Journal of Biological Control, 27(3): 176–183, 2013

Research Article

Molecular identification of yeast like microorganisms associated with field populations of aphid predator, *Chrysoperla zastrowi sillemi* (Esben-Petersen) (Neuroptera: Chrysopidae) and their role in fecundity

B. N. HEMALATHA, T. VENKATESAN,* S. K. JALALI, S. SRIRAM # and B. REETHA

National Bureau of Agriculturally Important Insects, H.A. Farm Post, Hebbal, Bangalore-560024, India

 # Present Address: Division of Plant Pathology, Indian Institute of Horticultural Research,Hessaraghatta, Bangalore-560089, India

**Corresponding author Email:* tvenkat12@gmail.com

ABSTRACT: Resident microflora of alimentary canal and fat bodies associated with eleven field collected *Chrysoperla zastrowi sillemi* (Esben-Petersen) adult females were characterized and their possible role in influencing the fecundity was studied. The isolated yeasts varied among different populations of the predator. Culturable yeasts *viz.*, *Wickerhamomyces anomalus*, *Pichia anomala*, *Candida blankii*, *C. apicola*, *C. pimensis*, *Torulaspora delbrueckii*, *Zygosaccharomyces rouxii* and *Kodamea ohmeri* were isolated from gut, diverticulum and fat bodies of the adult females and characterized by biochemical and molecular tools. The yeast isolate of *T. delbrueckii* in combination with honey and castor pollen grains were found to increase the fecundity of the adult females as compared to those that were reared on honey and pollen in different generations.

KEY WORDS: *Chrysoperla zastrowi sillemi*, yeast, molecular characterization, fecundity.

(Article chronicle: Received: 01-06-2013 Revised:17-08-2013 Accepted: 05-09-2013))

INTRODUCTION

Common green lacewing, *Chrysoperla zastrowi sillemi* (Esben-Petersen) (Neuroptera: Chrysopidae), is one of the important biological control agents and used effectively to manage various sucking pests in different agro-ecosystems (Cannard *et al.*, 1984; Athan *et al*., 2004; Venkatesan *et al.*, 2009; Henry *et al*., 2010). It has long been considered as a promising candidate for the pest management programs worldwide due to its wide prey range and geographical distribution, resistance to insecticides, voracious larval feeding capacity and commercial viability (Tauber *et al*., 2000; Sayyed *et al*., 2010).

Symbiotic yeast and bacteria have been reported from variety of insects. There is a growing interest in understanding yeast diversity associated with insect hosts. Yeasts might be playing some role in supplying amino acids, vitamins, degradation of xenobiotic compounds, fermentation of food (Veja and Doud, 2005; Peter *et al*., 2012) and killer activity against variety of harmful yeasts and bacteria of host insects (McCormack *et al*., 1994). Chrysopid predators are found to harbor many endo-symbiotic yeasts which may provide essential amino acids that are normally absent in their diet (Hagen and Tassan, 1966; 1972) and (Hagen *et al.*, 1970). Several chrysopid predators *viz*., *Chrysoperla carnea* (Stephens), *Nudita occidentalis* (Johnson), *Enemochrysa tibialis* (Banks) and *Enemochrysa punctinervis* (Macachlan) complex and *Mallada perfectus* Banks (Johnson, 1982) were found to harbor yeasts. Further, Tauber *et al*., (2000) reported that lacewing adults form an obligate symbiosis with yeasts. Hagen and Tassan (1972) reported that chrysopid adults from different locations possess different taxa of yeast in their crops and they play a role in their fitness attributes. The hypothesis of the study is to probe whether chrysopid form a symbiotic relationship with microbes? and if so, their influence on their fitness attributes. Chrysopids were found to occur on several crops in India and so far no information is available on the microbial diversity occurring on the adults and their role on the fitness attributes. Keeping this in view, a study has been undertaken to characterize the gut and fat body resident microflora associated with *C. z. sillemi* female populations collected from various geographical locations in India to understand their role on the fecundity.

MATERIALS AND METHODS

Collection of *Chrysoperla zastrowi sillemi*

Ten field populations of chrysopid (30 adults) were collected from cotton in eight states *viz.*, Tamil Nadu (Coimbatore), Gujarat (Anand), Pusa campus (Delhi), Uttar Pradesh (Varanasi), Rajasthan (Sriganganagar & Udaipur), Andhra Pradesh (Guntur), Dharwad (Karnataka), and Punjab (Ludhiana) and were reared in laboratory at National Bureau of Agriculturally Important Insects, Bangalore. Chrysopid predators collected were identified as *Chrysoperla zastrowi sillemi* (Esben-Petersen) (Henry *et al.*, 2010).

Isolation of microflora associated with *C. z. sillemi*

Part of field-collected *C. z. sillemi* adults were separated individually into sterilized glass tube (4.5 x 2.5 cm) and kept in 95% ethanol for 2-3 min to disinfect the surface; followed by a wash with saline (0.5%). Further, the adults were dissected in a sterile condition and intact tissues of gut, diverticulum and fat bodies were obtained which were used for isolation of microorganism. The tissues were placed in a micro centrifuge tube containing 100 µl of sterile saline (0.9% NaCl) and were then mashed. The suspension thus obtained was spread on to YPDA media (consisting of 10g of yeast extract, 20g of peptone, and 20g dextrose and15g of agar in 1L of distilled water) and was incubated for 48 h at 25°C. The colonies obtained were streaked in to media plates to obtain pure culture. Single yeast colonies were purified at least twice. Pure culture were then inoculated into YPD broth and incubated to obtain optimum growth.

Yeast were initially identified on morphological basis (e.g., size, shape and production of ascospores) and based on the ability of utilization of 12 carbohydrate (i.e., urease, melibiose, lactose, maltose, sucrose, galactose, cellobiose, inositol, xylose, dulcitol, raffinose and trehalose) [Hi Candida identification kit KB006] according to the manufacturers protocol (Table 1). Molecular characterization was performed for further identification and amplification of partial ribosomal RNA gene.

Molecular characterization of yeasts

Culture broth was centrifuged (10000 rpm) for 10 min and the pellet was collected. Extraction buffer (200 mM Tris Hcl/200mM NaCl/25mM EDTA /0.5% SDS) was added to the pellet and homogenized and incubated for 60 min at 55°C. Then, 40 µl polyvinyl pyrrolidone (pvp) (1%) was added and incubated at 25°C for 30 min and then chloroform (300 µl) was added, centrifuged and the supernatant containing DNA was transferred. For precipitation of DNA, isopropanol was added and allowed it to stand for about 30 min at –20°C and centrifuged at 4°C. Such DNA pellet was dissolved in 50 µl $T_{10}E_1$ buffer.

The primers ITS-1F (5'-TCCGTAGGTGAACCTG CGG-3') and ITS-1R (5'-GCTGCGTTCTTCATCGATGC-3') were subsequently used for the amplification of partial

Table 1: Biochemical test for the yeast isolates of different populations of *Chrysoperla zastrowi sillemi*

Carbohydrate fermentation	Population										
	CZS1	CZS 2	CZS 3	CZS 4	CZS 5	CZS 7	CZS 8	CZS 9	CZS 10	CZS 15	CZS16
Urease	–	–	–	–	–	–	–	–	–	–	–
Melibiose	+	+	+	+	+	+	+	w	+	+	w
Lactose	–	–	–	+	–	–	+	–	–	–	–
Maltose	+	+	w	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+
Cellobiose	+	+	–	+	+	+	+	+	+	+	+
Inositol	–	+	–	–	–	+	–	–	+	–	–
Xylose	+	+	+	–	+	+	+	–	+	+	–
Dulcitol	–	–	–	–	–	–	–	–	–	–	–
Raffinose	+	+	+	w	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+

+ = Positive reaction; – =Negative reaction; w = weak reaction

18s RNA genes, ITS-1 and partial 5.8S rRNA genes using standard PCR conditions. The total volume of 50µl PCR mixture consists of 10x-reaction buffer, 10mM dNTP, 2µl of each primer and 1U *Taq* polymerase. Each PCR was performed with a total of 10 µl of genomic DNA. The resulting PCR product electrophoresed in a 1.5% TBE – agarose gel and a 100 bp ladder was used to size products. The resulting PCR amplicons were sequenced and analyzed using BLAST. The partial rRNA gene sequence including sequences for the 18S rRNA, ITS 1 and 5.8S rRNA genes were deposited in GenBank (Table 2).

Phylogenetic tree of 18s rRNA region of the yeast isolates was constructed and the evolutionary history was inferred by using Maximum Likelihood method based on the Tamura 3-parameter model. Evolutionary analyses were conducted in MEGA5 the analysis involved 23 nucleotide sequences all positions with less than 95% site coverage were eliminated. The tree with the highest log likelihood (–1799.9842) is shown and the percentage of trees in which the associated taxa clustered together is shown next to the branches. When the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method would have been used with MCL distance matrix (Tamura, 1992; Tamura *et al*., 2011).

Role of endosymbiotic yeast on fecundity

Role of yeasts on the fecundity of adults was tested with already available lab population. Yeast *Torulospora delbrueckii* isolated from the adult gut and diverticulum were selected to find out their possible role on the fecundity based on the information that the yeast could be playing some role in improving the fecundity (Hagen *et al*., 1970).

Two pairs of adults were maintained in a pearl pet container (0.5 lit cap.) by providing 50% honey and adequate quantity of castor pollen grains and this treatment was taken as control and for the test the adults were fed with 1mg of yeast isolate (*Torulaspora delbrueckii*) per ml of 50% honey and castor pollen grains. These treatments were carried out in twelve replicates and the egg count was recorded for every alternative day and continued for about 30 days. The treatments were continued for four generations. Student's t-test was used for comparison of fecundity of *C. z. sillemi* reared on yeast plus castor pollen grains and honey (treatment 1) and castor pollen and honey (treatment 2). The entire study was conducted in an environmental chamber at a constant temperature of 26 ± 1°C and 60 ± 10% relative humidity and 14:10 h light: dark cycle.

Table: 2 Yeasts isolated from *Chrysoperla zastrowi sillemi*

Population	Isolation source	Yeast isolate	GenBank Acc. No.
Coimbatore CZS-1	Gut	*Wickerhamomyces anomalus*	JQ061140
	Diverticulum	*W. anomalus*	JQ061141
	Fat bodies	Not observed	–
Dharwad	Gut	*W. anomalus*	JQ241273
CZS-2	Diverticulum	Not observed	–
	Fat bodies	*Candida apicola*	JQ241274
Guntur	Gut	Not observed	–
CZS-3	Diverticulum	*Candida blankii*	JQ340778
	Fat bodies	*C. blankii*	JQ340779
Bengaluru CZS-4	Gut	*Torulaspora delbrueckii*	KC507191
	Diverticulum	*T. delbrueckii*	KC507190
	Fat bodies	*T. delbrueckii*	KC507192
Delhi	Gut	*W. anomalus*	JQ340782
CZS-5	Diverticulum	*W. anomalus*	JQ340781
	Fat bodies	Not observed	–
Ludhiana	Gut	*Zygosaccharo-myces rouxii*	JQ410174
CZS-7	Diverticulum	*Z. rouxii*	JQ410172
	Fat bodies	Not observed	–
Sriganga-nagarCZS-8	Gut	*W. anomalus*	JQ410175
	Diverticulum	Not observed	–
	Fat bodies	*W. anomalus*	JQ410176
Udaipur	Gut	*Kodamaea ohmeri*	KC473465
CZS-9	Diverticulum	*K. ohmeri*	KC473466
	Fat bodies	Not observed	–
Lab reared	Gut	*Candida pimensis*	KC473467
CZS-10	Diverticulum	*C. pimensis*	KC473468
	Fat bodies	Not observed	–
Anand	Gut	*Pichia anomala*	KC473463
CZS-15	Diverticulum	*P. anomala*	KC473464
	Fat bodies	Not observed	–
Varanasi	Gut	*Kodamea ohmeri*	JQ061139
CZS-16	Diverticulum	*K. ohmeri*	JQ061138
	Fat bodies	Not observed	–

RESULTS AND DISCUSSION

Seven yeast isolates were isolated and identified from gut, diverticulum and fat bodies of different populations of *C. z. sillemi* adults. The biochemical test kit specifically used for identification for *Candida* species. The identification was based on color changes i.e., from pink to yellow and orangish yellow to red. These changes

were compared with list of *Candida* species provided in the identification index of the kit. The colorimetric change which indicates pink to yellow is positive reaction, where as no change in colour is a negative reaction. For carbohydrate urease it is orangish yellow to red which indicates positive reaction and negative if no change in colour. The seven yeast isolates showed positive reactions to carbohydrate assimilation and some negative reactions (Table 1), thus indicating different yeast species. But positive reactions when compared to standard identification index was not matching to any of the *Candida* species which indicate the yeasts belong to different taxa. Hence, for further confirmation, molecular characterization was performed.

The amplicons of various yeasts, isolated from different populations of *C. z. sillemi* were given in Fig. 1 (fat bodies), Fig. 2 (gut) and Fig. 3 (diverticulum). The yeast isolates were found to be *Wickerhamomyces anomalus* (strain CZS-1 & 5), *Pichia anomala* (CZS-2, 8 &15), *Candida blankii* (CZS-3), *Candida apicola* (CZS-2), *Torulaspora delbrueckii* (CZS-4) *Zygosaccharomyces rouxii* (CZS-7), *Kodamea ohmeri* (CZS-9 &16), *Candida pimensis* (CZS-10) and their matching was 100%, 99%, 100%, 97%, 99%, 99%, 97% and 97%, respectively. *W. anomalus* was found in most of the populations of the predator (CZS-1, CZS-2, CZS-5 and CZS-8). In general, yeast taxa obtained in gut, was also observed in fat bodies or diverticulum, however, in CZS-2 (Dharward population), *W. anomalus* was isolated from gut and *C. apicola* was obtained from fat bodies. Chrysopid predators were found to have association with variety of microflora namely *Torulopsis* sp. (Hagen *et al*., 1970); *Candida multigemmis* (Buhagiar) Meyer and Yarrow; *Torulopsis multigemmis* (Johnson, 1982); *Metschnikowia chrysoperla*, *Candida picachoensis* and *Candida pimensis* (Suh *et al.*, 2004 and Gibon and Hunter, 2005); *Metschnikowia pulcherrima* (Woolfolk and Inglis, 2004), *Metschnikowia corniflorae* sp. no. (Nguyen *et al*., 2006). The yeast *T. delbrukii* was isolated from the gut of female *Chauliodes rastricornis* (Neuroptera) in LA, USA. *Kodamaea ohmere* was isolated from gut of female *Corydalus cornutus* (Neuroptera) (Nguyen *et al*., 2007). *Pichia guilliermondii* was found to be commonly occurring yeast from the host insects (Zacchi and Vaughan-Martini, 2002). Chrysopid adult from different region had different taxa of yeasts in their gut (Hagen and Tassan, 1972) and similar were observations obtained in our study.

All organisms are classified into 2 major clades representing that the first clade contains 17 species (*W. anomalus* to *C. pimensis*) further divided into 3 sub clades. All *W. anomalus* species except two are closely

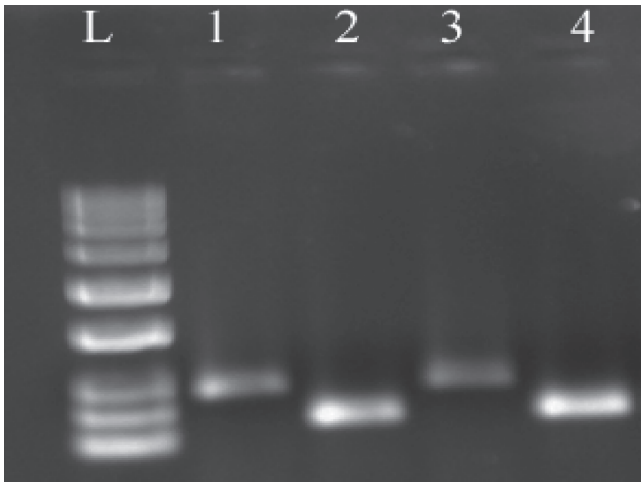

Fig. 1: Agarose gel showing the PCR product (175 bp to 300 bp) for partial ribosomal RNA gene of yeast isolates isolated from fat bodies of *C. z. sillemi* adults. Lane M:Molecular marker of size 100bp; lane 1 (CZS-3), 2 (CZS-2), 3 (CZS-4) and 4 (CZS-8).

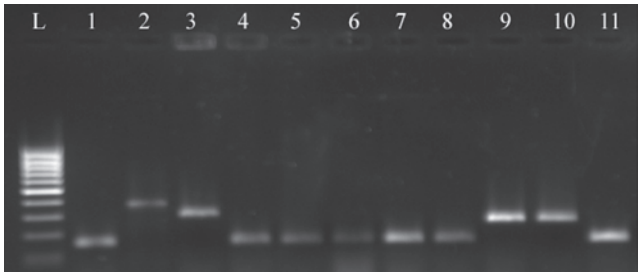

Fig. 2: Agarose gel showing the PCR products (175 bp to 300 bp) for partial ribosomal RNA gene of yeast isolates isolated from gut of *C. z. sillemi* adults. Lane M: Molecular marker of size 100bp; lane 1 (CZS-1), 2 (CZS-3), 3 (CZS-4), 4 (CZS-2), 5 (CZS-5), 6 (CZS-8), 7 (CZS-9), 8 (CZS-15), 9 (CZS-7), 10 (CZS-10) and 11 (CZS-16)

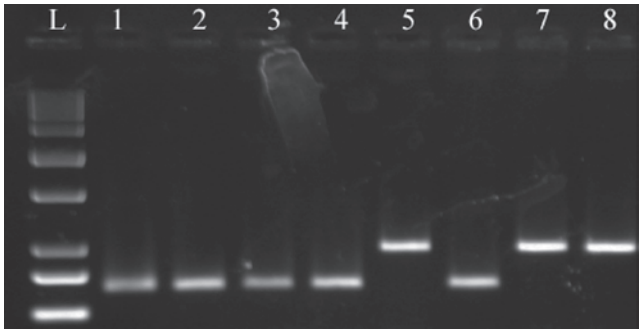

Fig. 3: Agarose gel showing the PCR products (175 bp to 300 bp) for partial ribosomal RNA gene of yeast isolates isolated from diverticulum of *C. z. sillemi* adults. LaneM:Molecular marker of size 100bp; lane 1 (CZS-1), 2 (CZS-5), 3 (CZS-9), 4 (CZS-15), 5 (CZS-4), 6 (CZS-16), 7 (CZS-7) and 8 (CZS-10)

related to each other with an average of 70% bootstrap support. The rest two *W. anomalus* species showing similarity to *C. apicola and C. pimensis* with 56% bootstrap support *T. delbrueckii* showing similarity to *Z. rouxii* by sharing common inter node *K. ohmeri* and *C. blankii* present close to each other with 94% bootstrap support (Fig. 4).

Yeast like microorganisms in general present in diverticulum of chrysopid adults (Hagen and Tassan, 1966, 1972; Hagen *et al*., 1970, Johnson, 1982). Woolfolk and Inglis (2004) found the occurrence of large population of yeasts in the foregut and diverticulum relative to the midgut and hindgut regions. Lacewings that fed on sugary substance have a much larger tracheal trunk around the diverticulum, a modification suggested as necessary to satisfy the oxygen demand of resident yeast (Canard *et al.*, 1984; Gibson and Hunter, 2005).

There was no instance of yeasts in the eggs and larvae. For further confirmation whether adults posses yeasts in their alimentary canals upon eclosion from pupae no instances of yeast were found. Only the adult chrysopids collected from different geographical localities showed the presence of yeast isolates. This indicated that the yeast were transients and acquired through the diet and can be indicated as facultative endosymbionts and transmission through is not obligatory. Hagen *et al.*, (1970)

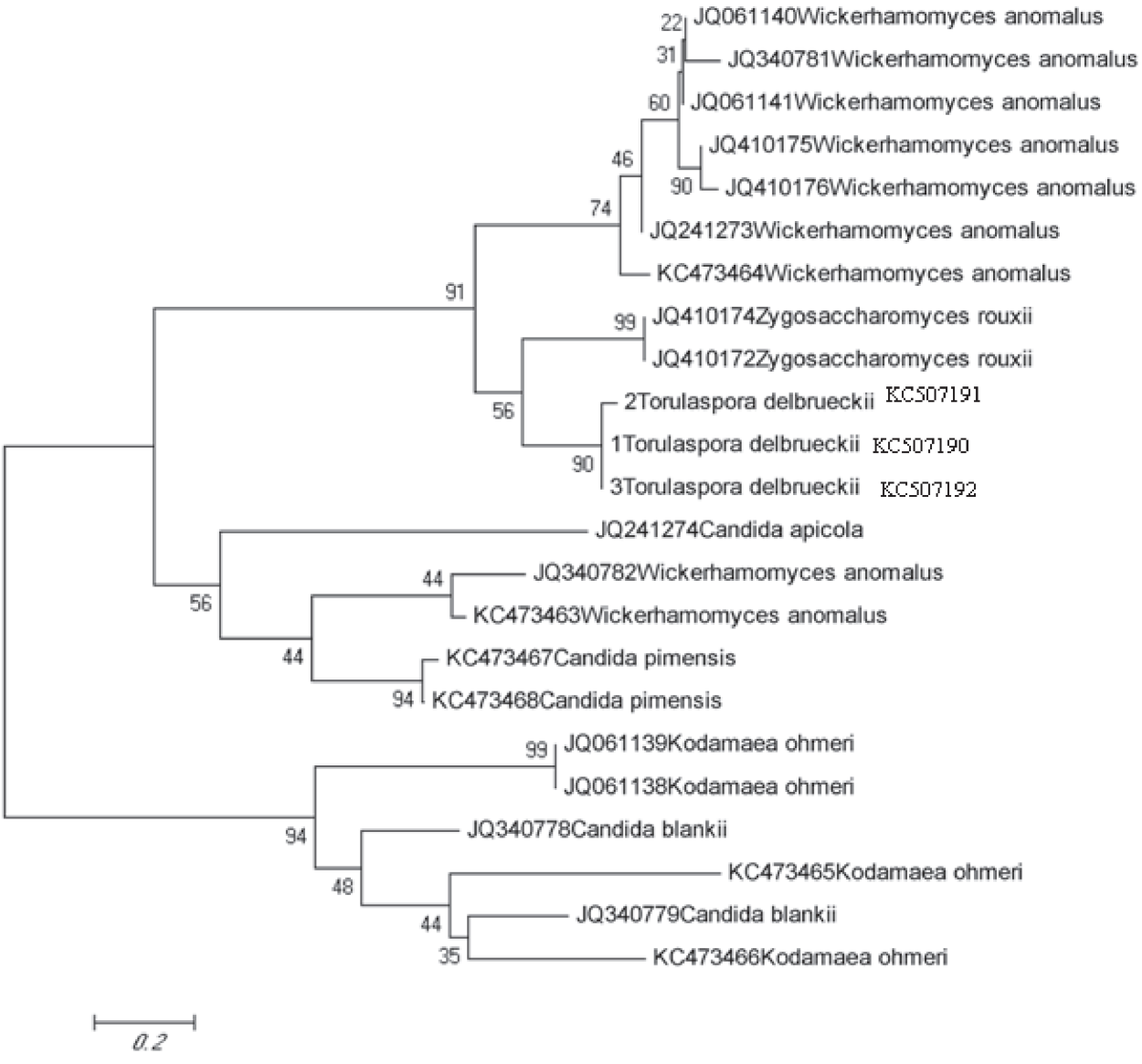

Fig. 4: Evolutionary relationships of yeast taxa constructed for internal transcribed spacer region containing 18srRNA gene, ITS-1 and 5.8r RNA gene of culturable yeasts in *Chrysoperla zastrowi sillemi* gut, diverticulum and fat body samples

suggested that adults of *C. carnea* obtain yeasts from environment and not the lenial. Suh *et al*., (2004) isolated yeasts *viz*., *M. chrysoperla*, *C. picachoensis* and *C. pimensis* from the egg surface of the several *Chrysoperla* sp. from Arizona. Hagen *et al*., (1970) concluded that the yeast symbionts in the diverticulum provide valine to adult females, imparting a significant positive effect on fecundity. Diverticulum in the alimentary canal of chrysopid adults act as reservoir for various food sources wherein yeast, bacteria and other obligate micro organisms are harboured (Marchini *et al*., 2002; Woolfolk *et al*., 2004). Woolfolk and Inglis (2003) found *Metschnikowia pulcherrima* Pitt & miller was predominant yeast found in the alimentary canal of *C. rufilabris* which are acquired from the environment. However, we did not observe *M. pulcherrima* in Indian populations of *C. z. sillemi* may be due to the difference in cropping pattern and diversity grown in different countries. Yeast is an excellent source of vitamins and sterols, and yeast is often used as a nutrient supplement in insect diets (Cohen, 1999). Habitat differences may explain the occurrence of phylogenetically distinct yeasts in their guts. *M. noctiluminum*, *C. picachoensis* and *C. pimensis* obtained from adult lacewings (Woolfolk and Inglis, 2003; Suh *et al*., 2004; Nguyen *et al*., 2006). Nguyen *et al*., (2007) isolated five novel *Candida* species in insect-associated yeast clades from Neuroptera and other insects. Further, they reported that they could not isolate yeasts from lacewing larvae; however they isolated from lacewing predators which feed on plant nectar or pollen. This shows that yeasts were picked from the plant nectar or pollen grains and not from the prey insects. Woolfolk and Inglis (2003) isolated bacteria from the adult females of *C. rufilabris* which are transients may be acquired from the field; however, we could not observe any bacteria from the adult females.

Fecundity studies with yeasts revealed that there was a significant increase in the fecundity levels in the adults provided with honey; pollen and yeast isolate (*T. delbrueckii)* for four generations when compared to control (honey + pollen). The same trend was observed for four generations (Table 3) which showed that *T. delbrueckii* might be supplying some amino acids or factors which increase the fecundity of the adult female and this needs further studies. Mean fecundity of *C. z. sillemi* reared on honey, pollen and *T. delbrueckii* for four generations was significantly higher (162. 85 eggs/ 2 females) than that of honey and pollen reared females (116.14) (Student's t = 17.33, P <0.05, df = 110). Endo-symbiotic yeast namely *Torulopsis multigemmis* was associated with adults of several chrysopid species (*Mallada perfectus* Banks, *Eremochrysa tibialis*, *E. punctinervis* (Johnson, 1982). Chrysopid predators were found to harbor yeast symbionts in their gut especially in the diverticulum supplies amino acids (valine) by which fecundity of the predator was significantly increased Hagen and Tassan 1966, 1972; Hagen *et al.*, 1970. This shows that the yeast acquired through the diet had a role in increasing the fecundity of the adults.

Table 3: Role of honey, pollen and endosymbiotic yeast *Torulaspora delbrueckii* on the fecundity

Generation	Honey + pollen	Honey + pollen + yeast	"t" stat 5%
I	118.23 ± 3.55[b]	155.69 ± 3.77[a]	7.22**
II	108.84 ± 2.22[b]	152.76 ± 4.38[a]	8.93**
III	110.38 ± 1.64[b]	166.07 ± 2.67[a]	17.7**
IV	127.23 ± 2.01[b]	177.0 ± 5.13[a]	9.02**

** Analysis of variance was significant (P<0.05). Means (+ SEM) accompanied by different same letter in a row are significantly different.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, National Bureau of Agriculturally Important Insects for providing necessary facilities.

REFERENCES

Athan R, Kaydan B, Ozgokce MS. 2004. Feeding activity and life history characteristics of the generalist predator, *Chrysoperla externa* (Neuroptera: Chrysopidae) at different prey densities. *J Pest Sci*. **77** (1):17–21.

Canard M, Semeria Y, New TRR. 1984. *Biology of Chrysopidae*, Dr. W. Junk, Publisher: The Haque, The Netherlands. 294.

Canard M, Kokubu K, Duelli P. 1990. Tracheal trunks supplying air to the foregut and feeding habits in adults of European green lacewing species (Insecta: Neuroptera: Chrysopidae). Pretoria, Republic of South Africa: Dept of Agricultural Development; 277–286.

Cohen AC. 1999. Artificial diet for rearing entomophages comprising sticky, cooked whole eggs (continuation in part). U.S. Patent. 51999; 945:271.

Coppel HC, Mertins JW. 1977. *Biological Insect Pest Suppression*. Spinger-Verlag. Berlin, Germany.

Fresitas S. 2002. *Controle biológico no Brasil: parasitóides e predadores*. São Paulo. Manole.

Gibson CM, Hunter MS. 2005. Negative fitness consequences and transmission dynamics of a heritable fungal symbiont of a parasitic wasp. *Appl Env Microbiol*. **75**: 3115–3119.

Hagen KS. 1950. Fecundity of *Chrysopa californica* as affected by synthetic foods. *J Econ Ent.* **43**: 101–104.

Hagen KS, Tassan RL. 1966. The influence of protein hydrolysates of yeasts and chemically defined upon the fecundity of *Chrysopa carnea* (Neuroptera). *Vestn Cesk Spol Zool.* **30:** 219–227.

Hagen KS, Tassan RL. 1972. Exploring nutritional roles of extracellular symbiotes on the reproduction of honeydews feeding adult chrysopids and tephritids p. 323–251. In: *Insect and Mite Nutrition. Significance and Implications in Ecology and Pest Management.*

Hagen KS, Tassan RL, Sawall EF. 1970. Some eco-physiological relationships between certain *Chrysopa*, honeydews and yeasts. *Boll Lab Entomol Agric Portici* **28**: 113–134.

Hassan SA. 1974. Mass-culturing and utilization of *Chrysopa* spp. (Neuroptera: Chrysopidae) in the control of insect pests. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz* **81**: 620–637.

Henry CS, Brooks SJ, Johnson JB, Venkatesan T, Duelli P. 2010. The most important lacewing species in Indian agricultural crops, *Chrysoperla sillemi* (Esben-Petersen), is a subspecies of *Chrysoperla zastrowi* (Esben-Petersen) (Neuroptera: Chrysopidae). *J Nat Hist.* **44**: 2543–2555.

Johnson JB. 1982. Bionomics of some symbiote using Chrysopidae (Insecta: Neuroptera) from the Western United States. Ph.D. dissertation. University of California, Nerkeley.

Marchini D, Rosetto M, Dallai R, Marri, L. 2002. Bacteria associated with oesophageal bulb of the med fly *Ceratitis capitata* (Diptera: Tephritidae). *Curr Microbiol.* **44**: 120–124.

McCormack PJ, Wildman HG, Jeffries P. 1994. Production of antibacterial compounds by phylloplane-inhabitating yeasts and yeast like fungi. *App Env Microbiol.* **60** (3): 927–931.

Nguyen NH, Suh SO, Erbil CK, Blackwell M. 2006. *Metschnikowia noctiluminum* sp. nov. *Metschnikowia corniflorae* sp. nov. and *Candida chrysomelidarum* sp. nov., isolated from green lacewings and beetles. *Mycol Res.* **110**: 346–356.

Nguyen NH, Suh SO, Blackwell M. 2007. Five novel *Candida* species in insect- associated yeast clades isolated from Neuroptera and other insects. *Mycologia* **99**: 842–858.

Peter W, Magali P, Elzbieta R, Paul GB, Stefanos A, Miryan C, Tobias, UTL, Lee JR, Arne H, Marie B, Cletus PK, Jure P, Alan K. 2012. This is not an Apple–Yeast Mutualism in Codling Moth. *J Che Ecol.* **38**: 949–957.

Sayyed AH, Pathan AK, Faheem U. 2010. Cross-resistance, genetics and stability of resistance to deltamethrin in a population of *Chrysoperla carnea* from Multan, Pakistan. *Pesticide Biochem Physiol.* **98**: 325–332.

Smith RC. 1922. *The biology of the Chrysopidae*. CornellUniv. Agr.

Suh SO, Blackwell M. 2004. *Metschnikowia chrysoperlae* sp. nov., *Candida picachoensis* sp. nov. and *Candida pimensis* sp. nov., isolated from the green lacewings *Chrysoperla comanche* and *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Intl J Syst Evol Microbiol.* **54**: 1883–1890.

Tamura K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition, transversion and GC content biases. *Mol Biol Evol.* **9**: 678–687.

Tamura K, Peterson N, Stechar G, Nei M, Kumar S. 2011. MEGA. Molecular evolutionary genetic analysis using maximum likelihood, *Mol Biol Evol.* **38**: 2731–2739.

Tauber MJ, Tauber CA, Daane KM, Hagen KS. 2000. Commercialization of predators: recent lessons from green lacewings (Neuroptera: Chrysopidae). *Am Entomol.* **46**: 26–38.

Vega FE, Dowd PF. 2005. *The role of yeasts as insect endosymbionts. Insect-Fungal Associations: ecology and evolution*, New York: Oxford University Press, 211–243.

Venkatesan T, Jalali SK, Murthy KS, Rabindra RJ, Lalitha Y. 2009. Occurrence of insecticide resistance in field populations of *Chrysoperla zastrowi arabica*

(Neuroptera: Chrysopidae) in India. *Indian J Agric Sci.* **79**: 910–912.

Woolfolk SW, Inglis GD. 2003. Microorganisms associated with field-collected *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) adults with emphasis on yeast symbionts. *Biol Control* **29**, 155–168.

Woolfolk SW, Cohen AC, Inglis GD. 2004. Morphology of the alimentary canal of *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) adults in relation to microbial symbionts. *Ann Ent Soc Am.* **97**: 795–808.

Zacchi L, Vaughan Martini A. 2002. Yeats associated with insects in agricultural areas of Perugia Italy. *Ann Microbiol.* **52**: 237–244.