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Black Soldier Fly (*Hermetia illucens*), a source of antimicrobial peptides

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Abstract. In the modern world, multi-drug antibiotic resistance is an increasingly serious problem which poses a serious threat to both plants, animals and humans. The unreasonable use of antibiotics has led to the spread and increase in the number of infectious diseases that existing antibiotics may not be able to cope with. Thus, there is a need for the development of new classes of antibiotics that do not induce resistance. It is necessary to find agents with new mechanisms of action for the development of such antimicrobial compounds. Antimicrobial peptides are excellent candidates for this role. Penetrating through the membranes, they affect the target protein without high specificity, which in turn reduces the probability of induced resistance to a minimum [13]. Scientists have identified about 57 active peptides belonging to various groups of antimicrobial peptides, including defensins, cecropins, attacins and lysozyme. Defensins form the largest group of antimicrobial peptides in insects. As a rule, a defensin-like peptide contains from 34 to 43 amino acids. Antimicrobial peptides obtained from *Hermetia illucens* can become a good alternative to antibiotics for the prevention and treatment of infectious diseases, as they differ in their antimicrobial properties and are less likely to induce resistance [8]. The lipid composition as well as the amino acid composition can be changed by different larvae diets. Larvae fat contains oleic, palmitic, lauric, myristic, stearic and palmitolic acids, which are saturated and unsaturated fatty acids, and have an intense effect against bacteria.

1 Introduction

Antimicrobial peptides (AMPs) are molecules of the innate immune defense of the body, consisting of 15 – 20 amino acid residues preventing the pathogen from entering the host. Antimicrobial peptides are able to selectively target bacteria, since their cationic molecules have a strong affinity for bacterial membranes enriched with negatively charged components – lipopolysaccharides and others. Due to their versatile nature of interactions with membranes, most AMPs have a wide spectrum of action and increased resistance to

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microorganism adaptation [2]. The development of antimicrobial peptides resistance in bacteria is hindered by the peculiarities of the bactericidal action mechanism: the rapid increase in membrane permeability of microorganisms, as well as the loss of their protective function, leading to osmotic cell disruption. Antimicrobial peptides do not accumulate in the body; they are bound and blocked by plasma proteins, and destroyed by proteases. AMPs are able to have various immunomodulatory effects (for instance, they stimulate the natural killer cells activity), without suppressing of the body immune system functions [1]. That is why they can provide an alternative to antibiotics and be used to develop new classes of antimicrobial drugs.

2 Main part

A huge diversity of insects with substantial reserves of amino acids enables us to use them in order to obtain antimicrobial peptide sources. *Hermetia illucens* (the Black Soldier Fly; BSF) is eminently suitable as a source of AMPs, as it has an outstanding ability to adapt and survive in conditions with a wide diversity of microorganisms (Fig. 1) [5].

Fig. 1. *Hermetia illucens* (Black Soldier Fly; BSF) [3].

Initially, *Hermetia illucens* was bred in order to develop and implement a closed-loop economy in the fields of biological purification, waste management, processing of industrial by-products and agricultural waste bioconversion. The BSF larvae breeding is an economical way to convert organic residues into a source of biomolecules [11].

Scientists have identified about 57 active peptides belonging to various groups of antimicrobial peptides, including defensins, cecropins, attacins and lysozyme (Fig. 2). 13 of the identified AMPs have antimicrobial activity; 22 antimicrobial and antitumor; 8 antimicrobial and antiviral; 2 antimicrobial and antifungal; 7 antimicrobial; 7 antimicrobial, antitumor and antiviral activity. Although attacins and lysozyme are proteins due to their high molecular weight, they belong to the AMP classes, since they exhibit antibacterial activity [4].

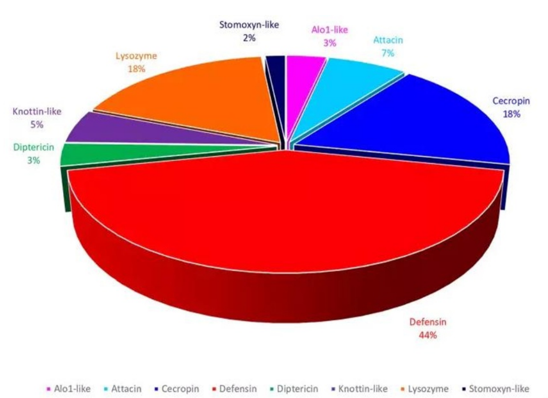

Fig. 2. Identified AMP groups from larvae and adult Hermetia illucens transcriptomes of [4].

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The natural bioactive compounds isolated from the larvae fat (Fig. 3) are capable of destroying drug-resistant bacteria prevalent in agriculture, medicine and veterinary medicine that harm plants, animals and people [7].

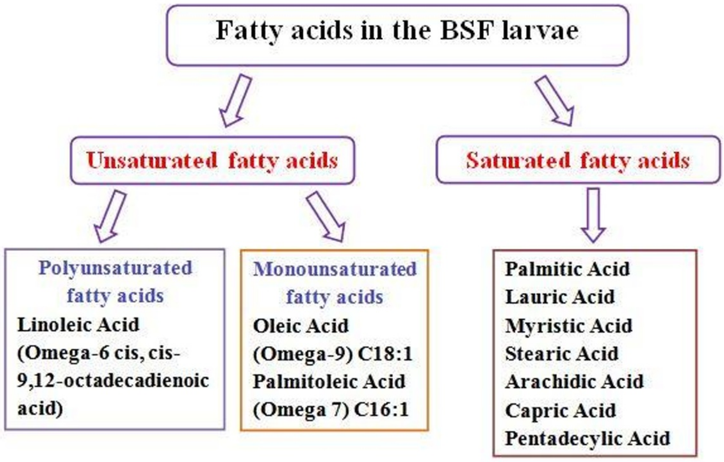

Fig. 3. The content of fatty acids in the Hermetia illucens larvae [7].

In addition to AMPs, the BSF larvae composition provides a valuable source of biologically active compounds enriched with lipids, such as oleic, lauric, myristic, linoleic, capric, palmitic fatty acids and others, with myristic acid having a wide range of antibacterial action, as well as larvicidal and repellent activity (Table 1).

Table 1. Fatty acid composition of a 15-day-old H. Illucens larva [6].

Parameters	**Result (% w/w)**	
	Raw	**Steam**
Fat content	**38.09**	**27.49**
Saturated fatty acids:		
Capric Acid (C10:0)	0.84	0.81
Lauric Acid (C12:0)	40.29	49.18
Tridecanoic Acid (C13:0)	0.02	0.03
Myristic Acid (C14:0)	6.76	8.09
Pentadecanoic Acid (C15:0)	0.12	2.70
Palmitic Acid (C16:0)	9.99	8.53
Heptadecanoic Acid (C17:0)	0.11	0.19
Stearic Acid (C18:0)	1.27	1.42
Arachidic Acid (C20:0)	0.04	0.05
Behenic Acid (C22:0)	0.02	0.04
Unsaturated fatty acids:		
Myristoleic Acid (C14:1)	0.16	0.23
Palmitoleic Acid (C16:1)	2.07	2.70
Cis-10-Heptadecanoic Acid (C17:1)	0.00	0.24
Elaidic Acid (C18:2n9t)	0.30	0.29
Oleic Acid (C18:1n9c)	7.99	5.94
Linolelaidic Acid (C18:2n9t)	0.00	1.41
Linoleic Acid (C18:2n6c)	4.02	0.03
v-Linoleic Acid (C18:3n6)	0.00	0.00
Cis-11,14-Eicosedienoic Acid (C20:2)	0.02	0.03
Cis-8,11,14-Eicosetrienoic Acid (C20:3n6)	0.03	0.02
Total fatty acids	74.04	79.41

Scientists have found that hexanedioic acid isolated from the BSF larvae successfully fights against gram-positive and gram-negative bacteria. The composition of lipids varies depending on the methods of larvae processing, which in turn leads to different fatty acid profiles [8,9,10]. The BSF larvae lipids are a high-quality product, not inferior to the competitors obtained from other plant and animal sources. The lipid composition as well as the amino acid composition can be changed by different diets of larvae. Lipids and their derivatives have antimicrobial properties [4].

The experiments conducted by scientists have demonstrated that the BSF larvae fat extract exhibit antibacterial activity against various bacteria (Table 2). During the research, a special solution (AWME – acidified water-methanol extract) was developed for the *Hermetia illucens* larvae extraction capable of extracting biologically active molecules [7].

Table 2. Content and published biological activity of major compounds of AWME of *H. Illucens* larvae fat [7].

NAME OF COMPOUNDS	CONTENT (%)	BIOLOGICAL ACTIVITY
Octadec-9-enoic acid (oleic acid)	22.22	Antibacterial
n-Hexadecanoic acid (palmitic acid)	20.34	Antibacterial
Dodecanoic acid (lauric acid)	18.48	Antibacterial
Tetradecanoic acid (myristic acid)	5.59	Antibacterial
Octadecanoic acid (stearic acid)	5.34	Antibacterial
cis-9-Hexadecenoic acid (palmitoleic acid)	3.02	Antibacterial
1,2,3-Propanetriol	6.88	Antimicrobial and antiseptic
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	0.51	Antimicrobial
cis-9-Hexadecene	0.15	Antimicrobial
9-Octadecenic acid (Z)-, methyl ester (oleic acid methyl ester)	1.62	Antimicrobial
Dodecanoic acid, 2,3-dihydroxypropyl ester (monolaurin)	1.32	Antimicrobial
Eicosanoic acid (arachidic acid)	0.36	Antibacterial, antifungal, antioxidant
Hexadecanoic acid methyl ester (palmitic acid methyl ester)	0.35	Antibacterial and antifungal
Octadecenic acid, methyl ester (stearic acid methyl ester)	0.34	Antimicrobial
9,12-Octadecadienoic acid (Z, Z) - (linoleic acid)	0.23	Antibacterial

Table 2 data confirms the antibacterial activity of a mixture of AWME the BSF larvae fat (obtained by pressing live 15-day-old larvae) against phytopathogenic bacteria. As previously reported, larval fat contains oleic, palmitic, lauric, myristic, stearic and palmitolic acids, which are saturated and unsaturated fatty acids, and have an intense effect against bacteria. That is consistent with the activity of the fatty acids listed in the table.

The BSF larvae proteins, fats and meal already act as additives in animal feed and cosmetics. Due to its antimicrobial properties the BSF may be used in the production of medicines and foodstuffs as well [12].

According to the data presented in Table 3, it is evident that biologically active substances of the larvae fat have antimicrobial activity. Using the agar diffusion method, the fat was found to inhibit the growth of Bacillus subtilis, Staphylococcus aureus, Escerichia coli and Candida albicans. Their content level in the samples is within the permissible range, and in some cases they were not detected at all.

Table 3. *Hermetia illucens* larvae fat antimicrobial activity [12].

Sample	Suppression zone diameter, mm			
	Bacillus subtilis*	***Staphylococcus aureus****	***Escherichia coli****	***Candida albicans****
Bright Fat Fraction (BFF)	12.5±1.5	0	0	10.5±0.7
Directly pressed fat (DPF)	0	0	0	12.5±0.9
Dark Fat Fraction (DFF)	16.5±0.5	12.5±1.5	10.0±0.0	12.5±0.9
Protein Fraction (PF)	10.0±0.3	0	0	0
Note: *Bacillus subtilis* – hay bacillus; *Staphylococcus aureus* – "golden staph"; *Escherichia coli* – E. coli; *Candida albicans* – diploid fungus.				

During the development of a model with the BSF larvae fat aimed to optimize the fatty acids composition and ratio, it was found that the total number of microorganisms increases when stored at a temperature of 5°C, but at the same time the number of microscopic fungi decreases (Table 4).

Table 4. The BSF larvae fat antimicrobial and antifungal activity in plant-based food matrix [12].

Indicators	Samples			
	№ 30, 1st day	**№ 30, 30th day**	**№ 50, 1st day**	**№ 50, 30th day**
Microorganism count, CFU/g	8.8×10^2	1.9×10^7	1.6×10^2	1.6×10^6
Coliform bacteria count, CFU/g	7.0×10^1	$< 4.0\times10^1$	$< 1.0\times10^1$	5.0×10^1
Total number of mesophilic lactic acid bacteria, CFU/g	1.1×10^2	$< 1.0\times10^1$	8.0×10^1	$< 1.0\times10^1$
Number of suspected bifidobacteria, CFU/g	$< 1.0\times10^1$	$< 1.0\times10^1$	$< 1.0\times10^1$	$< 1.0\times10^1$
Total number of sulfite-reducing bacteria (clostridia), CFU/g	$< 1.0\times10^1$	$< 1.0\times10^1$	$< 1.0\times10^1$	$< 1.0\times10^1$
Yeast count, CFU/g	9.0×10^1	2.2×10^4	$< 1.0\times10^1$	3.4×10^4
Number of mold fungi, CFU/g	1.1×10^2	1.7×10^2	4.7×10^2	$< 4.0\times10^1$
Microorganism count, CFU/g	8.8×10^2	1.9×10^7	1.6×10^2	1.6×10^6
Sample 30. 30% of the total fat content in the plant-based food matrix was replaced by larval fat; Sample 50. 50% of the total fat content in the plant-based food matrix was replaced by larval fat. The samples were stored at +5°C				

The data given in Table 4 shows that the BSF larvae fat antimicrobial properties increase microbiological safety and stability of microbiological parameters in food systems.

3 Conclusion

Thus, it can be concluded that Hermetia illucens larvae act as a natural biodegrader, since they are in an environment closely related to pathogenic microorganisms (bacteria and fungi). The conditions in which insects are grown have a significant impact on their innate immune systems. The BSF body immune system plays an important role in their survival: it gives them the ability to adapt to various environmental conditions, as well as to produce various kinds of antimicrobial peptides [6]. They, in their turn, can later be used in various spheres of our life: agriculture, animal husbandry, veterinary medicine, medicine and the food industry.

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