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Comparative analysis of 16S rRNA of bacteria associated with the ancient annelid *Platynereis dumerilii*: structural conservation across clades

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ABSTRACT: The study dealt with the comparative analysis of the 16S rRNA of bacteria associated with *Platynereis dumerii* belonging to five major phyla namely Proteobacteria, Actinobacteria, Cyanobacteria,Femicutes, and Bacteroidetes, integrating their phylogenetic reconstruction and delineation. Maximum likelihood trees reveal consistent clustering of bacterial taxa into five major phyla.The phylogenetic coherence and conserved 16S rRNA motifs across clades suggest functional constraints and possible co-evolution with the host. These findings provide a molecular framework for understanding the evolutionary ecology of *P. dumerilii*-associated microbiota and highlight conserved ribosomal features as phylogenetic markers across bacterial lineages.

KEYWORDS: Annelids, Bacteria, *Platynereis dumerilii*, 16S rRNA

INTRODUCTION

Platynereis dumerilii, a marine annelid widely used as a model organism, coexists with a diverse array of microorganisms in its natural and laboratory environments. These microbial communities play essential roles in the worm's development, immunity, and ecological interactions. *P. dumerilii* secretes mucus tubes that provide microhabitats for microbial colonization of microoragnisms responsible for polysaccharide degradation and anaerobic metabolism [1]. Similarly, Gammaproteobacteria like *Vibrio cholerae* and *Pseudomonas aeruginosa* may interact with *Platynereis* through nutrient cycling or opportunistic colonization, especially in laboratory cultures where environmental control is limited [2]. In addition, sensory integration of *Platynereis* supports their ecological coexistence.

The 16s rRNA gene is a ubiquitous gene of the prokaryotes with approximately 1500 base pair coding genes for the RNA that is a part of 30S ribosomal subunit [3]. It has regions of conserved and variable sequences that serves an important role in determination of both close and distant phylogenetic relationship (Figure 1). This characteristic allows the utilization of 16S rRNA to depict genetic relationship at genus and species level [4].

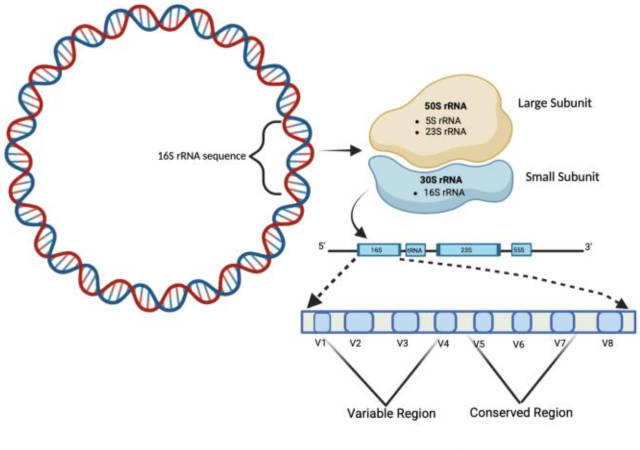

Figure 1. Schematic representation of the prokaryotic genome and ribosomal RNA gene organization. The circular prokaryotic DNA is illustrated, with the 16S rRNA gene region highlighted. Adjacent to the genome sequence, is the 50S (large ribosomal subunit) and 30S (small ribosomal subunit) are shown, emphasizing the structural role of the 16S rRNA within the 30S subunit. Below, the rRNA operon is depicted, showing the typical arrangement of 16S rRNA-tRNA-23SrRNA-5S rRNA. Within the operon is the variable region depicted by V1, V2 until V8 while in between the variable regions are the conserved regions which are the key to its dual role in structural integrity and phylogenetic resolution.

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In prokaryotes, the 16S rRNA gene encodes the RNA component of the small (30S) ribosomal subunit which plays a central role in mRNA translation and taxonomic studies. Its eukaryotic counterpart is the 18S rRNA, which forms a small 40S ribosomal subunit and shares the same conserved structure and function in protein synthesis across the eukaryotic domain [5,6,7].

In the present study, five major bacterial groups such as Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, and Cyanobacteria were selected to represent a broad phylogenetic range within prokaryotes in determining their relationship and interaction with *P. dumerilii.*

MATERIALS AND METHODS

Data Collection and Sequence Retrieval

Complete 16S rRNA sequences from five major bacterial phyla representing diverse lineages and ecological functions: Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, and Cyanobacteria were obtained from the National Center for Biotechnology Information (NCBI) GenBank database. Each representative species from each group were selected based on the availability of full length, high quality annotated sequences. The inclusion criteria required that each sequence be labeled as “complete” and taxonomically verified in the NCBI database to ensure the accuracy and comparability across taxa.

Table 1. Classification of bacteria used in this study.

Phylum	Class	Species	Accession Number
Proteobacteria	Gammaproteobacteria	*Escherichia coli*	D12649.1
Proteobacteria	Gammaproteobacteria	*Pseudomonas aeruginosa*	AB126582.1
Proteobacteria	Gammaproteobacteria	*Salmonella enterica* strain HC-2sh	CP185384.1
Proteobacteria	Gammaproteobacteria	*Vibrio cholerae*	AY292952.1
Proteobacteria	Alphaproteobacteria	*Cereibacter sphaeroides*	CP015216.1
Proteobacteria	Campylobacterota	*Helicobacter pylori*	U00679.1
Proteobacteria	Betaproteobacteria	*Neisseria meningitidis* strain N.544/03	AY735392.1
Proteobacteria	Alphaproteobacteria	*Bartonella henselae* str. Houston-1	NR_074335.2
Proteobacteria	Gammaproteobacteria	*Legionella pneumophila*	EU054324.1
Proteobacteria	Gammaproteobacteria	*Acinetobacter baumannii*	AY738399.2
Bacteroidetes	Bacteroidia	*Bacteroides fragilis strain* MDR_U01	NZ_CP119602.1
Bacteroidetes	Bacteroidia	*Prevotella melaninogenica*	L16470.1
Bacteroidetes	Flavobacteriia	*Flavobacterium frigidarium*	AF162266.2
Bacteroidetes	Bacteroidia	*Porphyromonas* gingivalis	L16492.1
Bacteroidetes	Flavobacteriia	*Capnocytophaga canimorsus*	L14637.1
Bacteroidetes	Flavobacteriia	*Chryseobacterium aahli* strain T68	JX287893.1
Bacteroidetes	Sphingobacteriia	*Pedobacter lusitanus* strain NL19	KJ579161.3
Bacteroidetes	Cytophagia	*Cytophaga* sp. SA1	AF414444.1
Bacteroidetes	Bacteroidia	*Odoribacter lunatus*	NZ_CALPCZ010000067.1
Bacteroidetes	Bacteroidia	*Parabacteroides distasonis*	CP042284.1
Firmicutes	Bacilli	*Bacillus subtilis*	AB501343.1
Firmicutes	Clostridia	*Clostridioides difficile* strain ST963	CP102403.1
Firmicutes	Bacilli	*Streptococcus pneumoniae*	MF578776.1
Firmicutes	Bacilli	*Lactobacillus acidophilus* strain	KU324919.1
Firmicutes	Bacilli	*Staphylococcus aureus* strain 06-16607	HQ260332.1
Firmicutes	Bacilli	*Enterococcus faecalis*	AB036835.1
Firmicutes	Bacilli	*Listeria portnoyi* strain FSL L7-1582	NR_181107.1
Firmicutes	Clostridia	*Clostridium perfringens* rrnD operon	AB045285.1
Firmicutes	Bacilli	*Leuconostoc mesenteroides*	DQ297412.1
Firmicutes	Bacilli	*Paenibacillus polymyxa* strain AD7	KP210082.1

Actinobacteria	Actinobacteria	*Mycobacterium sp.* TY59	LC645084.1
Actinobacteria	Mollicutes	*Mycoplasmopsis bovis*	U02968.1
Actinobacteria	Actinobacteria	*Streptomyces sp.* YSPA8	LC739529.1
Actinobacteria	Actinobacteria	*Propionibacterium acnes*	JF430009.1
Actinobacteria	Actinobacteria	*Actinomyces israelii* DSM 43320	NZ_JONS01000103.1
Actinobacteria	Actinobacteria	*Bifidobacterium longum* strain BG3	AY735403.1
Actinobacteria	Actinobacteria	*Nocardia asteroides* strain DSM 43255	AF430026.1
Actinobacteria	Actinobacteria	*Kocuria rosea* strain K29-01	EU333875.1
Actinobacteria	Actinobacteria	*Rhodococcus equi* strain BAB-1825	KC443097.1
Actinobacteria	Actinobacteria	*Micromonospora echinospora*	NZ_JBFAII010000139.1
Cyanobacteria	Cyanobacteria	*Synechococcus elongatus* PCC 6301	NR_074309.1
Cyanobacteria	Cyanobacteria	*Prochlorococcus marinus*	NZ_CVSV01000135.1
Cyanobacteria	Cyanobacteria	*Trichormus variabilis*	NZ_JACJRH010000085.1
Cyanobacteria	Cyanobacteria	*Nostoc punctiforme*	NR_074317.1
Cyanobacteria	Cyanobacteria	*Microcystis aeruginosa* strain	NR_074314.1
Cyanobacteria	Gloeobacteria	*Gloeobacter violaceus* PCC 7421	NR_074282.1
Cyanobacteria	Cyanobacteria	*Lyngbya sp.* CCY1209	NZ_JAJENU010000117.1
Cyanobacteria	Cyanobacteria	*Leptolyngbya sp.* isolate ULC077bin1	QBMH01000407.1
Cyanobacteria	Gammaproteobacteria	*Psychrobacter pacificensis*	NZ_JAQPZR010000046.1
Actinobacteria	Actinobacteria	*Williamsia* limnetica	NZ_QJSP01000046.1
Eukaryote	Polychaeta	*Platynereis dumerilii* clone 10	EF117908.1

Multiple Sequence Alignment Tool

The multiple sequence alignment was carried out using Clustal Omega by EMBL-EBI. The alignment process was conducted using default parameters to identify conserved motifs and divergent regions among the selected bacterial groups. Following the alignment, a phylogenetic tree (phylogram) was constructed to visualize evolutionary relationship and degree of sequence divergence across the dataset. This analysis enabled a comparative view of ribosomal RNA evolution between prokaryotic and eukaryotic taxa. In addition to Clustal Omega, MAFFT multiple sequence alignment was performed as secondary alignment of the rRNA sequences. This platform was selected for its high accuracy and customizable parameters which are especially advantageous when aligning a diverse sequences from both bacterial and eukaryotic origins. MAFFT's advanced algorithm allowed for a more nuanced comparison of conserved regions and secondary structure features, enhancing the reliability of the alignment and supporting the phylogenetic interpretation drawn from the dataset. For the visualization of the phylogenetic tree relationship among the aligned sequences, ETE3 viewer through genome.jp. ETE3 or Environment for Tree Exploration v3.

Phylogenetic Tree Analysis

To further validate and compare the phylogenetic outputs, MAFFT Archeopteryx Phylogenetic Tree Viewer was utilized, which provides interactive visualization of phylogram generated from MAFFT alignment.

RESULTS AND DISCUSSION

The phylogenetic tree depicts evolutionary relationships among bacterial taxa, organized into five major clades: Proteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria, and Firmicutes. The internal nodes of Proteobacteria which includes *H. pylori*, *E. coli*, and *P. aeruginosa* show moderate to strong bootstrap support indicating reliable grouping. However, the basal node linking *Helicobacter* to the rest of the clade has weak support. Meanwhile, Bacteroidetes indicates a highly confident evolutionary relationship with *Pedobacter lusitanus* as more distant among the species. Actinobacteria appears in multiple subclades. Cyanobacteria such as *S. elongatus* and *P. marinus*, with 100% bootstrap support, confirm their close evolutionary relationship and ecological similarity as marine photoautotrophs. Lastly, this suggests strong evolutionary linkage among these Gram-positive bacteria, many of which are host-associated or pathogenic.

Proteobacteria and Bacteroidetes are sister clades, diverging from a common ancestor. Similarly, Actinobacteria and Firmicutes appear as basal lineages, diverging earlier in bacterial evolution. Their separation from Cyanobacteria is supported by bootstrap values ranging from 80 to 100. The most ancient lineages in this tree are represented by Firmicutes and Actinobacteria, which occupy basal positions. Their early divergence is consistent with genomic studies suggesting that these clades retain ancestral traits such as simple cell wall structures and low GC content [8].

Structural conservation within these clades reflects deep evolutionary homology and functional constraints that have shaped bacterial diversification. Species such as *E. coli*, *S. enterica*, and *V. cholerae* cluster with high bootstrap support, indicating conserved structural features like outer membrane protein, flagellar basal bodies, and type III secretion systems which are necessary for motility, host interaction, and pathogenicity, and are conserved across γ-Proteobacteria [9]. The clade encompassing *B. fragilis*, *P. melaninogenica*, and *Porphyromonas gingivalis* exhibits strong phylogenetic cohesion sharing conserved polysaccharide utilization loci and β-barrel membrane proteins facilitating carbohydrate metabolism and niche adaptation in the gut microbiome [9]. Members such as *Streptomyces sp.*, *M. echinospora*, and *Mycobacterium sp.* retain type I polyketide synthases, mycolic acid biosynthesis enzymes, and regulatory domains involved in secondary metabolism. These structures underpin antibiotic production and cell wall integrity, and are deeply conserved across soil-dwelling and pathogenic Actinobacteria [10] *Prochlorococcus marinus*, *Synechococcus elongatus*, and *N. punctiforme* have a conserve photosystem II core proteins (D1/D2), phycobilisomes, and thylakoid membrane structures for oxygenic photosynthesis and are evolutionarily stable across aquatic Cyanobacteria [11]. Moreover, *Bacillus subtilis*, *Clostridium perfringens*, and *Streptococcus pneumoniae* show conservation of endospore-forming proteins, peptidoglycan biosynthesis enzymes, and two-component regulatory systems which is important to stress resistance, cell division, and environmental adaptation, and are conserved across both aerobic and anaerobic Firmicutes [12].

Figure 3 delineates the evolutionary relationships among diverse bacterial taxa across four major groups: Alphaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria, and Bacteroidetes. Most bootstrap values exceed 90%, suggesting high confidence in the inferred relationships. Alphaproteobacteria (*B. henselae* and *N. meningitidis)* form a distinct clade with a bootstrap value of 96. These species are known for their pathogenicity in humans causing cat scratch disease and responsible for meningitis [14]. Within the clade of Gammaproteobacteria, the strongest support (bootstrap 100) is observed between *V. cholerae* and *E. coli*, both enteric pathogens with facultative anaerobic metabolism. The relatively lower support (67) between *Pseudomonas aeruginosa* and *Acinetobacter baumannii* suggests either rapid divergence or horizontal gene transfer events, which are common in hospital-acquired pathogens [2].The Epsilonproteobacteria *Helicobacter pylori* stands alone in this group, branching with a bootstrap value of 97. Its distinct placement reflects its unique adaptations to the gastric environment and its divergence from other Proteobacteria[15]. Meanwhile the Bacteriodetes is the most taxonomically diverse in the tree which can be attribited to their exiting physical, and physiological roles.

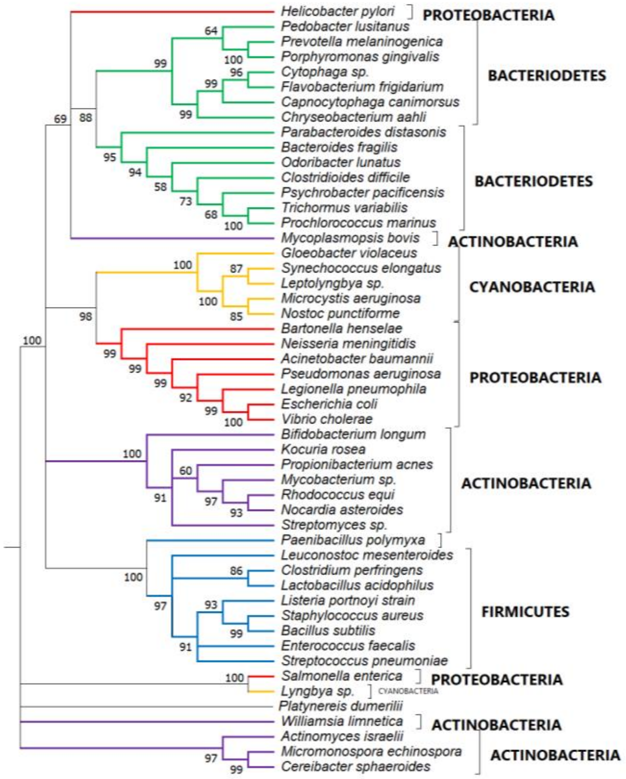

Figure 2. Phylogenetic Tree of the Selected Bacteria Associated with *Platynereis dumerilii* based from 16SrRNA Sequences.

The evolutionary relationships among Actinobacteria, Mycoplasmatales, and Cyanobacteria is presented in Figure 4. Actinobacteria Clade is well-supported, with bootstrap values ranging from 84 to 99. These taxa share high sequence similarity, consistent with their classification within Actinobacteria, a phylum known for its high G+C content and complex secondary metabolism [16]. *Bifidobacterium longum* and *Propionibacterium acnes* cluster with a bootstrap value of 100, indicating a very strong evolutionary relationship. *Mycoplasmopsis bovis* forms a distinct branch with a bootstrap value of 99, reflecting its divergence from the Actinobacteria and Cyanobacteria. They are characterized by reduced genomes and lack of cell walls, which is reflected in their distant placement from other taxa [17]. Cyanobacteria is also strongly supported,.The close relationship between *Prochlorococcus marinus* and *Trichormus variabilis* (bootstrap 99) reflects their shared photosynthetic capabilities and ecological niches in marine environments [18]. The Actinobacteria and Cyanobacteria clades are clearly distinct, reflecting their deep evolutionary divergence. The summary of the imporantce of the selected bacterial speices highlighting their roles in symbiosis, nutrient cycling, and microbiome composition relevant to the marine polychaete *Platynereis dumerilii* are presented in Table 2.

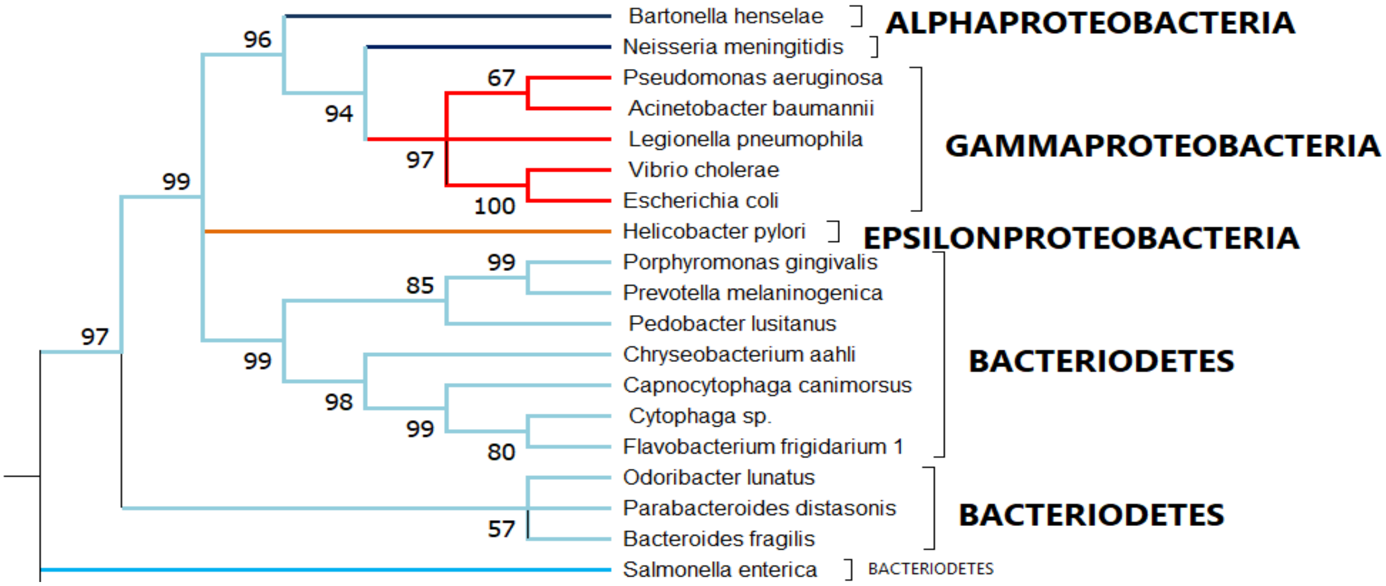

Figure 3. Phylogenetic Tree of the Selected Proteobacteria and Bacteriodetes Associated with *Platynereis dumerilii* based from 16SrRNA Sequences.

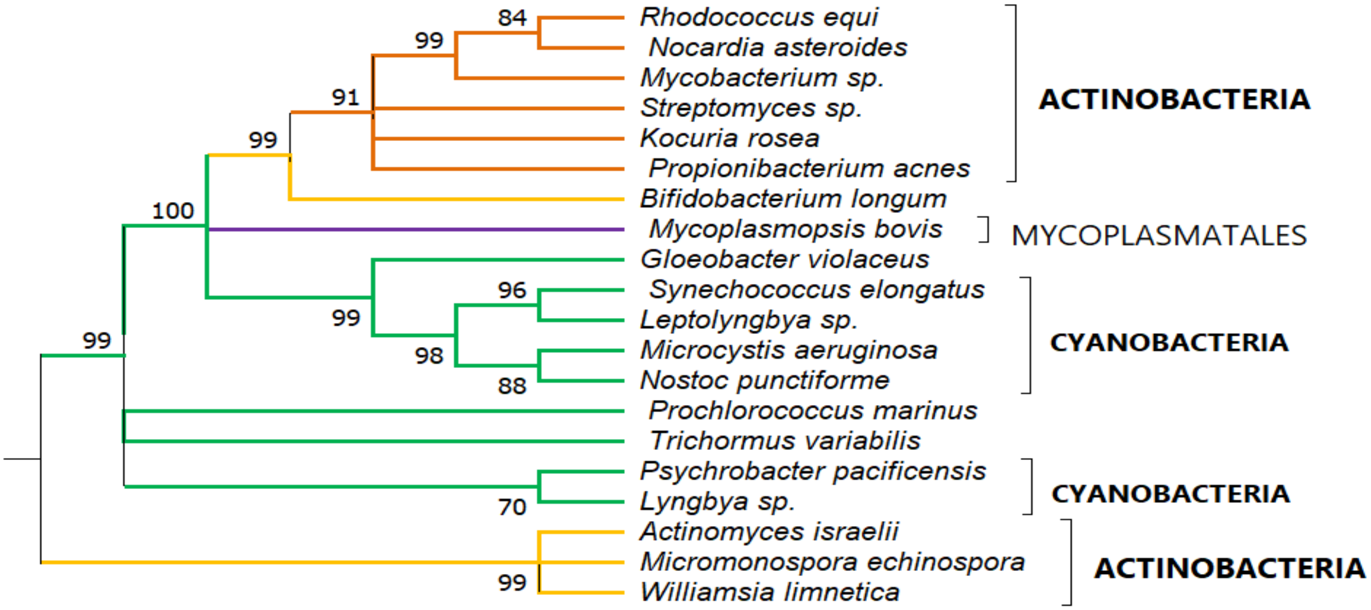

Figure 2. Phylogenetic Tree of the Selected Cyanobacteria, and ActinobacteriaAssociated with *Platynereis dumerilii* based from 16SrRNA Sequences.

Table 2. Summative features of bacteria associated with *P. dumerilli* [12, 13, 19, 20, 21, 22]

Species	**Functional Traits**	**Ecological Role**	**Physiological Features**	**Relevance to *Platynereis dumerilii***
Helicobacter pylori	Urease, flagella	Gastric pathogen	Microaerophilic	Comparative host-pathogen signaling models
Escherichia coli	Fermentation, toxins	Gut commensal/pathogen	Facultative anaerobe	Benchmark for gut microbiome structure
Salmonella enterica	Type III secretion	Enteric pathogen	Facultative anaerobe	Reference for innate immune activation pathways
Vibrio cholerae	Cholera toxin	Aquatic pathogen	Halotolerant	Marine microbial comparison; toxin evolution
P. aeruginosa	Biofilm, resistance	Opportunistic pathogen	Aerobic	Biofilm formation parallels in annelid mucus layers
Neisseria meningitidis	Capsule, pili	Nasopharynx pathogen	Aerobic	Capsule structure comparison in immune evasion
L.pneumophila	Intracellular replication	Amoebae/biofilms	Thermotolerant	Intracellular persistence models
Bartonella henselae	Angiogenic factors	Zoonotic pathogen	Intracellular	Comparative study of host vascular responses
A.baumannii	Multidrug resistance	Hospital-acquired	Desiccation-resistant	Resistance gene mapping in marine sediments
Pedobacter lusitanus	Polysaccharide degradation	Soil decomposer	Gliding motility	Enzymatic analogs in annelid gut symbionts
P. melaninogenica	Protease activity	Oral microbiota	Anaerobic	Protease comparison in annelid digestion
Porphyromonas gingivalis	Gingipains	Periodontal pathogen	Anaerobic	Host-microbe interface modeling
Bacteroides fragilis	PULs	Gut symbiont	Bile-resistant	Reference for annelid gut symbiosis
Parabacteroides distasonis	SCFA production	Gut microbiota	Anaerobic	SCFA analogs in annelid metabolism
Odoribacter lanatus	Butyrate synthesis	Gut microbiota	Anaerobic	Butyrate-linked immune modulation studies
Clostridioides difficile	Toxins A/B	Nosocomial pathogen	Spore-forming	Toxin structure comparison in host response
Clostridium perfringens	Gas gangrene toxin	Soil/gut pathogen	Anaerobic	Anaerobic toxin evolution studies
Bacillus subtilis	Endospores, antibiotics	Soil decomposer	Aerobic	Endospore formation parallels in annelid microbiome
Staphylococcus aureus	Coagulase, biofilm	Skin pathogen	Facultative anaerobe	Biofilm structure comparison
Streptococcus pneumoniae	Capsule, pneumolysin	Respiratory pathogen	Aerotolerant	Capsule-mediated immune evasion analogs
Enterococcus faecalis	Bacteriocins	Gut commensal	Facultative anaerobe	Bacteriocin analogs in annelid gut defense
Lactobacillus acidophilus	Lactic acid fermentation	Probiotic	Microaerophilic	Acid tolerance comparison in annelid gut
Leuconostoc mesenteroides	Dextran synthesis	Fermented foods	Facultative anaerobe	EPS production analogs in mucus layers
Paenibacillus polymyxa	Nitrogen fixation	Rhizosphere	Facultative anaerobe	Nitrogen cycling in benthic environments
Mycobacterium sp.	Mycolic acids	Soil/pathogen	Acid-fast	Cell wall structure comparison
Streptomyces sp.	Antibiotics	Soil decomposer	Filamentous	Secondary metabolite analogs in annelid symbionts
Micromonospora	Antitumor compounds	Soil	Spore-forming	Bioactive compound screening in annelid

Species	**Functional Traits**	**Ecological Role**	**Physiological Features**	**Relevance to *Platynereis dumerilii***
echinospora				microbiome
Actinomyces israelii	Sulfur granules	Oral/gut	Filamentous	Sulfur metabolism comparison
Propionibacterium acnes	Lipase, biofilm	Skin	Anaerobic	Lipid metabolism analogs
Bifidobacterium longum	SCFA production	Gut probiotic	Anaerobic	SCFA-linked immune modulation
Kocuria rosea	Pigments	Soil/skin	Aerobic	Pigment biosynthesis comparison
Rhodococcus equi	Cholesterol metabolism	Equine pathogen	Intracellular	Lipid metabolism analogs
Williamsia limnetica	Hydrocarbon degradation	Aquatic	Aerobic	Hydrocarbon cycling in marine annelid habitats
Cereibacter sphaeroides	Photosynthesis	Freshwater	Phototrophic	Phototrophic symbiont comparison
Gloeobacter violaceus	Photosystems I/II	Freshwater	Lacks thylakoids	Comparative photoreceptor evolution
Synechococcus elongatus	Circadian genes	Marine	Oxygenic photosynthesis	Circadian rhythm analogs in *P. dumerilii*
Prochlorococcus marinus	Light-harvesting proteins	Oceanic	Small genome	Comparative phototrophic gene clusters
Lyngbya sp.	Toxins	Benthic mats	Filamentous	Toxin evolution in benthic symbiosis
Microcystis aeruginosa	Microcystins	Algal blooms	Buoyant colonies	Toxin structure comparison
Nostoc punctiforme	Heterocysts	Symbiosis	Filamentous	Nitrogen-fixing symbiont analogs

CONCLUSION

This comparative phylogenetic analysis of 16S rRNA sequences from bacteria associated with *Platynereis dumerilii* reveals evolutionary conservation and clade-specific structural coherence across major bacterial phyla (Proteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria, and Firmicutes). The presence of conserved 16S rRNA motifs suggests functional constraints likely shaped by ecological interactions with the host.

DECLARATION

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Authorship Contributions.

Concept and Writing: Ms. Jae Ann Buenaluz, Dr. Mary Jhane G. Valentino; Critic and final revision: Ms. Jae Ann Buenaluz,, Dr. Mary Jhane G. Valentino, Mr. Wilhelm M. Javier; Dr. Eleonor S. Austria

Conflict of Interest.

There is no conflict of interect between the authors.

Authorship Contributions.

Concept and Writing: Ms. Jae Ann Buenaluz, Dr. Mary Jhane G. Valentino; Critic and final revision: Ms. Jae Ann Buenaluz,, Dr. Mary Jhane G. Valentino, Mr. Wilhelm M. Javier; Dr. Eleonor S. Austria.”

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