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Research article

Biodiversity of microbial communities on some species in the class Gastropoda common in coastal waters in Central Vietnam

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Abstract. Gastropoda is a large class in coastal waters in Central Vietnam. The interaction between microorganisms and Gastropoda mainly consists of symbiotic and parasitic relationships. In this study, biodiversity of microbial communities on some species of Gastropoda is evaluated, thereby predicting their interaction. From 12 samples of Gastropoda including 3 species: *Trochus maculatus*, *Cypraea eglantica*, *Chicoreus bruneus*, 101 microorganism strains including 79 bacterial strains (78.2%), 18 yeast strains (17.8%) and 4 actinomycetes strains (4.0%) were isolated. There were 15 strains (including 8 yeast strains, 5 bacterial strains and 2 actinomycetes strains) selected to identify based on sequence analysis of the D1/D2 region (yeast) and 16S rRNA gene (bacteria and actinomycetes). Based on the identification results, it is possible to predict the nutritional relationship between microorganisms and species of Gastropoda.

Keywords: Gastropoda; biodiversity; microorganisms; Central Vietnam

Introduction

Scientists have studied the relationship between bacteria and Gastropoda since the 1980s [10]. The interaction between microorganisms and Gastropoda mainly consists of symbiotic and parasitic relationships. Symbiotic relationships between microorganisms and Gastropoda include nutrient exchange, supply of organic compounds, production of vitamins and amino acids, resistance to pathogenic microorganisms, habitats, etc. Symbiotic bacteria usually belong to autotrophic bacteria such as *Thiopfundum lithotrophicum*, *Thiohalophilus thiocyanatoydans*, *Methylosphaera hansonii*, *Methylobacter luteus*, etc. [5]. Microorganisms can also produce toxins and cause serious diseases.

Ha Tinh and Ninh Thuan are two provinces in Central Vietnam. The coastal area of these two provinces is the habitat of many species in the class Gastropoda [7]. Research on the relationship between microorganisms and Gastropoda in coastal marine ecosystems contributes to building a database for the conservation, development and sustainable exploitation of these animals.

Materials and methods

Materials. Samples. 12 samples of Gastropoda were collected in coastal waters of Ha Tinh and Ninh Thuan.

Table 1

List of samples

No.	Species in the class Gastropoda	Code sample	Place
1	<i>Trochus maculatus</i>	H1, H3	Ha Tinh
		N1, N3	Ninh Thuan
2	<i>Cypraea eglantica</i>	H2, H5	Ha Tinh
		N2, N5	Ninh Thuan
3	<i>Chicoreus bruneus</i>	H4, H6	Ha Tinh
		N4, N6	Ninh Thuan
Total		12	

Media. Hansen medium for yeast [4]. MPA medium for bacteria [6]. SCA medium for actinomycetes [6].

Methods. Isolation method. Animal samples were peeled, collected the gastrointestinal tract, then diluted with salt water 9%. The diluent was cultured on a petri containing medium suitable for each microorganism group. Petri plates were put in incubators at the suitable temperature [8].

Grouping method. The strains were cultivated on suitable media and conditions. Then they were differentiated by their macro- and micro-morphologies according to C.P. Kurtzman et al. [8]

DNA extraction. The experiment was carried out using Zymo Research Kit (USA).

PCR. PCR is established for each microorganism group using the suitable primers and heating programs. Specifically, NL1/NL4 primers for yeast, 1492R/27F for bacteria and actinomycetes [3].

Sequence analysis. The sequencing results were compared to related data in Genbank by the BLAST search on NCBI.

Results and discussions

Isolation results. 101 microorganism strains were isolated from 12 samples collected from Ha Tinh and Ninh Thuan (Table 2).

Table 2 showed that bacteria had the greatest amount. Actinomycetes was the smallest of microorganism group. Fungi had not been found in isolated samples. Yeast was found mainly in samples collected from Ha Tinh, while actinomycetes were found mainly in samples in Ninh Thuan. Sample N1 (*Trochus maculatus*) had the existence of 3 microorganism groups. Sample N5 (*Cypraea eglantica*) only isolated bacteria. Other samples had the existence of two microorganism groups. The number of microbial strains isolated on each species in the class Gastropoda was also different (Figure 1).

Table 2

List of microorganism strains

No.	Sample	Strains code		
		Yeast	Bacteria	Actinomycetes
1	H1	H1.1Y, H1.4Y, H1.6Y	H1.2B, H1.3B, H1.7B, H1.8B	–
2	H2	H2.1Y, H2.2Y	H2.3B, H2.4B, H2.5B, H2.6B, H2.7B, H2.8B, H2.9B, H2.10B, H2.11B, H2.12B, H2.13B	–
3	H3	H3.8Y	H3.1B, H3.3B, H3.4B, H3.5B, H3.6B, H3.7B, H3.9B, H3.10B	–
4	H4	–	H4.1B, H4.3B, H4.4B, H4.5B, H4.6B	H4.2A
5	H5	H5.1Y, H5.2Y, H5.11Y	H5.3B, H5.6B, H5.7B, H5.8B, H5.9B, H5.10B, H5.12B, H5.13B, H5.14B	–
6	H6	H6.1Y, H6.2Y, H6.12Y	H6.3B, H6.4B, H6.5B, H6.6B, H6.9B, H6.11B, H6.15B, H6.16B	–
7	N1	N1.1Y, N1.2Y	N1.3B, N1.5B, N1.7B	N1.4A, N1.6A
8	N2	N2.3Y, N2.5Y	N2.1B, N2.2B, N2.4B, N2.6B	–
9	N3	N1.1Y	N3.2B, N3.3B, N3.4B, N3.5B, N3.6B, N3.7B, N3.8B, N3.9B, N3.10B, N3.11B	–
10	N4	–	N4.1B, N4.2B, N4.3B, N4.5B, N4.6B, N4.7B, N4.8B, N4.9B	N4.4A
11	N5	–	N5.4B, N5.5B, N5.15B, N5.16B	–
12	N6	N6.12Y	N6.7B, N6.8B, N6.13B, N6.14B	–
Total		18 (17.8%)	79 (78.2%)	4 (4.0%)

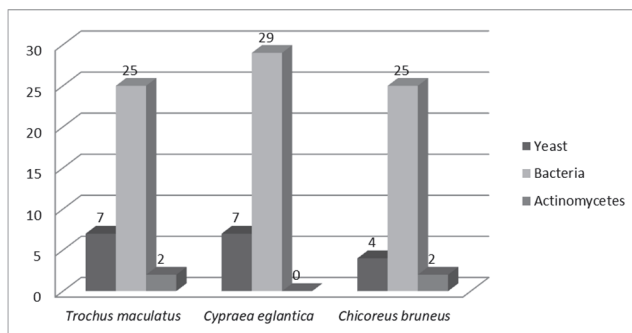


Figure 1. Number of microorganism strains in each species

Trochus maculatus and *Chicoreus bruneus* were 2 species with the existence of 3 microorganism groups (yeast, bacteria and actinomycetes). Actinomycetes had not been found in *Cypraea eglantica*.

Grouping based on characteristics of colonies and cells. Characteristics of colonies and cells play an important role in the classification of microorganisms. In this study, microorganism strains were grouped based on colony and cell characteristics in order to provide more accurate assessments of the diversity of microbial communities.

Bacteria. Based on colony and cell characteristics, 79 bacteria strains were divided into 5 groups (Table 3).

The isolated bacterial strains had a low biodiversity. The majority of bacteria group is the group with milky white colonies, slippery surface, viscous, spherical cells, separated (group 1 – 40.5 %). The results of bacteriological isolation are similar to the results of previous studies on the existence of bacteria in the species of Gastropoda [1].

Table 3

Grouping results of bacteria

Group	Quantity (percent)	Characteristic	
		Colony	Cell
1	32 (40.5%)	Milky white, smooth surface, viscous	Spherical cells, separated
2	8 (10.1%)	Red, smooth surface	Rod cells, separated
3	11 (13.9%)	White, smooth surface	Spherical cells
4	7 (8.9%)	Pink, smooth surface	Rod cells
5	21 (26.6%)	Yellow, smooth surface	Rod cells

Yeast. Yeast was the microorganism group with the highest biodiversity. 18 yeast strains were divided into 8 groups (Table 4).

Table 4

Grouping results of yeast

Group	Strains code	Characteristic	
		Colony	Cell
1	H6.1Y, H5.11Y	Milky white, spongy	Spherical cells, separated, reproduce by budding
2	N1.2Y, H1.6Y	White, smooth	Spherical cells, separated, reproduce by budding
3	N3.1Y, N6.12Y	Creamy white, spongy	Spherical cells, reproduce by budding
4	H1.1Y, H3.8Y, N1.1Y, H2.2Y	Black, wet, viscous	Spherical cells, arranged in chains
5	H6.12Y, N2.5Y	Dark brown, wet, viscous	Rod and spherical cells, arranged in chains
6	H2.1Y, H5.1Y, H5.11Y	White, wrinkled surface	Spherical cells, reproduce by budding
7	H1.4Y	Black, wet, viscous	Rod cells, arranged in chains
8	N2.3Y, H5.2Y, H6.2Y	Creamy white, spongy	Spherical cells, separated, reproduce by budding

Yeast is a polymorphic microorganism group, characteristics of colonies (color, shape) and cells that can change in the life cycle [3]. Therefore, it is necessary to use other classification methods (biochemical characteristics, molecular biology) to be able to more accurately assess the biodiversity of yeast.

Actinomycetes. 4 actinomycetes strains were divided into 2 groups according to the colony and cell characteristics described in Table 5.

Table 5

Grouping results of actinomycetes

Group	Strains code	Characteristic	
		Colony	Cell
1	N1.6A, N4.4A	Brownish, dry, hard, thick	Rod cells, branching
2	H4.2A, N1.4A	Brown, small, dry, thin	Rod cells, branching

According to the references, there are currently no reports of the existence of actinomycetes in the species of Gastropoda [9]. However, in this study, the number of actinomycetes isolated was very low, and the survey was only conducted on 12 animal samples. A larger number of animal samples should be surveyed to confirm the existence of actinomycetes in the species of Gastropoda.

Based on the grouping results, 15 microorganism strains representing each group were sequenced (including 8 yeast strains, 5 bacterial strains and 2 actinomycetes strains).

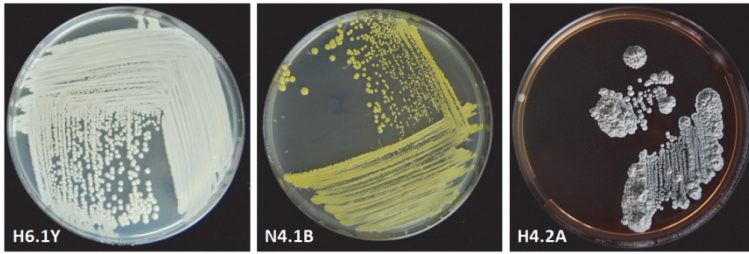


Figure 2. Colony characteristics of microorganism strains

Identification results. Microbial identification is considered a highly reliable classification method. Based on the identification results, it is possible to give the accurate assessment of biodiversity of microbial community. In this study, microorganism strains representing each group were sequenced with the corresponding genes. In yeast strains, DNA sequencing of the D1/D2 region of the large subunit of the 28S ribosomal RNA gene was evaluated with NL1/NL4 primers. 16S rRNA gene sequences were used to study bacteria and actinomycetes with 1492R/27F primers.

Bacteria. The results of bacterial identification showed that 5 bacteria strains identified could belong to 3 genera: *Burkholderia*, *Cellulosimicrobium*, *Rhodococcus*. Specially, genus *Cellulosimicrobium* includes cellulose-degrading bacteria. M.A. Dar et al. (2015) also published the existence of cellulose-degrading bacteria in *Achatina fulica*. He suggested that the interaction between *Cellulosimicrobium* and species of Gastropoda could be symbiotic relationships [1].

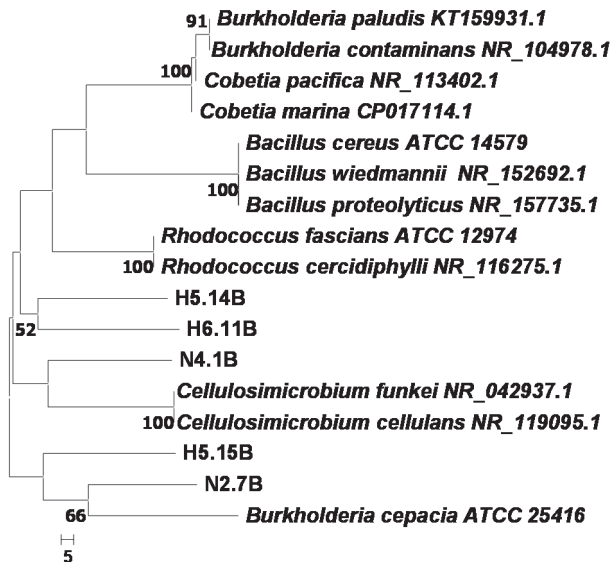


Figure 3. Phylogenetic tree of bacteria, based on 16S rRNA gene sequences

Yeast. In the grouping by characteristics of colonies and cells, yeast is a microorganism group with the highest biodiversity (they were divided into 8 groups). However, the yeast identification results showed that they consisted of 4 genera: *Meyerozyma*, *Aureobasidium*, *Pichia*, *Candida*. According to the references, these 4 yeast genera can be found in some marine animals including sea snails (a spe-

cies of Gastropoda) [3]. However, there has been no report on the interaction between these yeast genera and species of Gastropoda.

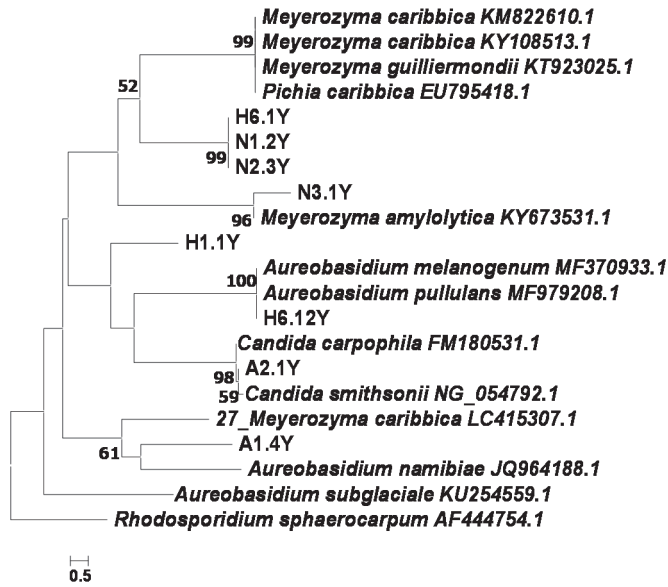


Figure 4. Phylogenetic tree of yeast, based on the D1/D2 region sequences

Actinomycetes. Combining the results of grouping based on characteristics of colonies and cells with sequencing results, it could be confirmed that 4 isolated actinomycetes strains belonging to genus.

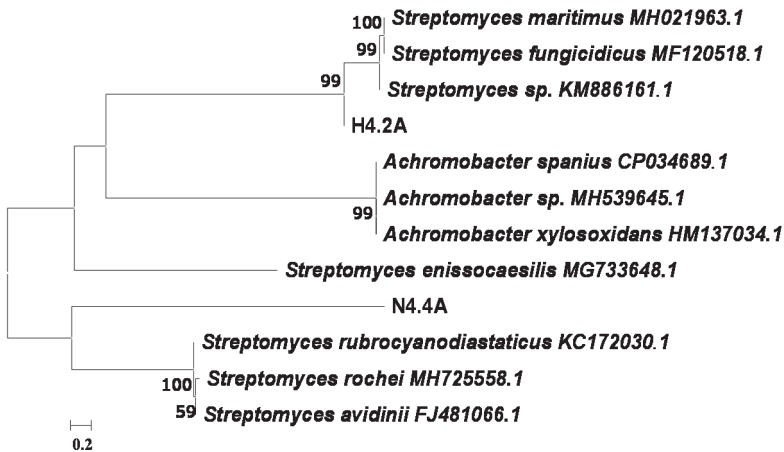


Figure 5. Phylogenetic tree of actinomycetes, based on 16S rRNA gene sequences

Streptomyces. Genus *Streptomyces* is a common genus in marine sediments [9]. As mentioned, there are currently no reports of the existence of actinomycetes in species belonging to Gastropoda. 4 isolated actinomycetes strains in this study might have entered the gastrointestinal tract of animal species along with their food.

Conclusions

Survey of 12 animal samples in the class Gastropoda (including species: *Trochus maculatus*, *Cypraea eglantica*, *Chicoreus bruneus*), 101 strains of microorganisms were isolated. Bacteria is the group with the highest number of strains, yeast is the group with the highest biodiversity.

Actinomycetes belong to genus *Streptomyces*; yeasts belong to the genera: *Meyerozyma*, *Aureobasidium*, *Pichia*, *Candida*; bacteria belong to the genera: *Burkholderia*, *Cellulosimicrobium*, *Rhodococcus*. Genus *Cellulosimicrobium* and species of Gastropoda may have symbiotic interaction.

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Научная статья

**Биоразнообразие микробных сообществ
некоторых видов класса Гастропода,
распространенного в прибрежных водах
в центральной части Вьетнама**

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Аннотация. Гастропода – это большой класс моллюсков в прибрежных водах в центральной части Вьетнама. Взаимодействие между микроорганизмами и гастроподой в основном состоит из симбиотических и паразитических отношений. В настоящем исследовании оценивается биоразнообразие микробных сообществ некоторых видов гастроподы, что позволяет прогнозировать их взаимодействие. Из 12 образцов гастроподы, включающих 3 вида: *Trochus maculatus*, *Cypraea eglantica*, *Chicoreus bruneus*, изолирован 101 штамм микроорганизмов, из них 79 штаммов бактерий (78,2 %), 18 штаммов дрожжей (17,8 %) и 4 штамма актиномицетов (4,0 %). Было отобрано 15 штаммов (вклю-

чая 8 штаммов дрожжей, 5 штаммов бактерий и 2 штамма актиномицетов) для идентификации на основе анализа последовательностей области D1/D2 (дрожжи) и гена 16S рРНК (бактерии и актиномицеты). На основании результатов идентификации можно предсказать питательную взаимосвязь между микроорганизмами и видами гастроподы.

Ключевые слова: гастропода; биоразнообразие; микроорганизмы; центральная часть Вьетнама

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