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BACTERIAL AND FUNGAL ISOLATES FROM LABORATORY-REARED AEDES AEGYPTI (LINNAEUS), MUSCA DOMESTICA (LINNAEUS) AND PERIPLANETA AMERICANA (LINNAEUS)

RIAZ I. ZUBERI, SAYADA HAFIZ and S.H. ASHRAFI

P.C.S.I.R. Laboratories, Karachi 32

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Eggs, larvae, pupae and adults of *Aedes aegypti* (Linnaeus), *Musca domestica* (Linnaeus) and *Periplaneta americana* (Linnaeus)were separately plated out on nutrient media for the isolation of the normal aerobic bacterial and fungal flora. Fifteen isolates from *Aedes aegypti*, 27 from *Musca domestica* and 15 isolates from *Periplaneta americana* were identified.

Introduction

Rearing of insects in a laboratory poses many problems, the most important of which are sporadic infections or disastrous outbreaks culminating in complete destruction of insect cultures. According to their habitat, the insects are infected from their environment, e.g., the houseflies reared in the laboratory get infected through food and water while the cockroach population falls prey to infection from bread and milk. Frequent examinations of the normal flora of reared insects, therefore, are very helpful in the early recognition of pathogenic microorganisms. The present findings report the generally occurring aerobic bacteria and fungi associated with eggs, larvae, pupae and adults of laboratory-reared *Aedes aegypti* (Linn.), *Musca domestica* (Linn.) and *Periplaneta americana* (Linn.).

Materials and Methods

Three insects, *Aedes aegypti* (Linnaeus), *Musca domestica* (Linnaeus) and *Periplaneta americana* (Linnaeus) were examined for their bacterial and fungal microflora. The eggs, larvae, pupae and adults of these insects were obtained from hatchery from time to time. Each experiment was divided into the four stages of the insect life cycle. At least 10 eggs of each of the three insects were plated out simultaneously for the isolation of bacteria and fungi for comparative study. Similarly, 10 larvae, pupae, and adults of each insect in each experiment were studied. In all experiments, the different stages of the insects were surface sterilised and macerated in 10 ml sterile distilled water. Serial dilution technique was followed and the original suspension was diluted to $1:10$ to $1:10^3$ for the isolation of fungi and $1:10$ to $1:10^7$ for bacterial isolation. The dilutions were plated out on sabourads and nutrient agar respectively. The nutrient agar plates were incubated at 25°C and 37°C for 24 to 48 hr while sabourads agar plates were incubated for a week at 30°C. Pure cultures of bacteria and fungi were obtained on nutrient agar and sabourads agar slants respectively. The bacterial cultures were studied for their morphological, cultural and biochemical reactions and were identified.[1] The fungal cultures were characterised on the basis of their morphology, sporulation and pigmentation.

Results

The eggs of *Aedes aegypti*, *Musca domestica* and *Periplaneta americana* yielded a variety of bacteria. Culture No. 1 from *Aedes aegypti* was identified as *Pseudomonas aeruginosa*, which were short rods $0.5–0.6 \times 1.5\mu$, gave greyish colonies with dark centres on nutrient agar and produced a diffusible green pigment. The culture Nos. 2, 3 and 5 from *M. domestica* were identified as *Alcaligenes faecalis*, *Diplococcus* sp and *Shigelle dysenteriae* respectively. *A. faecalis* were rods, 0.5×1.0 to 2.0μ and produced white glistening colonies on agar; the *Diplococcus* sp. were oval and spherical cocci which occurred singly and in pairs and were encapsulated. The shiny, raised opaque colonies on N. agar were characteristic of *Shigella dysenteriae*, the rods measured $0.4–0.6 \times 1.0–3.0\mu$ and generally occurred singly. The eggs of *P. americana* yielded four cultures; cocci that occurred singly and in clumps gave opaque smooth white colonies on agar and were identified as *stophylococcus albus* (6); *Shigella alkalescens* (8) were $0.5 \times 1–1.5\mu$ rods which gave circular and raised colonies on agar. *Klebsiella pneumoniae* (10) were 0.3 to $0.5 \times 5\mu$ rods with rounded ends, encapsulated and produced white shiny colonies on agar. Small pale homogenous and entire colonies on N. agar were produced by *Micrococcus flavus*, Table 1.

Flavobacterium arborescens (2) and *Bacillus cereus* (4) were isolated from the larvae of *A. aegypti*. *F. arborescens* produced dirty orange colonies on agar; the irregular colonies with whip-like outgrowth were characteristic of *Bacillus cereus*, these bacilli measured $1–1.2\mu \times 3–5\mu$. From *M. domestica*, the bacteria isolated were *Proteus rettgeri*,

TABLE 1.—BACTERIAL ISOLATIONS FROM EGGS OF AEDES AEGYPTI, MUSCA DOMESTICA AND PERIPLANETA AMRICANA.

Source	Culture No	Motility	Gram stain	Triple sugar iron agar: Butt	Triple sugar iron agar: Slant	Triple sugar iron agar: H_2S	Indole	M.R.	V.P.	Citrate	Gelatin liquification	NO_3	Urease	Gl	Lc	Su	Mnl	Xy	Mn	Ar	Milt
Aedes aegypti	1	+	—	NC	NC	—	—	—	—	+	rapid	+	—	A	—	—	—	A	—	—	—
Musca domestica	2	+	—	NC	NC	—	—	—	—	+	—	—	—	—	—	—	—	A	—	—	—
Musca domestica	5	—	—	A	NC	—	—	—	—	—	—	+	—	A	—	A	A	A			—
Musca dom stica	3	—	+				—				—			A		A	—	—	—	A	
Periplaneta americana	6	—	+				—	—		+	+	+		AG	AG	AG	AG	AG	AG	AG	AG
Periplaneta americana	12	—	+				—	—			Slow	—		—	AG	A	—	—			—
Periplaneta americana	8	—	—	A	NC	—	—	+	—	+	—	+	—	A	—	—	A	A			A
Periplaneta americana	10	—	—	AG	NC	—	—	—	+	+	—	+	—	AG	AG	AG	AG	AG	AG		AG

A acid; AG acid and gas; NC no change; Gl glucose; Lc lactose; Su sucrose; Mnl mannitol; Xy xyclose; Mn mannose; Ar arabinose; Mlt maltose; + positive and — Negative.

0.5–0.8μ rods and produced dirty white small colonies on agar. *Gaffkya tetragena* (7) were cocci occurring in fours and pairs with circular white colonies on agar; *Proteus mirabilis* (8) were rods 0.5×1.0μ to 3.0μ, occurred singly and in pairs and had irregular grey. swarming colonies on agar. The short rods which measured 0.3 –0.6μ× 0.8–2.5μ and produced moist greyish colonies on agar were identified as *Salmonella gallinarum* (9). From the nymphs of *P. americana*, *Sh. alkalescens* (13) *K. pneumoniae* (14) and *M. Iavus* (15) were isolated (Table 2).

B. cereus (7) was also isolated from the pupae of *A. aegypti*. The other isolates from surface-sterilized mosquito pupae were *Bacillus megaterium* (9) which were rods 1.2–1.5μ×2–3μ and produced round, convex, entire and creamy white colonies on agar; *Brevibacterium tegumenticola* (3) another isolate, produced small white convex colonies on agar and were small rods 0.6–0.8μ×1.0–1.3μ. *Proteus inconstantans* (12) was also isolated from the pupae of *A. aegypti*, these were short rods 0.5–0.8μ and produced shiny moderate size raised colonies on agar. The pupae of *M. domestica* gave cultures of *P. rettgeri* (10), *P. mirabilis* (12) *K. pneumoniae* (13) and *S. gallinarum* (14) Biochemical reactions are given in Table 3.

From the adults of *A. aegypti*, *Escherichia intermedium* (15) short rods, 0.5×1–2μ which produced entire smooth colonies on agar, were isolated. The other isolates from the adults of *A. aegypti* were *Aerobacter aerogenes* (18), rods 0.5–0.8μ×1–2μ which gave thick raised entire colonies on agar; *Aerobacter cloacae* (16) gave circular opaque entire colonies on agar; the rods, 0.5–0.6×3–3.5μ which gave greyish white glistening entire colonies on agar were identified as *Salmonella para typhi A* (19). *A. faecalis* (20) was also isolated from the adults of *A. aegypti*. Surface-sterilized adults of *M. domestica* yeilded cultures of *Escherichia freundii* (21), short rods with rounded ends 0.5–0.6×1–2μ which gave entire round smooth colonies on agar; *Proteus morgani* (19) were rods 0.4–0.6μ×1.0–2.0μ and gave greyish white smooth glistening colonies on agar; *Bacillus licheniformis* (25) which gave white, opaque and branching colonies on agar were 0.6–0.8μ×2–3μ rods. The other isolates from the adults of *M. domestica* were *K. penumoniae* (22) *P. mirabilis* (23) *A. aerogenes* (18), *A. cloacae* (15), *P. aeruginosa* (26). Out of the five isolates from adult *P. americana*, four belonged to the family Enterobacteriacae. These were *E. intermedium* (30) *K. pneumonae* (32), *A. Cloacea* (33) and *P. rettgeri* (34); the fifth culture isolated from the adults of *P. americana* was a member of the family Pseudomonadacae, *P. aeruginosa* (18) (Table 4).

Several species of fungi were isolated from

TABLE 2.—BACTERIAL ISOLATIONS FROM LARVAE OF *Aedas aegypti*, *Musca domestica* AND *Periplaeta amricana*.

Source	Culture No	Motility	Gram stain	Triple sugar iron agar			Indole	M.R.	V.P.	Citrate	Gelatin liquification	NO_3	Urease	Gl	Lc	Su	Mnl	Xy	Mn	Ar	Mlt
				Butt	Slant	H_2S															
Aedes aegypti	2	—	—				—				+	—		AG	AG	—	—	AG	AG	—	
Aedes aegypti	4	—	+				—	—	+	late +	rapid	+		AG	—	AG	—	—	—	—	
Musca domestica	6	—	—	A	NC	—	+	+	—	+	—	+	+	AG	—	AG	—	—	—	—	—
Musca domestica	8	+	—	AG	NC	+	—	—	+	+	—	+	+	AG	—	AG	—	AG	—	—	—
Musca domestica	9	—	—	A	NC	+	+	+	—	—	—	+	—	A	—	—	A	—	AG	—	A
Musca domestica	7	—	+				—	—	—	—	—	+		A	A	A	A	—	—	A	—
Periplaneta americana (Nymphs)	13	—	—	A	NC	—	—	+	—	+	—	+	—	A	—		—		A		A
Periplaneta americana (Nymphs)	14	—	—	AG	NC	—	—	—	+	+	—	+	—	AG	AG	AG	AG	AG	AG		AG
Periplaneta americana (Nymphs)	15	—	+				—	—			Slow	—		—	AG	A	—	—			—

A acid; AG acid and gas; NC no change; Gl glucose; Lc lactose; Su sucrose; Mnl mannitol; Xy xylose; Mn mannose; Ar arabinose; Mlt maltose; + positive and — negative.

TABLE 3.—BACTERIAL ISOLATIONS FROM PUPAE OF *Aedes aegypti* AND *Musca domestica*.

Source	Culture No	Motility	Gram stain	Triple sugar iron agar			Indole	M.R.	V.P.	Citrate	Gelatin liquification	NO_3	Urease	Gl	Lc	Su	Mnl	Xy	Mn	Ar	Mlt
				Butt	Slant	H_2S															
Aedes aegypti	7	—	+				—	—	+	late +	rapid	+		AG	—	AG	—	—	—	—	
Aedes aegypti	9	—	+				—	—	+	—	slow	—		A	A	A	A	A	A	A	A
Aedes aegypti	5	—	+	NC	NC	—	—	—	—	+	—	+		AG	—	—	—	—	—	—	—
Aedes aegypti	12	+	—	A	NC	—	+	+	—	+	—	+	—	AG Late (slight gas)	—	AG	—	—	—	—	—
Musca domestica	10	—	—	A	NC	—	+	+	—	+	—	+	+	AG	—	AG	—	—	—	—	—
Musca domestica	12	+	—	AG	NC	+	—	—	+	+	—	+	+	AG	—	AG	—	AG	—	—	—
Musca domestica	13	—	—	AG	NC	—	—	—	+	+	—	+	—	AG	AG	AG	AG	AG	AG		AG
Musca domestica	15	—	—	A	NC	+	+	+	—	—	—	+	—	A	—	—	A	—	AG	—	A

A acid; AG acid and gas; NC no change; Gl glucose; Lc lactose; Su sucrose; Mnl mannitol; Xy xylose; Mn mannose; Ar arbinose; Mlt maltose; + positive and — negative.

Table 4.—Bacterial Isolations From Adults of Aedes Aegypti. *Aedes aegypti*, *Musca domestic* and *Periplaneta americana*.

Source	Culture No	Motility	Gram stain	Triple sugar iron agar			Indole	M.R.	V.P.	Citrate	Gelatin liquification	NO_3	Urease	Gl	Lc	Su	Mnl	Xy	Mn	Ar	Mlt
				Butt	Slant	H_2S															
Aedes aegypti	15	—	—	AG	A	—	—	+	—	+	—	+	—	AG	AG	AG	AG	A	A	AG	AG
A. aegypti	18	—	—	AG	A	—	—	—	+	+	—	+	—	AG	AG	—	AG	AG		AG	AG
A. aegypti	20	+	—	AG	A	—	—	—	+	+	slow	+	—	AG	AG	A	AG	AG	AG	AG	AG
A. aegypti	16	+	—	AG	NC	—	—	+	—	—	—	+	—	AG	—	—	AG	—		AG	AG
A. aegypti	19	+	—	NC	NC	—	—			+	—	—		—	—	—	—	—	—	—	—
M. domestica	20	—	—	A	AG	+	—	—	+	+	+	+		AG	AG	AG	AG	AG	AG	AG	AG
M. domestica	21	—	—	AG	NC	—	—	—	+	+	+	+	—	AG	AG	AG	AG	AG	AG		AG
M. domestica	22	+	—	AG	NC	+	—	—	+	+	+	+	+	AG	—	AG	—	AG	—	—	—
M. domestica	23	+	—	AG	NC	—	+	+	—	—	+	+		AG	—	—	—	—	—	—	—
M. domestica	19	+	—	AG	A	—	—	—	+	+	slow		—	AG	AG	A	AG	AG	AG	AG	AG
M. domestica	18	—	—	AG	A	—	—	—	+	+	—	+	—	AG	AG	—	AG	AG		AG	AG
M. domestica	16	+	—	A	NC	—	+	+	—				—	AG	—	—	AG	AG			AG
M. domestica	24	+	+				—	—	+	+	rapid	+		A	AG	A		A		A	A
M. domestica	25	—	—				—				+	—		AG	AG	—	—	AG	AG	—	
M. domestica	26	+	—	NC	NC	—	—	—	—	+	rapid	+	—	A	—	—	—	A	—	—	—
P. americana	18	+	—	NC	NC	—	—	—	—	+	rapid	+	—	A	—	—	—	A	—	—	—
P. americana	32	—	—	AG	NC	—	—	—	—	+	—	+	—	AG	AG	AG	AG	AG	AG	AG	AG
P. americana	33	—	—	AG	A	—	—	—	+	+	—	+	—	AG	AG	—	AG	AG		AG	AG
P. americana	30	—	—	AG	A	—	—	+	—	+	—	+	—	AG	AG	AG	AG	A	A	AG	AG
P. americana	34	—	—	A	NC	—	—	+	—	+	—	+	+	AG	—	AG	—	—	—	—	—

A acid; AG acid & gas; NC no change; Gl glucose; Lc lactose; Su sucrose; Mnl mannitol; Xy xyclose; Mn Mannose; Ar arabinose; Mlt maltose; + positive and — negative.

TABLE 5.

Source	Size and morphology	Pigment and colony character	Identification
Eggs and larvae of *A. aegypti* and *P. americana*	Spores borne on sides of the conidiophores singly and in clusters at the tip containing a row of structures the protoplast. Conidiophores 500μ long × 3.5 to 4μ thick. Conidia 15–23 × 5–7μ	Colonies consist of the conidiophores and are irregularly valvety growth dark brown, with strict margin	*Helminthosporium sp.*
Larvae of *A. aegypti*, nymphs of *P. americana* and pupae of *M. domestica*	Conidiophores 400–700 μ or more long × 5–15μ in diameter. Conidia 2–5μ × 3–6μ	Dull green with light yellow reverse, scant growth in older areas, spreading growth	*A. flavus*
Eggs of *A. aegypti* and adults of *P. americana* and *M. domestica*	Conidia globose about 3–6μ in size	Yellowish brown growth with a greenish cast, reverse yellowish brown	*A. ustus*
Pupae of *M. domestica* adults of *M. domestica*	Spores ellipitic, columella round. Sporengiophores erect and of varying height 10–30 mμ highx 8–16μ wide Sporangia small globose unequal or ovoid	Fluffy, buff growth turning to grey in older cultures	*Mucor racemosus*
Larvae of *M. domestica*	Conidiophores about 2.5μ in diameter. Conidia elliptical 2.5 to 3μ long	Bluish green dry, selvety growth, reverse pale yellow strict margin	*Penicillium* sp.
Nymphs of *P. americana*, Pupae of *A. aegypti* & *M. domestica*, adults of *M. domestica* and *P. americana*	Conidia globose, blakish, 2.5μ–7μ in diameter stalk several millimeters in length and 12 to 18μ in diameter	Colonies at first white spreading rapidly with development of black pigment, reverse of the colony yellowish white	*Aspergillus niger*
Eggs of *M. domestica*	Oval budding cells 3–8μ × 4–15μ, Glucose, sucrose, Maltose fermented; growth profuse in ethanol as sole source of Carbon	Colonies round and whitish 2–3 mm diameter	*Sacchromyces* sp.

surface-sterilised stages in the life cycle of *A. aegypti*, *M. domestica* and *P. americana* (Table 5). A species of *Helminthosprium* was isolated from the eggs of *A. aegypti* while *Aspergillus ustus* was isolated from the eggs of *P. americana*. The eggs of *M. domestica* yielded almost a pure culture of a species of *Sacchromyces*.

The larvae of *A. aegypti* yielded the cultures of *Helminthosporium* sp. and *Aspergillus* I*avus*. A species of *Penicillum* was isolated from the larvae of *M. domestica* while from the nymphs of *P. americana*, cultures of *Aspergillus niger* and *A. flavus* were isolated.

A. niger was also isolated from the pupae of *A. aegypti*. The pupae of *M. domestica* harboured two species of *Aspergillus*, *A. niger* and *A. flavus*.

Surface-sterilised adults of *A. aegypti* were found to be free from usually occurring saprophytic fungi. Adult houseflies yielded cultures of two species of *Aspergillus* and one of *Mucor*. The *Mucor* sp. was identified as *M. racemosus* while the Aspergilli were *A. niger* and *A. ustus*. The adults of *P. americana* were found to harbour *A. ustus* and *A. niger*.

Discussion

Pseudomonas aeruginosa (Schroeter) Migula, a potential pathogen of grasshoppers[2] and locusts[3] was found to occur as part of the normal flora of mosquitoes, cockroaches and houseflies. It was isolated several times from the eggs of *A. aegypti* and *P. americana* and adults of *M. domestica*.

With the development of the eggs of *A. aegypti* into larvae, pupae and adults, there was found a gradual increase in the aerobic flora with a predominant enteric group. Different species of *Bacillus* were encountered during bacterial isolations from various stages of the life cycle of the three insects and these seemed to come with the food as saprophytic contaminants. *Shigella alkalescens* was found to be associated only with cockroaches and was not isolated in any experiment throughout the life cycles of *A. aegypti* and *M. domestica*.

Weistrich *et al.*4 reported *Alkalagenes sp.* from larvae and adults and *Brevibacterium* sp. only from larvae of *A. aegypti*. In the present findings, *Alkalagenes faecalis* was isolated only from the adults and *Brevibacterium tegumenticola* from the pupae of *A. aegypti*. *Sacchromyces* sp. reported from *A. aegypti* by Weisterich *et al.* was found associated only with the eggs of *M. domestica*.

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