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Predation of bacteria by *Lampito mauritii* (Kinberg) and *Eudrilus eugeniae* (Kinberg) reared in different substrates

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ABSTRACT

The quanti-qualitative nature of bacteria in the gut and casts of *Lampito mauritii* and *Eudrilus eugeniae* as influenced by different feeding substrates like soil, sawdust, pressmud and pressmud-sawdust mixture have been determined. Relatively high number of species types and population was found in pressmud and pressmud-sawdust mixture. A phenomenal rise in colony forming units of *Klebsiella pneumoniae* and *Morganella morganii* in the gut and casts of both worms was due to their indigestion, while remaining species like *Pseudomonas aeruginosa*, *Bacterium antitratum*, *Mima polymorpha*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Proteus mirabilis*, *Proteus rettgeri*, *Escherichia coli*, *Staph citreus*, *Micrococci*, *Bacillus subtilis* and *Streptomyces albus* were digested.

Key words: *Eudrilus eugeniae*, gut bacteria, *Lampito mauritti*

INTRODUCTION

During vermicomposting, the breakdown of complex organic compounds from organic wastes is accomplished through natural bacterial action. The 'hot spots' of these bacterial biomass include the gut of soil animals, freshly decomposing plant and animal residues and rhizosphere (Bowen 1980). Wright (1972) has reported that *Lumbricus terrestris* finds bacteria attractive as food and proposed that they could be an important source of dietary protein. Bacterial feeding by earthworms has been reported by many workers (Day 1950; Ponomareva 1962; Daniel and Anderson 1992).

The passage of soil through earthworm gut changes its physiological properties and the level of microbial activity. It is also generally considered that the earthworm increases bacterial population in soil patches by providing them with high quality substrates for growth, e.g. the earthworm gut, tunnels and casts (Lee 1985). A high level of bacterial activity in the gut of *Aporrectoidae caliginosa* (Scheu 1987), *Lumbricus rubellus* (Daniel and Anderson 1992) and *Lumbricus terrestris* (Pedersen and Hendriksen 1993) have been reported. The population of ingested bacteria increases while passing along the gut of earthworms (Parle 1963; Lee 1985; Thorpe *et al.* 1993 and Toyota and Kimura 1994).

Worm casts form a suitable base for free living beneficial microbes whose activity is essential for the release of nutrients to the medium for plants (Tomati and Galli 1995). Many investigators have shown increased fungal populations (Parle 1963; Cooke 1983), bacterial populations (Parle 1963; Edwards and Fletcher 1988 and Heijnen and Marinissen 1995), enzyme activities (Krishnamoorthy and Vijranabhiah 1986), hormone activities (Tomati *et al.* 1988) and NPK enrichment (Mulongoy 1986) in casts compared with the surrounding soils. Bacterial populations were found to be high in the casts of *Lumbricidae* spp. (Scheu 1987; Heijnen and Marinissen 1995), *Megascolides autrophyes, Metaphire houlleli* and *Nelloscolex strigosus* (Tiwari *et al.* 1989). Studies have also demonstrated a high bacterial population in the casts of earthworms compared with the underlying soil (Parle 1963; Satchell 1983; Lee 1985; Edwards and Fletcher 1988 and Pedersen and Hendriksen 1993) Earthworm activity has been shown to promote the dispersal of a variety of beneficial soil bacteria like *Pseudomonas* spp. through the soil (Madsen and Alexander 1982). However, studies on the bacterial analysis in the gut and casts of earthworms fed with various organic

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Abbreviations: BA- Blood agar; CFU- Colony forming unit; MA - Mac Conkey agar; NA - Nutrient agar

wastes like sawdust and pressmud in order to convert them into organic manure, have not been made so far. Hence the present work is aimed at qualitative and quantitative analysis of bacteria in the gut of two composting earthworms, *Lampito mauritii* (Kinberg) and *Eudrilus eugeniae* (Kinberg) and in their freshly laid casts, using different feeding substrates.

MATERIALS AND METHODS

Clay loam soil (collected from the Agricultural Experimental Farm, Annamalai University), sawdust (by product of logging and carpentry industry), pressmud (or filter cake - a sugar factory waste) and pressmud-sawdust mixture (1 : 1) were used as feeding substrates for earthworms - *Lampito mauritii* (Kinberg) and *Eudrilus eugeniae* (Kinberg). 1g each of them was suspended in 1 ml sterile saline (1g Na Cl_2 in 100 ml distilled H_2O) in a sterile test tube, shaken thoroughly in a Vortex mixer and was used as inoculum for isolation and enumeration of bacteria from different substrates. Using a standard Platinum loop, 0.01 ml of the inoculum was inoculated into Blood Agar (BA), Nutrient Agar (NA) and Mac Conkey Agar (MA) plates and incubated at 37°C for 18 - 24 hours. The different colony forming units (CFU) developing on the media were estimated and expressed as CFU x 10^3 according to the method of Baron *et al.* (1994). The bacterial colonies were identified using Gram's stain and biochemical reactions according to the method described by Mahon and Manuselis (1995).

The gut contents (3 to 4 cm of gut ranging from 20 to 100 segments in *L.mauritii* and 4 to 5.5 cm of gut ranging from 18 to 185 segments in *E.eugeniae*) of the three stages of worms (preclitellate, early clitellate and late clitellate) which were fed on different substrates were dissected out using sterile scissors and placed in sterile test tubes containing 2 ml of sterile saline. The tubes containing the gut contents were shaken thoroughly and 0.01 ml inoculum was spread on the surface of the BA, NA and MA plates and incubated as stated earlier. The casts were collected after 15 days of feeding and 1 g was transferred to 1 ml of sterile saline, shaken well and 0.01 ml taken as inoculum to spread on NA, BA and MA plates. After incubation, colonies were counted from the plates and expressed as stated earlier. Data represented in the tables are means of six samples of substrates, gut of different age groups and casts.

RESULTS AND DISCUSSION

Soil bacteria form an important nutrient (Edwards and Fletcher 1988) and a source of dietary protein (Wright 1972) to earthworms. *Eisenia foetida* acquires its minerals and vitamins in the form of microbial biomass (Neuhauser *et al.* 1980). It is therefore unavoidable for the earthworms to feed on bacteria in soil since they are omnipresent. Earthworms need a combination of microbes, cellulose and grit as a source of ingesta for maximum growth and reproduction (Flack and Hartenstein 1984). In such a context, of the four different substrates included in the present study, pressmud and pressmud-sawdust mixture with many types and populations of bacteria, could be expected to form better food source than soil and sawdust alone to the two composting earthworms *Lampito mauritii* and *Eudrilus eugeniae* (Tables 1 - 4).

Pedersen and Hendriksen (1993) reported qualitative and quantitative changes in the bacterial flora of ingested food materials during gut transit. Populations of some *Enterobacteriaecae* such as *Serratia marcescens*, *E.coli*, *Salmonella enteritidis* and *Bacillus cereus* var *mycoides* in *Lumbricus terrestris* (Day 1950 and Thorpe *et al.* 1993) and *E.foetida* (Brown and Mitchell 1981) have been observed to decrease during passage through gut. In the present study five bacterial species *i.e. Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia coli* (except in soil) and *Morganella morganii* were isolated in the gut of both earthworm species reared in all subtrates (Tables 1-4). Among them, the occurrence of CF units of *Enterobacter aerogenes*, *Enterobacter cloacae* and *Escherichia coli* have been observed to decrease in reproductively active older animals than growing preclitellate adolescent worms. This indicates that these bacterial species are digested by these worms.

Some of the bacterial species that are taken in with the soil are killed during their passage through the earthworm's digestive tract as evidenced by the mortality of *Bacillus cereus* var *mycoides*, *Serratia marcescens* and *E.coli* in the digestive tract of *L.terrestris* (Day 1950; Brusewitz 1959), *E.coli* in the digestive tract of *Pheretima* spp. (Khambata and Bhatt 1957) and *Enterobacter cloacae* in the digestive tract of *L.terrestris* (Pedersen and Hendriksen 1993). In the present study, the following bacterial species present in different substrates were not isolated from the gut and casts of *L.mauritii* and *E.eugeniae* when reared on them: i.e. *Pseudomonas aeruginosa*, *Bacterium antitratum*,

Table 1. Isolation and estimation of bacteria in the gut and casts of *Lampito mauritii* and *Eudrilus eugeniae* reared in soil

Sl. No.	Bacterial species	Soil	CFU x 10^3 /L. mauritii gut 3 - 4 cm^{-1}			CFU x 10^3 /E. Eugeniae gut 4 - 5.5 cm^{-1}			WormCasts	
		CFU x 10^3 g^{-1}	PC	EC	LC	PC	EC	LC	L.mauritii CFU x $10^3 g^{-1}$	E.eugeniae CFU x $10^3 g^{-1}$
	Gram negative									
1	*Klebsiella pneumoniae*	6	116	157	196	107	182	210	62	186
2	*Pseudomonas aeruginosa*	9	-	-	-	-	-	-	-	-
3	*Bacterium antitratum*	2	-	-	-	-	-	-	-	-
4	*Enterobacter aerogenes*	75	66	48	31	55	28	13	-	-
5	*Enterobacter cloacae*	26	16	13	7	13	9	6	-	-
6	*Morganella morganii*	17	78	112	185	92	119	217	290	980
	Gram positive									
7	*Bacillus subtilis*	5	-	-	-	-	-	-	-	-
	Total	140	276	330	419	267	338	447	352	1168

PC -Preclitellate Stage, EC- Early Clitellate Stage, LC-Late Clitellate Stage - denotes absence

Table 2. Isolation and estimation of bacteria in the gut and casts of *Lampito mauritii* and *Eudrilus eugeniae* reared in sawdust

Sl. No.	Bacterial species	Sawdust CFU x 10^3 g^{-1}	CFU x 10^3 /L. mauritii gut 3 - 4 cm^{-1}			CFU x 10^3 /E. Eugeniae gut 4 - 5.5 cm^{-1}			Worm Casts	
			PC	EC	LC	PC	EC	LC	L.mauritii CFU x $10^3 g^{-1}$	E.eugeniae CFU x $10^3 g^{-1}$
	Gram negative									
1	*Klebsiella pneumoniae*	1	221	264	294	316	372	375	80	148
2	*Enterobacter aerogenes*	21	18	11	8	15	8	4	-	-
3	*Enterobacter cloacae*	26	21	17	10	19	13	11	-	-
4	*Morganella morganii*	29	313	344	391	310	404	418	132	817
5	*Proteus mirabilis*	35	-	-	-	-	-	-	-	-
6	*Escherichia coli*	10	7	5	2	6	4	1	-	-
	Gram positive									
7	*Streptomyces albus*	7	-	-	-	-	-	-	-	-
	Total	129	570	638	705	666	801	809	212	965

PC-Preclitellate Stage, EC - Early Clitellate Stage, LC- Late Clitellate Stage - denotes absence

Table 3. Isolation and estimation of bacteria in the gut and casts of *Lampito mauritii* and *Eudrilus eugeniae* reared in pressmud

Sl. No	Bacterial species	Pressmud CFU x 10^3 g^{-1}	CFU x 10^3 /L. mauritii gut 3 - 4 cm^{-1}			CFU x 10^3 /E. Eugeniae gut 4 - 5.5 cm^{-1}			Worm Casts	
			PC	EC	LC	PC	EC	LC	L.mauritii CFU x $10^3 g^{-1}$	E.eugeniae CFU x $10^3 g^{-1}$
	Gram negative									
1	*Klebsiella pneumoniae*	37	386	397	418	391	398	415	142	241
2	*Pseudomonas aeruginosa*	61	-	-	-	-	-	-	-	-
3	*Bacterium antitratum*	40	-	-	-	-	-	-	-	-
4	*Mima polymorpha*	13	-	-	-	-	-	-	-	-
5	*Enterobacter aerogenes*	29	26	23	18	27	17	11	-	-
6	*Enterobacter cloacae*	65	55	51	48	44	32	18	-	-
7	*Morganella morganii*	23	615	644	689	687	711	740	679	1515
8	*Proteus mirabilis*	32	-	-	-	-	-	-	-	-
9	*Proteus rettgeri*	24	-	-	-	-	-	-	-	-
10	*Escherichia coli*	16	13	11	8	11	9	5	-	-
	Gram positive									
11	*Staph citreus*	3	-	-	-	-	-	-	-	-
12	*Micrococci*	8	-	-	-	-	-	-	-	-
13	*Bacillus subtilis*	18	-	-	-	-	-	-	-	-
	Total	369	1095	1126	1181	1160	1167	1189	821	1756

PC - Preclitellate Stage, EC- Early Clitellate Stage, LC- Late Clitellate Stage - denotes absence

Table 4. Isolation and estimation of bacteria in the gut and casts of *Lampito mauritii & Eudrilus eugeniae* reared in pressmud - sawdust mixture

Sl. No.	Bacterial Species	PSM	CFU x 10^4 /L. mauritii gut 3 - 4 cm^{-1}			CFU x 10^4 /E. Eugeniae gut 4 - 5.5 cm^{-1}			Worm Casts	
									L. mauritii	E. eugeniae
		CFU x 10^4 g^{-1}	PC	EC	LC	PC	EC	LC	CFU x $10^4 g^{-1}$	CFU x $10^4 g^{-1}$
	Gram negative									
1	*Klebsiella pneumoniae*	26	415	461	687	646	689	719	163	266
2	*Pseudomonas aeruginosa*	36	-	-	-	-	-	-	-	-
3	*Bacteriam antitratum*	27	-	-	-	-	-	-	-	-
4	*Mima polymorpha*	5	-	-	-	-	-	-	-	-
5	*Enterobacter aerogenes*	40	36	31	29	31	26	21	-	-
6	*Enterobacter cloacae*	26	21	18	13	20	19	12	-	-
7	*Morganella morganii*	35	617	902	981	432	486	611	606	1333
8	*Proteus mirabilis*	42	-	-	-	-	-	-	-	-
9	*Proteus rettgeri*	17	-	-	-	-	-	-	-	-
10	*Escherichia coli*	11	9	7	3	8	6	2	-	-
	Gram positive									
11	*Staph citreus*	1	-	-	-	-	-	-	-	-
12	*Micrococci*	3	-	-	-	-	-	-	-	-
13	*Bacillus subtilis*	10	-	-	-	-	-	-	-	-
14	*Streptomyces albus*	2	-	-	-	-	-	-	-	-
	Total	281	1098	1419	1713	1137	1226	1365	769	1599

PC - Preclitellate Stage, EC - Early Clitellate Stage, LC - Late Clitellate Stage PSM - Pressmud-sawdust mixture- denotes absence

Mima polymorpha, *Proteus mirabilis*, *Proteus rettgeri*, *Staph citreus*, *Micrococci*, *Bacillus substilis* and *Streptomyces albus*. This could be due to the complete digestion of these species.

Bacteria occur in high concentrations in the gut of earthworms than the food materials or bulk soil (Ineson and Anderson 1985). Toyota and Kimura (1994) isolated true autochthonous species like *Klebsiella oxytoca*, *Enterobacter cloacae*, *Serratia liquefaciens* and *Aeromonas hydrophilla* from the gut of *Pheretima* spp. There is much evidence of resident gut microflora and also that many microorganisms passing through the gut are unharmed. Bacterial populations showed a logarithmic increase during passage through the gut of *L.terrestris*, *Aporrectoidae caligenosa*, *Aporrectoidae longa* (Parle 1963). Kozlovskaya and Zhdannikova (1961) reported that the total density of bacteria in the gut of species of *Lumbricidae* and *Octolasium* was ten or more times larger than that in soil. In the present study, though undoubtedly there was an increase in the total population of bacteria in the gut as the food passes through it, there was variation in the diversity of bacteria as they occur in the different stages of worms, *L.mauritii* and *E.eugeniae*. It was quite interesting to note that *Klebsiella pneumoniae* and *Morganella morganii* increased in their population, with advancing age, in the gut of both the species of worms, whereas *Enterobacter aerogenes*, *Enterobacter cloacae* and *E.coli* were reduced with advancing age. These variations may be due to bacterial growth during gut transit, selective digestion of some bacteria and/or indigestability of others. The present study has conclusively established the dominant occurrence of *Klebsiella pneumoniae* and *Morganella morganii* in the gut of both *L.mauritii* and *E.eugeniae* in all three developmental stages and in all four substrates used to rear them. This observation assumes much significance as *Klebsiella pneumoniae* - a facultatively anaerobic nitrogen fixing species (Alexander 1978) - might, in part, contribute to the nitrogenous nutritional requirements of these worms. The significant role of *Klebsiella pneumoniae* and also that of *Morganella morganii* - and their dominant bacterial species in the gut of both species - needs a critical appraisal with respect to the growth and well being of these two earthworms. A symbiotic relationship between earthworms and their gut microflora have been proposed by Lavelle *et al.* (1983).

Ponomareva (1962) reported that the number of bacteria in earthworm faeces was thirteen times higher than in the surrounding soil. In the present study, the total number of bacterial colonies in the casts of *L.mauritii* and *E.eugeniae* were 2.51 and 8.32 times higher than in the soil, 1.64 and 7.48 times higher than in sawdust, 2.22 and 4.75 times higher than in pressmud and 2.83 and 5.90 times higher than in pressmud-sawdust mixture. Such high concentrations in earthworm casts is due to selective consumption of bacteria (Hendriksen 1990).

An increased occurrence of cellulolytic, hemicelluloytic and amylolytic bacteria in worm casts compared to the surrounding soil was reported

by Loquet *et al.* (1977). Kozlovskaya and Zhdannikova (1961) reported that the number of *Bacillus idozus* and *Bacillus cereus* were greater in casts than in soil. Casts of *L.mauritii* and *E.eugeniae*, in the present study exhibited greater population of *Klebsiella pneumoniae* than the substrates - soil, sawdust, pressmud and pressmud - sawdust mixture. So also *Morganella morganii* occur in greater population in the casts of *L.mauritii* and *E.eugeniae* than the substrates. Studies on the significance of the occurrence of these two bacterial species in high numbers in the casts of *L.mauritii* and *E.eugeniae* on soil fertility and crop growth will be rewarding.

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