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Short Communication

A possibly new *Rickettsia*-like genus symbiont is found in Chinese wheat pest aphid, *Sitobion miscanthi* (Hemiptera: Aphididae)

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ABSTRACT

In this study, we investigated *Rickettsia* infection in Chinese wheat pest aphid (*Sitobion miscanthi*), moreover detected a possibly new *Rickettsia*-like symbiont, provisionally named as SMLS[1] (*S. miscanthi* L type symbiont). The sequence of SMLS 16S rRNA gene is 94% similar to that of its presumed closest relative, *Orientia tsutsugamushi*. If levels of divergence indicate taxonomic distinctiveness, SMLS probably represents a new genus in the family Rickettsiaceae. SMLS occurs in most populations of *S. miscanthi*, and with divergent infection frequencies, from 5.0% to 93.8%.

1. Introduction

Almost all aphids (Hemiptera: Aphididae) harbor the obligate endosymbiont *Buchnera aphidicola*, which provides essential amino acids (Douglas, 1998). Aphids harbor a wide array of bacteria in addition to *B. aphidicola*. The three main secondary symbionts, *Serratia symbiotica* (R type), *Hamiltonella defensa* (T type) and *Regiella insecticola* (U type), occur in many aphid species (Haynes et al., 2003; Moran et al., 2005; Russell et al., 2003; Sandstrom et al., 2001). *Rickettsia* and a new X type symbiont are reported in the pea aphid, *Acyrthosiphon pisum* (Chen et al., 1996; Guay et al., 2009). The well-known bacterium *Wolbachia pipientis* is known from several species of aphids, including *Toxoptera citricida*, *Aphis craccivora*, *Cinara cedri* (Gomez-Valero et al., 2004; Jeyaprakash and Hoy, 2000) and *Sitobion miscanthi* (Wang et al., 2009).

S. miscanthi (Takahashi) is one of the important and widespread wheat pests in China (Zhang, 1999). It damages wheat by sap-sucking and indirectly by transmitting several plant viruses (Blackman et al., 1990; Brunt et al., 1996). This aphid, a member of the Macrosiphini, frequently contains secondary symbionts (Buchner, 1965; Sandstrom et al., 2001). In this study, we investigated *Rickettsia* symbiont infection in *S. miscanthi*, and detected a possibly new *Rickettsia*-like symbiont.

2. Materials and methods

2.1. Aphid sampling

Samples of *S. miscanthi* were collected from 19 localities covering the primary wheat farming regions of China (Table 1). To avoid collecting siblings, the offspring of a single mother, we took only one aphid from within a 10 m area (Wang et al., 2009). Aphids used for molecular analysis were initially immersed in 95% ethanol and subsequently maintained at −20 °C until DNA extraction. Aphids collected in the same locality were considered as a geographic population.

2.2. DNA extraction

Before DNA extraction, every aphid was washed with 70% ethanol and sterile water several times to remove surface contamination. Total DNA was extracted from each single aphid using an EasyPure Genomic DNA Extraction Kit (TransGen, Beijing) following the manufacture's recommendations. The elongation factor-1α gene was used as a reference to evaluate DNA quality.

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[1] SMLS = *Sitobion miscanthi* L type symbiont.

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Table 1
Wheat aphid, *Sitobion miscanthi* (Hemiptera: Aphididae), samples, *Rickettsia* and SMLS infections. SMLS = *S. miscanthi* L type symbiont.

Locality ID	Collection locality	Code	Total No. tested	*Rickettsia* sp.	SMLS	Accession No.	Infection frequency (%)
1	Qinghai, Xining	XN	17	5		HM156668	29.4
2	Xinjiang, Shihezi	SHZ	15				
3	Sichuan, Jiangyou	JY	15		9	HM156659	60.0
4	Yunnan, Honghe	HH	21				
5	Shaanxi, Yangling	YL	22				
6	Shaanxi, Hanzhong	HZ	10				
7	Shaanxi, Baoji	BJ	20		1	HM156655	5.0
8	Henan, Zhoukou	ZK	22		18	HM156658	81.8
9	Henan, Luoyang	LY	19		16	HM156656	84.2
10	Henan, Dengzhou	DZ	20		3	HM156657	15.0
11	Shandong, Taian	TA	22		2	HM156648	9.09
12	Hebei, Baoding	BD	18		9	HM156655	50.0
13	Hebei, Shijiazhuang	SJZ	18		3	HM156654	16.7
14	Hubei, Danjiangkou	DJK	10		2	HM156650	20.0
15	Hubei, Zaoyang	ZY	21		3	HM156653	14.3
16	Jiangsu, Zhenjiang	ZJ	15		1	HM156652	6.67
17	Jiangsu, Yancheng	YC	15		11	HM156649	73.3
18	Jiangsu, Nanjing	NJ	16		15	HM156651	93.8
19	Anhui, Hefei	HF	27		10	HM156647	37.0

2.3. Rickettsia detection and cloning

Forward primer 16SA1(5′-AGAGTTTGATCMTGGCTCAG-3′) (Fukatsu and Nikoh, 1998) and reverse primer Ric16SR (5′-TCCA CGTCACCGTCTTGC-3′) (Sakurai et al., 2005) were used to amplify the 16S rRNA gene (16S) of *Rickettsia* sp. Cycling conditions were 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 53 °C for 45 s, 72 °C for 1 min, and a final elongation for 10 min. The plasmid containing 16S sequences of *Rickettsia* was served as positive controls. Negative controls used sterile water as the template in the amplifications.

PCR products were purified using EasyPure PCR Purification Kits (TransGen, Beijing). Purified DNA was cloned with the pEASY-T1 vector (TransGen, Beijing) and transformed into the *Escherichia coli* TOP10 competent Cell (TransGen, Beijing). The positive clones were sequenced by BioSune Company (Beijing).

2.4. Sequence analysis

The software Pintail (Ashelford et al., 2005) was employed to detect the chimera structure in the sequences. The 16S sequences of the following bacteria were used in the phylogenetic analysis: *Rickettsia* from *A. pisum* (RPU42084, AB196668), *R. limoniae* from *Limonia chorea* (AF322443), *R. typhi* (L36221), and *R. bellii* (CP000087); *Orientia tsutsugamushi* (AF062074); *Ehrlichia ewingii* (M73227) and *Wolbachia* of *Culex quinquefasciatus* (AM999887) was chosen as outgroup. Multiple sequences were aligned using Clustal W as implemented in MEGA 4.0 (Tamura et al., 2007) with the default parameters. A Bayesian inference (BI) phylogenetic tree was constructed using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The best-fit nucleotide substitution model, GTR with gamma distribution, was selected using jModelTest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) based on the Akaike Information Criterion (AIC) (Akaike, 1974). Two independent runs of 4,000,000 generations with four chains were performed, and the sampling frequency was set as every 100 generations. For each analysis, the first 10,000 trees (as 25% of the total) were discarded as burn-in, and a majority rule tree was obtained.

3. Results and discussion

3.1. Rickettsia detection and phylogenetic analysis

The 343 individuals of *S. miscanthi* collected from 19 populations were subjected to diagnostic PCR analysis (Table 1). *Rickettsia* was only detected in XN population with 29.4% infection frequency, 16S sequences of it shared 99% similarity with those of *R. bellii* (CP000087) and the *Rickettsia* symbiont in *A. pisum* (RPU42084, AB196668). We also detected the gene of a previously unreported aphid symbiont, herein named SMLS (*S. miscanthi* L type symbiont). A BlastN search of GenBank found that the most similar relative of SMLS was *O. tsutsugamushi* (AF062074), which was only 94% similar. SMLS was detected in most aphid populations (14/19), and with divergent infection frequencies, from 5.0% to 93.8%. In the phylogenetic tree (Fig. 1), there were two high supported monophyletic clades, one including *Rickettsia* symbionts and other including *O. tsutsugamushi* and SMLSs.

In *Rickettsia* clade, *Rickettsia* from XN was clustered with the *R. bellii* group, which includes *Rickettsia* from *A. pisum*. In *O. tsutsugamushi* clade, SMLSs varied little among all populations.

3.2. SMLS possibly represents a new genus in the family Rickettsiaceae

Herein, we first reported SMLS in *S. miscanthi*. The closest relative of the SMLS found in GenBank is *O. tsutsugamushi*, formerly in the genus *Rickettsia* (Tamura et al., 1995). These two taxa share 94% for their 16S sequences, and this is lower than the 97% similarity cutoff for the identification of genera (Drancourt et al., 2000; Petti, 2007). Thus, it is plausible, albeit speculative, that SMLS represents a new genus in the family Rickettsiaceae. SMLS is not universally present in wild *S. miscanthi*. Thus, SMLS might not have an obligate association with *S. miscanthi*, it might occur in other aphids and this requires testing. The 16S sequences of SMLS from different populations are very similar (Fig. 1), indicating that the symbiont has undergone recent and frequent intraspecific transfer.

3.3. Further research in SMLS

SMLS would be treated as a new secondary symbiont in aphid, with high vertical transmission rate from the infected mother to the offspring under the laboratory rearing conditions (data not shown) (Russell et al., 2003). To date, there have about 11 secondary symbionts (Guay et al., 2009; Russell et al., 2003) been reported in aphids. Some of them harbor in special cells (Moran et al., 2005), and hold diverse roles on the aphids (Montllor et al., 2002; Oliver et al., 2003; Scarborough et al., 2005; Tsuchida et al., 2004). Further research in SMLS will focus on the tissue tropism, the role in the aphid's biology and the interaction with the obligate *Buchnera*.

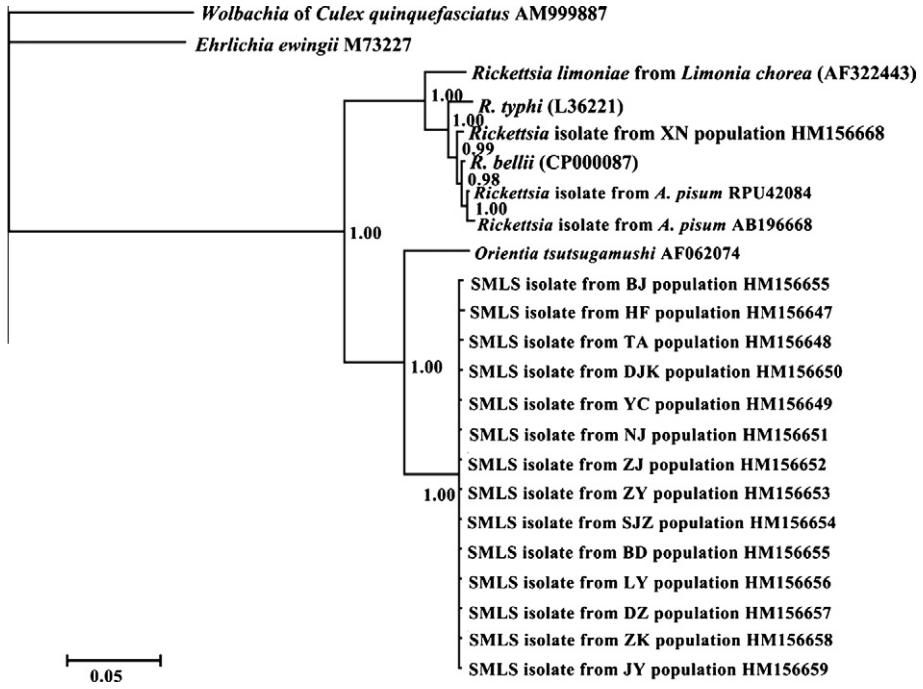

Fig. 1. A Bayesian inference tree based on 16S rRNA gene sequences depicting the phylogenetic relationships of the *Rickettsia* and SMLS of wheat pest aphids, *Sitobion miscanthi*, in China. Numbers near interior nodes indicate Bayesian posterior probabilities. The bar indicates the estimated number of substitutions per site.

Conflicts of interest

There are no conflicts of interest to be declared.

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