Article

Analysis of *Sogatella furcifera* (Horvath) soluble proteins by SDS-PAGE

ZiQiang Liang, **TingTing Liu**, **AnWen Liang**, **GuangHong Li**, **FangHai Wang**

State Key Laboratory for Biocontrol and Institute of Entomology, Sun Yat-sen University, Guangzhou, China

E-mail: lsswfh@mail.sysu.edu.cn

Received 26 August 2016; *Accepted 2 September 2016*; *Published 1 December 2016*

Abstract

The soluble proteins from nymphs and adults of *Sogatella furcifera* were analyzed by SDS-PAGE. The number of protein bands increased gradually as the nymphs developing, such as six and 14 protein bands were found in 3rd-instar nymphs and 5th-instar nymphs respectively. At the same time, we found that three bands expressed in each instar, two bands began to appear from 4th-instar, and six bands were specific in 5th-instar. There were four bands that their content in 5th-instar nymphs with long-winged disc was at least 65.61% higher than in 5th-instar nymphs with short-winged disc. There were 13 protein bands observed in male adults, while female adults had 13 corresponding protein bands and a specific band expressed only in tissue. Comparing between two wing-type adults, four bands were specific to long-winged adults, while the content of other three bands in long-winged adults was at least 72.54 % higher than in short-winged adults. Finally, these specific protein bands associated with wing or sex were discussed what kind role they played in wing or sexual differentiation. The results will be helpful to further explore the mechanism of wing or sexual differentiation about planthoppers.

Keywords SDS-PAGE; *Sogatella furcifer*; soluble protein; wing differentiation; sexual differentiation.

Arthropods
ISSN 2224-4255
URL: http://www.iaees.org/publications/journals/arthropods/online-version.asp
RSS: http://www.iaees.org/publications/journals/arthropods/rss.xml
E-mail: arthropods@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Sogatella furcifera (Horvath) is a kind of rice planthoppers and belongs to migratory pests with wing dimorphism, namely usually appearing macropterous or brachypterous individuals. Long-winged adults can make a long-distance migration and short-winged adults have a strong fecundity, so the ratio of long-winged and short-winged individuals is an important factor affecting the population dynamics of rice planthoppers (Zhou et al., 2014). In addition, sex ratio is also a key reason affecting planthoppers population (Shi et al., 2010; Li et al., 2014). Therefore, the depth study of *S.furcifera* wing and sexual dimorphisms is not only

beneficial to understanding of the mechanisms of these dimorphisms, but can help us make a more accurate prediction of rice planthoppers outbreak.

Juvenile hormone (JH) can regulate the development of insect. In the phenomenon of insect wing dimorphism, JH has been proved to be a key hormone promoting the emergence of short-winged individuals (Nijhout and Wheeler, 1982). A lot of researches have been done to explore the mechanism of wing differentiation in *Nilaparata lugens*, as a proximal species of *S.furcifera*, and found that JH and juvenile hormone esterase (JHE) were mainly biochemistry factors to regulate the wing development (Iwanaga and Tojo, 1986; Zhang et al., 2007; Xie et al., 2009). Additionally, yolk protein is another major aspect of the planthopper study (Dai and Yi, 2006). Therefore, in recent years, there are many studies on reproduction and wing dimorphism of planthoppers focusing on these hormones and proteins, but less research are reported to investigate other proteins involved in wing or sexual differentiation.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) can make a simple comparative analysis on differential protein of individuals or cells which are under different conditions. Even more, the relative molecular mass and semi-quantitative analysis of proteins can be done quickly by this method, and lay the foundation for further study of differential proteins.

In this study, the soluble proteins of *S.furcifera* during different developmental periods were analyzed by SDS-PAGE and then protein profiles were scanned and compared between different wing-typed or sex individuals. Several specific protein bands associated with wing or sex types were found, and the results will be helpful to further explore the mechanism of wing or sex differentiation about planthoppers.

2 Materials and Methods

2.1 Insects and culture conditions

Insects were collected from the rice field located in the South China Agricultural University, Guangzhou, China. They were bred indoor and the rearing conditions were controlled to 28±1℃, RH85% and 16L/8D. The 1^{st}-instar to 5^{th}-instar nymphs and two wing-typed female and male adults were taken out and preserved in -80℃.

2.2 Hemolymph protein samples preparation

The adult samples include four groups: long-winged males, long-winged females, short-winged males and short-winged females. Five adults were selected for each group, and placed in centrifuge tubes respectively. Adding insect physiological saline with Protease Inhibitor Cocktail Set Ⅰ (Merck), then insect bodies were cut into several pieces and extracted for 30 minutes. Extractions were centrifuged in 4 ℃ and the supernatants were collected as hemolymph protein samples. The remaining precipitations were washed and dried, preserved in -80℃finally.

2.3 Tissue protein samples preparation

Ten nymphs for each instar (1^{st}-instar to 4^{th}-instar), long-winged disc and short-winged disc 5^{th}-instar nymphs, and the precipitations of adult samples preserved by last step were grounded and extracted with RIPA lysis buffer for 30 minutes. After centrifuging in 4 ℃, the supernatants were collected as tissue proteins samples.

2.4 SDS-PAGE

Electrophoresis samples were based: V(2× loading buffer)： V(protein samples)= 1： 1. The mixtures were boiled for 5 minutes and then centrifuged for 5 minutes. 20 μl supernatants of each electrophoresis samples were placed onto 10% SDS-PAGE gels to run until the dye reached the bottom of the gel. After the electrophoresis, gels were dyed, bleached and photographed. Finally, the profiles were scanned and analyzed with BandScan software.

3 Results

3.1 Characteristics of soluble proteins in *S.furcifera* nymphs

Fig.1 shows protein profile of *S.furcifera* nymphs. The observed protein bands of 1st-instar nymphs are only three. The number of protein bands increases gradually as the nymphs developing, such as six and 14 protein bands are found in 3rd-instar nymphs and 5th-instar nymphs respectively. We speculate that the number of protein bands increases gradually from 1rd-instar nymphs to 5th-instar nymphs because the structure and function become complicated gradually during the development of *S.furcifera*.

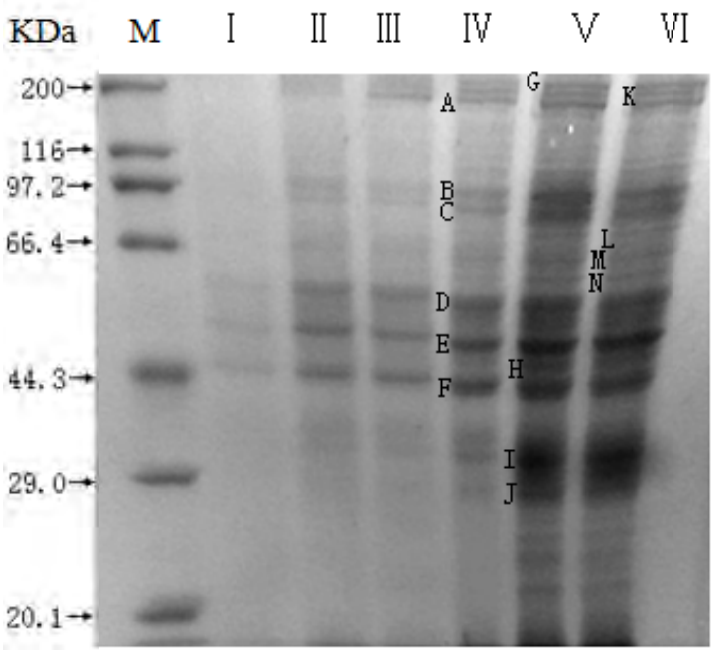

Fig.1 Analysis of the soluble proteins in *S.furcifera* nymphs by SDS-PAGE. M, protein marker; Ⅰ, 1st-instar nymphs; Ⅱ, 2nd-instar nymphs; Ⅲ, 3rd-instar nymphs; Ⅳ, 4th-instar nymphs; Ⅴ, long-winged disc 5th-instar nymphs; Ⅵ, short-winged disc 5th-instar nymphs. The observed protein bands are shown by letter A to N, respectively.

The observed protein bands were made a semi-quantitative analysis by software BandScan (Table 1). Bands D, E and F had rich content in each instar nymph, which can explain that these three protein bands are needed to participate in the development of *S.furcifera* nymphs all time. In addition, some specific proteins appeared in particular instar nymphs, such as band I and K were started to be observed in 4th-instar nymphs as well as bands G, H, J, L, M and N appeared only in 5th-instar nymphs. Comparing the protein contents between long-winged disc and short-winged disc 5th-instar nymphs, the content of band A,F,L,M and N in long-winged disc 5th-instar nymphs was at least 65.61% higher than in short-winged disc 5th-instar nymphs, and their molecular weights were 180, 44.3, 79, 66.4 and 62 KDa respectively.

3.2 Characteristics of soluble proteins in *S.furcifera* adults

Fig.2 shows protein profiles of *S.furcifera* adults. There are 13 protein bands observed in male adults, while female adults have 13 corresponding protein bands and a specific band (Band 10) expressed only in tissue. So the band 10 should be a female specific protein. These results show that the number of specific protein bands associated with sex or wing types is very low, but the content differences are exhibited for most protein bands between female and male individuals or between long wing and short wing individuals.

Table 1 The analysis of soluble protein contents (ng/μl) in *S.furcifera* nymphs.

Band Number	Molecular Weight (KDa)	1st instar	2nd instar	3rd instar	4th instar	5th instar (long-winged disc)	5th instar (short-winged disc)
A	180	—	—	6.347	6.989	11.644	7.031
B	97	—	1.787	1.102	7.836	14.969	10.323
C	89	—	1.853	1.093	6.998	14.321	11.771
D	58	1,349	8.112	7.350	9.945	13.231	11.450
E	52	2.101	8.948	7.633	15.034	30.847	26.739
F	44.3	1.961	8.357	7.056	15.849	27.358	12.824
G	200	—	—	—	—	6.347	4.993
H	48	—	—	—	—	13.275	11.474
I	31	—	—	—	6.122	28.476	23.595
J	28	—	—	—	—	15.409	12.221
K	190	—	—	—	4.393	5.012	4.392
L	79	—	—	—	—	6.921	1.987
M	66.4	—	—	—	—	7.985	2.345
N	62	—	—	—	—	7.412	2.102

—: non-existent or very low content

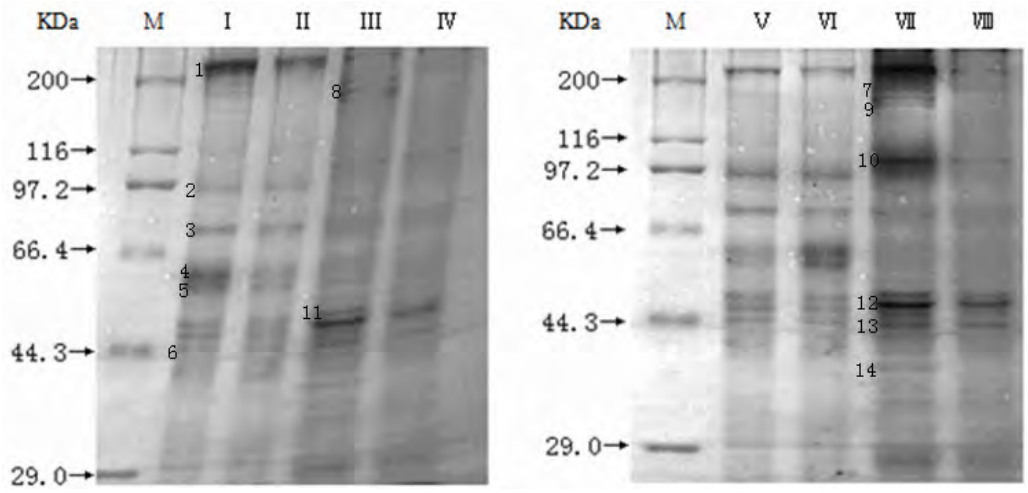

Fig. 2 The soluble proteins of *S.furcifera* adults on SDS-PAGE gel. M, protein marker; Ⅰ, long-winged male hemolymph protein; Ⅱ, short-winged male hemolymph protein; Ⅲ, long-winged male tissue protein; Ⅳ, short-winged male tissue protein; Ⅴ, long-winged female hemolymph protein; Ⅵ, short-winged female hemolymph protein; Ⅶ, long-winged female tissue protein; Ⅷ, short-winged female tissue protein. The observed protein bands are shown by number 1 to 14, respectively.

The observed protein bands were made a semi-quantitative analysis by software BandScan (Table 2). Whether in male or female tissue protein profiles, there were four specific protein bands (Band 7, 8, 9, 14) showed only in the long-winged individuals, corresponding to a molecular weight of 190, 180, 165 and 35 KDa. The content of band 1 in hemolymph from long-winged adults was nearly two times higher than from short-winged adults, while the contents of band 11 and 12 in tissue from long-winged adults were at least two times higher than from short-winged adults.

Table 2 The content analysis of soluble protein bands in *S.furcifera* adults.

Band Number	Protein Source	Molecular Weight (KDa)	Long-winged male (ng/μl)	Short-winged male (ng/μl)	Long-winged female (ng/μl)	Short-winged female (ng/μl)
1	hemolymph	>200	31.657	16.329	18.881	10.943
	tissue		—	—	42.307	3.378
2	hemolymph	95	4.768	4.845	13.034	15.175
3	hemolymph	80	8.662	8.375	11.081	10.279
4	hemolymph	62	15.909	7.956	10.043	15.849
5	hemolymph	58	15.625	8.208	10.112	15.609
6	hemolymph	44.3	4.509	3.988	4.799	3.865
7	tissue	190	7.754	—	8.343	—
8	tissue	180	7.824	—	7.999	—
9	tissue	165	6.079	—	8.712	—
10	tissue	99	—	—	24.653	2.530
11	hemolymph	53	5.925	5.341	5.565	5.007
	tissue		6.349	—	6.887	2.756
12	hemolymph	50	5.631	5.832	5.823	5.124
	tissue		26.649	9.630	26.208	12.298
13	tissue	42	6.467	5.913	7.396	7.045
14	tissue	38	4.756	—	5.004	—

—: non-existent or very low content

4 Discussion

This study is the first time to compare the soluble proteins from nymphs and adults of *S. furcifera*. The number of protein bands increase from 3 (1^{st}-instar) to 14 (5^{th}-instar), and show that protein bands in nymphs increase with growing up. Moreover, there are two protein bands begin to appear from 4^{th}-instar nymphs and six protein bands are observed only in 5^{th}-instar nymphs. In adults, one female special protein and four long-winged special proteins are found. The result reveals the variation of the developing nymph proteins as well as several specific proteins associated with wing-type or sex, it will be helpful to further study the mechanism of development or wing differentiation about rice planthoppers.

The differences of insect wing dimorphism are showed not only in the shape of wing, but also in flight-related muscles (Braendle et al., 2006). Comparing soluble proteins between long-winged disc and short-winged disc 5^{th}-instar nymphs, totally five protein bands were found to have a significantly higher content in long-winged disc 5^{th}-instar nymphs than in short-winged disc 5^{th}-instar nymphs, and the molecular weight of two protein bands among these are 180 and 44.3KDa respectively. Myosin rod protein (MRP) is the main component of flight muscle fibrils, besides it also exist in those muscle with strong contraction (e.g. muscle from insect body wall) (Polyak et al., 2003). Additional, troponin is a major regulator involved in muscle contraction under a control of calcium. The molecular weight of *Drosophila* MRP and *N.lugens* troponin T subunit is 155KDa (Standiford et al., 1997) and 45.7 KDa (Xue et al., 2013). In this study, the molecular weight of two protein bands (180 and 44.3KDa) are close to 155KDa and 45.7KDa, so the two proteins maybe belong to MRP and troponin T subunit respectively. Moreover, during the wing differentiation of *N.lugens*, indirect flight muscle (IFM) start development and differentiation first and wing disc differentiation followed (Du et al., 1998). 5^{th}-instar nymphs have finished the wing differentiation, so the other bands whose content is higher in long-winged nymphs maybe belong to IFM. However, these speculations need to be further explored and proved.

Long-winged adults have a strong ability to fly and can transport for a long distance, so they need a powerful flight muscles as a support. In this study, four proteins were found to exist only in long-winged adults and other four protein contents were much higher in long-winged adults than in short-winged adults. We believe that these proteins should be related with flight muscles.

The yolk protein of *N. lugens* exists only in fertile phase of female providing nutrition for the embryo development and one of its yolk protein subunit molecular weight is 124.5KDa (Dai and Yi, 2006). Comparing the protein profiles of female and male adults, one protein (99KDa) was found only in female tissue, this protein is very likely to come from the yolk protein subunit.

Acknowledgments

This research was supported by the Natural Science Foundation of Guangdong Province (2015A030313114) and National Natural Science Foundation of China (31171844).

References

Braendle C, Davis GK, Brisson JA, Stern DL. 2006. Wing dimorphism in aphids. Heredity (Edinb), 97: 192-199

Dai HG, Yi WX. 2006. Separation and characterization of vitellin of *Nilaparvata lugens* (Stal). Acta Entomologica Sinica, 49: 29-33

Du JG, Wang Q, Cheng XN. 1998. Histological studies on the wing bud development of the borwn planthopper *Nilaparvata lugens*. Journal of Nanjing Agricultural University, 21: 121-124

Shi CZ, Wang HR, Ge LQ, et al. 2010. Effect of low temperature on development and fecundity of *Nilaparvata lugens* (Stal). Journal of Shanghai Jiaotong University, 28: 292-295

Iwanaga K, Tojo S, 1986. Effects of juvenile hormone and rearing density on wing dimorphism and oocyte development in the brown planthopper, *Nilaparvata lugens*. Journal of Insect Physiology, 32: 585-590

Li XZ, Li SS, Wang JG, et al. 2014. Temporal dynamics of *Nilaparvata lugens* and *Sogatella furcifera* population structure in late rice fields. Acta Agriculturae Universitatis Jiangxiensis, 36: 1036-1041

Nijhout Fl, Wheeler DE. 1982. Juvenile hormone and the physiological basis of insect polymorphism. Quarterly Review of Biology, 57: 109-133

Polyak E, Standiford DM, Yakopson V, et al. 2003. Contribution of myosin rod protein to the structural organization of adult and embryonic muscles in Drosophila. Journal of Molecular Biology, 331(5): 1077-1091

Standiford DM, Davis MB, Miedema K, et al. 1997. Myosin rod protein: a novel thick filament component of Drosophila muscle. Journal of Molecular Biology, 265: 40-55

Xue J, Zhang XQ, Xu HJ, et al. 2013. Molecular characterization of the flightin gene in the wing-dimorphic planthopper, *Nilaparvata lugens*, and its evolution in Pancrustacea. Insect Biochemistry and Molecular Biology, 43: 433-443

Xie ZH, Su P, Liao XL. 2009. Research on wing dimorphism of brown planthopper. Acta Agriculturae Universitatis Jiangxiensis, 21: 95-99

Zhou CG, Feng YL, Lu CF, et al. 2014. Advances in wing dimorphism of Rice Planthoppers. Hubei Agricultural Sciences, 53: 3985-3990

Zhang QX, Sun ZX, Li GH, et al. 2007. The effects of exogenous hormones ZR-515 and RH-2485 on wing dimorphism of *Nilaparvata lugens.* Acta Scientiarum Naturalium Universitatis SunYat Sen, 46: 76-78