CHAPTER - 1

CULTIVABLE BACTERIA IN THE GUT, COELOMIC SPACE AND HABITAT OF *EISENIA FETIDA* WITH SPECIAL EMPHASIS ON CELL NUMBER OF VARIOUS *BACILLUS* SPP. IN A COW DUNG-MICROBE-EARTHWORM SYSTEM

1.1 Introduction

Microbes have seemingly endless capacity to transform the world around them. All life on Earth depends directly or indirectly on microbes for many essential functions. All living organisms including plants and animals have closely associated microbial communities which make life possible for them by means of making nutrients, metals, and vitamins accessible or neutralizing toxins from the host body and its environment or even making the host resistant to other pathogenic microbes. Eukaryotic invertebrate hosts, in many occasions, function or demonstrate phenotypes which are impossible unless supported by the physiological activities of the bacteria that are allowed purposefully to reside within the system of organs. On the other hand, the eukaryotic host thrusts an effect on the dynamics of free –living microbial community (Fraune and Bosch, 2010). The interactions of the host with the environmental microbes and the ephemeral microbial communities help not only in shaping the microbial landscape but also to understand how the obligate and symbiotic microbes influence phenotypic expression of the host as well. One of the classical examples of symbiosis in invertebrates where the bacterium in return of its permanent shelter and food-security renders protection against protozoan infection and synthesizes vitamin for the host is that of *Glossina morsitans* (tsetse fly) and *Wigglesworthia glossindia* (a Gram-negative bacterium) (Heller, 2011). In *Drosophila*, the presence or absence of specific *Wolbachia* species determines resistance or susceptibility to viral infection (Faria *et al.,* 2016). The actinobacterial symbionts, *Coriobacterium glomerans* and *Gordonibacter* sp., residing in the mid-gut of the cotton stainer *Dysdercus fasciatus* contribute to strengthening the immune status of the host by supplementing B vitamins (Salem *et al.,* 2014).

Biomass of soil, in many ecosystems, constituted of invertebrate components is largely dominated by earthworms. A vertically transmitted bacterium *Verminephrobacter*, made its presence in the excretory organ (nephridia) of nearly all earthworms from the evolutionary past. In the act of give-n-take policy of symbiosis, *Verminephrobacter* spp. utilizes waste products of digestion and in return provides support to host reproduction (Lund *et al.,* 2014). A second vertically transmitted symbiotic bacterium, *Flexibacter*-like, in some of the earthworms, has also been reported (Møller *et al.,* 2015). The

characteristic body cavities, gut and coelom, of the typical tube-within-tube architecture of earthworm have enabled the researchers to investigate the immune system and its communication with the dynamic microbial population present within the cavities (Engelmann *et al.,* 2011). Earthworm hosts quite a sizable number of bacteria in its gut and coelom from its habitat (soil). The gut contains more (many times) live bacteria than coelom (Edwards and Lofty, 1977). A certain fraction of the innumerable diverse bacteria present in the earthworm feed, during its passage through the gut, enjoys the selective advantage in the gut environment to contribute transformation of the soil chemistry (Govindarajan and Prabhaharan, 2014). The bacterial density in the coelomic fluid is regulated by various factors including the presence of phagocytic cells and antimicrobials (Schindler, 2004). Since expression of defence molecules, coelomic cytolytic factor (CCF) in particular, in the gut epithelial tissue is high; immune response to an incessant flow of high-density microbial presence is less affected. The scenario of the cellular immune response in the coelomic cavity is different because of the coelomocytes, liberated from the coelome-mesenchyma, are the major players of the immune system (Dvořák *et al.,* 2016). Within the coelomic cavity the freely suspended amoebocytes, also called as immunocytes can be regarded as functionally equivalent to that of macrophages present in the vertebrates (Dhainaut and Scaps, 2001). Chloragosomes stores riboflavin (vitamin B2). Incursion by soil-borne pathogens is thwarted by eleocytes resulting from chloragocytes. When there is a possibility of huge upsurge due to the mobilization of pathogenic bacteria, pathogens in mass are captured or in other words encapsulated by multicellular entities produced by amoebocytes and eleocytes (collectively called coelomocytes). The plausible immunomodulatory function of riboflavin (stored in chloragosomes) was demonstrated by establishing it as the chemo attractant for coelomocyte-taxis (Sulik *et al.,* 2012). Eleocytes accumulates free riboflavin in chloragosome. It was hypothesized earlier that the riboflavin content is perturbed under stress or else dependent on the balance between pathogen load and immune system of the earthworm (Plytycz and Morgan, 2011**)**. Several speculations were made to reveal how the level of riboflavin is maintained in chloragocyte and eleocytes in the light of the existing knowledge that riboflavin biosynthesis does not occur in animals. It was observed that even starvation for more than a month took a heavy toll on earthworm's

body weight and reproduction but coelomocytes and riboflavin storage or accretion remained largely unaffected (Sulik *et al.*, 2012). Hence, the suspect of endosymbionts or restricted harbouring of riboflavin-synthesizing bacteria in the coelomic cavity of the earthworm becomes very strong. Even if the host's compulsion for allowing a proliferation of those preferred bacteria in the coelomic cavity to obtain the sustained supply of riboflavin is assumed or hypothesized as the prime reason for symbiosis, solid microbiological evidence are necessary to accept the hypothesis. Several pertinent issues are to be addressed in order to establish this phenomenon of purposeful hosting of soil-borne bacteria in the coelomic fluid. What attracts these environmental bacteria to direct their senses to reach the organic destiny within a biologically active niche (coelomic cavity in the present study)? What is the host's compulsion to allow the proliferation of those preferred bacteria and promote relaxation to evade immune-barricade? This study has adequately addressed these questions.

In the present study, the interrelationship between habitat (processed cow dung) and the earthworm (*Eisenia fetida*) has been studied in the laboratory mimicking the *in-situ* conditions. The undigested residue of consumed food material excreted by the herbivorous cows, defined as cow dung, contains high titres of culturable *Bacillus* spp. ($> 10^{13}$ g^{-1} processed cow dung) during processing until it gets suitably processed for feeding the earthworms. Earthworms consume their own home/habitat and their growth is dependent on microbial associations. Since several *Bacillus* species present in the gut of *E. fetida* play a major role in the degradation of polymeric materials, they are predominant with titres $> 10^{11}$ g^{-1} gut content. We hypothesized that the journey of *Bacillus* population from cow dung to earthworm gut and back to the environment has an important regulatory effect on the overall dynamics of *Bacillus* population even in case of invasion of a pathogenic species, *Bacillus thuringiensis*, and re-entry or colonization into the host system. At least three different *Bacillus* species were found in differential numbers in the circulating coelomic fluid of the host. This phenomenon has enabled us to uncover the basis of symbiosis in the coelom of *E.fetida*.

1.2 Materials and methods

1.2.1 Rearing of *Eisenia fetida* in laboratory

Fresh *Eisenia fetida* specimens were collected from Centre for Floriculture and Agro-business Management (COFAM), University of North Bengal, Darjeeling. The worms were maintained in the laboratory with dried cow dung at 22°C and 70-80% humidity in plastic tubs (Edwards and Bohlen, 1996).

1.2.1.1 Processing of cow dung for feeding E. fetida

Raw cow dung (RCD) samples from healthy cows (*Bos taurus indicus*) were collected and made into chips; sun-dried (8 h /day) for two days in the open air to transform them into semi-dried, odourless chips. The chips were broken into small pieces, moistened by spraying sterile distilled water (in order to increase the moisture content to 70%) and then left in the incubator at 25°C for 48 h. A quantity of 100 g of this semi-processed RCD was taken per sterile Petri-plate (diameter = 14 cm) to prepare the processed RCD (PrCD) earthworm bed for further experiments.

1.2.1.2 Monitoring of cultivable bacterial load and major Bacillus spp. *diversity during making of processed RCD*

Set of three semi-processed RCD containing Petri-plates were taken for this experiment. The plates were incubated at 25°C. Pinch sampling of the content of Petri-plate was followed in order to achieve a representative coverage of the cultivable bacterial load and diversity of *Bacillus* spp. Four separate aseptic recoveries, approximately 0.1 g per recovery per quadrant, were combined (quantity of mixture was determined) to make one 'sample'. Each PrCD sample, taken in a 2.0 ml microfuge tube, was dissolved (50% w/v) in phosphate buffer saline, pH 7.2 and vigorously mixed for 1.5 min using a vortex mixer (Tarson). The insoluble part of the mixture was allowed to gravitate in order to collect the PrCD aqueous solution (stock solution; undiluted). A series of dilution tubes per sample was set up to obtain dilutions of 10^{-1} through 10^{-7}. Multiple dilutions and plating on Luria agar (LA), diluted (0.1x LA) Luria agar (DLA) and Hichrome Bacillus Agar (HBA; Cat. No. M1615; Himedia, India) were used to ensure about countable plates, after incubation

in aerobic and anaerobic chambers at 25 °C, for studying the population dynamics of cultivable bacterial load and major *Bacillus* spp. diversity. Day 0 sample is defined as the sample drawn on the day immediately after placing the semi-processed PrCD in the Petri-plates. Sampling after a passage of 24 h from the time of day 0 was considered as day 1 sample. Likewise, samples at regular 24 h intervals up to day 6 were analyzed.

1.2.2 Estimation of cultivable bacterial load and major *Bacillus* spp. diversity in habitat of *E. fetida*

Two sets, three PrCD containing Petri-plates per set, were taken for this experiment. Set I was kept without earthworms. In the other set, Set II, each Petri-plate was inoculated with twenty-five adult earthworms (having clitella; body weight 0.3 ± 0.05 g). The plates were incubated at 25°C and observed at regular intervals to check escape of any earthworm from their PrCD beds. Pinch sampling of PrCD (earthworm bed) was followed in order to achieve a representative coverage of the cultivable bacterial load and diversity of *Bacillus* spp. Four separate aseptic

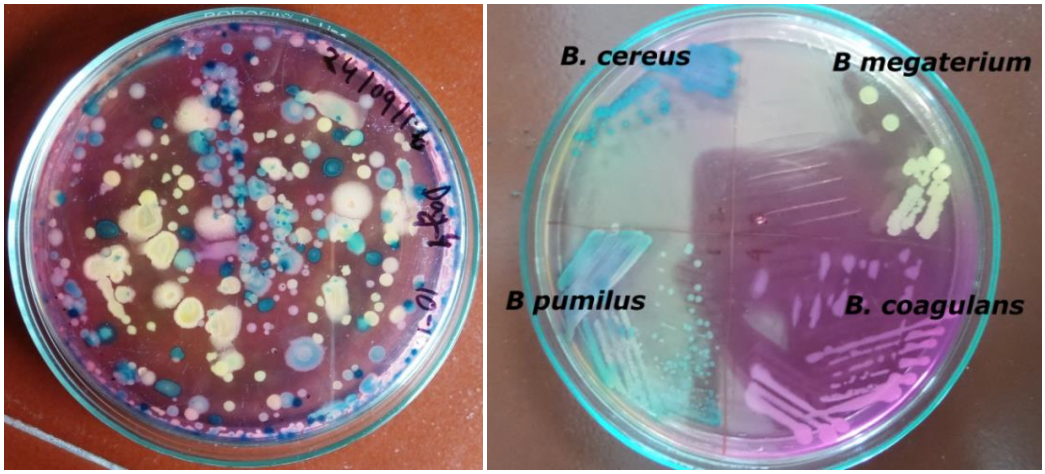


Fig. 1.1: Culture plates showing growth of major Bacillus spp. on HBA media

recoveries, approximately 0.1 g per recovery per quadrant, were combined (quantity of mixture was determined) to make one ‘sample’. Each PrCD sample, taken in a 2.0 ml microfuge tube, was dissolved (50% w/v) in phosphate buffer saline, pH 7.2 and vigorously mixed for 1.5 min using a vortex mixer. The insoluble part of the mixture was allowed to gravitate in order to collect the PrCD aqueous solution (stock solution;

undiluted). A series of dilution tubes per sample was set up to obtain dilutions of 10^{-1} through 10^{-7}. Multiple dilutions and plating on Luria agar (LA), diluted (0.1x LA) Luria agar (DLA) and Hichrome Bacillus Agar (HBA; Cat. No. M1615; Himedia, India) were used to ensure about countable plates, after incubation in aerobic and anaerobic chambers at 25 °C, for studying the population dynamics of cultivable bacterial load and major *Bacillus* spp diversity. Day 0 sample is defined as the sample drawn on the day immediately after the inoculation of *E. fetida* in PrCD bed. Sampling after a passage of 24 h from the time of day 0 was considered as day 1 sample. Likewise, samples at regular 24 h intervals up to day 6 were analyzed. Parallel samples (on the same sampling day) were drawn simultaneously from the Set I for evaluation of cultivable heterotrophic bacterial population along with the predominant *Bacillus* spp.

1.2.3 Estimation of cultivable bacterial load and major *Bacillus* spp. diversity in the gut of *E. fetida*

At different days, day 0 – 6, two earthworms (*E. fetida*) were taken from each of the three experimental plates of Set II and washed several times with sterile distilled water. Thoroughly washed earthworms (two from each PRCD plate) were transferred to sterile dry tissue paper kept inside the sterile Petri plates and observed for 20-30 min in the laminar hood. Fresh cast pellets of *E. fetida* expelled on the tissue paper were aseptically collected in pre-weighed sterile microfuge tubes. After measuring the quantity of the gut sample, dissolution of the same in PBS (50% w/v) was made. Dilution plating, spreading and incubation were done as described in the preceding section. The above-mentioned procedure was done on Day 0, 1, 2, 4 and 6.

1.2.4 Estimation of cultivable bacterial load and major *Bacillus* spp. diversity in coelomic fluid of *E. fetida*

Two earthworms (*E. fetida*), at different days (day 0 – 6), were taken from each of the three experimental plates of Set II and washed several times with sterile distilled water. Thoroughly washed individual earthworm was held firmly in a sterile tissue paper and coelomic fluid (≤ 20.0 µl) was collected in a fine- sharp sterile capillary tube (diameter ≤ 1.0 mm) by puncturing the coelomic cavity. The coelomic fluid content of the capillary

tube was serially diluted in sterile PBS. Multiple dilutions and plating on LA, DLA and HBA plates were done to enumerate the total number of heterotrophic bacteria (CFUs) and differentiation between various species of *Bacillus* along with viable count respectively.

1.2.5. Formulation of a medium -Coelomic fluid Mimic Broth (CMB)

The coelomic fluid from fifty earthworms was collected (procedure described earlier) in a sterile microfuge tube. Coelomocytes were discarded as pellet and the supernatant was collected in a fresh sterile microfuge tube after centrifugation for 10 min at 500 x g, 4 ^{0}C. Thus the CF devoid of coelomocytes was analyzed for carbohydrate, protein, fat, urea, mineral and free amino acid contents following standard methods. With reference to the observed contents of the cell-free coelomic fluid, a medium was formulated with the following composition: (in w/v.): 0.05% Dextrose, 0.77% Beef extract, 0.075% Tween 20, 0.002% Urea, 0.14% Yeast extract, 0.7% NaCl and 1.5% Agar (used only for making plates).

1.2.6. Culture-independent assessment of eubacterial diversity in the coelomic fluid of *E. fetida*

Since aseptically collected coelomic fluid, used directly as the source of target DNA from resident coelomate bacteria, repeatedly failed in PCR amplification of 16S rRNA gene sequences, enrichment procedure was followed not only to increase template numbers but also to decrease PCR inhibitory substances, present if any in the coelomic fluid. 5 ml of CMB medium was inoculated with 5 µl of aseptically collected coelomic fluid and incubated overnight at 25°C. The bacterial cell pellet was collected after centrifugation of the culture at 5000 x g for 5 min. The pellet was resuspended in 500 µl sterile distilled water, lysed by boiling at 100 ^{0}C in a water bath for 20 min. The lysate was taken as templates to PCR amplify 16S rRNA gene sequences following standard method (ref). The PCR product was gel-analyzed for specificity and yield before cloning. Blunt-end PCR products generated by proofreading DNA polymerase were directly ligated with pJET 1.2/ blunt cloning vector following protocols supplied by the manufacturer of Clone JET PCR Cloning Kit #K1231 (Thermo scientific). *E. coli* DH5α was directly

transformed with the ligation product following $CaCl_2$ transformation method. The recombinant colonies were picked up by sterile toothpicks to construct master plates for building the clone library. Each of the library members was assigned a number. A set of 14 random numbers ($1/10^{th}$ of total clones) were generated using research randomizer (http://www.randomizer.org/). Prior to clone analyses of the 14 colonies, as per numbers (random) from the previously marked colonies (1.2,3….140) on master plates, short strikes of individual colonies were propagated on ampicillin plates. Small amounts of each were used for colony PCR using pJET1.2 forward and reverse sequencing primer following protocols supplied by the manufacturer (Thermoscientific). Colony PCR products were digested with *Hae*III in accordance with the manufacturer's (Merck Genei) instruction, electrophoresed on a 2% (w/v, solution of agarose in TAE buffer) agarose gel, and band sizes were determined by using 100 bp DNA ladder as size standards. Amplified Ribosomal DNA restriction analysis (ARDRA) data from 14 clones were analyzed. The phylogenetic tree was generated by PyElph (Pavel and Vasile, 2012) (a software tool for gel images analysis) using neighbour joining method.

1.2.7. Culture-dependent isolation of *Bacillus* spp. from coelomic fluid of *E. fetida* and phylogenetic characterization of the isolates

At different time intervals, two earthworms (*E. fetida*) were taken from each of the three experimental plates, washed thoroughly several times with sterile distilled water. The coelomic fluid was collected in a sharpened capillary tube by carefully puncturing the body wall from each worm held firmly in a sterile tissue paper. The content of the capillary tube was liberated in PBS and multiple dilutions and plating on HBA plates were done to differentiate as per colour of colonies of *Bacillus* spp. The most frequently occurring colonies representing three different colours and morphology (yellow mucoid colonies for *B. megaterium*; blue flat colonies for *B. cereus*; light green to green colonies for *B. pumilis*) were isolated and dilution streaked on HBA to obtain pure cultures of the isolates. Pure cultures were maintained in Luria agar slants. Cell pellet (from 0.5 ml log-phase culture) suspended in 200 µl sterile distilled water, boiled for 1.5 minutes at 850 watts in microwave was centrifuged at 8,000 rpm for 2 min to obtain the supernatant; 2 µl of the resulting supernatant was used as template DNA for PCR amplification of 16S

rRNA gene using a set of primers as described by Lane (Lane, 1991). The PCR product was gel-analyzed for specificity and yield before cloning. The blunt-ended PCR product was ligated with pJET 1.2 vector and transformed into *E. coli* DH5α to obtain recombinant colonies, followed by sequencing of the cloned inserts in ABi3730XI by Sanger sequencing methodology.

1.2.7.1 Phylogenetic affiliations of three different strains of Bacillus, Ah4, BP, and BCR

Multiple alignments and phylogenetic analyses of the 16S rRNA gene sequences of Ah4, BP, BCR and all recognized species of genus *Bacillus* (ascertained from nBlast result against the query Ah4/BP/BCR sequence) were conducted in the software package MEGA (version 7.0) (Kumar *et al*., 2016). Multiple alignments of sequences were done with CLUSTAL W (Thompson *et al*., 1994) and the resulted multiply aligned sequences were corrected, edited and approximately 1423 base pair long nucleotide stretch of all the *Bacillus* sp. was selected for further analysis. Distances were calculated according to the Jukes-Cantor parameter. Phylogenetic analyses were performed using two tree-making algorithms: the neighbour-joining (N-J) (Saitou and Nei, 1987) and UPGMA (Unweighted Pair Group Method with Arithmetic mean) methods to ensure consistency of the clusters formed (data not shown) (Sneath and Sokal, 1973). All gaps and missing data in the aligned sequence were eliminated from the dataset (complete deletion option). Tree topology was evaluated by the bootstrap resampling method of Felsenstein (1985) based on 1000 replications.

1.2.8. Individual growth curves of *B. megaterium* (Ah4), *B. cereus* (BCR), *B. pumilus* (BP), *and B. coagulans* (BCO) [four predominant *Bacillus* species in RCD or PRCD] in CMB

Growth curves of four different strains of *Bacillu*s, Ah4, BP, BCR, and BCO in CMB medium were compared. A 100-ml flask containing 10 ml CMB medium was inoculated with Ah4 or BP or BCR or BCO. The culture was incubated overnight at 25°C. 10 ml of sterile CMB (in a 100 ml flask) was inoculated with 100 µl of the overnight grown culture and incubated at 25 °C for 4 h with shaking (80 rpm) to reach the log phase. An aliquot of 3.0 ml of log phase culture was then used to inoculate two 500 ml flasks each containing 300 ml sterile CMB. The contents of each flask were thoroughly mixed and

distributed aseptically into 60 sterile flasks such that each flask contains 10 ml of inoculated culture. One flask from the set was immediately placed on ice for dilution plating (0 h plating) and the rest were kept in the incubator at 25 °C. At different time intervals, flasks were withdrawn from the incubator, placed on ice for dilution plating on Luria agar (LA) plates. For each such culture, grown for a defined time at 25 °C, a series of dilution tubes was set up to obtain dilutions of 10^{-1} through 10^{-7} of the *Bacillus* strains. Multiple dilutions and plating were used to ensure countable plates for plotting the growth curves.

1.2.9. Dual-species competition among four *Bacillus* spp.; three most prevalent coelomic *Bacillus* species (*B. megaterium*, *B. cereus*, *B. pumilus*) and one chance intruder *B.thuringiensis*

Log phase cells from monocultures of four *Bacillus* species [*B. megaterium* (Ah4); *B. cereus* (BCR); *B. pumilus* (BP); *B. thuringiensis* (BT)] were centrifuged to remove the supernatants, and the cell pellets were resuspended in fresh CMB broth. The cell suspension was adjusted to OD_{600} 0.5, and mixed in 1:1 ratio of Ah4 : BCR; Ah4 : BP; Ah4 : BT; BCR : BP; BCR : BT; and BP : BT. The 1:1 mixture of dual-species contained approximately 10^{10}-10^{11} cfu ml^{-1} of each species. 1 ml of each mixture was inoculated into 99 ml CMB in a 250 ml Erlenmeyer flask. The contents of each flask were thoroughly mixed and distributed aseptically into 10 sterile 125 Erlenmeyer flasks such that each flask contains 10 ml of inoculated culture. One flask from the set was immediately placed on ice for dilution plating (0 h plating) and the rest were kept in the shaker incubator at 25 °C. At different time intervals, flasks were withdrawn from the incubator, placed on ice for dilution plating on HBA plates. For each such culture, grown for a defined time at 25 °C, a series of dilution tubes was set up to obtain dilutions of 10^{-1} through 10^{-7} of the *Bacillus* strains . Multiple dilutions and plating on HBA were used to ensure countable plates for plotting the growth curves. Competitive index (CI) values were determined for every combination of dual-species. CI is calculated as species A/species B ratio within the output sample(x) divided by the corresponding ratio in the inoculum (y). CI = x/y. If the CI value is 1, it would reflect that species A is able to grow as efficiently as the species B, and a CI < 1 would indicate that growth of species A is attenuated.

1.2.10. Growth of *B. megaterium* (Ah4), *B. cereus* (BCR), *B. pumilus* (BP), and *B. coagulans* (BCO) in mixed culture

Log phase cells from monocultures of four *Bacillus* species [*B. megaterium* (Ah4); *B. cereus* (BCR); *B. pumilus* (BP); *B. coagulans* (BCO)]were centrifuged to remove the supernatants, and the cell pellets were resuspended in fresh CMB broth. The cell suspension was adjusted to OD_{600} 0.5, and mixed in 1 : 1: 1: 1 ratio of Ah4 : BCR : BP: BCO. The 1:1:1:1 mixture of quadruple-species contained approximately 10^8-10^9 cfu ml^{-1} of each species. 2 ml of the mixture was inoculated into 198 ml CMB in a 500 ml Erlenmeyer flask. The contents of the flask were thoroughly mixed and distributed aseptically into 20 sterile 125 Erlenmeyer flasks such that each flask contains 10 ml of inoculated culture. One flask from the set was immediately placed on ice for dilution plating (0 h plating) and the rest were kept in the shaker incubator at 25 °C. At different time intervals, flasks were withdrawn from the incubator, placed on ice for dilution plating on HBA plates. For each such mixed-culture, grown for a defined time at 25 °C, a series of dilution tubes was set up to obtain dilutions of 10^{-1} through 10^{-7} of the *Bacillus* strains . Multiple dilutions and plating on HBA were used to ensure countable plates for plotting the growth curves.

1.2.11. Chemotaxis assay

For chemotaxis experiments, four PRCD dwelling Gram positive cells of different species of the genus *Bacillus* (*B. megaterium*, *B. cereus*, *B. pumilus*, *B. coagulans*), one soil-borne pathogen (*B. thuringiensis*) and one Gram negative soil inhabiting species (*Pseudomonas aeruginosa*) were used. Cells of *Bacillus* spp. were grown in CMB medium while *P. aeruginosa* cells were grown in LB. Overnight grown bacteria in CMB or LB medium (0.2 ml) were inoculated into 10 ml sterile CMB or LB in a 250 ml Erlenmeyer flask and kept on a rotary shaker at 30 ^{0}C until $O.D_{600}$ reached 0.2 to 0.4 to attain the log phase. Cells from log phase were transferred (1% v/v) to 5 ml sterile medium (CMB/LB) in a 125 ml Erlenmeyer flask and then incubated as described above until $O.D_{600}$ reached 0.5 to 0.6. Bacterial cultures were centrifuged at 8000 x g for 5 min at 4 ^{0}C. Pellets were washed twice with PBS buffer (= chemotaxis buffer) and the re-

suspended pellet was added up to 2 ml of PBS to bring bacteria to approximately 10^9 per ml. The microfuge tubes, each containing 200 µl bacterial suspensions, were horizontally placed in sterile Petri-plates under the laminar hood. Coelomic fluid (CF), ≤ 5 µl, was collected in a sharpened capillary tube (length, ≤ 4 cm; internal diameter of the piercing end to draw CF, ≤ 0.5 mm) by carefully puncturing the body wall from each worm. After drawing CF, the other ends of the capillary tubes were thoroughly sealed with molten wax. The control capillary contained sterile PBS instead of CF. The open end of each capillary (rinsed with PBS) was then inserted into the microfuge tube containing the bacterial suspension. The apparatus used in this study (Fig. 1.) was basically a hybrid of two earlier apparatus for chemotaxis assay (Adler, 1973; Chakraborty and Roy, 1990).

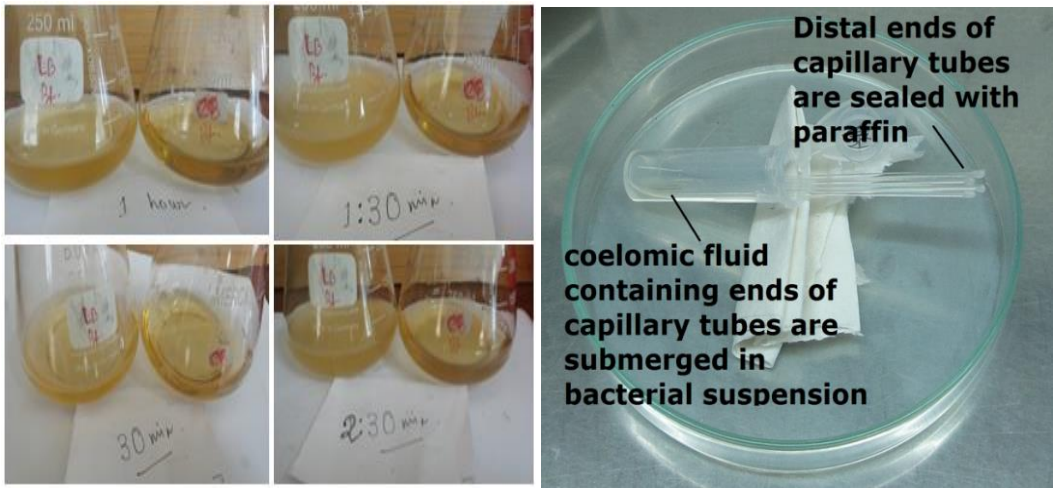


Fig. 1.2: Photographs of experiments on bacterial competition and chemotaxis. A- Culture flasks showing growth of major *Bacillus* spp. in coelom mimicking broth. B- Chemotaxis assay.

After incubation at different time intervals, the capillaries were removed and their exterior surfaces were washed with a thin stream of sterile water, the sealed ends were broken over sterile microfuge tubes containing 1.0 ml PBS such that contents of the open ends were fully released. Suitable dilutions were made in sterile PBS and spread plated in HBA plates (for *Bacillus* spp.) or LA (for *P. aeruginosa*). After incubation overnight at 30 ^{0}C, colonies were counted.

1.2.12. Riboflavin synthesis of *B. megaterium* (Ah4), *B. cereus* (BCR), *B. pumilus* (BP), and *B. coagulans* (BCO) in CMB

Overnight grown cultures of *B. megaterium* (Ah4), *B. cereus* (BCR), *B. pumilus* (BP), *and B. coagulans* (BCO) in CMB were inoculated (2% v/v) into 10 ml sterile CMB in a 250 ml Erlenmeyer flask, and kept on a rotary shaker at 30 ^{0}C until $O.D_{600}$ reached to 0.4 to attain the log phase. Cells from log phase were transferred (1% v/v) to 5 ml CMB kept in culture tubes. Four sets of culture tubes (one set per bacterial culture consisting of 10 culture tubes) wrapped in aluminum foil, to prevent photo-degradation of riboflavin produced by the bacteria, were grown under mild shaking condition (80 rpm) at 25°C. 2 ml cultures from one of each tube of four different sets were taken out at different time intervals in aluminum foil-wrapped microfuge tubes and centrifuged at 8000 x g for 5 min. From each microfuge tube, 1 ml of the supernatant was collected in a fresh aluminium foil-wrapped microfuge tube and mixed with 1 ml of sodium borate buffer. OD was measured at 440 nm in the nano-spectrophotometer (Make) and concentration of riboflavin was determined by plotting the OD value in a standard curve of riboflavin (prepared, prior to the experiment, by following standard method) (Bartzatt and Follis, 2014).

1.2.13. Statistical analysis

All the experiments were carried out in triplicate (n=3). The results are expressed as mean ± SD. The observed p-value corresponding to the F-statistic of one-way ANOVA was ≤ 0.01, which strongly suggested that one or more pairs of the variables were significantly different. One way ANOVA with post-hoc Tukey HSD Test (Kramer, 1956) was done where necessary to compare results of the initial (day 0 or day 1) and final day. The level of significance at 99% or 95% was indicated in the corresponding line or bar graphs as **, $p < 0.01$; and *, $p < 0.05$. Similar statistical analysis was performed and interpreted accordingly on experimental results described in other chapters also.

1.2.14 Isolation and taxonomic identification of bacteria from the gut of *E. fetida*

For isolation of the strains, the earthworms were washed repeatedly with sterile distilled water and then anesthetized by placing into 10% ethanol. The posterior (gut region in the last 1/3rd of the worm's body) gut was aseptically removed from the anaesthetized worm. After surface cleaning by sterile distilled water the gut along with its content was mixed

with 500µL of phosphate buffer saline (pH 7.2) using a micro pestle in a 1.5ml microfuge tube. Coelomic fluid did not require mixing with a pestle. The mixed content was serially diluted (in PBS) and spread on Luria agar and incubated at 25ºC for 48h in aerobic and anaerobic conditions. Distinct and unique colonies that developed on agar plates were picked, single colony purified, put in glycerol stock (80%) and refrigerated (-70 °C) for future study.

1.2. 14.1 Identification and phylogenetic analysis based on 16S rRNA gene

The 16S rRNA genes of strains were amplified from the genomic DNA prepared by standard protocol (Farlong *et al.* 1996). The purified amplicon was ligated to pJET 1.2 blunt vector (ThermoFisher, K1232) and cloned in *E. coli* DH5α. The inserted part was sequenced from both forward and reverse ends to obtain an almost complete 16S rRNA gene sequence. The sequence comparisons with entries in the GenBank and EMBL databases were performed with the BLASTn program (Pearson, 1990; Altschul *et al*., 1990; Altschul *et al*., 1997). To determine the phylogenetic affiliation, the 16S rRNA gene sequences of the strains were aligned with the sequences of members of the closely related bacteria with the CLUSTAL W program (Thompson *et al.*, 1994). Evolutionary relationships of members of the strains were inferred using three different tree-making algorithms: the neighbour-joining (Saitou and Nei, 1987), maximum likelihood (Felsenstein, 1981)and maximum-parsimony (Fitch, 1971) in Mega Ver 7.0 (Kumar *et al.*, 2016). Phylogenetic analyses and the fidelity of the tree topologies were evaluated by bootstrap analysis with 1000 replicates (Felsenstein, 1981; Tamura *et al.*, 2007).

1.2. 14.2 Phenotypic and chemotaxonomic study of selected novel bacterial strains

Growth of the strains were tested at 4 ºC, 10 ºC, 20 ºC, 28 ºC, 30 ºC, 35 ºC, 37 ºC and 42 ºC (±1 ºC). For salt tolerance tests 2%, 4%, 6%, 8%, 10%, 15% or 20 % (w/v) NaCl was added to peptone-yeast extract (PY) medium (composition: 10 g peptone, 5 g yeast extract) devoid of NaCl or KCl. To assess growth at different pH levels, the pH of the sterile LB medium was adjusted from pH 3.0 to 12.0 by using either 0.1 M HCl or 0.1 M NaOH. Results were obtained after 48 h incubation at 28 ºC. The Gram test was performed by the KOH lyses method (Murray *et al.*, 1994) and further confirmed by the

Gram-staining method of Claus (1992). Catalase activity was examined by the production of bubble after addition of few drops of 3% (v/v) H_2O_2 on the full grown colony of strains in LA slant. Voges-Proskauer test was performed by observation of colour development after addition of alpha-naphthol and potassium hydroxide to the Voges-Proskauer broth of the stain's culture. Ability to hydrolyze starch was determined by assessing the development of clear zones (after treatment with Grams iodine solution) around the streaked culture on starch agar plates (nutrient agar 2.3%; soluble starch 0.5%; pH 7.2). Urease test was performed by observing the development of red or pink colour at the periphery of the bacterial colonies on Christensen's urea agar plates (MacFaddin, 2000). Other biochemical characteristics or activities such as β-Galactosidase, nitrate reduction, arylsulfatase (3d), acid phosphatase, pyrazinamidase, α-esterase and β-esterase, citrate utilization, ONPG test, nitrate reduction, and abilities to ferment fructose, inulin, lactose, maltose, mannitol, raffinose, ribose, salicin, sorbitol, sucrose, trehalose etc. were examined by following standard methods (Gordon *et al.*, 1974; Lanyi, 1987; Smiberg and Krieg, 1994). Phenotypic characterization of ET03^T was performed using the Biolog GEN III MicroPlate following manufacturer's instructions. Briefly, bacterial suspension, prepared in special "gelling" inoculating fluid, was transferred to GEN III MicroPlate, (100 µl per well). Incubation was carried out in an aerobic atmosphere for 48 h. Increased respiration due to the growth of bacteria using the single carbon source provided in each well caused reduction of the tetrazolium redox dye, forming a purple colour. The reactions were read using the fully automated OmniLog system. Carbon source utilization assays were double-checked by using HiCarbohydrate kit parts A, B and C (Cat. No. #KB009; HiMedia) according to the manufacturer's protocol. Antibiotic susceptibility (specific for oligotrophic bacteria) was determined according to the method described by Kumar *et al*. (2010).

1.2.14.3 Determination of Respiratory Quinone, Polar lipid, FAME and GC mol% of the strains

For the study of quinones and polar lipids, two-stage lipid extraction method as described by Tindall *et al*. (1989) was followed with volumetric modifications. Extraction with methanol: hexane (2:1 v/v) followed by thin layer chromatography (TLC) was performed.

Briefly, the menaquinone part was purified by running the hexane fraction on TLC silica gel 60 F254 (Merck) using petroleum benzene: di-ethyl-ether (0.85:0.15) as the solvent. Further development of menaquinone components was done using acetone: water - 0.99:0.01 as the solvent and observed under UV_{254}. The methanolic part was taken for extraction of polar lipids using chloroform: methanol: 0.3%NaCl (1:2:0.8) as the extraction medium (Bligh and Dyer1959, Minnikin *et al*., 1984; Tindall, 1985). Polar lipids were separated by two-dimensional TLC. In the first dimension, chloroform: methanol: water (65: 25:4, v/v), and in the second dimension, chloroform: methanol: acetic acid: water (80:12:15:4, v/v) were used as the solvent to partition the polar lipids on silica gel plate. Lipid functional groups were identified using spray reagents specific for phospholipids (Mb-Blue), free amino groups (ninhydrin) and sugars (α-naphtol).

Fatty acids were extracted from 36 h old (exponentially growing) cells grown in tryptone-soy-agar (M290; HiMedia) at 28 °C and then esterified to form fatty acid methyl esters (FAMEs). The FAMEs were then analysed by GC (Hewlett Packard 5890 II plus) and the Sherlock Microbial Identification System using version 4.10 of the TSBA40 library (Microbial ID) as described previously (Kaur *et al.,* 2012).

1.2. 14.4 Scanning Electron Microscopy (SEM) of the strains.

Samples were fixed in glutaraldehyde (3%) and 2% osmium tetraoxide according to the standard protocol The fixed cells were mounted on small glass coverslips, dehydrated using increasing ethanolic gradients. After sputter gold coatingthe details of the cell shape were ascertained with help of a scanning electron microscope (EVO LS10, Zeiss, Germany; JS MIT 100, JEOL Ltd., Japan).

1.2. 14.5 Submission of the strains in recognized 'Type' culture collection centres

The novel strains were submitted to the internationally recognised 'Type' culture collection centres,with prior permission of the National Biodiversity Authority (NBA), Chennai, India.

1.3. Results and Discussion

1.3.1. Cultivable bacteria in cow dung during processing in the laboratory

On the zero day of the processing of RCD, the order of abundance of four different species of *Bacillus* was *B.coagulans* > *B. cereus* > *B. pumilus* > *B. megaterium* (Fig. 1.3-A). With time, up to 4th day of processing, there have been increases observed in case of *B. coagulans* (> 10^{13} cfu g^{-1} on 4 d) and *B. cereus* (> 10^{12} cfu g^{-1} on 4 d). The populations of the other two species, *B. pumilus and B. megaterium*, after getting increased up to the 2nd day of processing, have shown a progressive decrease in cell number on 4th and 6th day (Fig. 1.3-A).

1.3.2. Cultivable bacteria in the habitat of *E. fetida*

In absence of *E. fetida*, the fall and rise in populations of *B.coagulans*, *B. cereus,* and *B. pumilus* in PrCD resulted in a typical signature, fall on day 2↓ rise on day 4↑ fall on day 6↓, reflecting a net decline in numbers compared to their zero-day enumeration. The dynamics of *B. megaterium* numbers resulted into a signature (rise on day 2↑ fall on day 4↓ rise on day 6↑) opposite to other three *Bacillus* spp., showing a marginal increase in number on day 6 compared to day 1 (Fig. 1.3-C).

When earthworms (*E. fetida*) were released on PrCD beds, on 0 days, *B. megaterium* was least in numbers among the four *Bacillus* spp. With time, in presence of *E. fetida*, there has been an almost logarithmic rise in *B. megaterium* reaching highest cell density; while the population of *B. coagulans* declined logarithmically to become least in numbers among the four *Bacillus* spp. (Fig. 1.3-B). The cell density of *B. cereus,* after an initial fall in number on day 2, equalized *B. megaterium* on day 6. Till day 4, the population of *B. pumilus* decreased in orders (similar to that of *B. coagulans),* but has shown an increment on day 6 (Fig. 1.3-B). The results have shown that presence of *E. fetida* and their continuous feeding on PrCD influenced significant change in the population dynamics of four *Bacillus* spp. *E. fetida* had the remarkable positive effect on growth of *B. megaterium* providing the survival advantage. Conversely, the population of most dominant *Bacillus* species in RCD and PrCD, *B. coagulans*, faced extreme selection leading to the fast diminution in the viable count in the presence of the earthworms (*E. fetida*).

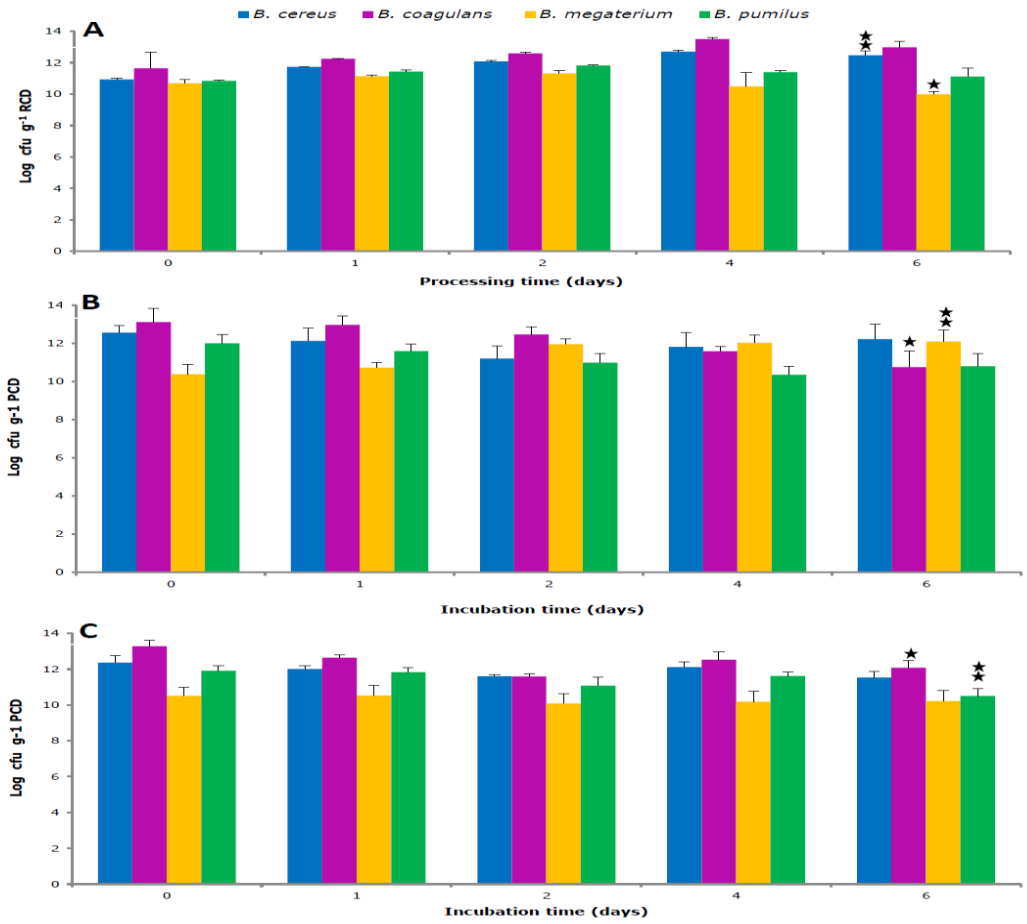


Fig.-1.3: Prevalence of the four most abundant bacilli present in cow dung that: (A) is under processing for 6 days without *E. fetida;* (B) had been processed for 6 days and was then incubated @25°C with *E. fetida* (100 animals KG^{-1}) upto 6 days; (C) had been processed for 6 days and was then incubated @25°C without *E. fetida.* [n=3, **, $p < 0.01$; and *, $p < 0.05$]. (RCD- Raw cow dung, PrCD- Processed cow dung).

1.3.3. Cultivable bacteria in gut of *E. fetida*

When earthworms (*E. fetida*) from the stock culture (maintained in vermiculture pit in the field) were thoroughly washed with sterile distilled water and released in fresh PrCD bed mixed with laboratory-grown cells of *B. thuringiensis*, gut sample collected immediately after the seeding (day 0 sample) contained no detectable number of *B. thuringiensis* while considerable number of it ($\approx 10^{10}$ cfu / g cast) was present on day 1. Population dynamics of *Bacillus* spp. in the gut sample of *E. fetida* on day 1 revealed an

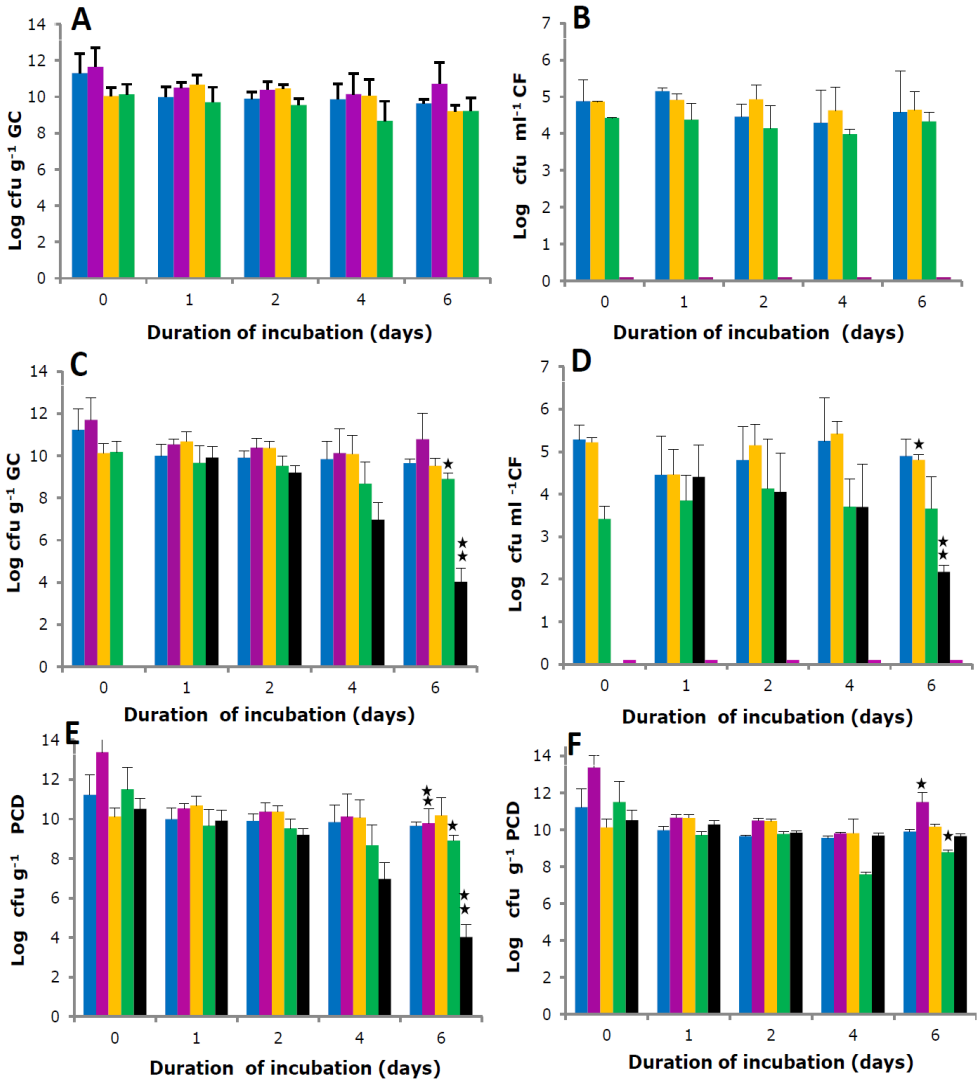


Fig.1.4: Prevalence of the dominant bacilli present in the: (A) gut of *E. fetida* inhabiting processed cow dung (PrCD); (B) coelomic fluid of *E. fetida* inhabiting PrCD; (C) gut of *E. fetida* inhabiting PrCD that had been supplemented with *Bacillus thuringiensis* (a chance pathogen of *E. fetida*); (D) coelomic fluid of *E. fetida* inhabiting PrCD that had been supplemented with *B. thuringiensis;* (E) PrCD that had been supplemented with *B. thuringiensis* and was inhabited by *E. fetida* and (F) PrCD that had been supplemented with *B. thuringiensis* but was not inhabited by *E. fetida.* Prior to supplementation with *B. thuringiensis* and/or introduction of *E. fetida*, all cow dung had been processed for 6 days. For inoculating dung with *B. thuringiensis*, a suspension of exponential phase cells (optical density [600 nm] 0.5) was used. For dung inhabited by *E. fetida*, the worm was immediately introduced (100 animals KG^{-1}) after processing. All dung samples were incubated (@25°C, in the dark) keeping in glass petriplates (diameter=15cm) for up to 6 days. The bacilli were *B. thuringiensis* [■], *B. megaterium* [■], *B. cereus* [■], *B. pumilus* [■] and *B. coagulans* [■].Values are means of three independent replicates (n=3), and bars indicate standard deviation [**, $p < 0.01$; and *, $p < 0.05$].

important fact that though *B. thuringiensis* was abundant, populations of *B. megaterium* dominated over other species of *Bacillus*. The dominance of *B. megaterium* continued on day 2 and 4 with a concurrent progressive reduction in *B. thuringiensis* populations (Fig. 2.4-C). Gut samples on day 6 have shown least representation of *B. thuringiensis* populations (reduced to 10^4 cfu g^{-1} cast) while the abundance of the other four *Bacillus* species was like this: *B. coagulans* > *B. megaterium* (≈ *B. cereus*)> *B.pumilus* (Fig. 1.4-E). Gut samples collected immediately from *E. fetida* just after the seeding (day 0 sample) in PrCD bed without the addition of laboratory grown cells of *B. thuringiensis*, contained no detectable number of *B. thuringiensis* as expected but abundances of *B. megaterium* and *B. pumilus* (≈ 10^{10} cfu / g^{-1} cast) were less by more than an order to that of *B. cereus* and *B. coagulans* (> 10^{11} < 10^{12} cfu g^{-1} cast) (Fig. 1.4 E). Decrease in the populations of *B. cereus, B. pumilus* and *B. coagulans* with the characteristic increase in *B. megaterium* was noted in the gut samples collected on day 1. The dominance of *B. megaterium* persisted on day 2 gut samples of *E. fetida*. Reduction in the population of *B. pumilus* was prominent in gut samples of day 4. Populations of *B. coagulans* exceeded other three Bacillus species in gut samples of day 6 (Fig. 1.4-E).

1.3.4. Cultivable bacteria in coelomic fluid of *E. fetida*

Coelomic fluids (CFs) aseptically withdrawn from multiple numbers of *E. fetida*, in different volumes, for different purposes, and examined many a time under the microscope or plated on rich medium (Luria-Bertani agar) have confirmed the presence of live bacteria. Colonies picked up randomly from such plates were clonally purified for studying their cell's Gram character, morphology, and few physiological and biochemical characteristics. After ascertaining that the majority of them could be putatively assigned to the genus- *Bacillus*, detailed enumeration of heterotrophic bacteria on LA and *Bacillus* spp. on selective HBA were done. Heterotrophic bacterial density in CF was in the range of $2.47 \times 10^5 \pm 1.9 \times 10^4$ cfu ml^{-1}. The density of *Bacillus* spp. in CF was in the range of $1.66 \times 10^5 \pm 1.4 \times 10^4$ cfu ml^{-1} which is approximately 66% of total bacterial load in the CF (Fig 1.5). The differential load of major *Bacillus* spp. was as follows: $6.31 \times 10^4 \pm 1.4 \times 10^3$ cfu ml^{-1} for *B. megaterium*, $4.77 \times 10^4 \pm 2.2 \times 10^3$ cfu ml^{-1} for *B. cereus*, and $1.81 \times 10^4 \pm 1.5 \times 10^3$ cfu ml^{-1} for *B. pumilus.*

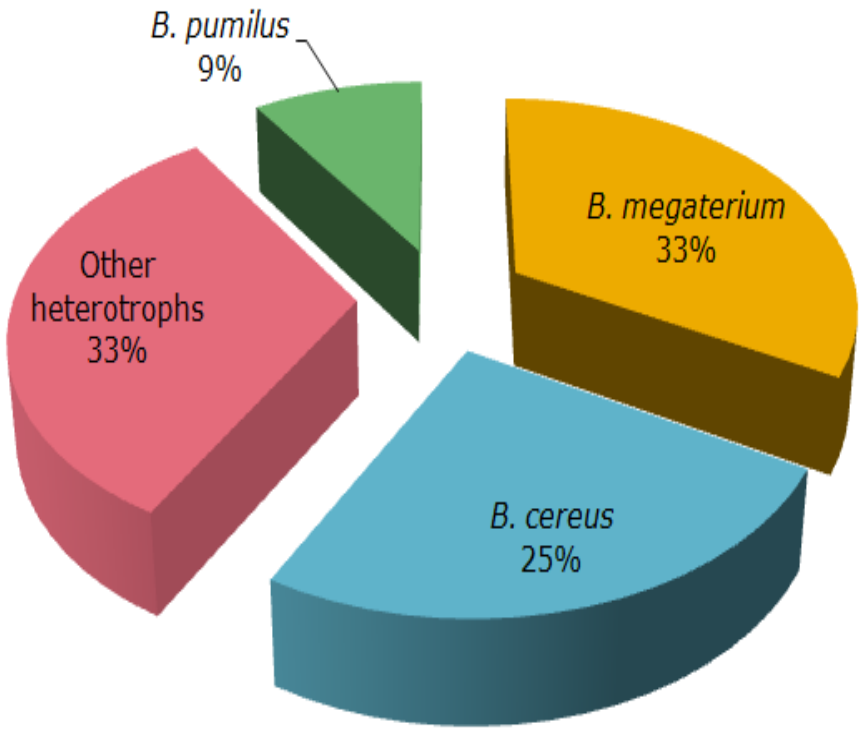


Fig. 1..5: Pie chart showing microbial load in coelomic fluid of *E. fetida* incubated at 25°C with processed cow dung (100 animals kg^{-1}).

The population densities of *B. megaterium* and *B. cereus* in the coelomic fluid of *E. fetida* were almost equal and found higher than *B. pumilus* on day 0. The cells of *B. coagulans*, although dominant in gut microbiota were completely absent in the CF of *E. fetida*. In numbers, *B. cereus* had a slight edge over *B. Megaterium* in CF collected on day 1 (Fig. 1.4-D). The most dominant population observed on day 2 and 4 was *B. megaterium*. The most variable population among the three residents was *B. cereus* (as adjudged by the standard deviation) but the mean value of *B. megaterium* had the slight edge over *B. cereus* on day 6 (Fig. 1.4-D). The population of *B. pumilus* remained always the least in the CF of *E. fetida*. The results indicate that the coelomic fluid appears selective and restrictive (rarely allowed cell densities of any of the three species to go above 10^5 cfu ml^{-1} CF) in hosting the three distinct species of *Bacillus*.

1.3.4.1. Population dynamics of three *Bacillus* species residing in the coelomic fluid of PrCD grown *E. fetida*

The population densities of *B. megaterium* and *B. cereus* in the coelomic fluid of *E. fetida* were almost equal and found higher than *B. pumilus* on day 0. The cells of *B. coagulans*, although dominant in gut microbiota were completely absent in the CF of *E. fetida*. In numbers, *B. cereus* had a slight edge over *B. megaterium* in CF collected on day 1 (Fig. 1.4D). The most dominant population observed on day 2 and 4 was *B. megaterium*. The most variable population among the three residents was *B. cereus* (as adjudged by the standard deviation) but the mean value of *B. megaterium* had the slight edge over *B. cereus* on day 6 (Fig. 1.4D). The population of *B. pumilus* remained always the least in the CF of *E. fetida*. The results indicate that the coelomic fluid appears selective and restrictive (rarely allowed cell densities of any of the three species to go above 10^5 cfu ml^{-1} CF) in hosting the three distinct species of *Bacillus*.

1.3.4.2. Dynamical changes in the population structure of the coelomic *Bacillus* species in the fate of forced introduction of *B. thuringiensis* in the coelome of PrCD grown *E. fetida*

When a high dose of *B. thuringiensis* (a known pathogen of invertebrates from *Bacillus* spp.) suspended in PBS, was injected into the coelomic cavity of PrCD grown *E. fetida*, the worms survived showing abundance of the intruding pathogen in good numbers (> 10^4 < 10^5 c. f.u ml^{-1}) in their CF, 8 h after injection on Day 0. There was a concomitant increase in the populations of *B. megaterium* and *B. cereus* (> 10^5 cfu ml^{-1}) and sinking down of *B. pumilus* population to marginally little above 10^3 cfu ml^{-1} (Fig. 1.6). On day 2, the populations of *B. thuringiensis*, *B. megaterium* and *B. cereus* almost equalized (>10^4 < 10^5 cfu ml^{-1}) and the population of *B. pumilus* got slightly elevated (10^4 cfu ml^{-1}) in the CF of *E. fetida*. Populations of *B. thuringiensis* dropped to 10^4 cfu ml^{-1} and came down to the level of *B. pumilus*, whereas cell density of *B. megaterium* reached the peak followed by *B. cereus* in CF on day 4. Further downsizing of the populations of *B. thuringiensis* in the CF took place on day 6, while the populations of *B. megaterium* and *B. cereus* were restricted to the level just below 10^5 cfu ml^{-1} (Fig. 1.6). The results have shown that coelomic fluid is not only capable of supporting the growth of *Bacillus* spp but also most likely host few bacteria selectively for its own defence against pathogens and/or to derive some other benefit.

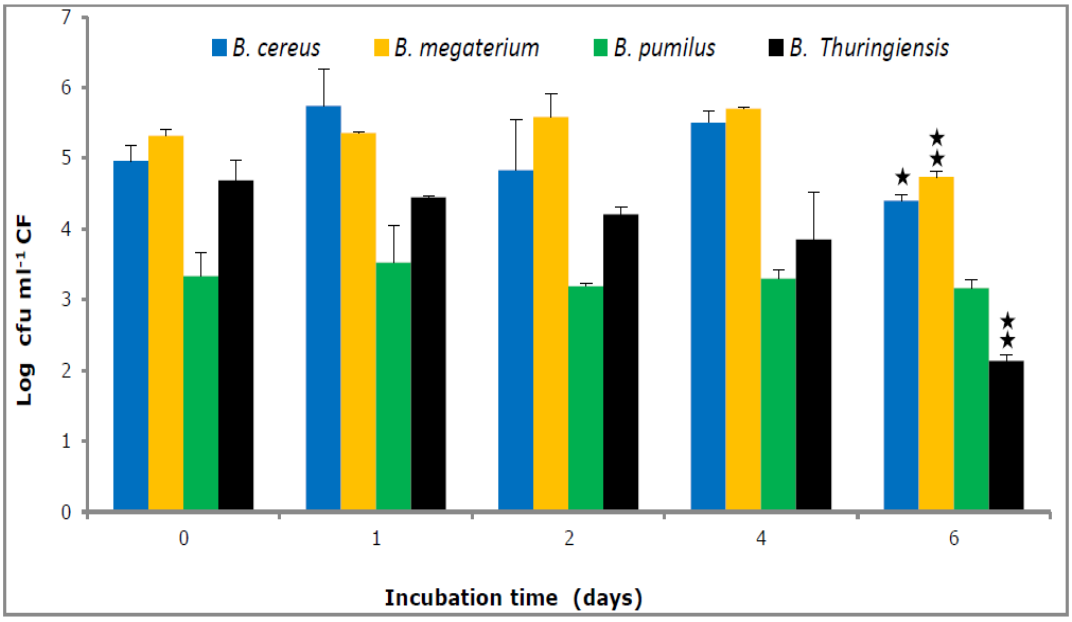


Fig. 1.6: Quantities of three dominant bacilli, *B. cereus*, *B. megaterium*, and *B. pumilus* in CF when coelom of *E. fetida* was injected with laboratory grown *B. thuringiensis* cells [n=3, **, $p < 0.01$; and *, $p < 0.05$].

1.3.5. Culture-independent assessment of eubacterial diversity in the coelomic fluid of *E. fetida*

A culture-independent molecular method based on the analyses of 16S rDNA obtained from total DNA has been used to better understand the diversity of bacteria inhabiting coelomic fluid of PrCD grown *E. fetida*. A random selection of 10 % clones, from the clone library, constituted of 140 clones from 16S rDNA, was made. All recombinant clones yielded a band of ≈ 1.5 kb in size after colony PCR amplification using pJET1.2 forward and reverse sequencing primers. Restriction digestion of the amplicons with *HaeIII* resulted in the number of pattern type. The comparison of ARDRA profiles was performed on the basis of the presence (1) or absence (0) of fragments was generated by PyElph 1.4 software[15]. The similarity between the DNA sequences was computed and used to generate a phylogenetic tree based on clustering methods applied to the distance matrix. The genetic distances are displayed on the branches (Fig. 1.7-A). Six clusters /groups or operational taxonomic units (OTUs) have resulted from this analysis; group I

(Ah4 group) comprised of lanes 12, 13, 14, 11* and 10*; group II (BCR group) consisting of lanes 4,3, and 6*; group III (BP group) comprised of lanes 1 and 7; group IV consisting of lanes 8 and 9; group V represented by lane 2; and group VI represented by lane 5. From the six OTUs, organism belonging to three groups, group I, II, and III were identified, from culture-dependent studies and phylogenetic characterization of the isolates, as *B. megaterium*, *B. cereus*, and *B. pumilus* respectively.

1.3.6. Culture-dependent isolation of *Bacillus* spp. from coelomic fluid of *E. fetida* and phylogenetic characterization of the isolates

Dilution plating of CF on HiChrome Bacillus agar (HBA) plates readily identified three types of colonies. The yellow mucoid colonies were characteristic of *B. megaterium* because of their property to ferment mannitol contained in the medium indicated by the change of the color of phenol red. The colonies of *B. cereus* are flat with distinct blue centers because of its possession of β-glucosidase that cleaves the chromogenic mixture present in the medium. The colonies of *B. pumilus* are raised and pink in colour. More than two colonies, from each of the three colony types, were repeatedly dilution streaked on HBA to obtain pure cultures. The strains, Ah4, BCR, and BP, representative of putative *B. megaterium*, *B. cereus*, and *B. pumilus* respectively, were phylogenetically characterized. During 16S rRNA gene sequence analysis, the pairwise comparison indicated that strain Ah4 shared similarity with *B. megaterium* NBRC 15308 (99.5%); strain BCR shared similarity with *B. cereus* ATCC 14579 (99.8%); strain BP shared similarity with *B. pumilus* NBRC 12092 (99.7%). Other species have shown a lower level of similarity to strain Ah4, BCR, and BP. Phylogenetic trees were constructed by using 16S rRNA gene sequences with neighbor-joining (Fig. 1.7-B), maximum likelihood and maximum parsimony methods (not shown) in MEGA (ver 7.0). Regardless of different evolutional comparisons, similar topology was obtained in all phylogenetic trees, which indicates that the strains Ah4, BCR, and BP belong to the *B. megaterium*, *B. cereus*, and *B. pumilus* species cluster respectively.

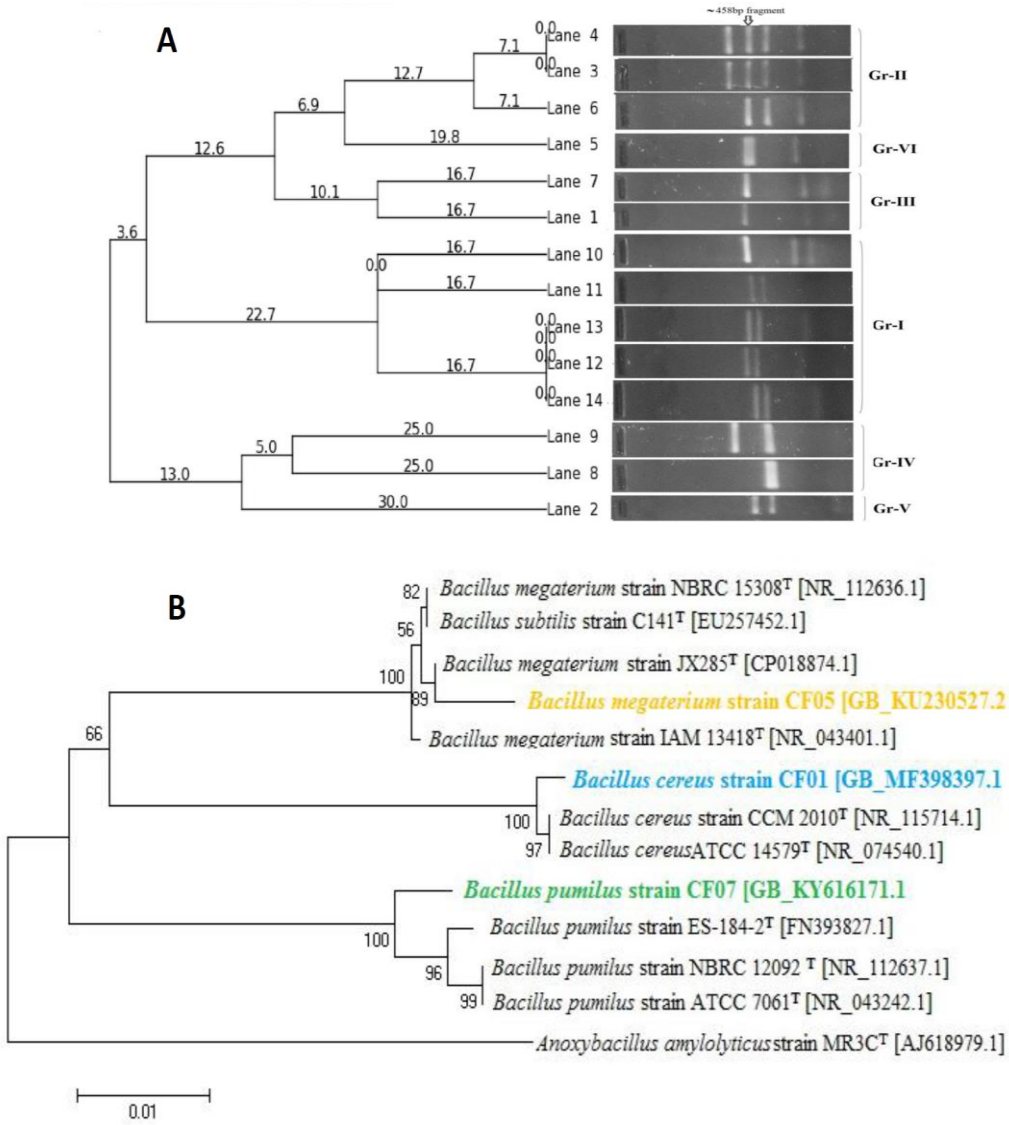


Fig. 1.7: Bacilli present in coelomic fluid of *E. fetida* assessed by: (A) Culture independent and (B) Culture dependent analysis. For culture independent assessment a phyllogram based on UPGMA matrices was computed (using PyElph software) from gel image of HaeIII digest of 16S rRNA gene amplicons derived from randomly selected 14 clones of the clone library created from metagenome extracted from coelomic fluid of *E. fetida.* Clusturing of homologous groups was based on genetic distance (expressed by numerical values on the dendrogram) in respect to sharing of restriction sites; Six groups have been identified- Gr-I [Lane numbers- 12,13,14, 11 and 10], Gr-II [Lane numbers- 4,3 and 6], Gr-III [Lane numbers- 1 and 7], Gr-IV [Lane numbers- 8 and 9], Gr-V [Lane number- 2] & Gr-VI [Lane number- 5]. Group-I,II and III have similar band patterns corresponding to HaeIII digest of 16S rRNA genes from *B. megaterium*, *B. cereus* and *B. pumilus* respectively (data not shown). For culture dependent assessment phylogeny of the three bacilli (indicated by coloured fonts) isolated from coelomic fluid of *E. fetida* has been computed based on Neighbour Joining matrices. Bootstrap values (expressed as percentages of 1000 replications) >50% are shown at branch points. Bar, 1 substitution per 100 nucleotide position.

1.3.7. Individual growth curves of *B. megaterium* Ah4, *B. cereus* BCR, and *B. pumilus* BP, and *B. coagulans* BCO (four predominant Bacillus species in RCD or PrCD) in CMB

It was evident that there is a dynamic presence of four *Bacillus* species, *B. megaterium*, *B. cereus*, *B. pumilus*, and *B. coagulans* in RCD, PrCD, and gut samples of *E. fetida*. On the contrary, coelomic fluid of the worms selectively hosted three of the four *Bacillus* spp., rejecting *B. coagulans*. Hence, carrying out *in-vitro* growth kinetics of each of the four *Bacillus* species in Coelomic fluid Mimicking Broth (CMB) became necessary to understand the cause of rejection. CMB was formulated (detailed composition given in the materials and methods) on the basis of determination of mean content (g ml^{-1}) of sugar, protein, free amino acids, triglycerides, urea, and uric acid in the coelomic fluid of *E. fetida* fed with PrCD, along with data from published literature on small molecule composition of CF (Bundya *et al.*, 2001).

The composition of the medium was largely determined by the objective of the experiment (to simulate mixed culture condition in the coelomic fluid of *E. fetida*), and the properties of the strains. Also, it was presumed that the metabolic activity of the cells of the individual species population shall modify the composition of CMB. It was observed that CMB supported the growth of all the four *Bacillus* spp. (Fig.1.8-Ai-iv). Taking cell densities, at two time points, 0.5 h (initial, t_1) and 3.0 h (final, t_2) as logA and logB respectively, and number of generations (n) worked out by the equation: n = (logB – logA)/log2, the generation time was calculated as: $(t_2 - t_1)/n$. The generation time of *B. cereus*, *B. megaterium*, *B. pumilus*, and *B. coagulans*, corresponding to their growth curves (Fig. 6 a, b and c), were 33.4, 24.6, 127.0, and 86.6 min respectively. Although the results have shown that in the CMB medium, *B. megaterium* and *B. pumilus* were the fastest and slowest growing *Bacillus* species respectively, yet the growth performance of *B. coagulans* was found better than *B. pumilus*. Hence, it appears that some other factor(s) like proneness to be phagocytosed by the immunocytes or any metabolic deficiency of *B. coagulans* in producing the specific product (upon which host may be benefitted) have had led to evolutionary selection/rejection in the coelom of *E. fetida*.

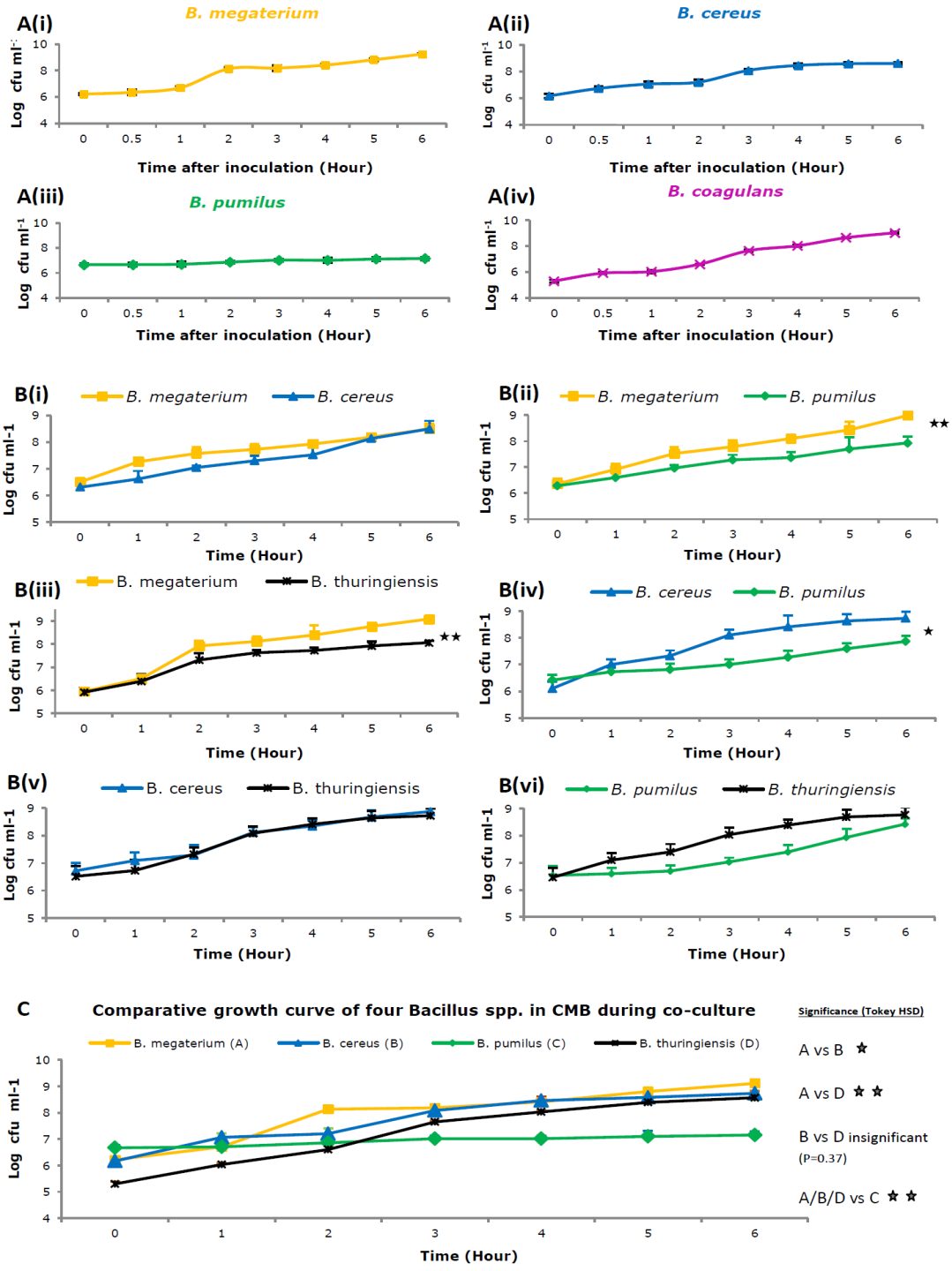


Fig. 1.8: Growth of pure cultures (of CF isolates) in Coelome mimicking broth(CMB) (6Ai to iv):Growth of cocultures in CMB (6Bi to vi): Growth of mixed culture in CMB (6C). [n=3, **, $p < 0.01$; and *, $p < 0.05$].

1.3.8. Dual-species competition among four *Bacillus* spp.; three most prevalent coelomic Bacillus species (*B. megaterium, B. cereus, B. pumilus*) and one chance intruder *B.thuringiensis*

Dual-species batch cultures, for all possible combinations (*B. cereus* and *B. megaterium*; *B. pumilus* and *B. megaterium*; *B. thuringiensis* and *B. megaterium*; *B. pumilus* and *B. cereus*; *B. thuringiensis* and *B. cereus*; & *B. pumilus* and *B. thuringiensis*) were studied to infer interaction among each other of the pair (Fig. 1.8). Competitive index (CI) value determined for the dual-species batch culture for *B. cereus* and *B. megaterium* or *B. thuringiensis* and *B. cereus*; was 1.0, which means that *B. megaterium* or *B. thuringiensis* is able to grow as efficiently as *B. cereus* in CMB (Fig. 1.8-A & E). For the dual-species batch-culture for *B. pumilus* and *B. megaterium* or *B. pumilus* and *B. cereus* or *B. pumilus* and *B. thuringiensis,* the C I value obtained was 0.87, 0.86, and 0.95 respectively (CI < 1.0 in all three cases), reflecting the fact that growth of *B. pumilus* is attenuated in all the three cases (Fig. 1.8-B, D, & F). CI value, obtained from dual-species batch culture for *B. thuringiensis* and *B. cereus,* was 0.93, which means that the growth of *B. thuringiensis* is attenuated (Fig. 1.8-C). The results obtained from dual-species competition have also revealed that the growth of *B. pumilus* was much better in dual-species batch culture, particularly combo with *B. thuringiensis,* than its performance as the solo or in mixed culture.

1.3.9. Growth of *B. megaterium* (Ah4), *B. cereus* (BCR), *B. pumilus* (BP), and *B. coagulans* (BCO) in mixed culture

The growth of the mixed culture of four different species of the genus *Bacillus* (Fig. 1.8-C) has supported that the complexity of CMB medium plays a role in determining inter-species interaction. At different time intervals, individual species in the mixed culture displayed differential growth rates. In the first one hour interval (0 -1h), highest growth rate was recorded for *B. cereus* (K = 2.96 h^{-1}) followed by *B. coagulans* (K = 2.40 h^{-1}), while in the following next hour (1 – 2h) *B. megaterium* displayed the highest growth rate (K = 4.75 h^{-1})compared to any other growth rate recorded for any of the remaining three species in successive time periods (Fig. 1.8-C). During 2-3h time period, *B. coagulans* (K = 3.44 h^{-1}) exceeded other three species in growth rate while in the following time period (3-4 h), *B. cereus* equalled the growth rate of *B. coagulans* (K = 1.25 h^{-1}). During 4-5 h

time period, *B. coagulans* maintained its dominance in growth rate (K = 2.06 h^{-1}). In the last lap, 5-6 h, the high growth rate was shown by *B. megaterium (*K = 1.42 h^{-1}) and *B. cereus (*K = 1.26 h^{-1}). Throughout the period of observation, the growth of *B. pumilus* remained least and inhibited, almost reached a plateau after 3 h of incubation. The results have clearly indicated the growth advantage of *B. coagulans* in mixed culture compared to its slow growth rate in solo culture. On the contrary, the growth of *B. pumilus* was at a high disadvantage, even found slower than its solo performance. Mixed culture of four different *Bacillus* species in CMB medium has exhibited synergism because it attained significantly higher cell density and produced more bacterial biomass (Fig. 1.8-C). Such synergistic growth may result when multiple species produce complementary enzymes and participate in the metabolite cross feeding enabling bacteria to consume substrates cooperatively. The problems of feedback inhibition and metabolite repression present in mono-species culture may be lessened in mixed culture.

1.3.10. Chemotactic responses of *B. megaterium*, *B. cereus*, *B. pumilus*, *B. coagulans*, *B.thuringiensis,* and *Pseudomonas aeruginosa* to coelomic-fluid of *E. fetida*

In order to investigate the phenotype involved in the establishment of cow-dung bacteria-*Eisenia* interactions and pathogenicity caused due to primary or secondary infection of soil-borne pathogens, chemotaxis has intuitively emerged in our study design. With the background information on small molecule composition of coelomic fluid of *E. fetida* being dominated by organic acids like succinate (being most prominent), malonate, acetate, α-ketoglutarate, and formate; amino acids like tyrosine and alanine; and other organic compounds like myo-inositol, glycerol, methanol, and nicotinamide mono nucleotide (Bundya *et al.,* 2001), taken together with earlier reports on taxis towards naturally occurring amino acids, sugars, or extracts of leaves of Birch, Poplar, Apple and Hawthorn by few species of *Bacillus* (Lebenko *et al.,* 2005)and identification of a chemoreceptor for TCA intermediates in *P. aeruginosa* (Lacal *et al.,* 2010) capillary assays were carried out to determine the repertoire of responses of the test organisms to coelomic fluid of *E. fetida*. The number of bacteria attracted into a capillary tube containing CF as attractant was measured. Kinetics of accumulation of the three different bacteria (*B. megaterium* strain Ah4, *B. cereus* strain BCR and *B. pumilus* strain BP isolated from CF of *E. fetida*) in capillaries was presented in Fig. 1.9. Highest

accumulation of cells in capillaries in 5 min was recorded for *B. megaterium* [significantly higher than *B. cereus* (**, $p < 0.01$) and *B. pumilus* (**, $p < 0.01$)]. Both *B. megaterium and B. cereus* have shown highest and 2nd highest accumulation up to fifteen minutes, although the difference was less significant (*, $p < 0.05$) at fifteenth minute; numbers decreased with time in *B. megaterium* while no characteristic change in

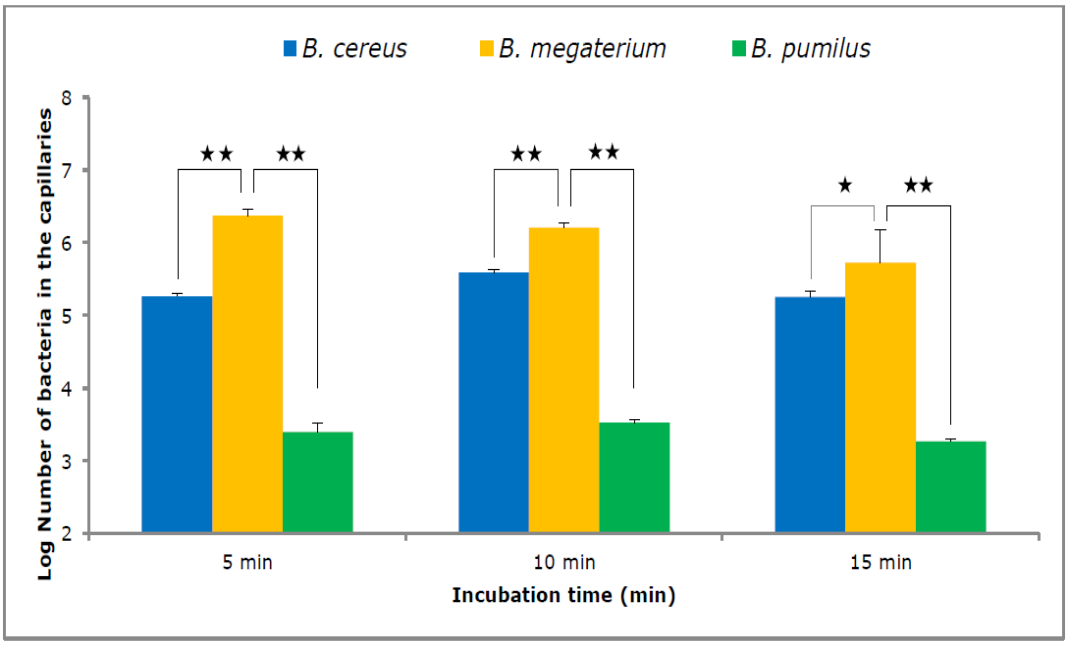


Fig. 1.9: Rate of accumulation of bacteria in capillaries containing coelomic fluid of *E. fetida* [n=3, **, $p < 0.01$; and *, $p < 0.05$].

numbers with time was noted in case of *B. cereus* The number of cells accumulated over time was least in case of *B. pumilus* (Fig. 1.9). To minimize error, testing with the blank was repeated at least 6 times. As a group, *Bacillus* exhibited differential levels of chemotaxis (chemotaxis of *B. megaterium* > chemotaxis of *B. cereus* > chemotaxis of *B. pumilus*) towards coelomic fluid of *E. fetida* where *B. megaterium* seems to be the most efficient in early Chemotaxis (Fig.1.9).

1.3.11. Riboflavin synthesis of *B. megaterium* Ah4, *B. cereus* BCR, *B. pumilus* BP, and *B. coagulans* BCO in CMB medium

Earthworms are vulnerable to invasion by bacterial pathogens because of their highly permeable integument. Yet, a high degree of bactericidal potency is also evident due to

the activities of the immune-competent cells which stores riboflavin to their advantage (Mazur *et al.*, 2011). Despite the inability of the host to synthesize riboflavin, its storage predominates in free coelomocytes of eleocyte-rich earthworm species like *E. fetida* (Santocki *et al.*, 2016). Thus, it appeared that riboflavin might be the key currency in host-bacteria symbiosis in the coelomic fluid. Agreements of symbiotic interactions between eukaryotic host and bacteria in several occasions have proven the involvement of bacterial riboflavin production and secretion (LeBlanc *et al.*, 2013). Hence, the ability of the *Bacillus* strains to secrete riboflavin in the CMB medium was assayed. *B. megaterium* exceeded the other three species (Fig. 1.10) In terms of secretion of riboflavin in the medium, with the characteristic cycle of release (highest secretion peak, > 3.0 µM, at 0.5 h) and uptake (signified by the decrease in the concentration). The other two strains, *B. cereus* and *B. pumilus*, which colonizes CF, have also shown their distinctive riboflavin secretion and uptake signatures.

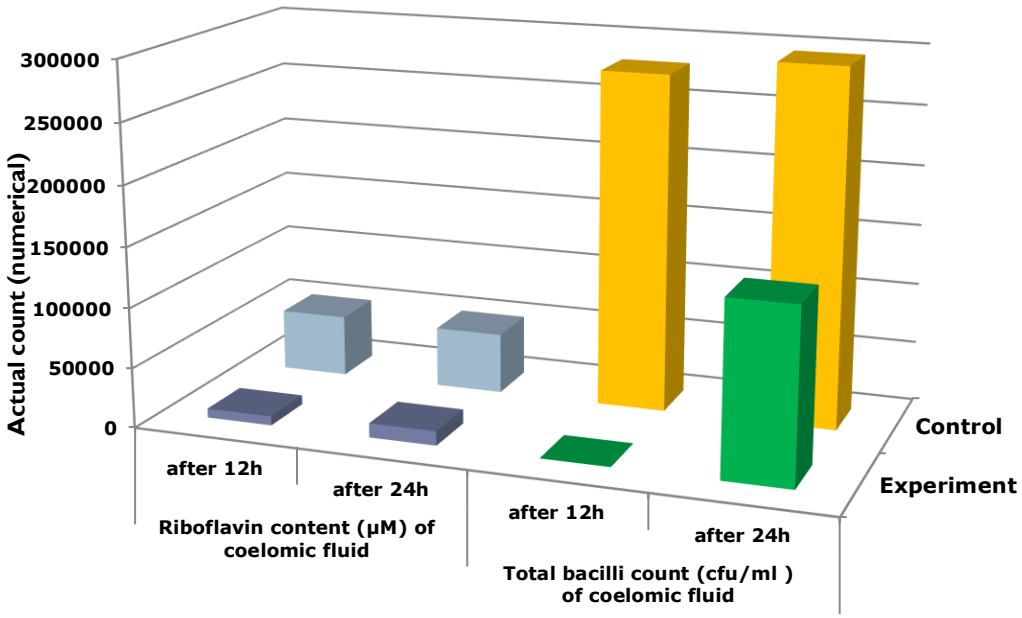


Fig. 1.10: Total bacilli count and concentration of riboflavin in the coelomic fluid of *E. fetida* after 12h and 24h of levofloxacin injection in comparison to the control animals without antibiotic treatment.

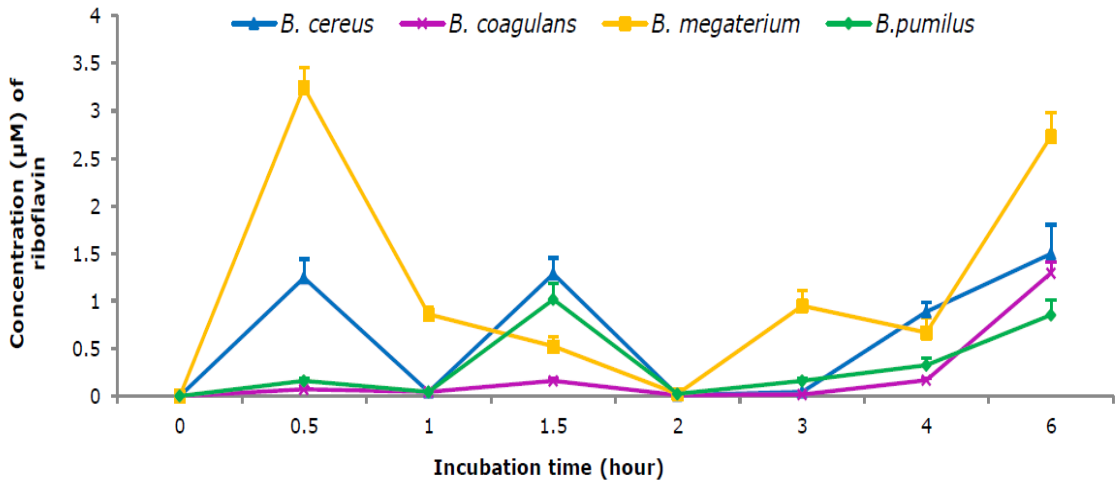


Fig. 1.11: Riboflavin secretion by pure cultures of three CF isolates (*B. cereus* CF01, *B. megaterium* CF05, and *B. pumilus* CF07) and one PrCD isolate (absent in CF), *B. coagulans* PrCD01, in CMB medium (n=3).

The secretion peaks (> 1.0 µM) at 0.5 and 1.5 h were noted for *B. cereus*, while only a conspicuous peak (< 1.0 µM) at 1.5 h was observed for *B. pumilus*. The species (which was not detected in CF but conspicuously present in PrCD and gut) *B. coagulans* secreted least or insignificant amount of riboflavin till 4 h of incubation in CMB (Fig. 1.11).

1.3.12. Isolation and taxonomic identification of bacteria from gut of *E. fetida*

Altogether 20 unique colonies (8 from cast, 3 from posterior gut, 5 from anterior gut and 4 from the whole gut in anaerobic condition) excluding 5 from coelomic fluid were isolated. 40% of the bacteria were Firmicutes; Proteobacteria and Actinobacteria were found in equal amount (30% each) (Fig. 1.12). A detailed phylogenetic tree (Fig. 1.13) shows the phylogeny of all the 20 isolates.

List of cultivable bacterial strains isolated from gut and coelomic fluid of *E. fetida* and their 16S rRNA gene accession numbers (NCBI) are provided in Table 1.1

Table 1.1: List of cultivable bacterial strains associated with *E. Fetida* and their 16S rRNA gene accession numbers.

Sl No.	Strain	NCBI Accession Numbers	16SrRNA gene sequence	Taxonomy
			From cast	
1	*Bacillus efetidiens* strain ET01 (=MCC3263, =LMG 29877, =JCM 32462, =KCTC 33945)	KU1751 76.2	>AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGATCGATGGGAGCTTGCTC CCTGAGATCAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTA ATACCGGATAATTTAGTTCCTCGCATGAGGAACTGTTGAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCAT TAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGA GACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGT GAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCCGTTCGAATAGGGCGGCACCTCGACGG TACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTG GGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGG GAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGATATTTGGAGGAACACCAGTGGCG AAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCAC GCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGA GTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAAC GCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTCCCCTTCGGGGGACAGAGTGACAGGT GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAG CATTCAGTTGGGCACTCTAAGATGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTA TGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGGGCAGCGAGACCGCGAGGTTTAGCCAATCCCATAAAACCGT TCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAAT ACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTGGAGC CAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAACCGTA	Phylum: Firmicutes Class:Bacilli Order: Bacillales Family: Bacillaceae (Sp. Nov.)
2	*Bacillus sp. strain ET02*	KU2305 22.2	>AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGATCGATGGGAGCTTGCTCCCT GAGATCAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACC GGATAATTTAGTTCCTCGCATGAGGAACTGTTGAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAG TTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCC CAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGAAGAAG GTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCCGTTCGAATAGGGCGGCACCTTGACGGTACCTGACCAGAA AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCG CAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTTGGGTCTAGAGTACGGCAC AGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGATATTTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGT AACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAA GTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTC AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC ATCCTCTGACAACCCTAGAGATAGGGCTTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCG TGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGATGACTGCC GGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACG GTACAAAGGGCAGCGAGACCGCGAGGTTTAGCCAATCCCATAAAACCGTTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGC ATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACC ACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTGGAGCCAGCCGCCTAAGGTGGGACAGTTGATTGGGGTGAAGT CGTAACAAGGTAACCGTA	Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae
3	*Chryseomicrobium excrementi* strain ET03^{T} (= KCTC 33943^{T} = LMG 30119^{T} = JCM 32415^{T})	KU2305 23.2	>AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGATGACGGAGGAGCTTGCT CCTCTAGATTCAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCCTGTAGATTGGGATAACTCCGGGAAACCGGGGC TAATACCGAATAATCCTTCGGACCTCATGGTCCGATGTTGAAAGACGGCTCCGGCTGTCACTACAGGATGGGCCCGCGGCGC ATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACT GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGC GTGAGTGAAGAAGGTTCTCGGATCGTAAAACTCTGTTGTGAGGGAAGAACAAGTACCGGAGTAACTGCCGGTACCTTGACG GTACCTCATTAGAAAACCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATCATT GGGCGTAAAGCGCGCGCAGGCGGTCCCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGG GGGACTTGAGTGCAGAAGAGGAAAGCGGAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGC GAAGGCGGCTTTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA CGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGG AGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA ACGCGAAGAACCTTACCAGGTCTTGACATCCCGCCGACCGCCCAGGAGACTGGGCCTTCCCTTCGGGGACGGCGGTGACAG GTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCC AGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCT TATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCGAACCCGCGAGGGGGAGCCAATCCCATAAAACC ATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGTGGATCAGCATGCCACGGTGA ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTATGG AGCCAGCCGCCGAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAACCGTA	Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Planococcaceae (Sp. Nov.)
4	*Bacillus eisenifilia* strain ET04 (=MCC 3370, =JCM 32463, =KCTC 33944, =LMG 30120)	KX8392 65.1	>GAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGATTGATGGGAGCCTGCTCCC TGATATCAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAAT ACCGGATAACTCATTTCCTCGCATGAGGAAATGTTGAAAGGTGGCTTTTAGCTACCACTTACAGATGGACCCGCGGCGCATT AGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACAGAGCAACGCCGCGTG AGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCCGTTCGAATAGGGCGGCACCTTGACGGT ACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGG GGGTAAAGCGCGCGCAGGTGGTTCCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGG AACTTGAGTGCAGAAGAGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGATATTTGGAGGAACACCAGTGGCGA AGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG CCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAG TACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG CGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTCCCCTTCGGGGGACAGAGTGACAGGTG GTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGC ATTCAGTTGGGCACTCTAAGATGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTAT GACCTGGGCTACACACGTGCTACAATGGACGGCACAAAGGGCAGCGAGACCGCGAGGTTTAGCCAATCCCATAAAACCGTT CTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATA CGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTGGAGCC AGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAACCGTAATCTTTCTAGAAGATTTCCCTCACCC	Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae (Sp. Nov.)
5	*Pseeudomonas aeruginosa strain ET05 (=MCC 3462)*	MF3983 95.1	>AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCTG GATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGC ATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGT AAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTA CGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATT GTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAA CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAG CAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAA TTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGA GGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGGTCCT TGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTT TCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA AGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCAG AGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTG CCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAAT CGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTT GCTCCAGAAGTAGCTAGTCTAACCGCAAGGGGGACGGTTACCACGGAGTGATTCACGACTGGGGTGAAGTCGTAACAAGGTAA CCGTA	Phylum: Proteobacteria Class: Gammaproteobacteria Order: Pseudomonadales Family: Pseudomonadaceae

6	*Bacillus sp. strain ET08*	KU2305 24	>AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGAAGGGAGCTTGCTCCC GGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGAGCTAATAC CGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTA GTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAA GGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCTTGCACCTTGACGGTACCTAACCAGAAA GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGC AGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGA GGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTA ACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGT GTTAGGGGGGGTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTC AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC ATCCTCTGACAACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTG AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGG TGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATAGA ACAAAGGGCTGCGAGACCGCAAGGTTTAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTG AAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACG AGAGTTTGCAACACCCGAGGTCGGTGAGGTAACCTTTATGGAGCCAGCCGCCGAAGGTGGGGCAGATGATTGGGGTGAAGTCG TAACAAGGTAACCGTA	Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae
7	*Bacillus sp. strain ET09*	KU2305 25	>AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGAAGGGAGCTTGCTCCC GGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGAGCTAATAC CGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTA GTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAA GGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCTTGCACCTTGACGGTACCTAACCAGAAA GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGC AGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGA GGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTA ACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGT GTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAA AGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT CCTCTGACAACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAG ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTG ACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAAC AAAGGGCTGCGAGATCGCAAGGTTAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAG CTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGA GTTTGCAACACCCGAAGTCGGTGAGGTAACCTTTATGGAGCCAGCCGCCGAAGGTGGGGCAGATGATTGGGGTGAAGTCGTAA CAAGGTAACCGTA	Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae
8	*Celullosaemicrobium bengalensis* strain ET10 (=MCC 3389, =LMG 30121, =KCTC 49085)	KU2305 26.2	>AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGATGCCCAGCTTGCTGG GTGGATTAGTGGCGAACGGGTGAGTAACACGTGAGTAACCTGCCCTTGACTTCGGGATAACTCCGGGAAACCGGGGCTAAT ACCGGATATGAGCCGCCCTCGCATGGGGGTGGTTGGAAAGTTTTTCGGTCAGGGATGGGCTCGCGGCCTATCAGCTTGTTGG TGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCC AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAGTGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGAA GGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCAGCTAACTACGT GCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCG TCTGGTGTGAAAACTCGAGGCTCAACCTCGAGCTTGCATCGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGACTGGAATT CCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCCGCAACTGACGCTGA GGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGCTC ATTCCACGAGTTCCGTGCCGCAGCAAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAA TTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGCA CGGGAAGCCACCAGAGATGGTGGTCTCTTTGGACACTCGTGCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGAT GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTCCATGTTGCCAGCGGGTTATGCCGGGGACTCATGGGAGACTGCCG GGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCG GTACAAAGGGCTGCGATACCGTAAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACC CCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC AAGTCACGAAAGTCGGTAACACCCGAAGCCCATGGCCCAACCGTTCGCGGGGGGAGTGGTCGAAGGTGGGACTGGCGATT GGGACTAAGTCGTAACAAGGTAACCGTA	Phylum: Actinobacteria Class: Actinobacteria Subclass: Actinobacteridae Order: Actinomycetales Suborder: Micrococcineae Family: Promicromonosporaceae (Sp. Nov.)
			from anterior gut	
9	*Pseudoxanthomonas* sp. strain EAG1	KX8392 66.1	>AGAGTTTGATCCTGGCTCAGAGTGAACGCTGGTGGTAGGCCTAACACATGCAAGTCGAACGGCAGCACAGGAGAGCTTGCTC TCTGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACTCTGTCGTGGGGGATAACGTAGGGAAACTTACGCTAA TACCGCATACGACCTACGGGTGAAAGTGGGGGACCGCAAGGCCTCACGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGG CGGGGTAAAGGCCCACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAG ACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATTCCAGCCATACCGCGTGAGTGAAGAAGGCC GCTCGGGTTGTAAAGCTCTTTTGTTGGGAAAGAAATCCTGCCGGTTAATACCTGGCAGGGATGACGGTACCCAAAGAATAAGC ACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAG GTGGTTGTTTAAGTCTGTCGTGAAAGCCCTGGGCTCAACCTGGGAATTGCGATGGATACTGGGCAACTAGAGTGTGGTAGAGG GTGGCGGAATTCCCGGTGTAGCAGTGAAAATGCGTAGAGGATCGGGAGGAACATCCGTGGCGAAGGCGGCCACCTGGGCCAA CACTGACACTGAAGGCACGAAAGCGTGGGGGAGCAGAACAGGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAA CTGGAATTGTTGGGTTCAACTTGGAACCCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTG AAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGT CTTGACATCCACGGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAG ACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTACTACAA TGGGAAGGACAGAGGGCTGCAATCCCGCGAGGGGAGCCAATCCCAGAAACCTTCTCTCAGTCCGGATCGGAGTCTGCAACTC GACTCCGTGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG TCACACCATGGGAGTTTGTTGCACCAGAAGCAGGTAGCTTAACCTTCGGGAGGGCGCTTGCCACGGTGTGGCCGATGACTGGG GTGAAGTCGTAACAAGGTAACCGTA	Phylum: Proteobacteria Class:Gamma-Proteobacteria Order: Xanthomonadales Family: Xanthomonadaceae
10	*Streptomyces lapidiformes* strain EAG2^{T} (=LMG 30118^{T} = KCTC 49082^{T} = JCM 32459^{T})	KY5939 24	>AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCCTTCGGGGTGG ATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCATTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCG GATATGACTGTCCACCGCATGGTGGATGGTGTAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTG AGGTAGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAG ACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGG CCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC CAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTC GGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCC TGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAG GAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAAC ATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAA TTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACA CCGGAAACGTCTGGAGACAGGCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATG TTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACC GCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAAT GGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACT CGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGG CGATTGGGACGAAGTCGTAACAAGGTAACCGTA	Phylum: Actinobacteria Class: Actinobacteria Order: Actinomycetales Family: Streptomycetaceae (Sp. Nov.)
11	*Pradoshia cofamensis* strain EAG3^{T} (=LMG 30312^{T} =JCM 32460^{T})	MF3983 91.1	>AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATCAGATGGGAGCTTGCT CCCGGATGATTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCCGTAAGATCGGGATAACTCCGGGAAACCGGTGC TAATACCGAATAGTTCTGACACATCATGGTGTCAGAGGGAAAGTTGGCTTCGGCTAACACTTACGGATGGGCCCGCGGCG CATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGAC TGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCG CGTGAGCGAAGAAGGCCTTCGGGTCGTAAAGCTCTGTTGTCAGGGAAGAACAAGTGCCACTCGAATAGGCTGGCACCTTGA CGGTACCTGACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTA TTGGGCGTAAAGCGCGCGCAGGCGGTCCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACT GGAGGACTTGAGTGCAGAAGAAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTG GCGAAGGCGACTCTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC CACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGG GGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAG	Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae (Gen. Nov.,

			CAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCCTTCCCCTTCGGGGGACAGAGTGAC AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTG CCAGCATTCAGTTGGGCACTCTAGGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCC CTTATGACCTGGGCTACACACGTGCTACAATGGGTGGTACAAAGGGCAGCGAGACCGCGAGGTTAAGCAAATCCCATAAAA CCACTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGT GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCCTTAC GGGAGCCAGCCGCCGAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAACCGTA	Sp. Nov.)
12	*Mumia enteroni* strain EAG4 (=LMG 30314, =KCTC 49083)	MF3 983 93.1	>AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGGCCCTTCGGGGTAC ACGAGCGGCGAACGGGTGAGTAACACGTGAGTAATCTGCCCTTCACTCTGGGATAACCGCTGGAAACGGTGGCTAATACCG GATATGACCTTTGCCCGCATGGGTGTTGGTGGAAAGTTTTTCGGTGGAGGATGAGCTCGCGGCCTATCAGCTTGTTGGTGGG GTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGAC TCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCAACGCCGCGTGAGGGATGACGGCC TTCGGGTTGTAAACCTCTTTCAGTAGGGACGAAGCACAAGTGACGGTACCTACAGAAGAAGGACCGGCCAACTACGTGCCA GCAGCCGCGGTAATACGTAGGGTCCGAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGTAGGCGGTTTGTCGCGTCGG GAGTGAAAACTTGGGGCTTAACCCCATTCGTGCTTCCGATACGGGCGGACTAGAGGCAGGCAGGGGAGAACGGAATTCCTG GTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGTTCTCTGGGCCTGTTCTGACGCTGAGGAG CGAAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTGTGGGATCCATTC CACGGGTTCCGTGCCGCAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGA CGGGGGCCCGCACAAGCGGCGGAGCATGCTGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATACACCAG AAGCCTGCAGAGATGTGGGTCTCTTTGGACACTGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG GGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTATGTTGCCAGCACGTAATGGTGGGGACTCATAGGAGACTGCCGGGGT CAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCGAGCATGCTACAATGGCCGGTA CAAAGGGCTGCGAAGCCGTAAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATTGGGATCTGCAACTCGACCCCA TGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAC GTCATGAAAGTCGGCAACACCCGAAGCCGGTGGCCTAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGGCTGGCGATTGG GACGAAGTCGTAACAAGGTAACCGTA	Phylum: Actinobacteria Class: Actinobacteria Subclass: Actinobacteridae Order: Actinomycetales Suborder: Propionibacterineae Family: Nocardioidaceae (Sp. Nov.)
13	Streptomyces sp. strain EAG5	MF3 983 914	>AGAGTTTGATCCTGGCTCAGGATCGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCGCTTTCGGGCGGG GATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGG ATATGACTGTCCATCGCATGGTGGATGGTGTAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGT AGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT ACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGG TTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGC CCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGA AATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGG GAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATCCCACGTTGTCCGTGC CGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAA GCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGTCTGGAGACAGG CGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC GCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGT GGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGC GAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGGTTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAA TCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGA AGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAACCGTA	Phylum: Actinobacteria Class: Actinobacteria Order: Actinomycetales Family: Streptomycetaceae
From posterior gut				
14	M*ycobacterium omicsense* strain EPG1^T (=LMG 30313^T =JCM 32461^T =KCTC 49084^T)	MF3 983 92.1	>AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGAAAGGCCCTTCGGGGTACTCG AGTGGCGAACGGGTGAGTAACACGTGGGTGATCTGCCCTGCACTTTGGGATAAGCCTGGGAAACTGGGTCTAATACCGAATAG GATCACTGGCTTCATGGTCGGTGGTGGAAAGCTTTTGCGGTGTGGGATGGGCCCGCGGCCTATCAGCTTGTTGGTGGGGTAATG GCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGATACGGCCCAGACTCCTACGGG AGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAA ACCTCTTTCGCCAGGGACGAAGCGCAAGTGACGGTACCTGGAGAAGAAGGACCGGCCAACTACGTGCCAGCAGCCGCGGTAAT ACGTAGGGTCCGAGCGTTGTCCGGAATTACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTCGCGTTGTTCGTGAAAACTCACAGC TTAACTGTGGGCGTGCGGGCGATACGGGCAGACTAGAGTACTGCAGGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGC AGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCAGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGTACTAGGTGTGGGTTTCCTTCCTTGGGATCCGTGCCGTAGCTAAC GCATTAAGTACCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCA TGTGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGCACAGGACGCCGGCAGAGATGTCGGTTCCCTTGTG GCCTGTGTGCAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCT CATGTTGCCAGCACGTAATGGTGGGGACTCGTGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCAT CATGCCCCTTATGTCCAGGGCTTCACACATGCTACAATGGCCGGTACAAAGGGCTGCGATGCCGTGAGGTGGAGCGAATCCTTT CAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTG CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCTAACCCTT GTGGAGGGAGCCGTCGAAGGTGGGATCGGCGATTGGGACGAAGTCGTAACAAGGTAACCGTA	Phylum: Actinobacteria Class: Actinobacteria Subclass: Actinobacteridae Order: Actinomycetales Suborder: Corynebacterineae Family: Mycobacteriaceae (Sp. Nov.)
15	*Bacillus anthracis* strain EPG2	KX8 392 67.1	>AGCAGATAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGAGCTTG CTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTA ATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCCTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGC TAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGAACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGC TTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCA CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGT TTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGG AATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAG GCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCC GCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGC CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGA TAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC GAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATG ACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGTACAAGGAGCTGCAAGACCGCGAGGTGGAG CTAATCTCATAAAACCGTTCTCGGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCA TGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACC TTTTTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAACCGTAACTTCTAGAAGTCTCCTACAA TTTTCCG	Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae
16	*Bacillus cereus* strain EPG3	KX8 392 68.1	>GGGATAATTGTATGAGACTTCTAGAAGATAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTC GAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGAT AACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGG ATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCC ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAA CGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGA CGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGG CGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTG AGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTC TGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGT GCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAA CTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGA CATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA GATGTTGGGTTAAGTCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGAC AAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAG AGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGA ATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTA ACACCCGAAGTCGGTGGGGTAACCTTTTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAACC GTA	Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae
Facultative anaerobic or microaerofilic isolated from gut				
17	*Acinetobacter desnitrificae* EDN1 (=MCC	MF 398 396.	>AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCTTAACACATGCAAGTCGAGCGGGGGAAGGTAGCTTGCTAC TGGACCTAGCGGCGGACGGGTGAGTAATGCTTAGGAATCTGCCTATTAGTGGGGGACAACATTCCGAAAGGAATGCTAATA CCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGG TGGGGTAAAGGCCTACCAAGGCGACGATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCA GACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGGAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAG	Phylum: Proteobacteria Class:

	3461, =KCTC 62305, =JCM 32455)	1	GCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTGAGACTAATACTCTTGGATAGTGGACGTTACTCGCAGAAT AAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCGAGCGTTAATCGGATTTACTGGGCGTAAAGCGT GCGTAGGCGGCCATTTAAGTCAAATGTGAAATCCCCGAGCTTAACTTGGGAATTGCATTCGATACTGGATGGCTAGAGTATG GGAGAGGATGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCATCT GGCCTAATACTGACGCTGAGGTACGAAAGCATGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATG TCTACTAGCCGTTGGGGCCTTTGAGGCTTTAGTGGCGCAGCTAACGCGATAAGTAGACCGCCTGGGGAGTACGGTCGCAAG ACTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTA CCTGGCCTTGACATACTAGAAACTTTCCAGAGATGGATTGGTGCCTTCGGGAATCTAGATACAGGTGCTGCATGGCTGTCGT CAGTTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTTTCCTTACTTGCCAGCATTTCGGATGGGAAC TTTAAGGATACTGCCAGTGACAAACTGGAGGAAGGCGGGGACGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACAC ACGTGCTACAATGGTCGGTACAAAGGGTTGCTACCTAGCGATAGGATGCTAATCTCAAAAAGCCGATCGTAGTCCGGATTG GAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGCCTT GTACACACCGCCCGTCACACCATGGGAGTTTGTTGCACCAGAAGTAGGTAGTCTAACCGCAAGGAGGACGCTTACCACGGT GTGGCCGATGACTGGGGTGAAGTCGTAACAAGGTAACCGTA	Gammaproteobacteria Order: Pseudomonadales Family: Moraxellaceae (Sp. Nov.)
18	*Klebsiella nitrificae* strain EN1	MF564193.1	>AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCT CTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGC TAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGCTG GTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACG GTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAA GAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGGTGAGGTTAATAACCTCATCGATTGACGTTACCCGCA GAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAA GCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGGCAGGCTAGA GCTTTGTAAAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCC CCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAAC GATGTCGATTTGGAGGTTGTCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGC AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACC TTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGT CGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGG AACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTA CACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGA TTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGC CTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCAC TTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCGTA	Phylum: Proteobacteria Class: Gammaproteobacteria Order: Enterobacteriales Family: Enterobacteriaceae (Sp. Nov.)
19	*Paracoccus rammohunii* strain ENF1	MF564191.1	>AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGAGATCTTCGGATCTAGCGG CGGACGGGTGAGTAACGCGTGGGAATATGCCCTTCTCTACGGAATAGCCTCGGGAAACTGGGAGTAATACCGTATACGCCC TACGGGGAAAGATTTATCGGAGAAGGATTAGCCCGCGTTGGATTAGGTAGTTGGTGGGGTAATGGCCTACCAAGCCTACG ATCCATAGCTGGTTTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGG AATCTTAGACAATGGGGGAAACCCTGATCTAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTCAGCT GGGAAGATAATGACGGTACCAGCAGAAGAAGCCCCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGGGCTAGC GTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGACCGGAAAGTTGGAGGTGAAATCCCAGGGCTCAACCTTGGAA CTGCCTTCAAAACTATCGGTCTGGAGTTCGAGAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGG AGGAACACCAGTGGCGAAGGCGGCTCACTGGCTCGATACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAG ATACCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGTCGTCGGGTAGCATGCTATTCGGTGACACACCTAACGGATTAA GCATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG GTTTAATTCGAAGCAACGCGCAGAACCTTACCAACCCTTGACATTACAGGACATCCCCAGAGATGGGGCTTTCACTTCGGTG ACCTGTGGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTCGGTTAAGTCCGGCAACGAGCGCAACCCACA CTTCCAGTTGCCAGCATTCAGTTGGGCACTCTGGAAGAACTGCCGATGATAAGTCGGAGGAAGGTGTGGATGACGTCAAGT CCTCATGGCCCTTACGGGTTGGGCTACACACGTGCTACAATGGTGGTGACAGTGGGCAGCGAGACCGCGAGGTCGAG GGATTGGGGTCTGCAACTCGACCCCATGAAGTTGGAATCGCTAGTAATCGCGGAACAGCATGCCGCGGTGAATACGTTCCC GGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGGTCTACCCGACGGCCGTGCGCTAACCCTTACGGGAGGCAGCG GACCACGGTAGGCTCAGCGACTGGGGTGAAGTCGTAACAAGGTAACCGTA	Phylum: Proteobacteria Class: Alphaproteobacteria Order: Rhodobacterales Family: Rhodobacteraceae (Sp. Nov.)
20	*Ensifer (=**Sinorhizobium**)nitrofacere* strain ENF4	MF564192.1	>AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCAGGCTTAACACATGCAAGTCGAGCGCCCCGCAAGGGGAGCGGCA GACGGGTGAGTAACGCGTGGGAACGTACCCTTTGCTACGGAATAACTCCGGGAAACTGGAACTAATACCGTATGTGCCCTT CGGGGGAAAGATTTATCGGCAAAGGATCGGCCCCGCGTTGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGAT CCATAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGA ATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCACCG ATGAAGATAATGACGGTAGTCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGC GTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGGTATTTAAGTCAGGGGTGAAATCCCGGAGCTCAACTCCGGAA CTGCCTTTGATACTGGGTACCTAGAGTATGGAAGAGGTAAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGG AGGAACACCAGTGGCGAAGGCGGCTTACTGGTCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAG ATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTAGCCGTCGGGCAGTTTACTGTTCGGTGGCGCAGCTAACGCATTAAA CATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT TTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGATCGCGGACAGTGGAGACATTGTCCTTCAGTTCGGC TGGATCGGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGCGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC TCGCCCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACGTC AAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAGTGGGCAGCGAGACCGCGAGGTCGAG CTAATCTCCAAAAGCCATCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCGCGGATC AGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTTTACCCGAAGGTAGTG TGCTAACCGCAAGGAGGCAGCTAACCACGGTAGGGTCAGCGACTGGGGTGAAGTCGTAACAAGGTAACCGTA	Phylum: Proteobacteria Class: Alphaproteobacteria Order: Rhizobiales Family: Rhizobiaceae (Sp. Nov.)
From coelomic fluid				
21	*Bacillus megaterium* strain Ah4	KU23052727	>AGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACTGATTAGAAGCTTGCTTCTAT GACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCGG ATAGGATCTTCTCCTTCATGGGAGATGTTTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGT GAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTC CTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGT CGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTACCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGT CTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACG TGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTCTTTTGGTCTGTAACTGACGCTGAGGTGCGAAA GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAG TGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAA GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGT TCCCCTTCGGGGGACAGAGTGACAGGCGTTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG CAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGACTCTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTC AAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGTATGGTACAAAGGGCTGCAAGACCGCGAGGTCAAGCCAAT CCCATAAAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCC GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGAGTAACCGTAA GGAGCTAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAACCGTA	Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae
22	*Bacillus pumilus* strain BP	KY616173.1	>AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACTGAAGGGAGCTTGCTCCCGGA AGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGAGCTAATACCGGAT ATTTTCTTGAACCGCATGGTTCAAGAATGAAAGACGGTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA GGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT ACGGGAGGCAGCAATAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCG TAAAGCTCTGTTGTTAGGGAAGAACAAGTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTAC GTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCT GATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTG TAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGC GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTG CTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC GGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTC CCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC CCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAAT CATCATCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCTGCGAGACCGCAAGGTTTAGCCAATCCCAC AAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGT GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGCAACACCCGAAGTCGGTGAGGTAACCTTTATGGAG CCAGCCGCCGAAGGTGGGGTAGATGATTGGGGTGAAGTCGTAACAAGGTAACCGTA	Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae
23	*Bacillus cereus* strain BCR	MF3983	>AGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAGCGAATGGATTGAGAGCTTGCTCTCAA GAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGG	Phylum: Firmicutes

		97	ATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGT GAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTC CTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGT CGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAAC TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAG TCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCAT GTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGTCACTGAGGCGCGAA AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTA GTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACA AGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCT TCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC AACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCA AATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATC TCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCG CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTTG GAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAACCGTA	Class: Bacilli Order: Bacillales Family: Bacillaceae
24	*Bacillus thuringiensis* strain BT	MF5743 66	>TATGAAGTTAACGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAGACTGGGATAACTCCGGGAAACCGGGGCTAATAC CGGATAATATTTTGAACTGCATGGTTCAAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTT GGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGA CTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCG GGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCT AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCACGCGCAGGTGGTTTCTT AAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTC CATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGC GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCT TTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGC ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAGG GCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG CGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACG TCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTA ATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGC CGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTT TTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAACCGTAA	Phylum: Firmicutes Class: Bacilli Order: Bacillales Family: Bacillaceae
25	*Escherichia coli* strain AG2	MH236 118.1	>AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGAAAGCAGCTTGCTGCTT TGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCG CATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTTGTTGGTGGGGTAA CGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGG AGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAG TACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCC AGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATG TGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCG GTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGG GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAG CTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGA GCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGG GAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAGTGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATC CTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCA TGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAA GTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAA TACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCG CTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCGTA	Phylum: Proteobacteria Class: Gammaproteobacteria Order: Enterobacteriales Family: Enterobacteriaceae

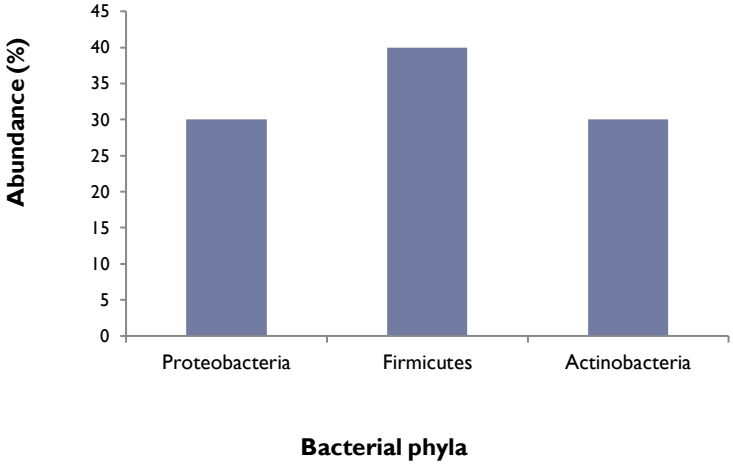


Fig 1.12. Major culturable bacteria in the gut of *E. fetida*

1.3.12. 1. Phylogenetic trese based on 16S rRNA gene sequences

Phylogenetic trees were constructed using MEGA vev7.0. For the strains ET03, EPG1, EAG2 and EAG3 detailed phylogenetic analysis have benn performed using the same protocol.

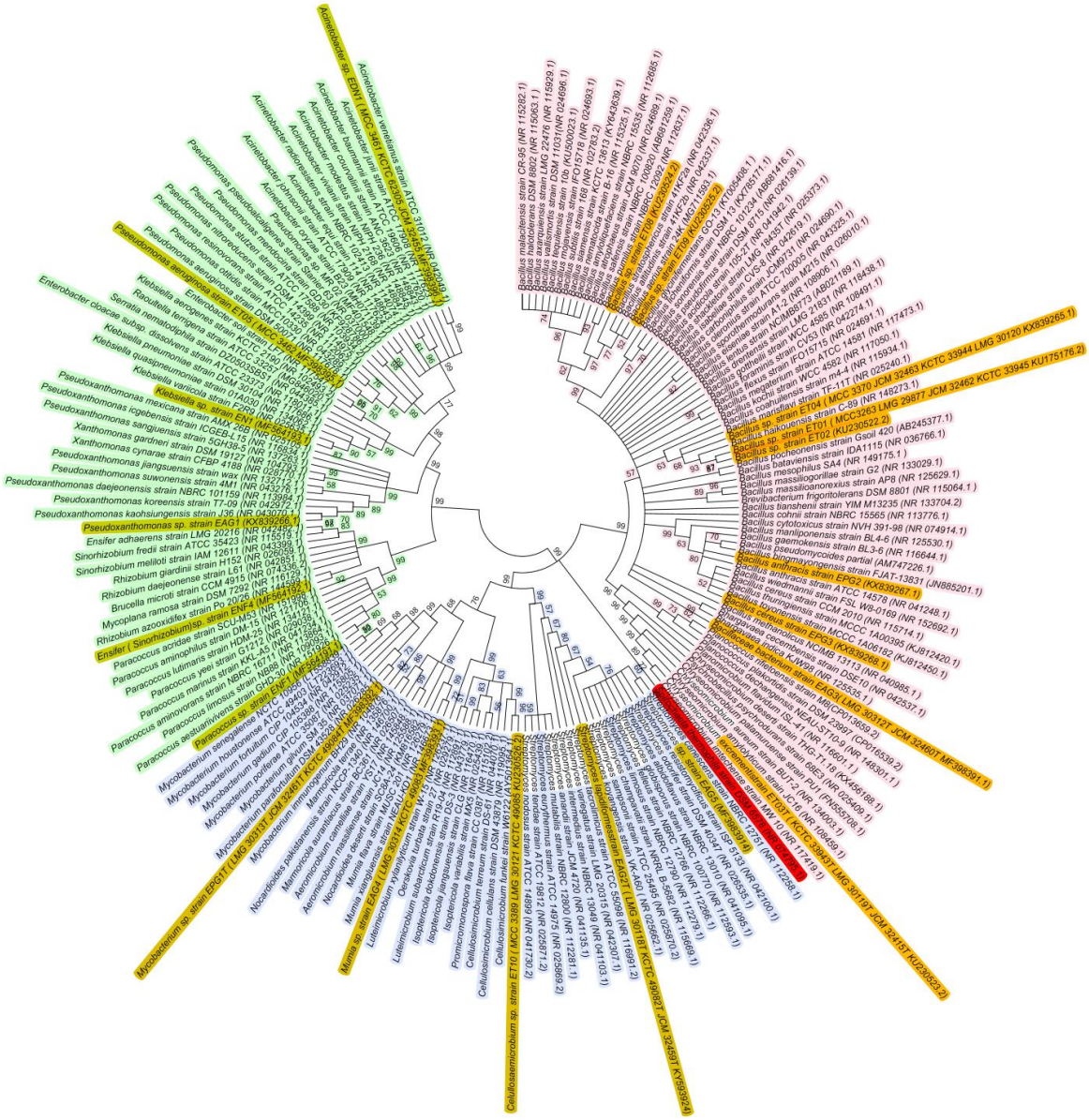


Figure.1.13- Phylogeny of the 20 isolates from the gut of *E. fetida* representing cultivable diversity from the phyla *Firmicutes* (pink), *Actinobacteria* (blue) and *Proteobacteria* (green).
The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches less than 50% bootstrap replicates have been collapsed. The evolutionary distances were computed using the Kimura 2-parameter and are in the units of the number of base substitutions per site. This analysis involved 185 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1152 positions in the final dataset. This evolutionary analyses were conducted in MEGA7. Firmicutes [ ], Actinobacteria [ ] and proteobacteria [ ] are the major bacterial phyla. The highlighted (Yellow) lines represent the 20 isolates; the red line dictates spirochaetes which was not found in the cultivable colonies.

According to the comparison of 16S rRNA gene sequences (using NCBI, Blastn), the closest relative of strain ET03^T were *C. palamuruens* strain PU1^T (99.1%), *C. aureum* strain BUT-2 (99%), *Psychrobacillus psychrodurans* strain BAB-2243 (99%), *C. amylolyticum* strain ID4 (98.9%), *C. imtechense* strain HWG-A7 (98.4%), *C.deserti* strain THG-T1 (96.3%), *Planococcus rifietoensis* strain M8 (95.7%), *Planococcus plakortidis* strain DSM 23997 (95.6%) sequentially followed by other members of the Family *Planococcaceae*. A tree depicting the phylogenetic position of strain ET03^T within the genus *Chryseomicrobium* is shown in Fig. 1.14. Based on 16S rRNA gene sequence comparison, strain ET03^T forms a distinct subclade with *C. palamuruens* PU1^T and other members of the genus *Chryseomicrobium*.

According to the comparison of 16S rRNA gene sequences, the closest relatives of strain EPG1^T were *Mycobacterium parafortuitum* strain DSM 43528^T (99.3% similarity), *Mycobacterium houstonense* strain ATCC 49403^T (99.0%), *Mycobacterium gilvum* strain PYR-GCK (98.9%). A tree depicting the phylogenetic position of strain EPG1^T within the family *Mycobacteriaceae* is shown in Fig. 1.15. Based on 16S rRNA gene sequence comparison, strain EPG1^T forms a distinct clade parallel to *M. parafortuitum.*

According to the comparison of 16S rRNA gene sequences, the closest relative of strain EAG2^T was *Streptomyces koyangensis* VK-A60^T (=KCCM 10555^T=NBRC 100598^T), showing 99.7 % sequence similarity. A tree depicting the phylogenetic position of strain EAG2^T within the genus *Streptomyces* is shown in Fig. 1.16. The strain EAG2^T forms a phylogenic line (clade) distant from the three clusters of *S. intermedius*, *S. koyangensis*, and *S. odorifer* in the tree. The position of the strain EAG2^T in the phylogenetic tree was unaffected by either the tree-making algorithm or the outgroup strains used. These findings suggest that strain EAG2^T represents a novel species that is closely related to *S. koyangensis*, *S. intermedius*, and *S. odorifer*, having a high 16S rRNA gene sequence similarity. The designation of the strain EAG2^T as a separate genomic species was suggested by the bootstrap value (85%) in the N-J tree based on nearly complete 16S rRNA gene sequence data.

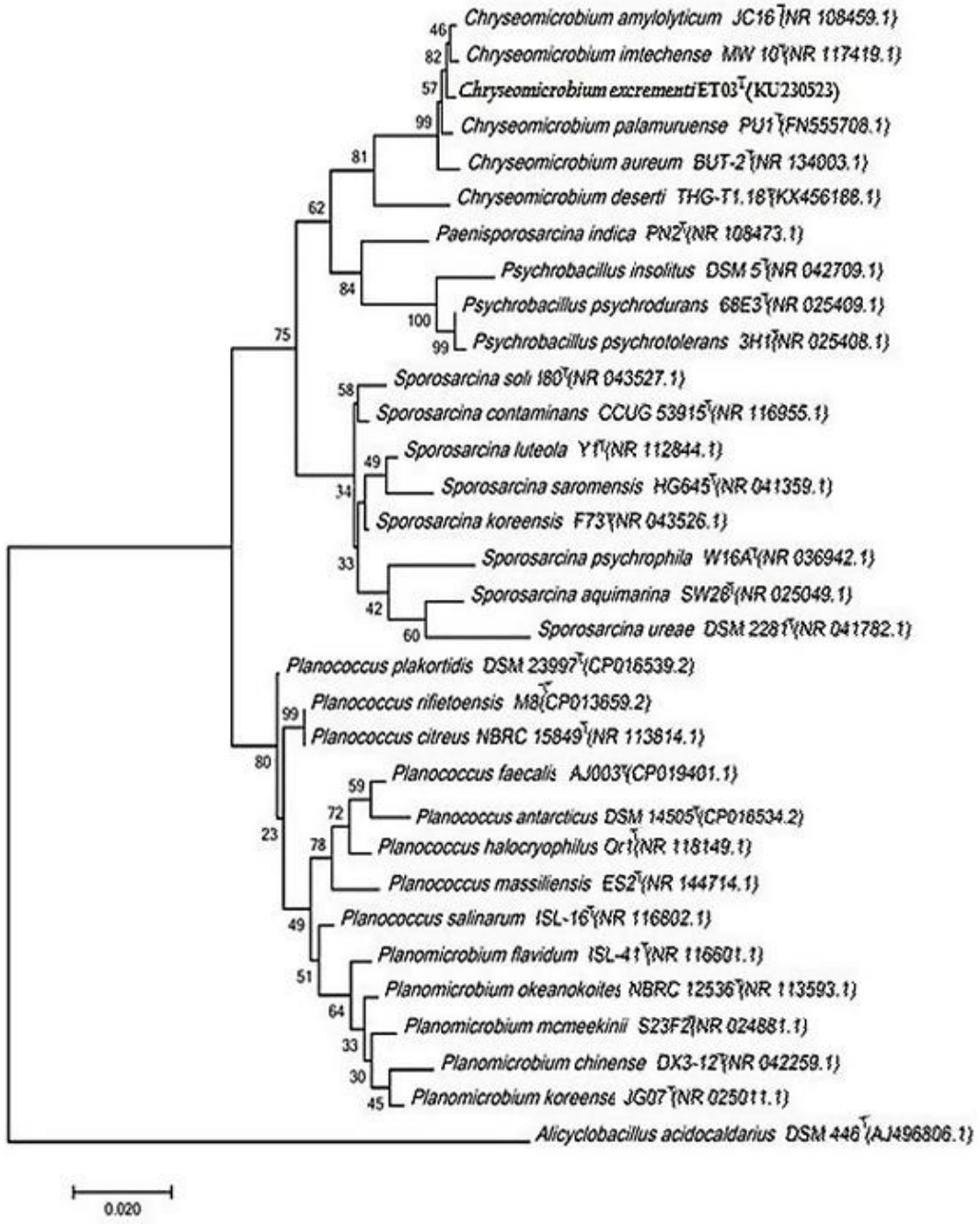


Fig. 1.14: Phylogenetic tree constructed by the neighbour-joining method based on 16S rRNA gene sequences showing the phylogenetic relationship between strain ET03^T and closely related species. Bootstrap percentages (based on 1000 replications) are shown at the nodes. The GenBank accession numbers for 16S rRNA gene sequences are shown in parentheses. *Alicyclobacillus acidocaldarius* strain DSM 446 (AJ496806.1) was used as the outgroup. Bar, 2 nt substitution per 100 nt.

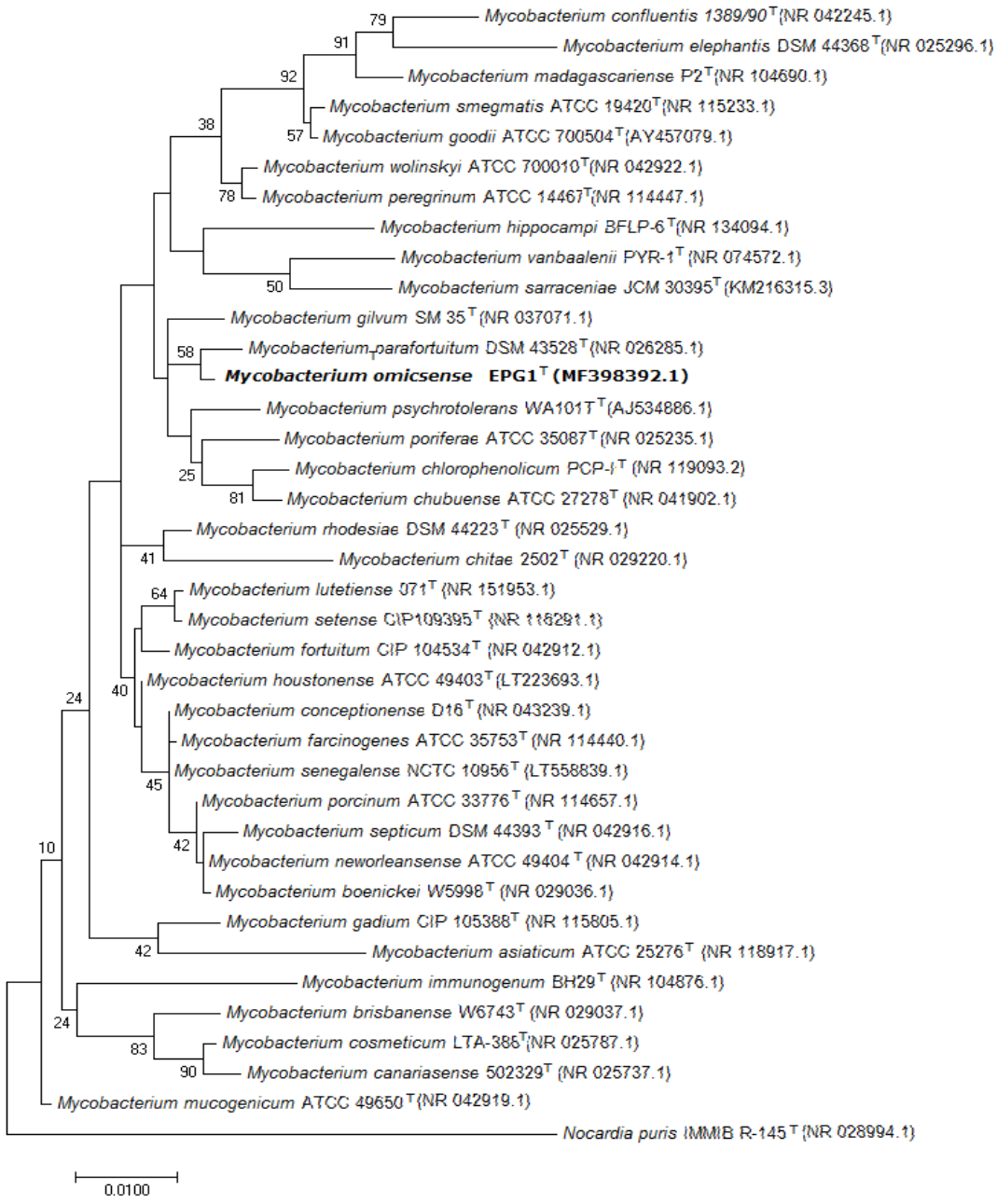


Fig. 1.15: Molecular Phylogenetic analysis of EPG1^T by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model. The tree with the highest log likelihood (-5467.4312) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 38 nucleotide sequences. There were a total of 1537 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

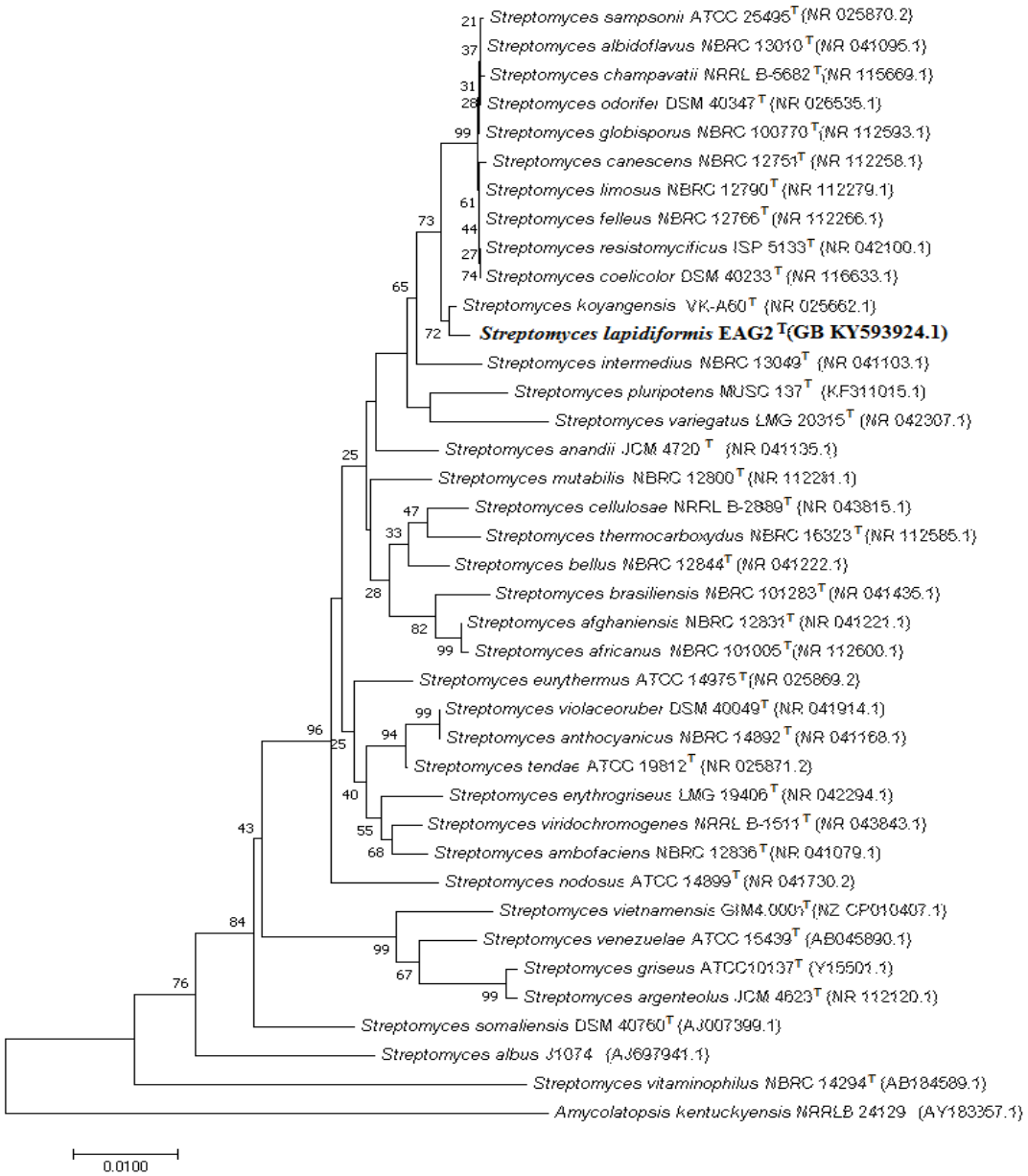


Fig. 1.16:. Evolutionary relationships of strain EAG2^{T}.

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.31523288 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown (>20) next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 39 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1557 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

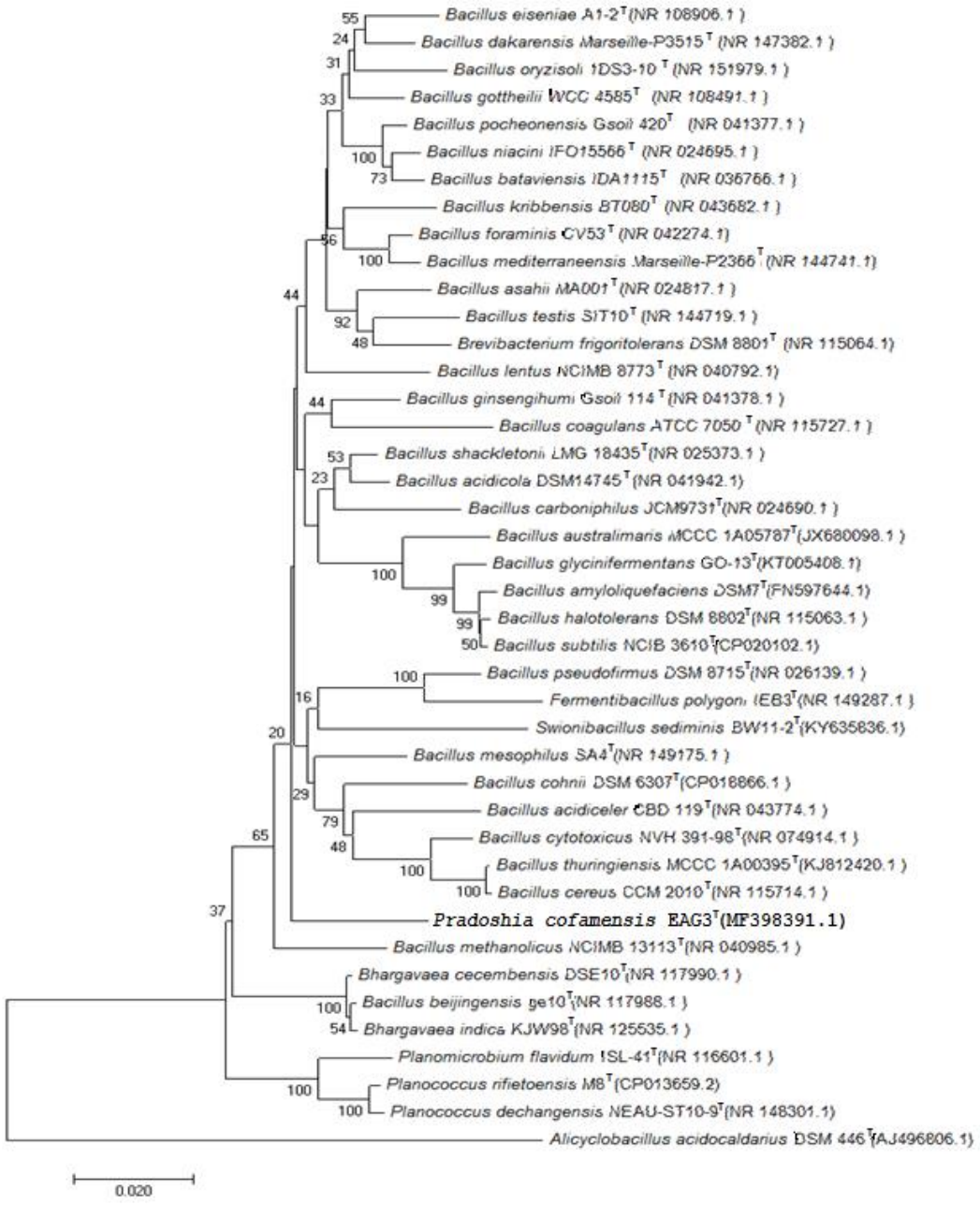


Fig. 1.17: Evolutionary relationships of strain EAG3^T. The evolutionary history was inferred using the Neighbor-Joining method . The optimal tree with the sum of branch length = 0.74518028 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. The analysis involved 42 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1414 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

According to the comparison of 16S rRNA gene sequences, the close relatives of strain EAG3^T from different genus of family *Bacillacae* were *Bacillus methanolicus* NCIMB 13113^T (95.7 % similarity), *Planococcus rifietoensis* M8^T (94.2% similarity), *Bhargavaea cecembensis* strain DSE10^T (94.2% similarity) *Planomicrobium flavidum* strain ISL-41^T (93.7% similarity) *and Fermentibacillus polygoni* strain IEB3^T (93.3% similarity). The 16S rRNA gene sequence comparisons showed sufficient differences so that the strain could be allocated to a separate species without the need for DNA–DNA hybridization experiments (Yarza *et al.*, 2014; Kim *et al*., 2014). A tree depicting the phylogenetic position of strain EAG3^T within the family *Bacillacae* is shown in Fig. 1.17. Based on 16S rRNA gene sequence comparison, strain EAG3^T forms a distinct uniramous clade.

1.3.12. 2. Biochemical characteristics of the novel bacterial strains

Strain ET03^T cells are Gram stain positive, non-motile rods, measuring 1.5±0.5µm in length and 0.5µm in width (Fig. 18-C). The physiological and biochemical test results have been enlisted in 'Table 1' in comparison to other close taxonomic neighbours. ET03^T contained MK-8 as the most predominant menaquinone; MK-7 and MK-6 were also detected (Table: 1.2). The cell-wall peptidoglycan of strain ET03^T contained L-Alanine, D-Glutamic acid, and 2,6,-Diaminopimelic acid in the peptide stem.

Strain EPG1^T cells were short spindle or rod-shaped measuring 1.2-2.2 µm in length x 0.6-0.9 µm in diameter as found in scanning electron microscope (Fig. 18-N). EPG1^T was negative for catalase, and β-Galactosidase activity, but showed nitrate reduction, arylsulfatase (3 d), acid phosphatase, urease, pyrazinamidase, α-esterase and β-esterase activity. The detailed physiological and biochemical test results have been enlisted in 'Table: 1.3' in comparison to other close taxonomic neighbours. EPG1^T contained MK-7, MK-8, and MK-9 as the most predominant menaquinones. TLC of methanolysates shows α-, α′-, and keto-mycolates, and wax esters.

Table.1.2**:** Characteristics that differentiate strain ET03^T from other members of the genus *Chryseomicrobium*.

Strains: 1, ET03^T; 2, *Chryseomicrobium imtechense* MW 10^T; 3, *Chryseomicrobium amylolyticum* JC16^T; 4, *Chryseomicrobium aureum*BUT-2^T; 5, *Chryseomicrobium palamuruense* PU1^T; 6, *Chryseomicrobium deserti* THG-T1.1B^T.

+, Positive; –, negative. AL, unidentified aminolipids; DPG, diphosphatidylglycerol; GL, unidentified glycolipid; PE, phosphatidyl ethanolamine; PG, Phosphatidyl-glycerol; phosphatidyl inositol (PI), phosphatidyl inositolmannoside (PIM) and PL, unidentified phospholipids; m-DAP-meso-diaminopimelic acid; ND, Not detected.

		Strains					
Sl. No.	**Characters**	**1**	**2**	**3**	**4**	**5**	**6**
1	Cell shape and size (µm) (length x diameter)	Rods, 1.5-2.2 x 0.5-0.6	Rod, 1.7–2.9 x 0.3–0.7	Rods, 2.0–3.0 x 1.0	Rods, 1.5–2.0 x 0.5 – 0.86	Rods, 1.6–2.0 x 0.6–0.7	Rods, 2.4-2.7 x 0.5-0.7
2	Motility	non-motile	non-motile	non-motile	non-motile	motile	non-motile
3	Growth temp. range (optimum)	20–40 ℃ (35–37)	4–45 ℃	25–40 ℃ (30–37)	20-35℃	18 - 40 ℃	20–35 ℃ (28–30)
4	pH range (optimum)	6-9	6–9	7–11 (7–8)	7-10	7 to 10	5-7 (7)
5	NaCl tolerance limit (%, w/v)	8	6	5	7	9	3
6	Catalase reaction	-	-	+	-	+	+
7	nitrate reduction	+	-	-	-	-	-
8	VP reaction	-	+	-	-	-	-
9	Urease activity	-	-	-	+	+	+
10	Oxidase	-	-	-	-	+	-
	Hydrolysis of:						
11	Starch	-	-	+	-	+	-
12	Gelatin	+	-	-	+	-	- ?
	Organic substrates utilized for growth:						
13	Citrate	-	+	-	-	+	+
14	Glycerol	-	+	+	-	+	-
	Acid production from various carbohydrates						
15	Glucose	+	+	-	-	+	-
16	Salicin	+	+	-	+	-	-
17	Mannose	-	-	+	-	+	+
18	Fructose	+	+	-	-	-	-
19	Maltose	+	+	-	-	+	+
20	Sucrose	-	+	-	-	-	+
21	Inulin	-	+	-	+	-	-
22	Trehalose	-	-	+	-	-	+
23	Melibiose	-	+	-	-	+	+
24	Cellobiose	-	+	-	-	+	-
25	**Menaquinones** (Descending abundance)	MK-8, 6, 7	MK7, 8, 7_{H2}, 6	MK7, 8, 6	MK7, 6, 8	MK8	MK-7, 8, 6
26	**Polar lipids**	PE, PI, PIM , PG	DPG, PG, PE, PC, GL	DPG, PG, PE, AL, PL,	DPG, PG, PE, PL	DPG, PG, PE	DPG, PE, PG, GL, AL
27	**Peptidoglycan types**	L-Ala-D-Glu	L-Lys-D-Asp	L-Orn-D-Glu	L-Orn-D-Glu	m-DAP	L-Orn-D-Glu
28	**DNA G+C content** (mol%)	42.9	53.4	57.6	48.5	48.5	50.4

Table 1.3: Characteristics that distinguish strain EPG1^T from other closely related Mycobacteria.
Strains: 1, EPG1^T; 2, *M. parafortuitum* DSM 43528^T*; 3, M. gilvum* PYR-GCK*; 4, M. houstonense* ATCC 49403^T and *5, M. fortuitum* CT6.
+, Positive; –, negative, NK- Not Known. aSymbols: +, 90% or more of strains are positive; −, 90% or more of strains are negative; d, 11–89% of strains are positive; v, variability of reaction; w, weak reaction; N, non- chromogenic; S, scotochromogenic; P, photochromogenic. nd, data not available.

No.	Characters	1	2	3	4	5
Cell morphology-						
1	Cell shape, size (µm) (length x diameter)	Short spindle or rods 1.2-2.2 x 0.6-0.9	Short - medium Rods, 2-3 x 1	Cocci 4 x 3.5	Long filamentous	Cocci/ rod 0.4-1.6 x 0.4-0.8
2	Pigmentation	P	P	S	N	N
Culture condition-						
3	Growth at 42°C	−	v	nd	+	v
4	Growth on 5% (w/v) NaCl	−	nd	nd	+	+
Enzyme activity-						
5	Nitrate reduction	+	d	+	+	+
6	Arylsulfatase (3 d)	+	−	+	+	+
7	Acid phosphatase	+	−	nd	Nd	+
8	Catalase	-	nd	+	+	nd
9	Urease	+	+	nd	+	+
10	Pyrazinamidase	+	nd	nd	Nd	d
11	α-Esterase	+	−	nd	Nd	d
12	β-Esterase	+	v	nd	Nd	d
13	β-Galactosidase	-	nd	−	Nd	−
Utilization of :						
14	Acetamide	V	nd	nd	+	d
15	Citrate	+	+	−	−	−
16	Xylose	+	v	nd	-	−
17	Mannitol	-	+	+	+	d
18	Sorbitol	-	nd	nd	+	-
19	Trehalose	-	nd	nd	+	d
Acid from:						
20	l-Arabinose	-	+	nd	-	nd
21	d-Mannitol	-	+	+	+	−
22	l-Rhamnose	-	v	−	-	−
23	d-Sorbitol	-	−	+	+	−
24	**Menaquinones** (Descending abundance)	MK-7, 8, 9	MK7, 8, 7_{H2}, 6	MK7, 8, 6	MK7, 6, 8	MK8
25	**Polar lipids**	PG, DPG, PE	DPG, PG, PE, PC, GL	DPG, PG, PE, AL, PL,	DPG, PG, PE, PL	DPG, PG, PE
26	Mycolates	α-, α′-, keto and wax ester	α-, α′-, keto and wax ester	α-, α′-, keto and wax ester	54-60 carbon mycolic acid	α-, α′-, and epoxy
27	**DNA G+C content** mol% (method)	68.3(WGS)	68.5(WGS)	67.9 (WGS)	64 (T_m)	66.2(WGS)

Table 1.4: Characteristics that distinguish strain EAG2^T from other closely related species of the genus *Streptomyces*.
Strains: 1, EAG2^T; 2, *Streptomyces koyangensis* VK-A60^T; 3, *Streptomyces sampsonii* ATCC25495^T; 4, *Streptomyces albus* ATCC 25426^T. +, Positive; –, negative, NK- Not Known.

Sl. No.	Characters	1	2	3	4
Colony morphology-					
1	Aerial mass colour	White	White/Yellow	Yellow/Grey	White/Yellow
2	Colour of substrate mycellium	Grey	Brown	Yellow/Orange/ Brown	colorless/pale yellow
3	Spore chain morphology	Closed spiral	Rectiflexibile	Rectiflexibile	Spiral
4	Number of spores per chain	10-30	10–50 or more	>50	10-50
5	NaCl tolerance (%, w/v)	7	10	7	7
6	Melanin production	-	+	-	-
7	nitrate reduction	+	+	ND	-
8	Urease activity	+	ND	ND	+
Hydrolysis of:					
9	Starch	+	+	+	+
10	Aesculine	Weakly +	+	+	+
11	Gelatin	+	+	+	+
Organic substrates utilized for growth:					
12	D-Glucose	+	+	+	+
13	Arabinose	+	+	+	Variable
14	Fructose	+	-	+	+
15	Rhamnose	+	-	-	-
16	Sucrose	-	-	-	-
17	DNA G+C content (mol%)	73.1	67.8	NK	NK
18	Fatty acid	$C_{14:0}$ 0.93 C14:0 iso 1.80 $C_{15:0}$ iso 13.38 C15:0 anteiso 51.83 C16:0 3.89 C16:0 iso 1.10 C17:0 iso 1.29 C17:0 anteiso 3.76	$C_{14:0}$ 1.33 C14:0 iso 8.84 C15:0 iso 7.02 C15:0 anteiso 16.54 C16:0 11.60 C16:0 iso 28.77 C17:0 iso 1.94 C17:0 anteiso 9.01	NK	NK

Table 1.5: Characteristics that distinguish strain EAG3^T from other closely related species of the genus Fam. *Bacillaceae*.

Strains: 1, EAG3^T; 2, *Bacillus methanolicus* NCIMB 13113^T; 3, *Planococcus rifietoensis* M8^T; 4, *Bhargavaea cecembensis* DSE10^T; 5, *Planomicrobium flavidum* ISL-41^T and 6, *Fermentibacillus polygoni* IEB3^T. +, Positive; –, negative, NK- Not Known.

		Strains					
Sl. No.	**Characters**	**1**	**2**	**3**	**4**	**5**	**6**
Cell morphology-							
1	Cell shape, size (µm) (length x diameter)	short rods 1.2-1.7 x 0.6-0.8	Rods 4-6 x >1	Cocci 4 x 3.5	Rods 2 x 0.6	Cocci/ rod 0.4-1.6 x 0.4-0.8	Rods 0.6–1.0 x 1.3–4.5
2	Motility	Motile	Motile	Non-motile	Non-motile	Motile	Motile
3	Sporulation	spore forming	Spore forming	No spore	No spore	No spore	Spore forming
Culture condition-							
4	Growth temp(°C) (optimum)	**4-42** (28)	35–60 (55)	30-42 (37)	15-55 (37)	4-37 (30)	12-40 (32)
5	pH range (optimum)	7-10 (7-8)	7-10	6-10	(7-7.5)	6-9 (7-8)	7.5-12 (9-10)
6	NaCl tolerance (%, w/v)	4	2	15	6	13	5
Enzyme activity-							
7	Catalase	+	+	+	+	+	+
8	Oxidase	-	+	+	+	+	+
9	nitrate reduction	+	-	-	+	-	-
10	Urease	-	-	-	+	-	-
Fermentation of-							
11	Fructose	+	-	-	-	+	-
12	Inulin	+	-	-	-	NK	-
13	Lactose	-	-	-	-	-	-
14	Maltose	+	+	-	-	-	-
15	Mannitol	-	+	-	-	-	-
16	Raffinose	-	+	-	-	-	-
17	Ribose	-	+	NK	-	-	+
18	Salicin	+	-	-	-	NK	NK
19	Sorbitol	-	+	-	-	-	-
20	Sucrose	+	-	-	-	-	-
21	Trehalose	-	-	-	-	-	-
Hydrolysis of-							
22	Esculine	-	+	-	+	-	+
23	Casein	-	-	-	+	+	-
24	Gelatin	-	ND	+	-	-	+
25	Starch	-	-	-	+	-	-
26	**G+C (mol%)**	41.82	48-50	**NK**	59.5	45.9	49.1
27	**Major isoprenoid quinones**	MK-7, MK-8	MK-7	Mk-7, Mk-8	MK-6, MK-8	MK-6, MK7, MK8	MK-7

Table 1.6: Cellular fatty acid profile (indicated numerically as percentages) of novel isolate EAG3^T in comparison to other closely related genus of the Fam. *Bacillaceae*.
Strains: 1, EAG3^T; 2, *Bacillus methanolicus* NCIMB 13113^T; 3, *Planococcus rifietoensis* M8^T; 4, *Bhargavaea cecembensis* DSE10^T; 5, *Planomicrobium flavidum* ISL-41^T and 6, *Fermentibacillus polygoni* IEB3^T.
ND- Not detected; '-' indicate 'data unavailable/not reported'.

*Summed feature 4 represents iso-$C_{17:1}$ I and/or anteiso/iso-$C_{17:1}$ B, which could not be separated by GC with the MIDI-Sherlock
Identification system.

Fatty acid	**Bacterial strains**					
	1	**2**	**3**	**4**	**5**	**6**
Branched chain						
iso-C14 : 0	7.5	-	2.8	8.5	8	10.2
iso-C15 : 0	30.7	27	6.9	12.8	1.8	12.2
anteiso-C15 : 0	12.9	16	37	31.2	39	47.1
iso-C16 : 0	11.5	12	7.6	10.7	11.5	7.8
iso-C17 : 0	2.6	4	4.2	-	2.8	2.9
anteiso-C17 : 0	5	14	8.6	6.2	11.3	2.4
Straight chain						
C14 : 0	0.6	-	0.6	-	-	1.8
C15 : 0	ND	-	2.5	-	-	8.1
C16 : 0	1.5	-	6.8	4.7	-	4.9
C17 : 0	0.3	-	4.4	-	-	0.8
C18 : 0	0.6	-	5.9	-	-	-
Unsaturated						
C16 : 1ω7c OH	13.7	-	4	4.5	11	-
C16 : 1 ω 11c	1.6	-	0.8	-	1	-
Summed feature 4*	4.2	-	1.9	-	8.4	-

The growth of EAG2^T colonies on LA is lichenoid, hard (stony), densely textured, chalky, raised, adhering to the medium and formed clear concentric rings with time. In liquid media, especially in shaken culture, the growth of EAG2^T is in the form of spherical growths or puffballs. Strain EAG2^T can be readily differentiated from the closest relatives, *Streptomyces koyangensis* VK- A60^T *Streptomyces albus* ATCC 25426^T and *Streptomyces sampsonii* ATCC25495^T with reference to physiological and biochemical characteristics including spore pattern, production of melanin, cellular

fatty acids and DNA G+C content (Table: 1.4). EAG2^T contained MK-8(60%) and MK-9(40%) as the most predominant menaquinones.

Strain EAG3^T cells are short bacilli as found in scanning electron microscope (Fig. 1.18-K). The physiological and biochemical test results have been enlisted in 'Table 5' in comparison to other close taxonomic neighbours. EAG3^T contained MK-7 and MK-8 as the major isoprenoid quinones. MK-6 and MK-7H_2 are also present in small amount.

1.3.12. 3. Determination of Polar lipid, FAME and GC mol% of the strains

Phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI) and phosphatidyl inositolmannoside (PIM) were identified as the phosplolipids present in strain ET03^T along with at least three spots of unknown lipids. Phosphatidyl glycerol (PG) was characteristically found in strain ET03^T as the primary glycolipid.

Major cellular fatty acids were 13-Methyltetradecanoic acid or iso-15:0 (45%), (9Z)-9-Hexadecenoic acid or 16:1 ω7c alcohol (13%), 14-Methylpentadecanoic acid or iso-16:0 (11.8%) and 12-Methyltridecanoic acid or iso-14:0 (6.2%). Trace amount of 12-Methyltetradecanoic acid or anteiso-15: 0 (3.4%), 15-Methylhexadecanoic acid or iso-17: 0 (2.2%) and 14-Methylhexadecanoic acid or anteiso-17: 0 (1.6%) were present which is typical of members of the genus *Chryseomicrobium*, but the proportions differed from those reported for other members of the genus.

The predominant polar lipids were phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylinnocitol (PI) and phosphatidylinnocitol mannoside (PIM). Major cellular fatty acids were hexadecanoic acid or $C_{16:0}$ (22.9%), (9Z)-9-octadecenoic acid or $C_{18:1}$ ω9c (21.1%) and 10-methyloctadecanoic acid or $C_{18:0}$ 10-methyl (11.5%). Small amount of tetradecanoic acid or $C_{14:0}$ (5.4%) and Octadecanoic acid or $C_{18:0}$ (1.9%) were also present. Fatty acids of strain EPG1^T were typical of other members of the Genus *Mycobacterium*, but the proportions differed.

Phosphatidyl-ethanolamine (PE), and phosphatidyl-inositol (PI) were identified as the major phosplolipids present in strain EAG2^T. Trace amount of phosphatidylinositol mannoside (PIM), and unidentified phospholipid (PL) were also detected. Major cellular fatty acids were 12-methyltetradecanoic acid (C15:0 anteiso), 13-Methyltetradecanoic acid (C15:0 iso), Hexadecanoic acid (C16:0) and 14-Methylhexadecanoic acid (C17:0

anteiso). The major fatty acid groups found in EAG2^T are enlisted in Table1 which differed from those reported for other members of the genus and showed best similarity index of 0.386 supporting claim of novel species

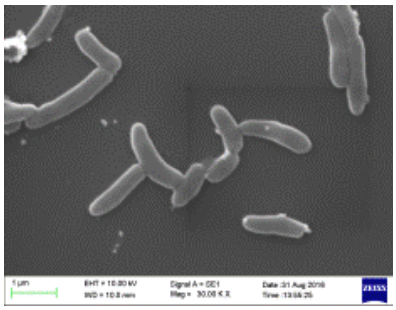
The predominant polar lipids present in strain EAG3^T were phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE). Major cellular fatty acids were 12-Methyltridecanoic acid ($C_{14:0}$ iso 7.5%), 13-Methyltetradecanoic acid ($C_{15:0}$ iso 30.7%), 12-Methyltetradecanoic acid (C15:0 anteiso 12.9%), 14-Methylpentadecanoic acid (C16:0iso 11.5%), (9Z)-9-Hexadecen-1-ol (16:1 ω7c alcohol 13.7%), 14-Methylhexadecanoic acid (C17:0 anteiso 5%) and 15-Methylhexadecanoic acid (C17:0 iso 2.6%). Fatty acids of strain EAG3^T were typical of other members of the family *Bacillacae*, but the proportions differed (Table: 1.6).

1.3.12 4. Scanning Electron Microscopy (SEM) of the strains.

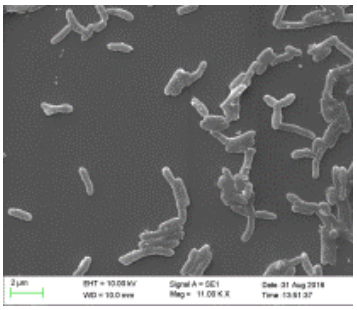
Details of the cell shape of the strains were ascertained with help of a scanning electron microscope. Figure 1.18-A-Y depicts the images.

1.3.12.5. Accession number & submission certificates from 'Type' culture collection centres

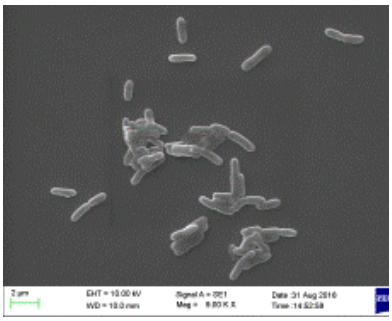
Accession numbers acquired for some of the unique bacterial strains from different internationally recognized Type culture collection centres are mentioned in Table 1.1 within parenthesis. Culture submission certificates describing their availability from these centres authenticate the submission.



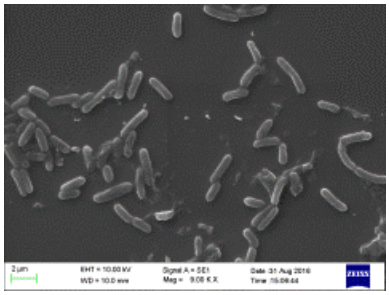
A.*Bacillus efetidiens* strain ET01



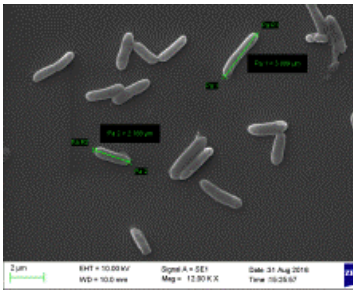
B. *Bacillus* sp. strain ET02



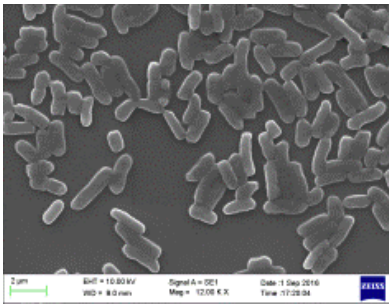
C.*Chryseomicrobium excrementi* strain ET03



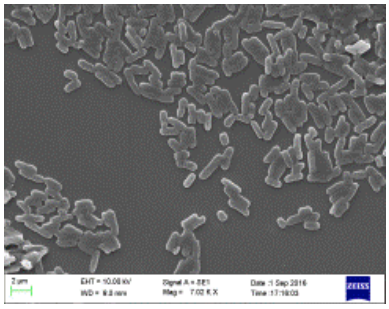
D. *Bacillus eisenifilia* strain ET04



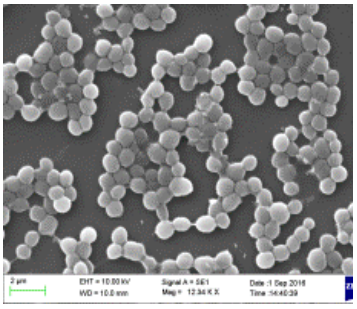
E. *Pseeudomonas aeruginosa* strain ET05



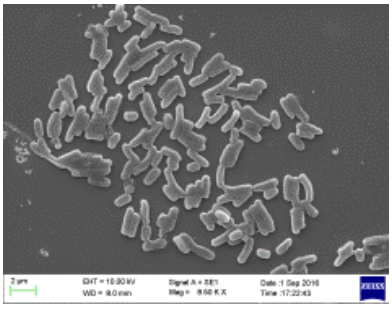
F. *Bacillus* sp. strain ET08



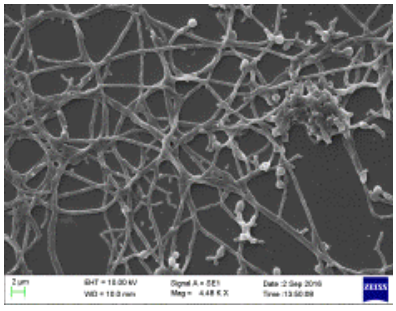
G. *Bacillus* sp. strain ET09



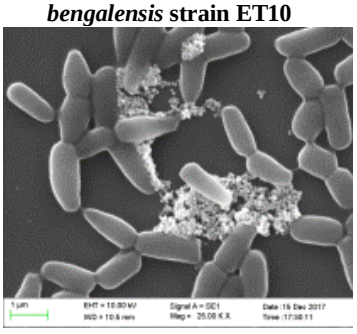
H. *Celullosaemicrobium bengalensis* strain ET10



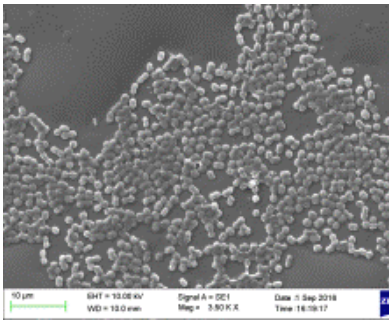
I. *Pseudoxanthomonas* sp. strain EAG1



J.*Streptomyces lapidiformes* strain EAG2

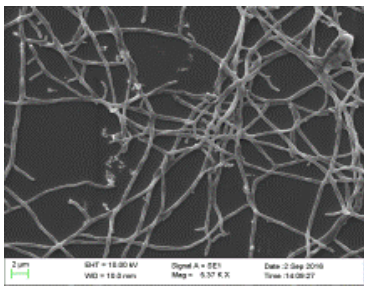


K. *Pradoshia cofami* strain EAG3

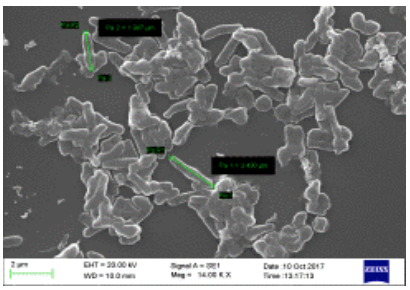


L. *Mumia enteroni* strain EAG4

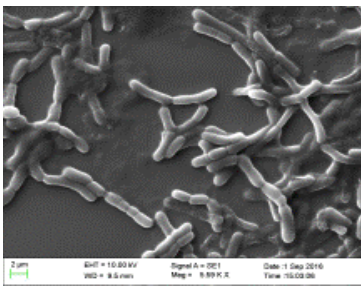
Cont….



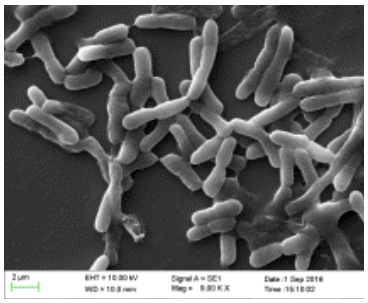
M. *Streptomyces* sp. strain EAG5



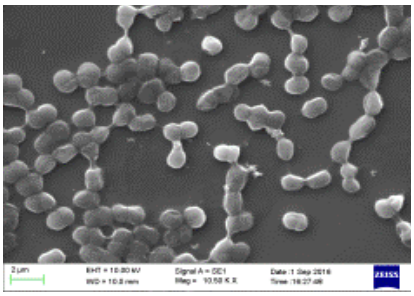
N. *Mycobacterium omicsense* strain EPG1



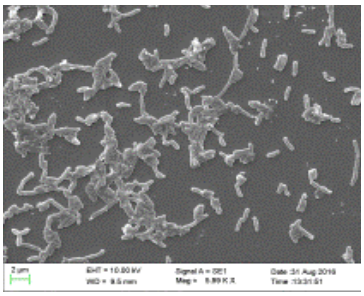
O. *Bacillus anthracis* strain EPG2



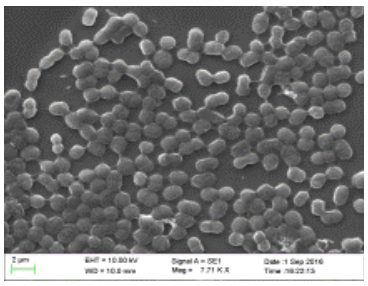
P *Bacillus cereus* strain EPG3



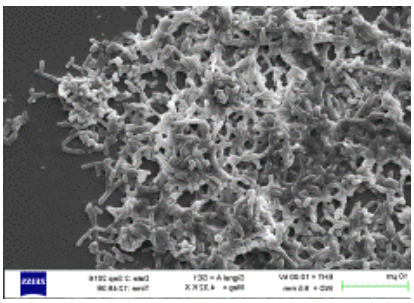
Q.*Acinetobacter desnitrificae* stain EDN1



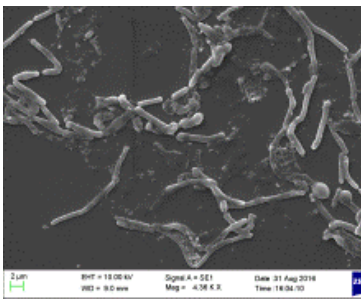
R. *Klebsiella nitrificae* strain EN1



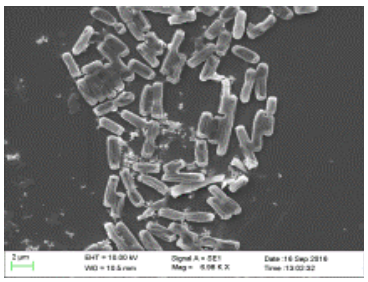
S. *Paracoccus rammohunii* strain ENF1



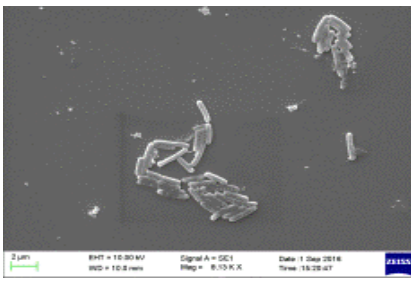
T. *Ensifer nitrofacere* strain ENF4



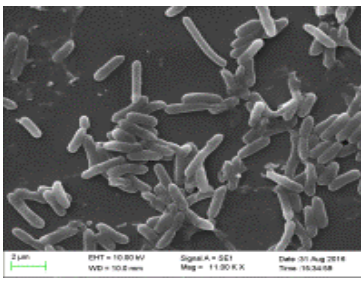
U. *Bacillus megaterium* strain Ah4



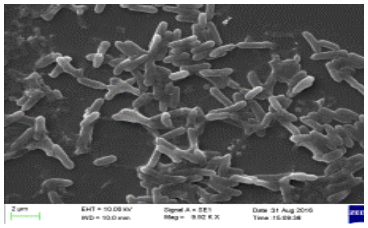
V. *Bacillus pumilus* strain BP



W. *Bacillus cereus* strain BCR



X. *Bacillus thuringiensis* strain BT



Y. *Escherichia coli* strain AG2

Fig. 1.18: (A-Y). Electron micrographs of log phase cultures of the bacterial strains isolated from the gut (A-T) and coelomic fluid (U-Y) parts of *E. fetida.*