Chapter 9

Gut Microbiome Analysis of Snails: A Biotechnological Approach

Mudasir A. Dar, Kiran D. Pawar and Radhakrishna S. Pandit

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Abstract

Mollusks are a diverse group of animals not only at the species level but also with respect to their habitat and behavior. Gastropods comprise 80% of the mollusks with approximately 62,000 living species including snails. Over the period of time, snails have evolved into marine, freshwater and terrestrial forms with a transitional shift in their feeding habits. From prehistoric times, mollusks have established an intimate relationship with humans. These animals are used as food, medicine, offering to gods and are also responsible for economic losses in the form of agricultural pests. As most of these animals feed on plant biomass, their guts have evolved to digest such lignocellulosic biomass with extraordinary efficiency. The plant fiber digestion in their guts depends predominantly on the metabolic activities of the gastro-intestinal microflora. Besides digestive functions, the seasonal dynamic and spatial distribution of bacterial gut community largely influences cold hardiness and many other metabolic properties in snails. Here, we assessed an overview of the various bacterial populations dwelling in digestive tracts of snails. This chapter provides insights into the gut microbiome of various snails that can be exploited for various industrial applications such as biomass degradation, production of biofuel, paper, wine and laundry detergents.

Keywords: Mollusca, snails, gut microbiome, symbiont, bacteria, industrial uses

1. Introduction

The phylum Mollusca is one of the most diverse groups of animals on earth that comprises 50,000 living species. Mollusks are soft-bodied animals that inhabit almost every kind of habitat. These are dominantly free-living metazoans that possess a calcareous exoskeleton to provide structural support for a muscular foot and enclose mantle cavity which is generally used for

feeding, respiration and sometimes locomotion [1]. It constitutes the second largest, and most variable, invertebrate phylum. The living species of the mollusks are divided into seven classes, that is aplacophora, polyplacophora, monoplacophora, gastropoda, cephalopoda, pelecypoda and scaphopoda [2]. Gastropods are the largest group of mollusks, comprising about 80% of the living mollusks with ca. 62,000 living species. The first gastropods originated during the late Cambrian period and over 500 million years ago. Since then, gastropods have radiated into marine, freshwater and terrestrial environments, changing their food preferences from herbivorous to carnivorous, endo-parasitism or symbiont-mediated chemoautotrophy [3].

The class gastropoda is the most speciose among animals to inhabit a variety of habitats such as oceans, rivers, etc. and are the ones that have inhabited the land among mollusks [4]. The aquatic forms have adapted to benthic forms while others remained pelagic. The life span ranges from months to decades [5, 6] and in some cases life is marked by varying periods of dormancy [7]. All gastropods are commonly called head-foot or cephalopodium which is a typical character of all gastropods because the head and foot arise from the same region making it very difficult to differentiate where the head ends or the foot begins [8]. The head of gastropods typically has two or four sensory tentacles with eyes and a ventral large foot, which gives them their name (in Greek, *gaster* is stomach and *poda* is feet). The anterior division of the foot, that is, propodium, is used for crawling. The shell in the larval stage is called protoconch. Most gastropods have a shell that typically opens on the right-hand side. Several species have operculum that is used to close the shell opening.

Most species of gastropoda include slugs and snails where the snails possess coiled shells on their body. The term snail is often used to describe marine and freshwater snails, along with terrestrial ones. More generally, the term is applied to land snails than to those from the sea or freshwater [9]. Snails generally thrive in habitats rich in calcium, limestone, marl and places with concrete and cement. They are hermaphrodite but reciprocal copulation is required to produce viable eggs. Eggs are laid 8 days after copulation producing about 400 to 1000 eggs per year [10]. Cool and moist soil is necessary for the egg hatching producing juvenile snails that eat their egg shells and remain burrowed for 2 weeks. The juveniles feed on tender shoots of plants while the adult can also digest detritus. Under unfavorable conditions, snails can bury themselves under soil and remain inactive from months to years [11].

The terrestrial snails like *Achatina fulica*, *Achatina achatina* and *Archachatina marginata* are large-sized terrestrial mollusks that can grow up to 20 cm in length and 10 cm in diameter. In these snails, the brownish shell having dark stripes generally covers half of the body [12]. Among these, the shell of *A. fulica* is smaller that can grow up to 3–4 inches, while *A. achatina* has a larger shell size of 10–11 inches [9].

Snails are both ecologically as well as economically very important animals. In the modern era of technology, the utility of snails is largely neglected, particularly in developed countries. Since snails dwell in a variety of niches, they could harbor a militia of micro-biota which could be exploited for various biotechnological purposes. This work provides insights into the microbiome of various snails. Furthermore, for the first time, we assessed the probable applications of snails in general and their gut micro-biota in particular for various biotechnology-based industries.

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2. Origin and distribution of snails

The families Lymnaeae and Planorbidae originated from the common ancestor approximately 250 million years ago during the Permian period. Some fossils belonging to family Bulininae and Planorbinae of the upper cretaceous have been obtained from Africa and India [13, 14]. The first fossil record for the family Achatinidae was obtained from the Pleistocene in Africa [15, 16] but the family clearly evolved much earlier. In the 1950s, Mead described the earliest achatinids that originated in Cameroon and Gabon, northward of the river Zambezi in Africa, which later spread to both arid and the sub-arid areas of the southern continent and other moist parts east of the great watershed [17, 18]. This indicated that temperate species were directly evolved from tropical ancestors. Nonetheless, little is known about the evolutionary history of the achatinids.

The habitats of terrestrial snails range from dense tropical forests in Africa to the fringing riparian forests of Savannah [19, 20]. The members of the family Achatinidae comprise more than 200 species in 13 genera that are native to Africa. Several species have attained pest nature within their native African range when the habitat was modified by human activities and cropping. Furthermore, due to the increased mobility of humans and globalization of trade and travel, several alien species have been accidentally or purposefully transported to areas outside of the African continent. In these new areas, Achatinidae have caused significant economic and ecological impacts [21]. Due to its invasive capacity, *Achatina fulica* has spread from East Africa to many regions around the globe including rainforests, tropics, subtropics, etc. Apart from anthropogenic activities [22], the higher adaptability of this snail to variety of habitats is often contributed by its gut micro-biota that it selectively chooses from the favorable environments for successful dispersion [23]. However, terrestrial species have a great capacity of adaptation, survival and may contain an intriguing micro-biota serving in the efficient degradation [24] of ingested lignocellulosic plant biomass into many useful products. Due to its fast distribution and voracious feeding, this species is now considered as the most destructive terrestrial gastropod [12]. The *A. fulica* has been blamed as an intermediate vector of many worms and microorganisms, causing a variety of ailments [25]. The species was introduced to the USA in 1939, to India in 1947 near Kolkata and to Brazil in 1980s.

The widespread distribution of *A. fulica* is caused by a number of factors [26]. Sometimes, it has been deliberately introduced by humans as pet and in some cases as a source of food or for ornamental and medicinal purposes (**Figure 1**). It is also transported unintentionally with agricultural, horticultural and other commercial products or in containers in which they are shipped. They were also transported accidentally with military equipment in many countries [27]. The land snail, *A. fulica,* spreads extensively along rivers and streams, either on floating mats of vegetation or by surviving long enough in the water to float downstream.

The pulmonate snails are native to Africa but are currently found in Asia, the Pacific, Madagascar, Indonesia, Australia, the Caribbean Basin, the United States and South America (Colombia, Venezuela, Ecuador, Brazil and Argentina) [28, 29].

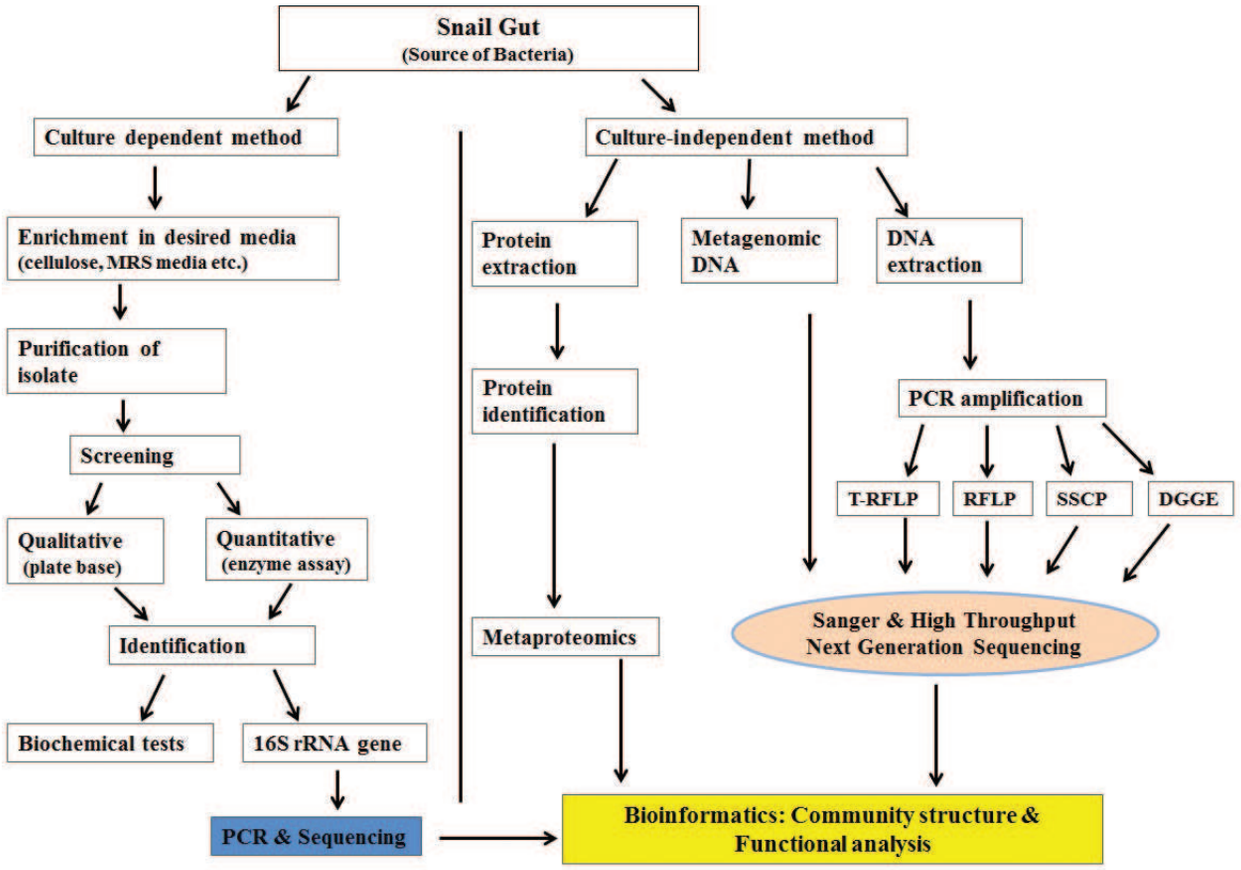

Figure 1. Different methods involved in the isolation and identification of microbes of the snail gut.

3. Uses of snails

From prehistoric times, it is quite evident that mollusks have a precarious relationship with humans. Many snails are known to damage the wooden ships and poison scuba divers. Researchers have also found that snails actually harbor a secret that could help humans to stay healthy and pain free. Even some authors quoted that guts of mollusks contain a unique set of microorganisms that might save human lives. During evolution, snails have also coevolved with ancient bacteria that reside in their guts. In return, the bacteria also express some drug-like molecules that help the snail's proper functioning and ward off diseases [30]. For example the *Leuconostoc mesenteroides* strain isolated from the gut of *Cornu aspersum* produced some bacteriocins-like substance that inhibited the growth of the pathogen *Propionibacterium acnes* [31].

3.1. Snails as food

The gastropods, particularly snails, have been used both as food as well as treatment for a variety of human diseases. The fossil remains of prehistoric shellfish found in caves indicate that snails have served as a delicacy for humans for thousands of years. The snails are easy to culture and majorly composed of muscles. Snails are a rich source of proteins containing high amounts of essential amino acids [32]. From the twentieth century, the food qualities of snails

were so appreciated that it was a highly sought-after food. They are preferred as a food source in certain parts of Africa, Asia and South America. In recent decades, the snail's consumption has increased throughout European countries, which consequently lead to their gradual disappearance from freely dwelling areas. This decrease in population contributed to predation of the species and introduction of pathogens that harmed the productivity of snails [33, 34]. The inedible parts of snails are also used in animal-feed preparations as shown in **Figure 1**.

3.2. Medicinal uses

Hippocrates reportedly said that crushed snails can be used to relieve inflamed skin and pain. Two decades ago, slime of the Chilean snail was reported to quickly heal the skin lesions with no scars. This innovation later led to the production of "*Elicina*" which is snail slime-based fairness products. Recently in 2010, Missha, a USA-based cosmetic company launched a branded fairness cream, "Aqua Cell Renew Snail Cream", containing 70% slime. The company also claimed that this cream reduces pigments, acne, scars and combats wrinkles [35]. Though snail slime contains unusual crystals of calcite, it may find some use in orthopedics also. This is because scientists at the Herriot-Watt University stated that calcite may be used for the development of bone cement by using inorganic crystals in organic matrix [36].

3.3. Religious importance of snails

In southern Miami, snail invasion is very severe because they are linked to religious rituals. In Candomblé religion, coloration of the shell is considered very important for offerings to their gods, Orishas, and symbolizes the personality of an Orisha (e.g., red indicates fire and fury, white indicates tranquility and age while yellow is for prosperity and wealth). However, the color preference can vary between nations of different areas of the religion. For example, sacrificial animals or their parts that are offered to Obatala (white Orisha) should be completely white such as the white blood of *A. fulica* [37].

3.4. Ecological importance

Some snails that climb the trees rasping on the surface of leaves can influence biosphere community succession and nutrient cycling. Snails also provide some antimicrobial barriers to the plants by secreting the wax layer which contains antimicrobial compounds [38]. Mucus secreted by the gastropods has been shown to have selective antimicrobial properties as well [39, 40]. Moreover, some snails are also used for monitoring the environmental pollution. Such is the species of *Arianta arbustorum*, which can tolerate higher concentrations of the heavy metals, like cadmium, lead and copper, indicating elevated levels of metal pollution in their niches [41].

4. Impact of snails on agriculture

Many researchers have reviewed the impacts of invasive mollusks on agriculture [42, 43] biodiversity and human health. However, the annual costs associated with damage to the environment and agriculture due to alien species in the USA have been recently estimated to be

US$120 billion. The combined costs associated with damage for the United States, the United Kingdom, Australia, South Africa, India and Brazil have been estimated as US$314 billion per year [43]. In the tropics, the loss caused by the snails is threefold. Primarily, there is loss of the agricultural products followed by the cost of labor and materials associated with the management of such pests. Lastly, there is opportunity losses related to the changes in agricultural practice such as cultivation of pest-resistant species only.

Among mollusks, the giant African land snail, *A. fulica* tops the list of agricultural pests. *A. fulica* (*Lissachatina fulica*) is a herbivore, feeding primarily on vascular plants [21] and plant tissues containing high protein and calcium content [44, 45]. All *Achatinidae* species need calcium for the formation of shell and reproduction. Thus, environments rich in calcium carbonate, such as limestone landscapes having a pH of 7.0–8.0, and urban areas with abundant concrete are preferred [28].

The adult snail of *A. fulica* daily consumes large quantity of plant material approximately 10% of its weight [46]. The seedling stage of plants is most preferred and vulnerable. The extent of damage is based on the chemical composition of the plant and varies spatially as well as temporarily [47]. Many researchers have stated that infestations by snails to the nursery stage are so severe that demands change in cultivation practice. For example, in Malaysia, Guam and Indonesia, during the season of peak infestations of *A. fulica*, it is almost impossible to grow vegetables [27, 48, 49].

A. fulica is considered the most damaging land snail in the world as it can dwell on over 500 different crop species. It is a non-host specific pest of crops like peanuts, beans, peas, cucumbers and melons. If fruits and vegetables are not available, snails can feed on variety of ornamental plants, tree barks and even paint on houses [21]. The snail also allies with other soil invertebrates to decompose the leaf litter [50] and is the most destructive pest; it is ranked second among the 100 worst alien invasive species [51]. It affects tropical and subtropical areas, causing large damages to farms, commercial plantations and domestic gardens. It can also be found on trees, decaying materials and next to garbage deposits [17]. In urban areas, the deposition of solid waste by humans is primarily responsible for the proliferation of pests [12]. This species has attained pest status also due to its voracious feeding, competing for physical space with the native fauna resulting in disequilibrium of biodiversity [12]. Apart from being an agricultural nuisance, snails can thrive in cities, crawl up the walls of buildings and skid cars on highways [27].

5. Control strategies for snails

Snails are important both ecologically as well as economically due to a variety of factors. The prolific breeder *A. fulica*, soon after the introduction to a new habitat, reproduces at alarming rates making the control strategy very difficult. The control strategy of the pest is based on physical, chemical as well as biological methods. The physical control includes collection and destruction of snails and their eggs from the infested site or campaigns organized by local agencies voluntarily supported by health service officials, local people, students and teachers. After collection, snails are crushed and buried deep into the soil, covered with kaolin. Eradication of the species involves a huge amount of chemicals, hand collection and extensive

public awareness programs like posters, documentaries, etc. Metaldehyde is the principal component of molluscicides and is indiscriminately used for the control of the snail *A. fulica,* consequently causing loss of productivity of local crops. For example, in Sao Paulo, farmers unknowingly used the molluscicide "metaldehyde" in banana fields to target snails, which killed many species including bats, skunks, lizards and small rodents which were beneficial as natural control agents of agricultural pests [12]. The physical methods are very time-consuming and tedious while the chemicals have resistance problems, killing the non-target flora and fauna. Therefore, biological control is the option that seems very fruitful and ecofriendly. But predatory snails (e.g., rosy wolf snail: *Euglandina rosea*) and flatworms have also failed to control some species such as *A. fulica* [52, 53]. As snails are ecologically and economically important due to the pest nature, the bacterial flora present in the gastrointestinal (GI) tract of snails may have an important role in digestion. These functionally specialized GI tract regions may be unique microenvironments and could harbor unusual bacterial communities.

6. Process of digestion in snails

In an ecosystem, the ability to procure enough food is pivotal for the survival of an organism. Feeding is necessary for the maintenance of metabolism, growth and reproductive success of animals. The process of digestion is characterized by a specific set of enzymes that often break the refractory food substances [54]. The alimentary tract of land snails is remarkably simple, possibly because of terrestrial life styles. The alimentary canal is usually divisible into buccal mass, esophagus, crop, stomach, intestine and rectum along with appendages like salivary and digestive glands (hepatopancreas) [55]. In *A. fulica,* like other gastropods, the food scraped by radula and ingested by the buccal mass is mixed with the secretions of the salivary gland and accumulates in the crop (ingluvius), a distensible muscular compartment. The crop and stomach are filled via two cannaliculi with the juice produced by the digestive glands. The medial part of the gut is surrounded by the digestive gland, which secretes more enzymes into the mid-gut lumen and also absorbs nutrients. The epithelium of the digestive tube is ciliated along almost its entire length, allowing the food to mix with digestive juices and helping to transport the alimentary mass. The ciliated epithelia also allow the microbial flora to anchor on the digestive tube [56]. The gut of the giant African land snail, *A. fulica,* is large enough to act as a fermentation vessel where a number of metabolic reactions are mediated by the host symbionts. The unabsorbed part of the alimentary mass (bolus) is compacted and passed directly into the rectum. The snail's digestion is primarily extracellular [55].

7. Role of the gut bacteria in snails

The gastrointestinal tracts of animals are modified as per their food requirement and physiological adaptations. All the herbivores that feed on lignocellulosic feed stock share two common features, that is enlarged digestive tract and gut micro-biota. Digestive tract is usually long enough having different regions such as esophagus, crop, rumen, caecum and rectal paunch while gut microbes provide the host with a unique set of necessary enzymes for the digestion of plant materials [57, 58]. The guts of herbivores that largely feed on lignocellulosic

rich plant materials act as natural bioreactors for the degradation of plant biomass making them efficient sources of industrially important bacteria [59]. In many herbivores and omnivores, the digestion of the plant biomass is of immense importance for the energy capture [60]. Therefore, bacterial flora present in the GI tract of these animals may have an important role in digestion. These functionally specialized GI tract regions may be unique microenvironments and could harbor unusual bacterial communities.

7.1. Abundance of bacterial symbionts in snails

During the past century, scientists have focused on microbes that secrete the cellulose hydrolyzing enzymes. For instance, Seillière [61] pioneered the isolation of bacterial cellulases from the gut of the terrestrial gastropod *H. pomatia*. Similarly, Florkin and Lozet [62] studied the cellulases, whereas Jeuniaux [63] observed that chitinases from *H. pomatia*, of microbial origin, played a major role in the digestion of plant components in all phytophagous snails.

Charrier et al. [64] observed that density of bacteria in *C. aspersum* and *H. pomatia* was up to 5.10^9 CFU g^{-1} fresh tissue in the distal intestine, while in proximal region it was from 10 to 1000 fold lower than in the distal part. The *H. pomatia* was the least colonized by bacteria. The *C. aspersum* that fed on carboxymethyl cellulose (CMC) harbored approximately 10^7 g^{-1} bacteria and while those fed on native cellulose contained 10^6 g^{-1} [65]. In another study carried out in aerobic and anaerobic conditions by the same authors, it revealed that gram-positive bacteria were in the range of $1.57 \times 10^9 \pm 0.10 \times 10^9$ $CFUg^{-1}$ in the intestine. Although the score of gram-negative aerobic bacteria accounted for $5.77 \times 10^8 \pm 1.35 \times 10^8$ $CFUg^{-1}$ in the intestine, but it comprised only 27% of the total bacterial load in *H. aspersa* [66]. However, Simkiss observed only 0.71×10^6 CFU g^{-1} body weight in *H. aspersa* [67]. In a similar report, researchers [68] noted less than 10^6 g^{-1} bacteria growing on sterile paper. In the intestine of *Tegula funebralis*, the number of culturable bacteria was 25×10^5 only [69].

Several strains growing on chitin have been isolated from different species of snails such as *C. gillenii*, *B. agrestis*, *B. noackiae* and *E. malodoratus*. The presence of chitinolytic bacteria in *H. pomatia* has been reported by Jeuniaux [63] where he observed the bacterial density in the range of 10^6 $CFUg^{-1}$ of the tissue. By culture-dependent method, Pawar with his coauthors [70] enumerated from 10^3 to 10^6 CFU from the whole GI tract of *A. fulica*. Koleva et al. [31], while studying the gut bacteria of *C. aspersum*, stated that bacterial diversity varies with the different stages of life cycle and accounted for maximum 1.6×10^9 CFU ml^{-1} gut extract during the active stage. Since more than 95% of the bacteria in any environment including guts of animals are un-culturable, their composition and community structure cannot be studied completely by culture-dependent approaches. As most of these studies were done using culture-dependent approaches, they might have not revealed much of bacterial composition and community structure in the GI tract of snails. More research is needed to study the bacterial diversity of snails by using advanced *in-silico* and meta-genomic approaches, harnessing the vast diversity of microbes in the snail guts. Very few studies have been carried out to analyze the bacterial populations in snails by using metagenomic methods. The complete details of the processes and protocols involved in the isolation and identification of the gut microbes are beyond the scope of this chapter, however, briefing the outline of most of these methodologies would be helpful. The brief outline of all these methodologies is given in **Figure 2**.

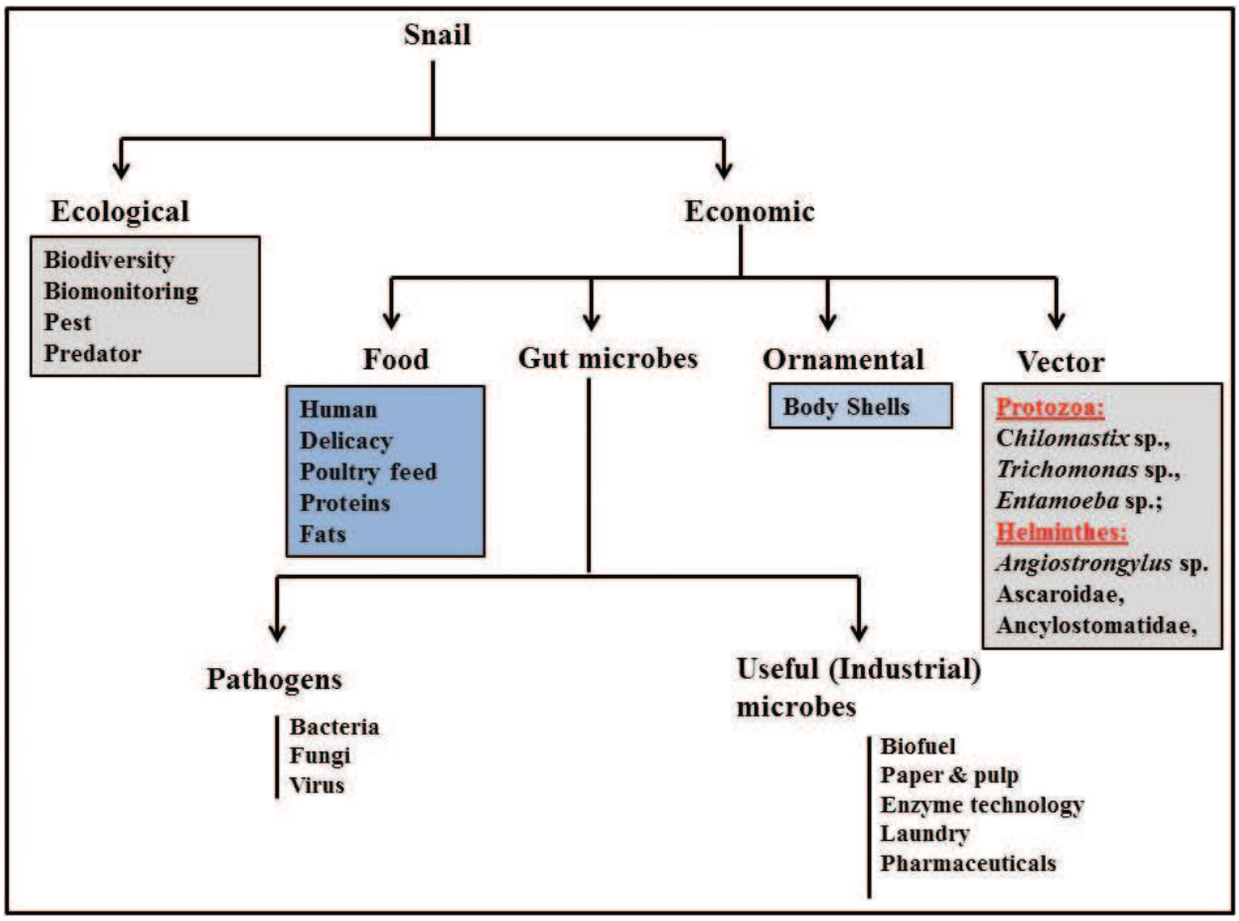

Figure 2. Ecological, economic and industrial utility of snails.

8. Host-symbiont interactions

Recent evidence for the presence of various kinds of bacteria in the snails suggested that a symbiotic relationship is developed between the host and the microbes during the course of evolution. Hitherto, a large number of eukaryotic symbionts have been isolated from snails in the families particularly, Achatinidae, Ampullariidae, Helicidae, Planorbidae, etc. as given below in **Table 1** [71]. Further, identification of the isolated gut bacteria has been done in vetigastropods of the genus *Haliotis* and in several other pulmonates. Among pulmonates, representatives of the genera *Biomphalaria*, *Bulinus*, *Helisoma* [72], *Helix*, *Cornu* [64, 66] and *Achatina* [70, 73, 74] have also been studied.

The advanced techniques like meta-genomics have proved that the gut bacteria perform many beneficial activities for the host. These resident bacteria help the host in processes such as digestion of complex molecules into simpler forms, generating energy, synthesis of cofactors, amino acids for basic metabolism as well as preventing the growth of pathogens. Some of the bacteria isolated from the snail caused the fermentation of sugars like glucose, lactose, mannitol, rhamnose, arabinose, maltose, etc. showing the positive interaction of the snails with their gut flora [75]. Some authors [40] reported the presence of several bacterial OTUs belonging

Sr. No.	Snail	Habitat of snail	Family	Microbes studied	Methodology	References
1	*Achatina achatina*	Terrestrial	Achatinidae	Bacteria	Biochemical	[9]
2	*Achatina fulica*	Terrestrial	Achatinidae	Bacteria, fungi, virus, protozoa	16Sr RNA/ metagenomics/ Microscopic	[23, 70, 73, 74, 137]
3	*Achatina mustelina*	Terrestrial	Achatinidae	Bacteria and fungi	Metagenomics	[40]
4	*Archachatina marginata*	Terrestrial	Achatinidae	Bacteria and fungi	Biochemical	[119, 138]
5	*Batillus cornutus*	Marine	Turbinidae	Bacteria	16S rRNA	[139]
6	*Helix aspersa/Cornu aspersum*	Terrestrial	Helicidae	Bacteria and yeast	16S rRNA/ Biochemical	[31, 67, 68, 140, 141]
7	*H. pomatia*	Terrestrial	Helicidae	Bacteria	Metagenomics	[131]
8	*Indoplanorbis exustus*	Freshwater	Planorbidae	Bacteria	Biochemical	[75]
9	*Lymnaea stagnalis*	Freshwater	Lymnaeidae	Bacteria	Biochemical	[75]
10	*Pomacea canaliculata*	Freshwater	Ampullariidae	Bacteria	16S rRNA	[71]
11	*Pila globosa*	Freshwater	Ampullariidae	Bacteria	16S rRNA	[142]
12	*Pila ovata*	Freshwater	Ampullariidae	Bacteria	Biochemical	[115]
13	*Tegula funebralis*	Marine	Tegulidae	Bacteria	Biochemical	[69]
14	*Trochus niloticus*	Marine	Tegulidae	Bacteria	Biochemical	[143]

Table 1. Species of snails that have been used for isolation of microorganisms.

to oceanospirillales, enterobacteriaes, alteromonadales, along with α-Proteobacteria and Rhizobiales in the fecal samples of *Achatinella mustelina*. Some snails thrive in toxic habitats like deep sea vents due to energy provided by the bacteria. The scaly foot snail, *Chrysomallon squamiferum*, discovered from the Kairei vent of Indian Ocean, flourishes by using a similar strategy, exploiting energy harnessed by the gut symbionts. That is why this snail can grow to up to 45 mm in size, when most of its close relatives did not grow beyond 15 mm in the absence of endosymbionts [76].

The physiology and diet of the host are the main components that determine the community structure of an organism. The gut microbiome of many animals including snails has been characterized recently [23, 70]. Animals are known to choose their gut microbes selectively/ functionally, and the microbial cells outnumber their hosts by many folds [77, 78]. Snails, like other invertebrates, eat soil to get the useful microbes that may augment in digestion. In turn, micro-biota provides important implications to the host's immune system [79] preventing invasion by exogenous pathogenic microbes [80, 81]. This in other words indicates that changes in microbial flora of the snail could have a negative impact such as without which they may stop feeding and ultimately die [82].

8.1. Cellulose-degrading bacteria

The plant biomass is comprised of three major components that is cellulose (50%), hemicellulose (30%) and lignin (20–30%). All herbivores do not possess the ability to digest plant polysaccharides and instead depend on their gut symbionts to derive the nutritionally important compounds from the ingested material [83–85]. Therefore, many researchers have extrapolated the gut microbiomes of many animals by using meta-genomics approach. Such studies have revealed that the gut of herbivores is a home to a consortium of microbes that have evolved to efficiently degrade and ferment the plant cellulose ingested by the host [86, 87]. These organisms possess a complex enzyme system known as cellulosome, and the complete enzymatic system includes three different enzyme types, that is exo-β-1, 4-glucanases (EC 3.2.1.91), endo-β-1, 4-glucanases (EC3.2.1.4) and β-1, 4-glucosidase (EC 3.2.1.21) along with several cofactors [88]. Cellulases act by hydrolyzing the β-1, 4 bonds in cellulose, releasing some small chains of oligosaccharides which are concurrently broken into monosaccharides by β-glucosidases [89]. The hydrolysis of lignin occurs due to the concomitant action of a specific set of enzymes such as laccase, lignin peroxidase, etc. In lignin degradation, the ligninolytic enzymes primarily alter the structural conformation of lignin by breaking several stable bonds resulting in production of free radicals [90]. From application point of view, bacteria are generally preferred over the fungi due to their higher growth rate allowing fast production of recombinant proteins [91]. Additionally, some glycoside hydrolases (GHs) of bacterial nature form multi-enzyme complexes called cellulosome provide increased synergy, stability and catalytic efficiency [92], while others are multifunctional, harboring both endoglucanase and xylanase activities [93]. A list of different groups of bacteria can be isolated from snails and thereby exploited for industrial applications. Therefore, enzymes of bacterial origin could offer specific biotechnological interests due to their less dependency on mediators. However, the lignocellulose-hydrolyzing enzymes secreted by bacteria are inducible, extracellular and cell associated [90]. Recently, Chang and his team [94] isolated a *Bacillus* strain that has a repertoire to remove lignin from rice straw; this biomass can be subsequently treated with lactic acid bacteria (LAB) to improve the sugars yield. These sugars can be further utilized for the production of bioethanol, biogas and bio-hydrogen by fermentations [70].

Some of the microbes such as bacteria *Fibrobacter succinogenes*, *R. flavefaciens* and *R. albus* [95] and some fungi are primarily responsible for degradation of plant cell walls. *R. albus*8 is anaerobic, fibrolytic and gram-positive bacterium present in herbivores and can degrade both cellulose and hemicellulose [60, 96]. But *R. flavefaciens* and *R. champanellensis* are very efficient cellulose degraders due to their cellulosome secretion which is lacking in case of *R. albus*8 [97].

The symbiotic bacteria from the gut of gastropods are considered to participate in the digestion of carbohydrates, such as cellulose and hemicellulose comprising the major part of the plants (**Table 2**). Recently, we reported the presence of lignocellulolytic bacteria in the GI tract of *A. fulica* [73]. However, Koch et al. [71] reported that *P. canaliculata* can survive till 56 days on a cellulose-rich diet and concluded the existence of bacterial endoglucanases that helps the snail to utilize cellulose polymer. Earlier studies [65, 68] showed that *H. aspersa* contains very few cellulose-degrading bacteria though some authors [64] claimed the complete absence of these bacteria in the gut. Many authors have demonstrated the degradation of native cellulose, mannan and laminarin by the snails [98, 99], thereby a large set of bacteria producing hydrolytic enzymes may be involved. The cellulases of animal origin were first studied by

Sr. No.	Snail species	Bacteria	NCBI accession no. (16S rRNA)	Gram stain	References
1	*Achatina fulica*	*Klebsiella pneumoniae*	AB680060	−ve	[23, 73, 74]
2		*Sphingobacterium mizutaii*	NR042134	−ve	
3		*Sphingobacterium multivorum*	FJ459994	−ve	
4		*Microbacterium* sp.	AB646581	+ve	
5		Uncultured *Flavobacterium* sp.	DQ168834	−ve	
6		*Aeromonas punctata*	NR029252	−ve	
7		*Microbacterium* sp.	AB646581	+ve	
8		*Klebsiella variicola*	NR025635	−ve	
9		*Aeromonas caviae*	AB626132	−ve	
10		*Aeromonas caviae*	JF920485	−ve	
11		*Streptomyces kunmingensis*	NR043823	+ve	
12		*Cellulosimicrobium* sp.	AB188217	+ve	
13		*Cellulosimicrobium funkei*	JQ659848	+ve	
14		*Klebsiella* sp.	AB114637	−ve	
15		*Enterobacter* sp.	JQ396391	−ve	
16		*Stenotrophomonas* sp.	DQ242478	+ve	
17		*Cellulosimicrobium cellulans*	AB166888	+ve	
18		*Cellulosimicrobium* sp.	HM367604	+ve	
19		*Agromyces allii*	NR_04393	+ve	
20		*Nocardiopsis* sp.	HQ433551	+ve	
21		*Microbacterium binotii*	JQ659823	+ve	
22		*Bacillus subtilis*		+ve	
23		*Ochrobactrum* sp.	KJ669202	−ve	
24		*Achromobacter xylosoxidans*	KJ669206	−ve	
25		*Klebsiella* sp.	KJ669189	−ve	
26		Enterobacter sp.	KJ669197	−ve	
27		*Enterobacter cloacae*	KJ669195	−ve	
28		*Bacillus.* sp.	KR866144	+ve	
29	*Archachatina marginata*	*Bacillus subtilis*	NA	+ve	[119]
30		*E. casseliflavus*	NA	+ve	
31		*Streptococcus faecalis*	NA	+ve	
32		*Staphylococcus aureus*	NA	+ve	
33	*Pomacea canaliculata*	*Nostoc* sp.	NA	−ve	

Sr. No.	Snail species	Bacteria	NCBI accession no. (16S rRNA)	Gram stain	References
34	*Helix aspersa*	*Pseudomonas* sp.	NA	−ve	[68, 133]
35		*Xanthomonas* sp.	NA	−ve	
36		*Acinobacter* sp.	NA	−ve	
37		*Vibrio* sp.	NA	−ve	
38		*Enterobacteriae* sp.	NA	−ve	
39		*Bacillus* sp.	NA	+ve	
40		*Staphylococcus* sp.	NA	+ve	
41		*Micrococcus* sp.	NA	+ve	
42	*Bulinus africanus*, *Biomphalaria pfeifferi*, *Helisoma duryi*	Chloroacidobacteria	NA	−ve	[72]
43		Chryseobacterium	NA	−ve	
44		Comamonadaceae	NA	−ve	
45		*Bacillus* spp.	NA	+ve	
46		*Aeromonas* spp.	NA	−ve	
47		Verrucomicrobiae spp.	NA	−ve	
48	*Batillus conutus*	*Bacillus* sp. JMP A	HM776393	+ve	[139]
49		Bacillus sp. JMP B	HM776394	+ve	
50		*Staphylococcus* sp. JMP-C	HM776395	+ve	
51	*Pila globosa*	*Klebsiella oxytoca*	KF017601	−ve	[142]

NA: not available.

Table 2. Cellulose degrading bacteria isolated from the digestive tract of different snails.

Biedermann and Moritz [100], in *Helix* spp., at the end of nineteenth century. Further, snails possess a micro-biota specialized in a variety of functions, thus contributing to an extraordinary (up to 80%) efficiency to digest plant biomass [24]. The abundance of carbohydrate-secreting bacteria and the rate of enzyme activity in various parts of the herbivorous guts are inversely proportional to each other, therefore, bacteria have become complementary for digestion of food. However, Payne et al. [101] also reported that wherever the enzyme production is less or nil, the enzymes released by the gut microflora would be of much help for digestion. The bacterial glycoside hydrolase (GH) genes and carbohydrate-binding modules (CBMs) are abundant in the digestive tract of animals [84, 102–106] which suggest the potential role of microbial symbionts in the hydrolysis of plant material to help extract nutrients [107]. The metagenomic and *in silico* studies have proved that gut symbionts perform useful functions to the host such as production of amino acids, energy generation and act as a barrier against diseases [108]. Recent works by researchers [23, 72] using advanced microbiological techniques elucidated that snails contain a vast array of microbial diversity within their guts.

8.2. Lactic acid bacteria

The lactic acid bacteria (LAB) comprise a significant proportion of the gut-bacterial communities of many animals including pigs, fowls, rodents, chicken, horses, gastropods and insects. These bacteria are vital for the host as they behave as protagonists in maintaining the ecological equilibrium between the different species of microorganisms inhabiting these environments. This microbial community takes part in the fermentation of the food, providing energy to the host [64]. Koleva et al. [31] isolated 55 strains of LAB from the gut of *C. aspersum* (**Table 3**). Based on 16S rRNA sequencing, *Lactobacillus* (18), *Enterococcus* (17), *Lactococcus*

Sr. No.	Snail	Bacteria	NCBI accession no. (16S rRNA)	Gram stain	References
1	*Helix pomatia*	*Buttiauxella agrestis*	DQ223869	−ve	[64]
2		*Citrobacter gillenii*	DQ223882	−ve	
3		*Buttiauxella agrestis*	DQ223871	−ve	
4		*Lactococcus lactis*	DQ223875	+ve	
5		*Kluyvera intermedia*	DQ223868	−ve	
6		*Lactococcus sp.*	DQ223877	+ve	
7		*Obesumbacterium proteus*	DQ223874	−ve	
8		*Enterobacter amnigenus*	DQ223879	−ve	
9		*Enterococcus raffinosus*	DQ223885	+ve	
10		*Enterococcus malodoratus*	DQ223886	+ve	
11	*Cornu aspersum,*	*Buttiauxella noackiae*	DQ223870	−ve	[66]
12		*Clostridium* sp.	DQ223883	+ve	
13		*Raoultella terrigena*	DQ223873	−ve	
14		*Enterobacter amnigenus*	DQ223878	−ve	
15		*Citrobacter gillenii*	DQ223881	−ve	
16		*Enterococcus casseliflavus*	DQ223887	+ve	
17		*Citrobacter sp.*	DQ223880	−ve	
18	*Helix aspersa*	*Lactobacillus brevis*	NA	+ve	[31]
19		*Lactobacillus plantarum*	NA	+ve	
20		*Lactococcu lactis*	NA	+ve	
21		*Weissella confusa*	NA	+ve	
22		*Lactobacillus* curvatus	NA	+ve	
23		*Enterococcus* mundtii	NA	+ve	
24		*E. faecium*	NA	+ve	

NA: not available.

Table 3. List of lactic acid bacteria used by snails in fermentation of digested food.

(12) and *Leuconostoc* (7) accounted for 33, 32, 21 and 13% of the bacterial diversity, respectively, including the strains belonging to genus *Weissella*. Among these genera, *Enterococcus* and *Lactococcus* exhibited the lactic acid activity, thereby indicating their role in the digestive physiology of the snail. However, the LAB are also reported to have a stimulatory response in a marine gastropod *Nassarius obsoletus* [109]. The epiphyte enterococci being the dominant lactic acid bacterium in the snail's intestine is quite interesting. *Lactococcus lactis* is a nonpathogenic bacterium that has been extensively used in the dairy industry for the manufacture of buttermilk, yogurt and cheese. These microbes are also routinely used in the fermentation process of wines, beer, bread and pickles.

Enterococcus, a LAB, inhabiting the gut of many herbivores, is considered as beneficial for the hosts because it forms a biofilm-like structure on the gut epithelium which could prevent the host gut from colonization of pathogenic microbes [110]. The members of the genus *Enterococcus* also produce some bacteriocins. The synergistic effect of this biofilm formation and production of antimicrobial compound probably impedes the entrance and establishment of perilous pathogens in the snail gut [111, 112].

8.3. Proteolytic bacteria

Proteases are enzymes that perform proteolysis, that is, hydrolysis of peptide bonds between two amino acids of a polypeptide chain. Protease enzymes are ubiquitous [113] in nature. Some proteases determine the lifetime of functional molecules like hormones, antibodies, or other enzymes that are very important for physiological processes. In the present era of advanced technology, more research is being done on eco-friendly products replacing the chemical processes by using enzymatic methods. Proteases have a high demand in industries like bread and meat industry, pharmaceuticals and agro-waste disposal management [114]. They are widely used in the film industry for recovery of silver from X-ray films, in the chemical industry for peptide synthesis, in the feed and food industry for production of protein hydrolysates, by waste processing companies, in the field of textile processing for degumming of silk and processing of wool and in the manufacture of detergents, pharmaceuticals and leather [115].

Though produced by many microorganisms, that is fungi, yeast, actinomycetes and molds, the proteases of bacterial origin are considered as most significant [116] because bacteria can be manipulated genetically to generate new enzymes with desired properties for the specific applications [117]. The bacterial proteases constitute about two-thirds of the industrially important enzymes and account for about 60% of the total worldwide sale in markets. Protease-producing bacteria are also useful for the ecosystem as these microbes decompose the dead and decaying animal or plant matter that is primarily composed of proteins. They can create pollution-free environment and are responsible for the recycling of nutrients.

Ariole and Ilega [115] isolated the proteolytic *Pseudomonas aeruginosa* from the gut of freshwater snail, *Pila ovata*. They concluded that this bacterium augmented the snail in degradation of nutrients showing a maximum proteolytic activity of 372 U/ml at pH 9. The saprophagous nature of *H. pomatia* suggests that its gut can be a site for protein digestion [118]. Proteolytic

activity contributed by the bacteria was also reported by Koleva et al. [31] in the gut of *C. aspersum* during the actively feeding stage.

In the African snail, *A. marginata*, the five-cellulase-and-protease-positive bacteria, belonging to genus *B. subtilis, S. aureus, S. casseliflavus* and *S. faecalis*, have been studied [119]. Few researchers have reported the protein digestion augmented by the gut symbionts in case of gastropods [120–122], with a 32-kDa protease present in gut lumen and midgut gland of *P. canaliculata*.

Snails are cheap, easy to rear and collect and contain copious microbes in their guts that can be exploited for various industrial purposes. The industrially important enzymes, like cellulases and proteases, can be isolated, extracted and purified from the gut microbes of snails thereby reducing the cost of imported materials. These enzymes are not only used in biofuel production but also harvested for other industries like pharmaceutical, waste disposal and detergent industries [119].

8.4. Chitinolytic bacteria

The omnivorous snails feed on insects that are a rich source of chitin, and in some cases, traces are often detected in gastropod feces. The body of phytophagous gastropods consists of 10% nitrogen, while food plants dined by snails contain only 4% of nitrogen. Chitin and its derivatives like chitosans could serve as a readily available nitrogen source for the gut bacteria and ultimately their host can take advantage of chitin-derived products [123].

Functional studies described extensively the importance of bacterial gut flora for the snail's digestion and nutrient supply [124]. Since the endogenous enzymatic activity in the intestine of the snail is very low, the snails may use their allochthonous and autochthonous bacteria for organic matter degradation [23, 99]. The digestive tract also harbors bacteria with special functions like metal chelation [67] and fermentation activity [64, 66], particularly on chitin and soluble cellulose, thereby providing nitrogen, lactate and acetate that are used as precursors as well as energy sources [70]. The DGGE fingerprinting technique along with NMDS analysis have revealed that intestine of the land snail *H. pomatia* harbors a unique set of bacterial flora. These authors also stated that sequences related to Pseudomonadaceae and Enterobacteriaceae spp. dominated the intestinal and digestive gland of snail populations. However, Kiebre-Toe et al. [125] and Charrier et al. [64] also reported the dominance of *Pseudomonas* sp., *Pantoea* sp. and *Buttiauxella* sp. in the intestine of *Helix* sp.

Lesel et al. [65] isolated the chitinolytic bacteria from the *H. pomatia* where chitinolytic bacteria were 10 times more abundant in the stomach and intestine than in the crop. In *Redix peregra*, the chitobiase activity was reduced when fed on antibiotic-treated diet, which also resulted in the loss of bacteria. This dual reduction indicates the synthesis of chitobiase by the bacteria inhabiting the gut [54]. Same conclusion was recounted by the Jeuniaux [126] and Donachie et al. [127] for the pulmonate *H. pomatia* and krill (*Megunyctiphunes norvegicu*) by showing a reduction in the enzymatic activity of the gut after the treatment of antibiotics.

8.5. Sulfate-reducing bacteria

Snails are copper-dependent animals as they use copper for the formation of the respiratory pigment haemocyanin. They also contain pore cells that can recycle the copper within the body. The sulfate-reducing bacteria increase the availability of copper to their snail hosts possibly by the effect of their metal-chelating activities [67]. The sulfate-reducing bacteria *Desulvibrio* sp. found in the crop of *H. aspersa* chelates the metals like Cu, Zn, Fe and Ni and make them ready for absorption. Similarly, some authors [128] concluded that digestive gland of the pulmonate *H. aspersa* acts as the store of Pb, Zn and Cd, which would represent a detoxification system. On the other hand, Simkiss [67] demonstrated the presence of sulfate-reducing bacteria in the crop of the snail *C. aspersum*.

Recently, Koch et al. [72] isolated the *Pseudomonas*, *Enterobacter* and *Lactococcus* bacterial species that were capable to degrade uric acid. However, in snails, uricase is found in several tissues, shuts down during estivation and does not participate in uric acid oxidation during arousal from this state [129]. However, tissue uricase along with bacterial uricase plays a role in nitrogen recycle of animals. In *P. canaliculata,* many bacteria not only help in digestion but also take part in recycling of uric acid like in arthropods.

9. Effect of gut physiology on the bacteria

The community structure of the microbes inhabiting the gut is predominantly altered by physiological states like hibernation and aestivation of the host [126, 130]. The physiological states like aestivation or hibernation are characterized by marked decrease in bacterial diversity due to expulsion of gut contents where some phylotypes are intentionally eliminated from the body. This gut clearance and other physico-chemical modifications may be responsible for the restructuration of the bacterial community like absence of mollicutes and α-proteobacteria in *H. pomatia* [131]. The snails also choose their gut biota as per physiological requirements. At the beginning of hibernation, certain groups are reduced and disappear while those that were meager during active stage may gain in space and become dominant. Further, during aestivation, the snails also lose large quantities of water, which may affect the viability of the gut bacteria and eventually their number and metabolism [31]. This could also be reason for the loss of allochthonous bacterial populations. During hibernation, there is a noticeable reduction of water content of the body along with reduction of food and low temperatures, which induce the snail to select the psychotropic bacteria only. These studies indicate that the gut flora is altered by different life stages and related physiological processes of the snails [132].

Though the bacteria survive during different physiological states like starvation, aestivation and hibernation of the snails, there is always a reduction in their number [64, 68] and these bacteria can be considered as autochthonous members of the snail gut. During these stages, mucous ribbon acts as the main nutritive medium for the bacterial growth [133]. In *C. aspersum*, amylolytic bacteria are adopted by vertical transmission [31] whereas proteolytic and cellulolytic bacteria were seen only during the adult stages of the animal. The higher cellulolytic

and proteolytic activity within the snail were predominately exhibited in active stage only indicating the transient nature of these bacteria, that is being ingested with the food from the environment thereby augmenting and improving digestion processes [65]. However, proteolytic bacteria were completely absent during hibernation, aestivation and in juvenile stages. The hibernation was marked with the decline of cellulolytic bacteria.

In *H. pomatia*, γ-proteobacteria and α-proteobacteria were the most abundant classes in all populations of snails. Only one phylotype of firmicutes has been reported during hibernation of snail populations. In non-hibernating snails, firmicutes were found only in the proximal intestine and digestive gland. In active snails, firmicutes were observed in distal intestine, with Mollicute specimen established abundantly in all three gut regions. However, they were restricted to the distal intestine and digestive gland at the beginning of hibernation [131].

The changes in the pH of the gut have serious effects on the microbial community. During anaerobioses, these bacteria in turn change the pH of the gut through fermentative reactions [119] producing end products that affect the acid-base balance of the digestive tract. But Churchill and Storey [134] postulated that in dormant snails, there is no accumulation of end-products (lactate and succinate) in dormant snails.

Besides all these functions that are contributed by the bacteria to their hosts, they also influence cold hardiness in their hosts. In snails such as *H. pomatia* and *C. aspersum,* the gut bacteria participate in ice-nucleating activity thereby reducing the cold hardiness in these snails [131, 135]. *H. pomatia* is known to decrease its supercooling point ca. by 3°, from –2 during its active state to –7°C in hibernation depending on the geographic location [136]. Lastly, enzymes secreted by the gut microbial community are very suitable for various biotechnological applications within the food, pharmaceutical and chemical industries along with detoxification of many hazardous chemicals.

In conclusion, snails present a vast diversity among mollusks with inherent industrial importance. Snails provide benefits not only as food for humans but are also routinely used in agriculture for the control of many insect pests. Though there are pros and cons associated with mollusks, a key need is better knowledge of the basic biology of these useful animals, with rigorous documentation of their habitats for the possible conservation. Little is known about the composition of snail micro-biota because a large number of species have been underestimated. Understanding the microbial ecology of snails may illustrate many useful processes like development of medicines from mucus or utilization of gut symbionts to challenge the emerging issues of environmental pollution and energy crisis. There is a dire need to explore more and more diversity of microbes that is encrypted in extreme environments like digestive tracts of snails. To accomplish this, many advanced techniques like high throughput next generation sequences (NGSs) along with other metagenomic techniques can be employed to unleash the role of these microbes in the host physiology.

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Author details

Mudasir A. Dar[1], Kiran D. Pawar[2] and Radhakrishna S. Pandit[1]*

*Address all correspondence to: rspandit@unipune.ac.in

1 Department of Zoology, Savitribai Phule Pune University, Ganeshkhind, Pune, Maharashtra, India

2 School of Nanoscience and Biotechnology, Shivaji University, Vidyanagar, Kolhapur, Maharashtra, India

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