2. REVIEW OF LITERATURE

The literature pertaining to the survey, economic importance, biodiversity, associated gut bacteria of fruit flies and role of gut bacteria in fruit flies management have been reviewed under the following heads:

2.1 Geographical distribution and economic importance

2.2 General status of tephritid taxonomy

2.3 Biodiversity of fruit flies

2.4 Molecular characterization of fruit flies

2.5 Gut bacterial diversity in fruit flies

2.6 Molecular characterization of gut bacteria

2.7 Bacterial odours as attractants for fruit flies

2.8 Role of bacteria in the IPM of fruit flies

2.1 Geographical distribution and economic importance

The family Tephritidae is one of the largest families of Diptera (Drew 1989a), comprising of predominantly medium sized, pictured-winged and highly ornamented flies often referred to as 'peacock flies' due to their habit of strutting and vibrating their wings (Kapoor 1993; Agarwal and Sueyoshi 2005; Satarkar *et al*. 2009; De Meyer *et al.* 2010). The tephritid flies are commonly known as "fruit flies" because a number of species infest a wide variety of fruits, vegetables, flower heads, seeds, leaves and other plant parts (White and Elson-Harris 1992; Agarwal and Sueyoshi 2005).

They are found in nearly all habitats with suitable plant life. Their distribution is cosmopolitan covering tropical, subtropical and temperate regions and they occupy habitats ranging from rainforests to open savannah except in Arctic and Antarctic regions (Kapoor *et al*. 1980; Drew 1989a; 1989b; McPheron

and Steck 1996; Norrbom *et al.* 1998; Michaux and White 1999; Agarwal and Sueyoshi 2005; De Meyer *et al*. 2010). These flies are widespread over the entire world and richly predominant in the tropical and subtropical areas.

They occupy a predominantly important place in the list of enemies of plants and among the world's most notorious agricultural pests, both because of their widespread presence and broad larval host range, the enormous direct and indirect damage by the major species of the fruit fly complex and thus have a grave effect on agricultural economy (Michaux and White 1999; Agarwal and Sueyoshi 2005; Satarkar *et al*. 2009; De Meyer *et al*. 2010).

Several tephritids are critically important as fruit crop pests (Thompson 1998). About 35% of fruit fly species attack soft fruits, including many commercially important ones (White and Elson-Harris 1992). Economic impacts can be enormous, and control or eradication requires substantial budgets. Dowell and Wange (1986) had rightly stated that establishment of major fruit fly threats to the Californian fruit industry would cause crop losses of US $ 910 million yearly, and an eradication program would cost US $ 290 million. Annual losses in the eastern Mediterranean (Israel, Palestinian Territories, Jordan) linked to fruit fly infestations are estimated at US $ 192 million (Enkerlin and Mumford 1997). In India, fruit flies caused annual estimated losses to the tune of $ 855.40 million (Sardana *et al*. 2005; Prabhakar *et al*. 2009a). Indirect losses resulting from quarantine restrictions imposed by importing countries to prevent entry and establishment of unwanted fruit fly species, however would exaggerate this figure enormously. Most economically important fruit fly pests belong to four genera: *Anastrepha* Schiner (New World Tropics), *Bactrocera* Macquart, *Ceratitis* MacLeay and *Dacus* Fabricius (Old World Tropics) (De Meyer *et al*. 2010).

Among fruit flies, *Bactrocera* species in particular are native to tropical Asia, Australia and South Pacific regions, with a few species being found in African and warm temperate areas of Europe and Asia. These are mainly polyphagous pests, having widespread distribution, wide climatic adaptation, high reproductive potential, high mobility and cause losses in fruit and vegetable crops (Muraji and Nakahara 2002).

In India, fruit flies have been identified as one of the ten most serious problems of agriculture as a whole and nine species in particular *viz*. melon fly, *B. cucurbitae*; oriental fruit fly, *B. dorsalis*; peach fruit fly, *B. zonata*; pumpkin fly, *B. tau*; guava fruit fly, *B. correcta*; lesser pumpkin fly, *Dacus ciliatus*; ber fly, *Carpomyia vesuviana* and seed fly, *Acanthiophilus helianthi* are major and economically important (Sardana *et al*. 2005). Among these the melon fly, *Bactrocera cucurbitae* is a polyphagous fruit fly that attacks more than 125 plant species, mostly belonging to the Cucurbitaceae and Solanaceae (Dhillon *et al.* 2005; Pinero *et al.* 2006), and include some species of significant agricultural interest. The first report on the melon fly was published by Bezzi (1913), who listed 39 insect species from India and considered India as its native region. Besides India, today the melon fly is distributed throughout the Pakistan, Nepal, China, New Guinea, Philippines, Mariana and Hawaii Islands, and throughout most of Southeast Asia (Hu *et al.* 2008). The species has also been reported from Egypt, Kenya and Tanzania (Weems and Heppner 2001) where it is a recent invader.

Two important pest species, *Bactrocera tryoni* (Froggatt) and *Ceratitis capitata* (Wiedemann) have not yet been reported from India. However, Munro (1938) recorded the later from Pusa, Bihar in 1907 and 1908, when he reared them on peach. Kapoor *et al.* (1980) related this as a case of accidental introduction which could not establish in India. Three more fruit fly species, *B. caryeae* (Kapoor), *B. caudate* (Fabricius) and *Rhagoletis cingulata* (Loew) are waiting to enter India or have doubtful presence (Sardana *et al*. 2005).

In Himachal Pradesh, *B. zonata* and *B. dorsalis* as pests of stone fruits, guava and mango (Bhalla and Pawar 1977); *B. cucurbitae* and *B. tau* on cucurbits (Sood and Nath 1999; Prabhakar *et al.* 2009a) are the most serious pests. *B. tau* in particular has been reported on many fruit and vegetable crops by Narayanan and Batra (1960) in India, Yang *et al*. (1994a; 1994b) in China and Huque (2006) in Bangladesh. This species was reported as a serious pest of

cucurbitaceous vegetables (Bhalla and Pawar 1977; Gupta *et al*. 1992; Sood and Nath 1999; Prabhakar *et al.* 2007; Prabhakar *et al.* 2009a) and also of solanaceous vegetables in Himachal Pradesh and plains of Punjab (Kapoor and Agarwal 1983). Recently, *B. scutellaris* (Bezzi) was reported as pest of many cucurbit crops in Himachal Pradesh (Sunandita and Gupta 2007; Prabhakar *et al.* 2007; Prabhakar *et al.* 2009a).

2.2 General status of tephritid taxonomy

Tephritid taxonomic research was pioneered by forefathers of biology, Linnaeus and Fabricius. Tephritid taxonomy has a long history (over-two centuries) with some 4,500 species having been described since mid-1700s (Drew and Romig 2000), distributed throughout the temperate, subtropical and tropical areas of the world.

The Dacinae fruit flies, one of the major subfamilies of the Tephritidae, are economically important group of Diptera. Drew (1989a) estimated that there are at least 800 species distributed in Africa (200), the Asian region (300) and throughout the South Pacific (300). This group is mainly found in subtropical and tropical areas. The rate of discovery of new species indicates that there may be up to a thousand species in total. Economically important species of fruit flies belong to the genera; *Anastrepha*, *Rhagoletis*, *Bactrocera* and *Ceratitis*. The Dacinae fruit flies have traditionally been divided into two main genera; *Bactrocera* and *Dacus*. Other major pest tephritids of the genera *Anastrepha*, *Rhagoletis* and *Ceratitis* belong to the subfamilies Trypentinae and Ceratinae.

Based on previous studies, the *Dacus* genus which includes a large number of fruit fly species is now renamed as *Bactrocera* (Drew 1989a). *Bactrocera* as a genus is one of the largest within family Tephritidae with about 500 described species arranged in 28 subgenera (Drew 1989a; Drew and Hancock 2000) whose members extend throughout Asia, Oceanic region and Australia. There are very few recorded species in Africa and only *B. oleae* is found in North Africa and Southern Europe.

In Indian subcontinent, the knowledge of family Tephritidae has been based largely upon the monumental monograph of Bezzi published in 1913 (Kapoor *et al.* 1980). During 1960s to 1990s, lots of work has been carried out on taxonomy of tephritid fruit flies in India by Kapoor and his associates. In 2005, Agarwal and Sueyoshi published catalogue of Indian fruit flies, listing 243 species in 79 genera. This is a grand gift to the most neglected group of insects in India (Anonymous 2010).

2.3 Biodiversity of fruit flies

The Tephritidae (*i.e.* the “true fruit fly”) is a medium sized insect family with about 4,448 recognized species or subspecies of fruit flies, classified in 481 genera (Agarwal and Sueyoshi 2005). It has a worldwide distribution with a considerable number of pest species and some beneficial members which are used as biocontrol agents (Zwolfer 1987; White 1987; Sardana *et al*. 2005).

Zaka-ur-Rab (1984) listed 60 genera and 138 species of tephritids in the Indian subcontinent, out of which 56 genera and 102 species belong to sub families other than Dacinae. However, Sardana *et al*. (2005) enlisted 207 species of fruit flies under 71 genera, 13 tribes and 4 subfamilies from India which infest a wide range of vegetable and fruit crops. Agarwal and Sueyoshi (2005) published catalogue of Indian fruit flies, listing 243 species in 79 genera, which have been arranged in 4 subfamilies and 18 tribes. Of the 4,448 recognized species or subspecies of fruit flies of family Tephritidae known so far, only 243 species of fruit flies have so far been reported from India , where as it is generally accepted that from 8-12 per cent of the world species of acalyptrate dipterans are represented in India. This indicates that more than 400 species of tephritid flies are estimated to occur in India and many of them are yet to be discovered from the biodiversity rich habitats of India (Anonymous 2010).

In Himachal Pradesh, 41 species distributed in 27 genera, 3 subfamilies and 10 tribes were listed in catalogue of Indian fruit flies by Agarwal and Sueyoshi (2005). List of tephritid fruit flies species reported from Himachal Pradesh is as follows :

Subfamily	Tribe	Genus	Species
DACINAE	**1. DACINI**	**I *Bactrocera*** Macquart	**1. *correcta*** (Bezzi)
			2. *dorsalis* (Hendel)
			3. *zonata* (Saunders)
			4. *diversa* (Coquillett)
			5. *cucurbitae* (Coquillett)
			6. *scutellaris* (Bezzi)
			7. *tau* (Walker)
		II *Dacus* Fabricius	**8. *discophorus*** (Hering)
			9. *ciliatus* Loew
TRYPETINAE	**2. ADRAMINI**	**III *Adrama*** Walker	**10. *apicalis*** Shiraki
			11. *austeni* Hendel
		IV *Meracanthomyia* Hendel	**12. *kotiensis*** Kapoor
		V *Pelmatops* Enderlein	**13. *ichneumoneus*** (Westwood)
	3. CARPOMYINI	**VI *Carpomya*** Costa	**14. *vesuviana*** Costa
	4. TRYPETINI	**VII *Anomoia*** Walker	**15. *immsi*** (Bezzi)
		VIII *Acidiella* Hendel	**16. *rioxaeformis*** (Bezzi)
		IX *Cornutrypeta* Han & Wang	**17. *melanonotum*** (Brunetti)
		X *Stemonocera* Rondani	**18. *cervicornis*** (Brunetti)
			19. *discalis* (Brunetti)
TEPHRITINAE	**5. NOEETINI**	**XI *Ensina*** Robineau-Desvoidy	**20. *sonchi*** (Linnaeus)
	6. PLIOMELAENINI	**XII *Elaphromyia*** Bigot	**21. *pterocallaeformis*** (Bezzi)
		XIII *Pliomelaena* Bezzi	**22. *quadrimaculata*** Agarwal & Kapoor
			23. *zonogastra* (Bezzi)
	7. SCHISTOPTERINI	**XIV *Rhabdochaeta*** Meijere	**24. *pulchella*** Meijere
	8. TEPHRELLINI	**XV *Metasphenisca*** Hendel	**25. *reinhardi*** (Wiedemann)
		XVI *Oxyaciura* Hendel	**26. *monochaeta*** (Bezzi)
			27. *xanthotricha* (Bezzi)
		XVII *Sphaeniscus* Becker	**28. *atilius*** (Walker)
	9. TEPHRITINI	**XVIII *Campiglossa*** Rondani	**29. *absinthii*** (Fabricius)
			30. *cribellata* Bezzi
			31. *lyncea* (Bezzi)
		XIX *Dioxyna* Frey	**32. *sororcula*** (Wiedemann)
		XX *Scedella* Munro	***33.* *spiloptera*** (Bezzi)
		XXI *Spathulina* Rondani	***34.* *acroleuca*** (Schiner)
		XXII *Sphenella* Robineau-Desvoidy	***35.* *sinensis*** Schiner
		XXIII *Acanthiophilus* Becker	***36.* *helianthi*** (Rossi)
		XXIV *Actinoptera* Rondani	**37. *carignaniensis*** Kapoor & Grewal
			38.* *formosana Shiraki
		XXV *Trupanea* Schrank	***39.* *pteralis*** Agarwal, Grewal, Kapoor, Gupta & Sharma
	10. TERELLIINI	**XXVI *Chaetostomella*** Hendel	**40. *completa*** (Kapoor, Malla & Ghosh)
		XXVII *Terellia* Robineau-Desvoidy	***41.* *Sarolensis*** (Agarwal & Kapoor)

2.4 Molecular characterization of fruit flies

Homoplasmy in morphology, great economic importance, adaptation to varied climatic conditions, a wide host range and little work on the genetic relationship among the members of tephritid fruit flies make these flies an excellent candidate for the study of species diversity and evolutionary processes.

Genetic markers and sequences from the mitochondrial genome in particular, have proven to be very informative in the study of species diversity and evolutionary processes (Shi *et al.* 2005; Xie *et al.* 2006). This is due to some of its peculiarities, such as strictly maternal inheritance, absence of recombination, a relatively high mutation rate and last but not least, the availability of efficient PCR primers (Simon *et al.* 1994) and a wealth of comparative data (Boykin *et al.* 2006; Mun *et al.* 2003; Shi *et al.* 2005; Nardi *et al.* 2003; 2005; Jamnongluk *et al.* 2003; Reyes and Ochando 2004; Xie *et al.* 2006). Mitochondrial *cytochrome oxidase subunit I* (*COI*) sequences were shown to be appropriate for intra-specific analysis because of the high degree of polymorphism observed.

Additionally, *COI* sequences are at the base of the barcoding identification system (Hebert *et al.* 2003; Hajibabaei *et al.* 2006) that, besides being a valuable tool for species identification and discovery, has been proposed as a powerful methodology in biosecurity and invasive species identification (Armstrong and Ball 2005). Currently, this tool has been applied in pest monitoring and quarantine (Armstrong and Ball 2005; Ratnasingham and Hebert 2007) and its usefulness has been confirmed in several hexapod orders: Coleoptera (Lobl and Leschen 2005), Diptera (Scheffer *et al*. 2006), Ephemeroptera (Ball *et al.* 2005), Hemiptera (Foottit *et al.* 2008; Lee *et al*. 2011), Hymenoptera (Smith *et al.* 2008) and Lepidoptera (Hajibabaei *et al.* 2006). Species identification is achieved by comparing the sequence of an unknown sample to a reference database through similarity methods such as BLAST (Altschul *et al.* 1990). The reliability of identification depends on the extent of taxonomical coverage of the group of

interest and an understanding of the degree of variation within species (Lee *et al.* 2011). A case study on tephritid fruit flies (Armstrong and Ball 2005) reported high rates of success, but also mentioned some difficulties with the identification of a few species (e.g. *B. dorsalis, B. cucurbitae, A. fraterculus*), where the occurrence of cryptic species, inadequate sampling of all genetic subgroups, and high levels of geographic differentiation might complicate identification. However, broader *ad hoc* surveys of the phylogeography and geographic variability in species might provide valuable additions to the barcoding dataset and its applicability in difficult groups. Modern control strategies, such as the use of semiochemicals, sterile insect techniques, and foreseeable genetic tools, are strictly species/strain specific, and thus require a deep knowledge of the taxonomy and population structure of the target. This necessity becomes even more sensible when dealing with insect groups characterized by the presence of sibling species, such as mosquitoes and tephritid fruit flies (Hu *et al.* 2008).

Recently, Zhang *et al.* (2010) studied 689 bp nucleotide sequences of the mitochondrial *cytochrome oxidase I* gene of thirty-five individuals representing 7 *Bactrocera* species found in the Chongqing region in China and GenBank submitted sequences for another 20 *Bactrocera* species and 2 tephritid species, *Anastrepha ludens* and *Ceratitis capitata,* which were used as outgroups for the phylogenetic analysis. They reported *Bactrocera* (*Tetradacus*) *minax* and *Bactrocera* (*Zeugodacus*) *diaphora* sequences for the first time, and the subgenus *Bactrocera* (*Tetradacus*), represented by *B.* (*T.*) *minax* and *B.* (*T.*) *tsuneonis,* was included for the first time in an analysis of the genus *Bactrocera* phylogeny. Zhang *et al.* (2010) observed that nucleotide diversity within subgenus ranged from 9.1 to 19.0% among the subgenera, and the net divergence among subgenera ranged from 4.6 to 12.7%. Phylogenetic analysis based on maximum parsimony method supported that subgenus *Bactrocera* (*Bactrocera*) and *Bactrocera* (*Zeugodacus*) are paraphyletic. The subgenus *Zeugodacus*, *Bactrocera* (*Zeugodacus*) *caudate*, *Bactrocera* (*Zeugodacus*) *diaphora,* and *Bactrocera* (*Zeugodacus*) *scutellata* are closely related to

Bactrocera (*Zeugodacus*) *tau* and *Bactrocera* (*Zeugodacus*) *cucurbitae*. These results indicated that subgenus *Austrodacus* and *Zeugodacus*, which attack cucurbit plants, are closely related to species of the subgenus *Afrodacus*, *Bactrocera*, and *Gymnodacus*, which attack plants of numerous families. Earlier phylogenetic relationships among 24 *Bactrocera* species belonging to 9 subgenera were studied by Smith *et al.* (2003) with DNA sequence of portions of the mitochondrial *16S rRNA*, *cytochrome oxidase II*, *tRNALys*, and *tRNAAsp* genes suggested (1) the genus *Bactrocera* is monophyletic, (2) the subgenus *Zeugodacus* is paraphyletic, (3) the subgenus *Daculus* is a sister group to subgenus *Bactrocera* and (4) the subgenus *Bactrocera* is monophyletic.

Asokan *et al.* (2007) reported the *mtCOI* based identification of three fruit flies, *B. dorsalis*, *B. correcta* and *B. zonata* where molecular identification has corroborated the morphological identification. A single fragement of approximately 500 bp was amplified for *B. dorsalis, B. correcta* and *B. zonata*. Sequencing results showed that the total nucleotide length obtained was 440 bases, for all the three species of fruit flies. Alignment of the above sequences in Bioedit revealed that there was 92% similarity between *B. dorsalis* and *B. correcta* and also between *B. correcta* and *B. zonata*. The number of nucleotides that were different between *B. dorsalis* and *B. correcta* and between *B. correcta* and *B. zonata* was 32 and 28, respectively. Highest variation (11%) was observed between *B. dorsalis* and *B. zonata*, where there was difference in 45 nucleotides.

Bactrocera cucurbitae populations sampled throughout Southern China, Thailand and the Philippines by Hu *et al.* (2008) observed that these populations were genetically very similar, and most likely constitute a single phyletic unit with no sign of cryptic species or historical separation on the basis of the mitochondrial *cytochrome oxidase I* gene analysis. They also observed that a single haplotype predominates throughout this region. However, interspecific distances with outgroups ranged from 0.051 between *B. cucurbitae* and *B. tau* to 0.167 between *B. cucurbitae* and *B. dorsalis*.

Shi *et al.* (2005) conducted an analysis of population genetic structure of *B. dorsalis* from China using mitochondrial cytochrome oxidase (*COI*) gene sequences. They observed twentyeight haplotypes among 37 individuals with up to 13 mutations and genetic distances reached 2.2% between haplotypes. They also observed many haplotypes were missing in the sampled populations in the haplotype network. However, 43 haplotypes were observed in the six *Bactrocera dorsalis* populations (71 individuals) with up to 12 mutations from China using (*COI*) gene sequences by Liu *et al.* (2007).

B. tau is a major cucurbit pest, morphologically members of the *B. tau* complex show differences in the three yellow stripes on the thorax, along with size and shape of dark bands on the dorsal abdomen. However, some species of the *B. tau* complex could not be easily distinguished morphologically. Mitotic karyotype and electrophoresis analyses of the *B. tau* complex have been demonstrated to be useful tools for separation of these closely related species, although the methods are somewhat tedious and time consuming (Baimai *et al.* 2000b).

Analysis of mitotic karyotypes of the larvae belonging to the same species of adult fruit flies morphologically identified as *B. tau s.s.* and *B. tau*-like species has revealed seven distinct chromosomal forms which are most likely to represent seven closely related species within the *B. tau* complex. All members of the *B. tau* complex in this study exhibited mitotic karyotype 2*n*=12, conforming to the other species groups of the genus *Bactrocera* as previously described (Baimai *et al.* 1995; 1999; 2000a).

Jamnongluk *et al.* (2003) compared sequences of the mitochondrial *cytochrome oxidase I* gene of eight species of the *Bactrocera tau* complex from Thailand using *Bactrocera dorsalis*, *Bactrocera pyrifoliae*, *Ceratitis capitata*, *Anopheles gambiae*, and *Locusta migratoria* as outgroups.. The sequence divergence between species in the *B. tau* complex ranged from 0.06 to 28%, and up to 29% between the complex and its tephritid outgroups, *B. dorsalis* and *C. capitata*.

2.5 Gut bacterial diversity in fruit flies

Insects represent one of the largest reservoirs of bacterial diversity on Earth and about 15% of all insects harbour diverse communities of bacteria (Brooks 1963; Buchner 1965; Douglas 1989; 1998; Stouthamer *et al.* 1999; Moran *et al.* 2005; 2008; Wernegreen 2002; Prabhakar *et al*. 2009b). The insect –bacterial association has co-evolved for more than 250 million years and have resulted in manifold interactions between insects and bacteria, ranging from pathogenicity to highly sophisticated symbiotic relationships (Smith and Szathmary 1995; Werren and O'Neill 1997; Douglas and Beard 1997; Wernegreen 2002; Oliver *et al.* 2003; 2005) and may be extracellular or intracellular and may play a role in the nutrition, the physiology or the reproduction of the insect host (Dale and Moran 2006). One of the most striking interactions is that bacteria have extended the nutritional range of insects by supplying nutrients as endosymbionts (Douglas *et al.* 2001) and by accessing otherwise indigestible substrates, such as lingo- cellulose-derived organic matter from soils, with the help of gut bacteria (Brune 1998). Considering the extent of the dependence between the insect and the symbiont and the age of the association, symbionts can be classified in two groups; the obligate primary (P) endosymbionts, which have a long evolutionary history with their hosts and they are required for host survival and fertility, and the facultative secondary (S) symbionts, which have established a more recent association with the host and they have retained their ability to return to a free-living condition (Moya *et al.*, 2008). Petri (1909; 1910) described one of the first bacterial symbiotic associations in an insect species, the olive fly, *Bactrocera* (*Dacus*) *oleae* (Kounatidis *et al.* 2009, Prabhakar *et al.* 2009b).

Vast range of gut bacteria have been isolated and identified from different orders of insects. Descriptions of new symbionts identified in insects are frequent in the literature. In the present review, a summary of association between bacteria and insects except fruit flies are presented in Table 2.1.

Table 2.1: Summary of a selected number of examples of insect-bacteria interactions

Insect order, common name and species name	Bacterial species (group)	Type of interaction	References
Orthoptera Desert locusts *Schistocerca gregaria* (Forskal)	*Pantoea agglomerans* (γ-proteobacteria)	Symbiont	Dillon and Charnley 1995; Dillon *et al*. 2000
Thysanoptera Western flower thrips *Frankliniella occidentalis* (Pergande)	*Pantoea agglomerans* (γ-proteobacteria)	Symbiont	de Vries *et al.* 2001a; de Vries *et al*. 2001b
Callembola *Folsomia candida* Willem	*Alcaligenes facecalis*	Symbiont	Thimm *et al.*1998
Anoplura Human body louse *Pediculus humanus* Linnaeus	*Rickettsia prowazekii* (α-proteobacteria)	Obligate intracellular	Andersson *et al.* 1998
Hemiptera Sharpshooters *Homalodisca coagulata* (Say)	*Baumannia cicadellinicola* (γ-proteobacteria) *Sulcia muelleri* (Bacteroidetes)	P-endosymbiont	Wu *et al.* 2006
Stinkbugs *Megacopta punctatissima* (Montandon) Blood sucking bug *Rhodnius prolixus* (Stal)	*Ishikawaella capsulate* (γ-proteobacteria) *Rhodococcus rhodnii*	Extracellular symbiont Gut symbiont	Hosokawa *et al.* 2005; Hosokawa *et al.* 2006 Douglas 2006 Shigenobu *et al.* 2000
Sap-sucking insects, Aphids *Acyrthosiphon pisum* (Harris) *Schizaphis graminum* Rondani *Baizongia pistaciae* (Linnaeus) *Cinaria cedri* Borner	*Buchnera aphidicola* Bap *Buchnera* BSg *Buchnera* BBp *Buchnera aphidicola* BCc (γ-proteobacteria) *Hamiltonella defensa*	P-endosymbiont S-symbiont	Tamas *et al.* 2002 van Ham *et al.* 2003 Perez-Brocal *et al.* 2006 Oliver *et al.* 2003;
Aphids *Acyrthosiphon pisum* (Harris) Sap-sucking insects, Psyllids *Pachypsylla venusta* (Osten-Sacken)	(γ-proteobacteria) *Carsonella ruddii* (γ-proteobacteria) *Portiera aleyrodidarum*	Endosymbiont P-endosymbiont	Grenier *et al.* 2006 Thao *et al.* 2004;
Sap-sucking insects, Whiteflies *Bemisia tabaci* (Gennedius) Sap-sucking insects, Mealybugs *Planococcus citri* (Risso)	(γ-proteobacteria) *Tremblaya princeps* (β-proteobacteria)	Endosymbiont	Baumann *et al.* 2002

Insect order, common name and species name	Bacterial species (group)	Type of interaction	References
Neuroptera Antlion *Myrmeleon bore* Tjeder	*Enterobacter aerogenes* *Bacillus cereus* *Bacillus sphaericus* *Morganella morganii* *Serratia marcescens* *Klebsiella spp*	Temporal association	Nishiwaki *et al.* 2004; Nishiwaki *et al.* 2007; Yoshida *et al.* 2001
Coleoptera Rice weevil *Sitophilus oryzae* (Linnaeus)	SOPE P-endosymbiont (γ-proteobacteria)	P-endosymbiont	Heddi *et al.* 1998
Lepidoptera Tobacco horn worm *Manduca sexta* (Linnaeus)	*Burkholderia sp.*, *Ralstonia sp.*, *Cupriavidus sp*., *Enterococcus gallinarum*, *Enterococcus casseliflavus*, *Enterococcus saccharolyticus, Citrobacter sedlakii, Caulobacter sp*., *Pseudomonas* spp., *Enterobacter cloacae*, *Enterobacter aphidicola*, *Enterobacter aerogenes, Sphingomonas* sp., *Flavobacterium hydatis*, *Flavobacterium* spp., *Delftia acidovorans, Bacillus licheniformis*	Gut symbiont	Brinkmann *et al.* 2008
Hymenoptera Carpenter ant *Camponotus floridanus* (Buckley) *Camponotus pennsylvanicus* (De Geer)	*Blochmannia floridanus* (γ-proteobacteria) *Blochmannia pennsylvanicus* (γ-proteobacteria) *Brevibacillus formosus* *Stenotrophomonas maltophilia*	Endosymbiont	Gil *et al.* 2003; Zientz *et al.* 2006; Degnan *et al.* 2005
Honey bee *Apis mellifera* Linnaeus	*Brevibacillus brevis* *Bacillus fusiformis* *Acinetobacter calcoaceticus* *Bacillus* spp.	Symbiont	Evans and Armstrong 2006

Many studies on the dacinae fruit flies have provided valuable data on the role of microorganisms in host plant relationships and fruit fly biology (Drew *et al*. 1983; Courtice and Drew 1984; Drew 1987; Drew and Lloyd 1987; Jang and Nishijima 1990). The role of extra cellular bacteria associated with the alimentary tract of dipteran larvae and/ or adults is only partially understood at present. Most specific studies on the relationship of bacteria and fruit flies relate back to the early work of Petri (1910) in which the association of organism *Bacillus (Pseudomonas) savastanoi* Smith (the known cause of olive knot disease) with *Bactrocera oleae* (Gmelin) was reported. He also described another bacterium *Ascobacterium luteum* in association with *Bactrocera savastanoi*.

Stammer (1929) isolated bacteria from 37 species of tephritidae and further described the bacterial transfer system through each stage of the life cycle of *B. oleae*; but the bacteria associated were not identified. Yamvrias *et al*. (1970) could not isolate either *P. savastanoi* or *A. luteum* from eggs and oesophageal bulbs of field collected *B. oleae* adults as earlier reported by Petri (1910). Girolami (1973) defined two different types of symbiosis in tephritidae; one with bacteria in adult oesophageal bulb and other with bacteria in the blind sacs at the anterior end of the larval midgut. The release of compact masses of bacteria from the oesophageal bulb into the midgut has been described; however identity of the microorganisms was not reported (Girolami 1983).

Pseudomonas (Phytomonas) melophthora was found to be associated with all stages of *Rhagoletis pomonella* (Walsh), oviposition punctures, larval burrows and exit holes in apple fruits (Allen and Riker 1932; Allen *et al*. 1934; Baerwald and Boush 1968). Contrary to this, Rossiter *et al*. (1983) identified bacteria associated with *R. pomonella* as *Klebsiella oxy*toca and *Enterobacter cloacae*, and reported that the bacteria are necessary for normal development in most tephritid species. The bacterium associated with *B. cucurbitae* adults and larval stages, was identified as *Pseudomonas pseudomalaii* (Gupta *et al*. 1982a; 1982b; Gupta and Pant 1983).

Tsiropoulos (1976) found 15 morphologically different bacteria associated with the walnut husk fly, *R. completa* (Cresson), but only the *Pseudomonas* spp. and *Xanthomonas* spp. were found associated with all stages of the fly. Howard *et al*. (1985) also characterized the oesophageal bulb inhabitants of six *Rhagoletis* spp. and discovered a diverse microbial flora, although *K. oxytoca* predominated in every species of fly. Twenty different strains of bacteria from laboratory reared *B. dorsalis* and 23 strains from wild adults, characterized as members of family Enterobacteriaceae have been reported (Jang and Nishijima 1990). Most common bacteria associated with *Bactrocera* flies were *Citrobacter freundii*, *E. agglomerans*, *E. cloacae*, *K. oxytoca* and *Kluyvera* spp. (Lloyd *et al*. 1986; Jang and Nishijima 1990). These bacteria were collectively referred as "Fruit fly type" bacteria. Studies suggested that the flies were colonized by the bacteria which were fed and that the dinitrogen fixing activity associated with the flies was localized within these colonizing bacteria (Murphy *et al*. 1994). In tephritidae, specific bacteria belonging to Enterobacteriaceae (*Erwinia herbicola, E. cloacae and K. oxytoca*) are believed to mediate interactions between the adult fruit flies and the larval host plant. The general pattern of results suggested that female flies coming to oviposit on fruiting hosts were spreading Enterobacteriaceae (Raghu *et al.* 2002). Eighteen different bacterial species belonging to the family Enterobacteriaceae, Pseudomonadaceae, Vibrionaceae, Micrococcaceae, Deinococcaceae, Bacillaceae and the genus *Listeria, Enterobacter, Providencia, Serratia* and *Staphyloccocus* spp. were most frequently isolated from the gut of Mexican fruit fly, *Anastrepha ludens* (Loew). Some isolates were resistant to penicillin and ampicillin probably having ecological significance with respect to intra- and inter-specific competition within host cadavers (Kuzina *et al*. 2001). Sood and Nath (2002) studied bacterial association in two species of fruit flies, *B. tau* and *B. cucurbitae* in Himachal Pradesh (India) and isolated 11 types of bacteria associated with *Bactrocera* spp. out of which five were common to both the species *viz., Pseudomonas putida*, *Erwinia herbicola* (*Pantoea agglomerans*), *Cedacea davisae*, *Arthrobacter* spp.

and *Xanthomonas maltophilia* (*Stenotrophomonas maltophilia*). Jamnongluk *et al.* (2002) reported endosymbiotic bacteria of the genus *Wolbachia* (widespread among arthropods) from tephritid fruit flies. Belcari *et al.* (2003) isolated nine species of bacteria from the oesophageal bulb of the olive fruit fly (*B. oleae*) and eight species from the phylloplane of the host plant. Only three species viz. *Agrobacterium radiobacter*, *Pseudomonas putida* and *Serratia marcescens* were isolated from both. A range of bacteria belonging to different genera *viz. Acetobacter*, *Agrobacterium*, *Arthrobacter*, *Listeria, Enterobacter, Pantoea*, *Pectobacterium*, *Klebsiella, Citrobacter, Erwinia, Bacillus, Lactobacillus, Kluyvera*, *Micrococcus, Pseudomonas, Staphylococcus, Streptococcus, Proteus, Providencia, Hafnia, Serratia and Xanthomonas* have been isolated and characterized from the fruit fly gut. (Lloyd *et al*. 1986; Drew and Lloyd 1987; Jang and Nishijima 1990; Lauzon *et al*. 1998; 2000; Zinder and Dworkin 2000; Bergey *et al*. 2001; Kuzina *et al.* 2001; Marchini *et al.* 2002; Sood and Nath 2002; Belcari *et al.* 2003; Behar *et al*. 2005; 2008; 2009; Capuzzo *et al.* 2005; Sacchetti *et al.* 2008; Kounatidis *et al.* 2009; Prabhakar *et al.* 2009b). However, tephritid gut bacteria mostly belong to family Enterobacteriaceae and two species viz. *Klebsiella* and *Enterobacter* are the predominant ones (Drew and Lloyd 1987; Zinder and Dworkin 2000, Behar *et al.* 2005; Prabhakar *et al.* 2009b). Recently, Crotti *et al.* (2010) reported that microbe-insect symbiosis had established acetic acid bacteria (AAB) as symbionts of several insects of the orders Diptera including fruit fly *Bactrocera oleae*, Hymenoptera, Hemiptera, and Homoptera.

An increasing number of reports of associations of bacteria with insects in general and fruit flies in particular cannot be considered just environmental microorganisms but are indeed symbionts of the host body, where they occupy a specific favourable niche. But, still there is a paucity of information on this aspect and research in this particular area with modern molecular tools is essential in order to strengthen knowledge on fruit fly ecology and clarification of the function(s) exerted by the bacteria in and for their hosts will be a major step toward understanding the bacterium-fruit fly association.

2.6 Molecular characterization of gut bacteria

Molecular approaches for the detection and characterization of microbes have resulted in dramatic change in our understanding of microbial diversity. It is now recognized that approximately 99 per cent of the microbes in the environment can not be cultivated (Amann *et al*. 1995). However, nucleic acid based approaches for the characterization of microbes have provided new informations inspite of their own limitations (Head *et al*. 1998). Nucleic acid sequence approaches, particularly those using 16S rRNA genes, are enabling the identification of the microbial community of insects (Brauman *et al*. 2001; Toth *et al*. 2001)

RAPD-PCR analysis has been used to compare the strains of bacteria between insects and within the generations. Some bacteria in the thrips persisted for two years through 50 generations and were therefore indigenous bacteria, whereas transient bacteria ingested with food did not pass to the next generations (de Vries *et al*. 2001a; 2001b).

Profiling the insect gut microbiota is now feasible using methods based on the *16S rRNA* gene. Molecular and cultural techniques were used to examine the *Sitophylus oryzae* Linn. principal endosymboites (SOPE) and were compared with proteobacteria (Heddi *et al*. 1998) and found that SOPE belongs to Enterobacteriaceae family and share 95.0 and 94.1 per cent sequence homology with *Escherichia coli* and *Salmonella paratyphi*, respectively. The closest symbiotic bacteria to SOPE are the primary endosymbiotes of *S. zeamais* Motsch (97.8 per cent) and closest free living bacteria to SOPE is *Erwinia herbicola* (96.00 per cent) (Heddi *et al*. 1998). In another study, Bauer *et al*. (2000) showed significant genetic diversity in enumerated lactic acid bacteria using ERIC-PCR (enterobacterial repetitive intergenic consensus).

For the first time, five distinct strains of *Wolbachia* in *Bactrocera ascita* based on *wsp* (*Wolbachia specific primers*) gene sequence were reported by Jamnongluk *et al*. (2002). It was also stated that four of the five *Wolbachia*

strains were in the same group as those found in other tephritid fruit flies, suggesting possible horizontal transmission of *Wolbachia* from other fruit flies into *B. ascita*.

Waleron *et al*. (2002) studied the genotypic characteristics of *Erwinia* based on PCR-RFLP analysis (polymerase chain reaction- restriction fragment length polymorphism) of the *recA* gene fragment. The results indicated that PCR-RFLP analysis of *recA* gene fragment is a useful tool for identification of species and subspecies of *Erwinia*. Whereas. Sood and Prabhakar (2009) studied genotypic characteristics of gut bacteria of fruit fly, *Bactrocera tau* with RAPD and PCR-RFLP using *recA* gene and *rrs* gene and reported PCR–RFLP profile of three symbionts was more authentic than RAPD–REP–PCR profile as PCR–RFLP profile was based on the specific bacterial gene than profile generated through RAPD–REP–PCR where, amplification of DNA can occur anywhere in the genome. The PCR–RFLP profile of three symbionts of fruit fly was also supported by the antibiotic sensitivity pattern of different symbionts of fruit flies.

PCR amplification and nucleotide sequencing of the entire *16S rRNA* gene of symbiotic bacteria of the olive fruit fly (*B. oleae*) consistently yielded a single sequence that displayed marked similarity with enterobacterial lineages, with closest matches (97%) to *Erwinia persicina* and *E. rhapontici*. The symbiont's identity was also distinct from *Pseudomonas savastanoi*. A novel species was proposed, by virtue of its unique properties, under the designation '*Candidatus Erwinia dacicola*' (Capuzzo *et al*. 2005). However, Kounatidis *et al.* (2009) investigated the association between *Acetobacter tropicalis* and *B. oleae* with cultivation-dependent and -independent techniques. Using an *A. tropicalis* specific PCR assay, the symbiont was detected in all insects tested originating from laboratory stocks or field collected from different locations in Greece. This acetic acid bacterium was successfully established in cell-free medium, and typing analyses, carried out on a collection of isolates, revealed that different *A. tropicalis* strains are present in fly populations. Three symbionts were

characterized from fruit fly *Bactrocera tau*, with traditional microbiological techniques as well as modern PCR based tools with 16S rDNA (*rrs* gene) sequence analysis by Prabhakar *et al.* (2009b). They observed two bacteria from family Enterobacteriaceae i.e. *Klebsiella oxytoca* and *Pantoea agglomerans* and one bacterium from family Staphylococcaceae namely *Staphylococcus sp*.

2.7 Bacterial odours as attractants for fruit flies

The attractancy of protein solutions containing bacteria to fruit flies was first reported by Gow (1954), when *B. dorsalis* in Hawaii responded strongly to solutions containing a *Proteus* species. Cultures of fruit fly type bacteria growing on peptone yeast extract agar (Drew *et al*. 1983) and hydrolyzed protein solutions inoculated with these bacteria are strong attractants for *Bactocera* species (Drew and Fay 1988). When plates inoculated with these bacteria were exposed on the host trees, wild flies were attracted to and fed on bacteria.

The attractant emitted by hydrolyzed protein solution, with and without bacteria are not known, although various protein bait formulations have been used in fruit fly control programmes for many years (Bose *et al*. 1978). Drew and Fay (1988), on the other hand, deduced that ammonia was only a weak attractant and that certain bacterial metabolites were the primary attractants. Drew (1987) proposed that bacterial volatiles such as 2-butanone were important attractants in dacinae and served as a feeding attractant to females and a sex attractant to mature males.

Evidence from field studies supported the theory that bacterial odours enhance host attractancy. An extended field study of a wild fly population in a peach tree in Queensland indicated that the host tree become more attractive to flies after a short period of occupation by a small population of flies (Drew and Lloyd 1987). Jang and Nishijima (1990), in a laboratory experiment, studied the attractancy of bacteria and PIB-7 (Protein hydrolyzate) and observed significantly higher response of flies (*B. tryoni*) to the bacteria in the absence of PIB-7, but relatively lower response of flies to bacteria alone when PIB-7 was also a treatment.

Certain components of bacterial odours serve as either feeding or ovipositional stimulants (Drew and Lloyd 1987). Under laboratory conditions, flies frequently returned to the same spot, regurgitate and reingest several times (Lloyd 1988). This behaviour involved some form of host marking system and bacterial odours were reported to be evolved. Robacker and Flath (1995) identified ammonia, trimethylamine, isoamylamine, 2-methyl-butylamine, 2, 5-dimethylpyrazine and acetic acid from the culture of *Staphylococcus aureus*. In contrary to this, Lee *et al*. (1995) identified 3-methyl-1-butanol, phenethyl alcohol, 2, 5-dimethylpyrazine, 2-methyl-1-propanol and 3-(methylthio)-1-propanol as volatile components from bacteria, *K. pneumoniae*. All the chemicals attracted Mexican fruit flies. However, the attractiveness of *E. agglomerans* isolated from apple maggot and Mexican fruit fly towards Mexican fruit fly did not vary significantly despite the variation in volatiles produced by them (Robacker *et al.* 2004). It was concluded that combinations of attractive chemicals sometimes are not attractive. Sood *et al.* (2010) studied washed and fermented bacterial preparation of two predominant *B. tau* symbionts, *Klebsiella oxytoca* and *Pantoea agglomerans* (reported in earlier publication Prabhakar *et al.* 2009b) for their attractancy to two fruit fly species (*B. cucurbitae* and *B. tau*) under laboratory conditions. *Pantoea agglomerans* (washed bacterial preparation) in combination with sugar attracted maximum number of *B. cucurbitae*, while protein hydrolyzate in combination with sugar attracted maximum number of *B. tau*. All the combinations of washed bacteria proved superior to control (sugar alone) in terms of attractancy for both species. As fermented bacterial preparation, *Klebsiella oxytoca* in combination with jaggary attracted maximum fruit flies of both the species when applied on potted cucumber plants (Sood *et al.* 2010).

The response of fruit flies to their type bacteria suggests that a system of bacterial attraction for fruit flies probably exists in nature and may play a vital role in fruit fly behaviour.

2.8 Role of bacteria in the IPM of fruit flies

Control of fruit flies by manipulating its symbiotes has been proposed long back in 1929-30. Foliar application of copper carbonate was recommended for killing the symbiotic bacteria and later on this preparation was improved by adding sugar (Baker *et al*. 1944; Fytizas and Tzanakakis 1966a; 1966b). The use of antibiotics like streptomycin has also been proposed which rapidly kill symbiotes and the resultant larvae die soon after hatching from the eggs. Recontamination of the adults with microorganisms can certainly occur but the progeny of recontaminated parents have a considerably reduced rate of survival.

The antibiotics, oxytetracyclin and sulphanilamide administrated to the larvae of the gourd fruit fly, *B. cucurbitae*, destroyed the symbiotic microorganisms in the mycetocytes of the mid gut region. The depopulated mycetocytes were with prominent vacuoles, and the treated larvae had reduced survival rates (Chinnarajan *et al*. 1972). However, all these approaches could not be commercialized because of low efficacy of copper carbonate and residual hazards of streptomycin and other antibiotic treatments. Sood and Nath (1998) evaluated some insecticide attractant solutions containing bacteria in yellow traps for mass trapping of fruit flies. Jaggery trap attracted maximum number of flies followed by ethyl methyl ketone + ammonium acetate + sugar, ethyl methyl ketone + sugar, *Erwinia herbicola* + sugar. Copper could play an important role as symbioticide, in destroying the fruit fly associated bacteria and thus helpful in managing first and second instar larvae of olive fruit fly (Belcari and Bobbio 1999). Application of fruit fly symbionts under field conditions at Palampur (Himachal Pradesh) during 2006-07 as foliar application and as bait in combination with insecticide, *K. oxytoca* resulted in significant reduction in fruit fly infestation (65.46 %) over untreated control (79.56 %) (Sood *et al.* 2010). The attractancy of gut bacterial symbionts to fruit fly species in spite of low field efficacy indicate that symbiotic bacteria could be exploited for its surveillance and management (Sood *et al.* 2010). Endosymbiotic bacteria of the genus *Wolbachia*

induce cytoplasmic incompatibility, thelytokous parthenogenesis, male-killing or feminization in their hosts, thus may be useful in IPM (Jamnongluk *et al*. 2002). The symbiotic bacteria modified with toxin genes can be used in the management of fruit flies (Sood and Nath 2005). Symbiont biology receives increasing attention because insect symbionts can potentially be used to control vector borne diseases or suppress insect pests (Crotti *et al.* 2010).