----------------|--------------------------------------------------|----------------------------------|---------------------------|------------------------------------|---------------------------------------------------------------|------------------------------------------------|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|------------|
| Macrolides      | Erythromycin, Roxithromycin, Tilmicosin, Tylosin | Pig manure                       | 6 d                       | -                                  | 12 days of BSFL bioconversion + 16 days of natural composting | 100.00%                                        | -         | The absolute abundance of total ARGs in the substrate decreased by 93.20%, while the relative abundance decreased by 37.80%.                                                                                                                                                          | The relative abundance of plasmids, integrons, and insertion sequences in the substrate decreased by 5.50%, 41.30%, and 47.80%. | -                                                                        | -                                                                                                       | 203        |
|                 | Tylosin                                          | 50% Wheat bran + 50% Wheat flour | 6 d                       | 100 mg/kg                          | 12 d                                                          | 93.90% (10.10% higher than the control group)  | -         | -                                                                                                                                                                                                                                                                                     | -                                                                                                                               | Providencia, Morganella                                                  | -                                                                                                       | 254        |
|                 | Lincomycin                                       | Wheat bran, Lincomycin           | 4 d                       | 1000 mg/kg, 1500 mg/kg, 2000 mg/kg | 12 d                                                          | 84.90% (3 times higher than the control group) | -         | The abundance of <i>InuA</i> , <i>InuC</i> , and <i>InuE</i> in the gut increased during the first 4 days, while the abundance of <i>InuC</i> and <i>InuE</i> decreased after Day 4. In contrast, the abundance of <i>InuB</i> , <i>InuD</i> , and <i>InuF</i> increased after Day 4. | -                                                                                                                               | Alcaligenes faecalis action of (GLD-1), Ochrobactrum intermedium (GLD-2) | With the synergistic action of isolated strains, the degradation rate of lincomycin by BSFL was doubled | 187        |
| Sulfonamides    | Sulfamethoxazole                                 | 50% Wheat bran + 50% Wheat flour | 6 d                       | 100 mg/kg                          | 12 d                                                          | 88.40% (12.29% higher than the control group)  | -         | -                                                                                                                                                                                                                                                                                     | -                                                                                                                               | Pseudomonas, Dysgonomonas, Morganella, Klebsiella                        | -                                                                                                       | 254        |

Table 2. (Continued)

| Antibiotic Type | Name         | Substrate                        | Inoculation Concentration | Initial Antibiotic Concentration | Treatment Duration | Degradation Rate                                                                                                                            | Half-Life | Changes in ARGs and Detection Sites                                                                                                                                                                                                                        | Changes in MGEs and Detection Sites | Functional Microbes in the Gut                                                                                                                                                                                                                                               | Extracellular Antibiotic Degradation Capability of Gut Microbiota | References |
|-----------------|--------------|----------------------------------|---------------------------|----------------------------------|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|------------|
| Sulfonamides    | Sulfadiazine | 50% Wheat bran + 50% Wheat flour | -                         | 100 mg/kg, 500 mg/kg, 1000 mg/kg | 6 d                | 73.99% (25.58% higher than the control group), 70.96% (48.94% higher than the control group), 25.89% (13.23% higher than the control group) | -         | The absolute abundance of <i>sul1</i> and <i>sul2</i> in the substrate decreased by 79.26% and 79.89%, respectively, compared to the control group. The relative abundance of <i>sul1</i> and <i>sul2</i> in the gut was lower than that in the substrate. | -                                   | <i>Clostridium sensu stricto</i> 1, <i>Nesterenkonia</i> , <i>Bacillus</i> , <i>Halomonas</i> , <i>Dysgonomonas</i> , <i>Caldalkalibacillus</i> , <i>Enterococcus</i> , <i>g_unclassified_f_Xa</i> , <i>nthomonadaceae</i> , <i>g_unclassified_f_Mi</i> , <i>crococaceae</i> | -                                                                 | 198        |
|                 |              |                                  |                           |                                  |                    |                                                                                                                                             |           |                                                                                                                                                                                                                                                            |                                     |                                                                                                                                                                                                                                                                              |                                                                   |            |
|                 |              |                                  |                           |                                  |                    |                                                                                                                                             |           |                                                                                                                                                                                                                                                            |                                     |                                                                                                                                                                                                                                                                              |                                                                   |            |
| Aminoglycosides | Gentamicin   | 50% Wheat bran + 50% Wheat flour | -                         | 100 mg/kg                        | 12 d               | 80.54%, 89.19% and 92.22%, respectively                                                                                                     | -         | -                                                                                                                                                                                                                                                          | -                                   | <i>Firmicutes</i> , <i>Bacillus</i>                                                                                                                                                                                                                                          | -                                                                 | 256        |
|                 |              |                                  |                           |                                  |                    |                                                                                                                                             |           |                                                                                                                                                                                                                                                            |                                     |                                                                                                                                                                                                                                                                              |                                                                   |            |
| Aminoglycosides | Gentamicin   | 50% Wheat bran + 50% Wheat flour | -                         | 100 mg/kg                        | 12 d               | 50.70% (24.20% higher than the control group)                                                                                               | -         | -                                                                                                                                                                                                                                                          | -                                   | <i>Dysgonomonas</i>                                                                                                                                                                                                                                                          | -                                                                 | 254        |
|                 |              |                                  |                           |                                  |                    |                                                                                                                                             |           |                                                                                                                                                                                                                                                            |                                     |                                                                                                                                                                                                                                                                              |                                                                   |            |

tution and cleavage, quinoline ring cleavage, hydrolysis, oxidation, deamination, demethylation, ring opening, and modification.<sup>64,186</sup> These results indicate that the BSFL gut microbiota have a strong capacity for the direct degradation of antibiotics.

### BSFL gut microbes reduce substrate ARGs

ARGs are critical tools for microorganisms to resist ABs. With the assistance of mobile genetic elements (MGEs), ARGs can be replicated from the genome of the original microorganism or directly transferred to another bacterial DNA fragment, conferring antibiotic resistance.<sup>200,201</sup> Recent studies have shown that BSFL gut microbiota can effectively inhibit the generation of ARGs in composting systems (Figure 4).<sup>135,202,203</sup> Compared to systems without BSFL gut microbiota, those containing BSFL gut microbiota demonstrated a 6.3% higher reduction rate of ARGs (*tet A*, *tet C*, *tet G*, *tet M*, *tet O*, *tet Q*, *tet T*, *tet W*, *tet X*) in chicken manure.<sup>135</sup> In another study on pig manure, BSFL gut microbes such as *Clostridium butyricum* and *C. bornimense* were found to compete with antibiotic-resistant bacteria like *Selenomonas* and *Paenicaligenes* for nutrients (e.g., nitrogen and lipids), thereby suppressing the proliferation of antibiotic-resistant bacteria.<sup>203</sup> Concurrently, the relative abundances of plasmids, integrons, and insertion sequences in pig manure decreased by 39.7%, 47.6%, and 43.9%, respectively, while the absolute abundance of ARGs declined by 78.4%.<sup>203</sup> These findings were further supported by Zhao et al.,<sup>202</sup> who observed that BSFL larvae treatment of fresh chicken manure significantly reduced the abundance of antibiotic-resistant bacteria, including *Corynebacterium glutamicum*, *S. aureus*, *Enterococcus faecium*, *Enterococcus thailandicus*, *Lactobacillus reuteri*, *Escherichia coli*, and *Pseudomonas aeruginosa*, by 52.6%, 29.7%, 52.2%, 100%, 28.8%, 52.6%, and 1.4%, respectively, compared to the control group without BSFL. Additionally, the abundance and relative abundance of plasmids decreased by 4.9% and 12.4%, respectively, while those of insertion sequences declined by 17.1% and 32.4%, and integrons by 16.8% and 46.6%. The relative abundance of ARGs also decreased significantly by 53.3%, with transferable ARGs associated with MGEs (e.g., macrolide-lincosamide-streptogramin B resistance genes, aminoglycoside resistance genes, and tetracycline resistance genes) showing an 18.5% reduction in relative abundance. These results suggest that BSFL gut microbiota may reduce the mobility and abundance of ARGs by inhibiting the proliferation of antibiotic-resistant bacteria and the generation of MGEs in composting systems.

However, the use of BSFL to process organic waste containing complex pollutants may increase the risk of ARGs accumulation in the BSFL gut (Figure 4). For instance, during the BSFL-mediated bioconversion of sewage sludge, the relative and absolute abundances of ARGs such as *blaCTX-M-32*, *ermB*, *tetA*, *tetQ*, and *tetW* in the substrate significantly decreased (with absolute abundances reduced by <97%, <97%, <64%, <97%, and <99%, respectively), the relative abundances of *Int11*, *su11*, and *tetW* in BSFL significantly increased. Concurrently, the abundance of potential ARGs hosts, including *Gordonia*, *Mycobacterium*, *Pseudochrobactrum*, and *Romboutsia* in the BSFL gut rose markedly. In contrast, no ARG accumulation risk was observed in BSFL reared on wheat bran.<sup>204</sup> Sewage sludge, which are organic waste rich in HMs, MPs, pharmaceuticals, cosmetics, and other pollutants, may exacerbate this risk.<sup>205</sup> Certain contaminants, such as MPs, can elevate intestinal inflammatory factors (ROS, IL-1, IL-7, IL-11) in BSFL, induce gut barrier damage, and promote the colonization of antibiotic-resistant bacteria (e.g., *Enterococcaceae*, *Hungateiclostridiaceae*, and *Clostridia*), thereby facilitating ARGs accumulation in BSFL.<sup>206</sup> Current studies suggest that short-term starvation treatment or high-temperature treatment of BSFL contaminated with ARGs can effectively reduce ARGs and mitigate their transmission risk to other organisms.<sup>207</sup> Nevertheless, it is recommended to classify and separate pollutants by type prior to BSFL-mediated organic waste processing to avoid synergistic toxicity and cumulative amplification of contaminants, which may exceed the bioremediation capacity of BSFL.

### BSFL GUT MICROBES REDUCE HMs BIOTOXICITY AND PROMOTE MPs DEGRADATION

#### BSFL gut microbes reduce HMs biotoxicity

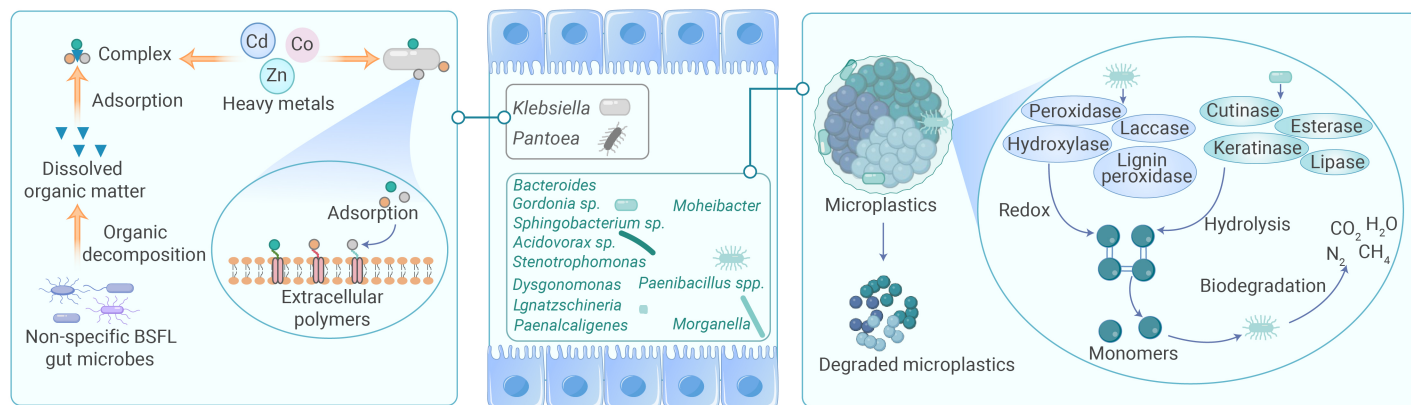
Excessive accumulation of HMs in the environment can severely impact soil microbial structure, crops, and groundwater.<sup>208-212</sup> In recent years, it has

been discovered that BSFL not only facilitates the efficient transformation of organic waste but also reduces the toxicity of HMs (Figure 5). For instance, *Klebsiella* and *Pantoea* isolated from the BSFL gut can reduce up to 62.30% Zn, 62.36% Co, and 77.2% Cd in the substrate, probably through bacterial adsorption.<sup>65,66</sup> This is because a significant amount of extracellular polymers on the surface of most biofilms contains carboxyl, hydroxy, mercapto, amino, amide, and phosphate groups. These extracellular polymers can directly bind free HMs, adsorbing them to the cell membrane surface, thereby reducing the concentration of mobile HMs in the environment.<sup>213</sup> In addition, through the action of BSFL and their gut microbes, substrates can produce DOM characterized by anionic complex groups such as humic acid, and tryptophan. Such DOM can bind HMs, forming stable complexes, which in turn reduces the biotoxicity of HMs.<sup>65,214</sup> However, while BSFL gut microbiota can effectively transform and detoxify HMs, certain HMs may still accumulate in the larval body, posing contamination risks. For instance, BSFL fed seaweed containing 36 mg/kg arsenic accumulated 22.5 mg/kg arsenic in their bodies,<sup>215</sup> far exceeding the Chinese feed hygiene standard limit (GB 13078-2017: 2 mg/kg). Similarly, when consuming corn leaves contaminated with 50 mg/kg Cd and Zn, BSFL accumulated 502.16 mg/kg Cd and 373.25 mg/kg Zn, respectively.<sup>216</sup> In wheat bran substrates with high copper (800 mg/kg) and Cd (80 mg/kg) levels, BSFL accumulated 549.0 mg/kg copper and 25.3 mg/kg Cd, with Cd levels severely exceeding regulatory limits.<sup>214</sup>

Current strategies to mitigate HMs accumulation in BSFL primarily include short-term starvation and substrate optimization. Studies demonstrate that fasting BSFL for 1.8–2.3 days after exposure to Cd (50–700 mg/kg) reduces larval Cd content by 50%.<sup>217</sup> Similarly, fasting BSFL for 0.98–1.6 days following mercury exposure (0.1–1 mg/kg) decreases mercury content by 50%.<sup>218</sup> Thus, short-term starvation represents a feasible strategy to prevent heavy metal transfer through BSFL and enhance their biosafety. Additionally, optimizing substrate composition to promote the formation of colloidal particles through interactions between HMs and DOM components (e.g., fulvic acid, humic acid) during composting can reduce HMs mobility and mitigate transfer risks.<sup>65,219</sup>

### BSFL gut microbes accelerate plastic degradation

MPs are small plastic particles (<5 mm) formed from the decomposition of plastic products. They exhibit very low degradation efficiency in their natural state<sup>220</sup> and can adsorb bacteria, viruses, and HMs in the environment, forming a virulence group. When ingested by living organisms, this group can increase the risk of organ damage and metabolic diseases.<sup>221</sup> Additionally, plasticizers, such as phthalates, which are used to optimize and modify the physico-chemical properties of plastics, can disrupt the human endocrine system and cause endocrine-related diseases.<sup>222,223</sup> The effective degradation of MPs and plasticizers, while ensuring biosafety and adhering to green standards, is a significant concern. Recent studies have shown that the intestinal microorganisms of BSFL can degrade MPs and plasticizers. When BSFL ingest polyethylene or polystyrene, the intestinal organisms, including *Gordonia* sp., *Sphingobacterium* sp., *Paenibacillus* spp., and *Acidovorax* sp., enhance the oxidation of the aromatic ring structures of these MPs. This process reduces the structural stability of MPs through the production of enzymes such as alkane hydroxylases, monooxygenases, multicopper oxidases, and laccases.<sup>67</sup> Furthermore, with the assistance of gut microbes such as *Ignatzschineria*, *Paenicaligenes*, *Moheibacter*, *Morganella*, *Dysgonomonas*, *Stenotrophomonas*, *Bacteroides*, and *Sphingobacterium*, BSFL can degrade up to 25.52% polyethylene, 22.21% polypropylene, 97.04% dimethyl phthalate, and 88.99% diethyl phthalate in the substrate within 8 days (Figure 5).<sup>129</sup> In general, gut microorganisms degrade MPs primarily through fragmentation, biodegradation, and mineralization.<sup>224-226</sup> These microbes adhere to the microplastic surface and form a biofilm, during which hydrophilic functional groups are transferred to the microplastic surface by microbial extracellular enzymes (such as oxidoreductases and hydrolases), which weaken the stability and resistance of the MPs.<sup>227</sup> Oxidoreductases (such as laccase, lignin peroxidase, hydroxylase, and peroxidase) produced by bacteria catalyze the formation of free radicals from the chemical groups on the surface of MPs, oxidising oligomers and monomers. Conversely, hydrolytic enzymes (including keratase, keratinase, esterase, and lipase) cleave chemical groups on the surface of polymers into oligomers and monomers, facilitating the degrada-



**Figure 5. Effect of BSFL gut microbes on substrate HMs and MPs** The gut microorganisms of BSFL, specifically *Klebsiella* and *Pantoea*, may be capable of adsorbing free HMs present in the substrate through various ionic groups on the biofilm surface. Additionally, DOM generated during organic matter decomposition by these gut microorganisms effectively adsorb free HMs. Meanwhile, other intestinal microorganisms such as *Gordonia* sp., *Sphingobacterium* sp., *Paenibacillus* spp., *Acidovorax* sp., *Ignatzschineria*, *Paenibacillus*, *Moheibacter*, *Morganella*, *Dysgonomonas*, *Stenotrophomonas*, and *Bacteroides* could potentially degrade MPs and destabilize their structure through various hydrolytic reductases and hydrolase pairs. These microorganisms might then be able to utilize the resulting small molecules from the cleavage.

tion of MPs.<sup>228-230</sup> The resulting short-chain polymers and smaller molecules (oligomers and monomers) can serve as carbon or energy sources for microorganisms. These microorganisms can aggregate intracellularly through semi-permeable membranes and mineralize these compounds into inorganic products such as  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ ,  $\text{CH}_4$ , and  $\text{N}_2$ .<sup>229,231</sup>

However, excessive concentrations of MPs in substrates can alter the structure of the BSFL gut microbiota, increase MPs accumulation in the larval body, and elevate biotoxicity.<sup>67,232,233</sup> Although studies confirm that MPs in the gut are effectively excreted during the prepupal stage,<sup>233</sup> whether MPs accumulate in organs other than the gut or pose risks of product contamination remains uninvestigated. Therefore, when processing substrates containing polyvinyl chloride, polystyrene, polypropylene, or polyethylene, it is advisable to maintain their concentrations below 10% to avoid significant adverse effects on BSFL growth and development.<sup>129,232,234</sup> For substrates containing dimethyl phthalate and diethyl phthalate, concentrations should be limited to <0.4%.<sup>129</sup> Similarly, substrates containing polyamide and polylactic acid should be restricted to concentrations below 0.22%.<sup>233</sup>

#### DEVELOPMENT AND APPLICATION OF BSFL GUT MICROORGANISMS

Recent studies have demonstrated that specific microbial strains isolated from the BSFL gut exhibit significant potential in enhancing bioconversion efficiency, nutrient synthesis, and pollutant degradation (see Table 3). For instance, Mazza et al.<sup>235</sup> found that inoculating *Bacillus subtilis* (isolated from the gut of BSFL) into fecal heaps increased the fecal reduction rate by 4.35%, the biotransformation rate by 1.32%, and the larval weight by 17.79%. Additionally, supplementing microorganisms such as *Candida tropicalis* and *Pichia kudriavzevii* (isolated from the gut of BSFL) increased BSFL production by approximately 36%.<sup>145</sup> Similarly, Liu et al.<sup>138</sup> found that inoculating *Bacteroides ovatus*, isolated from the gut of BSFL, into a compost pile increased lauric acid synthesis by 25%, palmitic acid synthesis by 18%, and oleic acid synthesis by 25%. Furthermore, reintroducing *Klebsiella pneumoniae* and *Proteus mirabilis*, isolated from the BSFL gut, into the compost heap increased ciprofloxacin degradation in the substrate by 60.60%.<sup>64</sup> These findings highlight the potential of BSFL gut microorganisms to enhance larval quality, accelerate substrate degradation, improve the biosafety of compost, and produce high-value products.

Microorganisms isolated from the gut BSFL also have the potential to serve as probiotics in livestock and poultry farming. Four strains of lactic acid bacteria were recently identified from the BSFL gut, including *Lactiplantibacillus pentosus* 5P2i1, *Lactocaseibacillus paracasei* 5P2i5, *Lactiplantibacillus pentosus* 5P2i9, and *Lactiplantibacillus plantarum* 6P1i1. These strains exhibit significant antagonistic effects against intestinal pathogens, as well as strong tolerance to pH and bile salts.<sup>236</sup> These findings suggest that BSFL could be a valuable source of natural probiotics, offering additional health benefits for livestock and poultry. However, ensuring the biosafety and technical viability of these functional microorganisms is essential for their

safe and effective applications.

#### CONCLUSION

Despite the great potential of BSFL in organic waste treatment and resource recovery, there are still many challenges and limitations. First, the types of organic waste that can be bioconverted by BSFL are diverse, such as food waste, chicken manure, pig manure, cow manure, slaughterhouse waste, blue-green algae, human feces, and even sewage sludge.<sup>84,85,237</sup> However, the growth and development of BSFL vary greatly with different organic wastes, and the optimal substrate combinations for BSFL growth are still unknown, significantly limiting the efficiency of BSFL bioconversion. Second, when treating organic waste containing high concentrations of toxic and harmful substances, BSFL may disrupt the gut environment stability, induce the colonization of PB carrying harmful substances (such as ARGs) in the gut, and lead to the accumulation of harmful substances in BSFL, affecting their safety as feed or fertilizer.

As one of the most promising insect species for resource development, the future development of BSFL bioconversion technology needs to be standardized and diversified. First, it is necessary to clarify the main production purposes of current BSFL bioconversion (such as eliminating organic waste, producing animal edible protein and fat, and biofertilizers), and to formulate and unify BSFL bioconversion technology according to different production purposes. For example, developing the best substrate formulas suitable for BSFL growth for different organic wastes, ensuring balanced nutrition, and pre-treating substrates through physical or chemical methods (such as grinding, fermentation) to improve cellulose utilization and overall treatment efficiency. Given the increasing importance of BSFL-derived protein in animal protein feed substitution, strengthening the safety assessment of BSFL bioconversion products, especially monitoring for harmful substances such as ABs, ARGs, and HMs, is particularly critical. It is necessary for practitioners to vigorously promote the formulation of hygiene standards for insect protein obtained from BSFL bioconversion and to standardize the use of BSFL-derived protein. Second, the functional fatty acids, AMPs, chitin, and its derivative chitosan found in BSFL are known beneficial substances.<sup>101,102</sup> By adjusting the substrate or adding probiotics and nutritional supplements, the synthesis of functional nutrients in BSFL can be specifically promoted.<sup>138,180,182</sup> This not only helps produce high-value products and improves economic benefits but also promotes health and sustainable development in the medical and agricultural fields. In addition, the in-depth development of BSFL bioconversion technology is also very important. Strengthening the research and application of the BSFL gut microbiota, exploring their functions and mechanisms in organic waste degradation, PB inhibition, ABs, ARGs and MPs degradation, and the reduction of HMs activity, and screening and cultivating gut microbial strains with high degradation capabilities, are of great significance for further improving the efficiency of BSFL bioconversion and ensuring product safety.

Table 3. Functional microbial strains from BSFL gut and their biotechnological applications

| BSFL Gut Isolates                                                                                                                                                                                    | Gut Microbiota Functions                                                                                                                                                            | References |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <i>Bacillus subtilis</i>                                                                                                                                                                             | The reduction rate of organic waste increased by 4.35%, the bioconversion rate improved by 1.32%, and the larval weight increased by 17.79%.                                        | 235        |
| <i>Candida tropicalis</i> ,<br><i>Pichia kudriavzevii</i>                                                                                                                                            | Compared to aseptic BSFL, the protein degradation rate of BSFL was increased by approximately 20%.                                                                                  | 56         |
| <i>Bacteroides ovatus</i>                                                                                                                                                                            | Compared with aseptic BSFL, the synthesis of lauric acid in BSFL was promoted by 25%, palmitic acid by 18%, and oleic acid by 25%.                                                  | 138        |
| <i>Serratia marcescens</i> ,<br><i>Bacillus subtilis</i>                                                                                                                                             | Inhibit the proliferation of <i>Escherichia coli</i> , <i>Salmonella</i> , and <i>Staphylococcus aureus</i> .                                                                       | 59         |
| <i>Lactobacillus casei</i>                                                                                                                                                                           | Stimulate the expression of cecropin 1 (HiCec1) and defensin 1 (HiDef1) in the hemolymph of BSFL.                                                                                   | 180        |
| <i>Enterococcus faecalis</i> ,<br><i>Proteus mirabilis</i>                                                                                                                                           | Degraded more than 54.40% of tylosin and 57.20% of enrofloxacin.                                                                                                                    | 63         |
| <i>Klebsiella pneumoniae</i> ,<br><i>Proteus mirabilis</i>                                                                                                                                           | Degraded 84.22% of ciprofloxacin.                                                                                                                                                   | 64         |
| <i>Serratia sp.</i> ,<br><i>Trichosporon asahii</i> ,<br><i>Pichia kudriavzevii</i> ,<br><i>Candida rugosa</i> ,<br><i>Galactomyces geotrichum</i> ,<br><i>Serratia marcescens</i>                   | Degraded 50.00%, 48.20%, 28.90%, 44.20%, 39.20%, and 28.50% of tetracycline.                                                                                                        | 186        |
| <i>Klebsiella</i> ,<br><i>Pantoea</i>                                                                                                                                                                | Reduced 62.30% of zinc, 62.36% of cobalt, and 77.20% of cadmium in the substrate, thereby mitigating heavy metal pollution.                                                         | 65         |
| <i>Lactiplantibacillus. pentosus</i><br>5P2i1,<br><i>Lacticaseibacillus. paracasei</i><br>5P2i5,<br><i>Lactiplantibacillus. pentosus</i><br>5P2i9,<br><i>Lactiplantibacillus. plantarum</i><br>6P1i1 | The good pH and bile salt tolerance, along with antagonistic activity against intestinal pathogens, indicate the potential for development as probiotics for livestock and poultry. | 236        |

ABBREVIATIONS

Antibiotics (ABs); Heavy metals (HMs); Antibiotic resistance genes (ARGs); Microplastics (MPs); Black soldier fly larvae (BSFL); Pathogenic bacteria (PB); Antimicrobial peptides (AMPs); Zinc (Zn); Cobalt (Co); Cadmium (Cd); Dissolved organic matter (DOM).

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## AUTHOR CONTRIBUTIONS

J.P. and D.W. conceived the original idea and provided critical revisions. J.P., S.Z. and R.L. wrote the manuscript and designed the figures. M.Y. designed the figures. Y.H. and Y.Y. reviewed and revised the manuscript. R.Q. and W.W. revised the manuscript. W.C. and S.M. contributed to the table creation and data compilation. All authors contributed to the manuscript and approved the final version.

## DECLARATION OF INTERESTS

Yulong Yin is an Editorial Board member of *The Innovation Life*. He was blinded from reviewing or making final decisions on the manuscript. The article was subject to the journal's standard procedures, with peer review handled independently of these Editorial Board members and their research groups. The other authors declare that they have no conflicts of interest.

## ETHICAL STATEMENT AND PATIENT CONSENT

Not applicable.

## DATA AND CODE AVAILABILITY

Not applicable.