様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Investigating natural product biosynthetic pathways in the microbiomes associated with long-lived aquatic vertebrates

研究課題名（英文）Investigating natural product biosynthetic pathways in the microbiomes associated with long-lived aquatic vertebrates

研究代表者

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研究成果の概要（和文）：私たちはまず、水生動物の長寿に寄与する可能性のある細菌、代謝産物、生合成遺伝子を特定するために、ヒザラガイ（寿命約40年）などの長寿動物から細菌分離株を取得しました。次に個々のヒザラガイの様々な部分から約430の細菌株を分離しました。特定の抗生物質に対して耐性を示す株は、生合成についての知見を得るためにPCRクローニングに供しました。その後のメタゲノム解析のにより、ヒザラガイサンプル５つのうち２つにおいて有望な生合成遺伝子を有する細菌を同定しました。本研究は微生物化学に関する研究を補完するために、Piel Lab (ETH Zurich) と共同で行われています。

研究成果の学術的意義や社会的意義
Through metagenomic analyses, we identified biosynthetically promising chiton-associated acidobacteria with the ability to produce bioactive sponge-type natural products. This could be a basis for exploring the biosynthetic potential of acidobacterial members from diverse environmental types.

研究成果の概要（英文）：To identify key bacteria, metabolites and biosynthetic genes that may contribute to the longevity of aquatic animals, we initially obtained bacterial isolates from long-lived animals, such as chitons (lifespan ~40 years). We isolated ~430 bacterial strains from different parts of individual chitons. Strains showing resistance to certain antibiotics were subjected to PCR-cloning to detect those with biosynthetic potential. Subsequent metagenomic analyses led to the identification of biosynthetically promising bacteria in two of six studied chiton samples. This is in collaboration with Piel Lab (ETH Zurich) to complement their work on bacterial chemistry (Chem 2023, 9, 12: 3696-713). Using known sponge-derived compounds, we optimized the screening protocol for testing the ability of such compounds to stimulate lifespan in the organism model Saccharomyces cerevisiae. This was based on monitoring proteasome activity of compound-treated yeast lysates.

研究分野：Microbiology and Biochemistry

キーワード：long-lived animals　chitons　potential bacteria　metagenome　metagenomic analyses　sponge-derived compounds　Saccharomyces cerevisiae　proteasome activity

様　式　Ｃ－１９、Ｆ－１９－１（共通）

１．研究開始当初の背景（Background）

It has recently been recognized the important contribution of beneficial microbiomes to the prolonged lifespan of their animal hosts. This raises important questions about the identity of key bacteria, the metabolites or compounds produced, and the encoding genes responsible for longevity and healthy lifespan in long-lived aquatic vertebrates and invertebrates. We hypothesize that certain bacteria inhabiting long-lived aquatic animals may produce metabolites with anti-aging, anti-inflammatory or antimicrobial properties, thereby protecting their hosts from age-related diseases and infection. We believe addressing these questions could give great scientific impacts, because natural products with such pharmacological properties can be developed as pharmaceutical agents that slow ageing or treat age-related diseases, thereby contributing to human healthy lifespan.

２．研究の目的（Purpose）

Our research aims to identify key bacteria, metabolites/natural products, and biosynthetic genes responsible for longevity and healthy lifespan in long-lived aquatic vertebrates or marine animals.

３．研究の方法（Methods）

Chiton (*Acanthopleura japonica*) individuals were initially collected along the Pacific Ocean coast near the Natural History Museum and Institute at Katsuura, Chiba, Japan. Ten chiton individuals were obtained and placed in 50-ml facon tubes containing seawater from the sampling sites and transferred to Faculty of Pharmaceutical Sciences at Hokkaido University for analyses. Baterial strains were isolated from different body parts of chitons, which were cultivated in media containing artifical seawater. To identify the presence of the target bacterium *Acanthopleuribacter pedis* associated with the Chiba Chiton [1], we initially designed numbers of oligonucleotides/probes based on taxonomic 16S rRNA gene as well as biosynthetic genes. We subsequently prepared metagenome from each chiton individual. The PCR products obtained from chiton metagenomes with such probes were cloned into *Escherichia coli*, and the resulting correct-sized amplicons were sequenced (Figure 1). In addition to chitons, we also obtained Japanese rockfish (lifespan >90 years) and isolated the fish-associated bacterial strains. Compounds were tested for their ability to stimulate lifespan extension in the organism model *Saccharomyces cerevisiae* based on protocols described by Maresh et al (2021) [2] with slight modifications (Figure 2).

4．研究成果（Results）

To identify key bacteria, metabolites and biosynthetic genes that may contribute to the longevity of aquatic animals, we initially obtained bacterial isolates from long-lived animals, such as chitons (lifespan ~40 years) and Japanese rockfish (lifespan >90 years). We isolated ~430 bacterial strains from different parts of individual chitons. Strains showing resistance to certain antibiotics were subjected to PCR-cloning to detect those with biosynthetic potential. We isolated metagenomes of some chiton samples. Subsequent metagenomic analyses led to the identification of biosynthetically promising bacteria in two of six studied chiton samples (Figure 1, Table 1) [3]. This is in collaboration with Piel Lab (ETH Zurich) to complement their work on bacterial chemistry (*Chem* 2023, 9, 12: 3696-713).

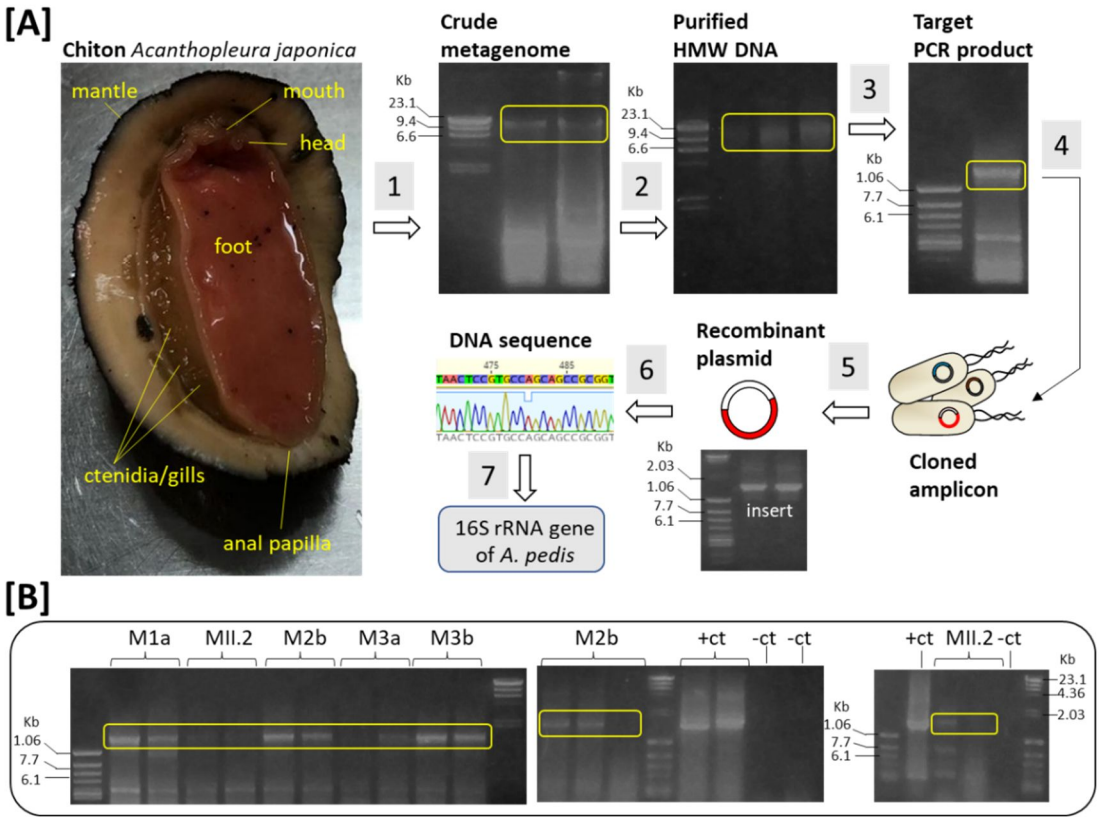

Figure 1. Metagenomic ananlyses of the Chiba chiton *Acanthopleura japonica* for the presence of the biosynthetically potential bacterium *Acanthopleuribacter pedis*. **[A]** Metagenomic DNA was initially extracted from the body of a chiton [step **1**], which was then purified by gel-extraction [step **2**]. The purified HMW metagenomic DNA was used as a PCR-template to target 16S rRNA gene of *A. pedis* [step **3**]. The target PCR product of ~1.4 Kb was cloned into *E. coli* DH5α [step **4**]. The resulting clones were screened by colony PCR using M13 primers [step **5**]. Recombinant plasmids were prepared from three positive clones and subjected to DNA sequencing [step **6**]. The resulting DNA sequence was queried against the non-redundant nucleotide database using BLAST (blastn) [step **7**]. **[B]** The DNA electrophoretic gels, showing correct-sized amplicons of ~1.4 kb in five of the six different metagenome samples, but not in the negative controls. Metagenomes of the five chiton individuals used as the PCR templates were labelled as M1a, MII.2, M2b, M3a, and M3b. The HMW DNA marker: λ DNA digested with *Hind*III, and LMW DNA marker: ΦX174 virion DNA digested with *Hinc*II (Nippon Gene). Abbreviations: +ct, positive control (16S amplicon of *A. pedis*); -ct, negative control (without DNA template); HMW, high-molecular-weight; and LMW, low-molecular-weight.

Through metagenomic approach, we particularly identified biosynthetically promising acidobacteria in the chiton metagenomes [3]. The chiton-associated Acidobacteria of the family Acanthopleuribacteraceae [1] contain large sets of biosynthetic gene clusters responsible to produce bioactive sponge-type natural products and other metabolites [3]. This suggests the possible ecological contribution of this talented acidobacterial producer to the longevity, survival, or defense of the animal hosts.

Table 1. Summary about detection of *A. pedis* 16S rRNA gene in chiton metagenomes.

Chiton label	Synonim label	Corrected-size band	Sequence of *A. Pedis* 16S rRNA gene
M1a	M1	+	+
MII.2	M2	+	+
M2b	M3	+	ND
M3a	M4	+	-
M3b	M5	+	-
M2a	M6	-	-

ND = not determined.

Using known marine sponge-derived natural products, we optimized the screening protocol for testing the ability of sponge-type compounds to stimulate lifespan extension in the organism model *S. cerevisiae*. This was based on monitoring proteasome activity of yeast lysates after being treated with tested compounds in comparison with the positive control (proteasome-stimulating compounds) and the negative control (proteasome inhibitor) [2] (Figure 2). It was found that two compounds slighly stimulated the yeast endogenous proteasomal activity.

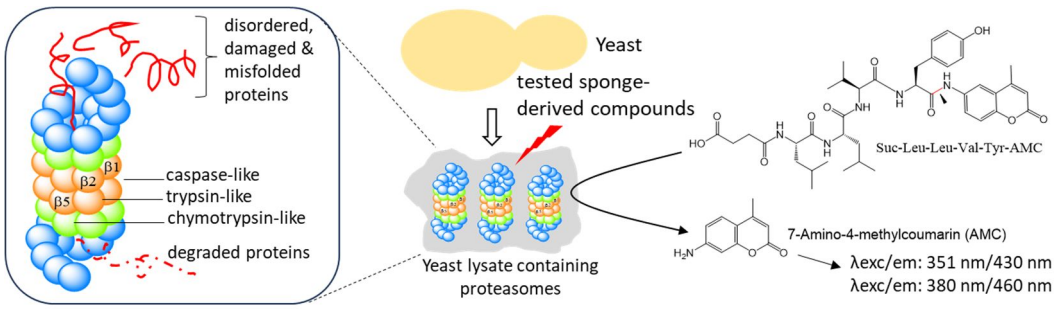

Figure 2. Preliminary testing for the proteasome-stimulating or -inhibiting activity of some sponge-derived natural products using yeast lysates. Proteasome activity in a yeast lysate was measured using the fluorogenic substrate Suc-LLVY-AMC. The caspase-like, trypsin-like, and chymotrypsin-like hydrolytic activities of the yeast proteasomes release free fluorophore AMC molecues that were detected by the plate reader Tecan i-control at the excitation/emission wavelengths of 351 nm/430 nm or 380 nm/460 nm, respectively. The experimental controls for this proteasomal activity assays were 1% DMSO as the negative control, Bortezomib (BTZ) as the proteasome inhibitor, and ursolic acid (UA) as the proteasome stimulator.

References:

[1] Y. Fukunaga, M. Kurahashi, K. Yanagi, A. Yokota, S. Harayama. *Acanthopleuribacter pedis* gen. nov., sp. nov., a marine bacterium isolated from a chiton, and description of Acanthopleuribacteraceae fam. nov.,

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[3] S. Leopold-Messer, C. Chepkirui, M.F.J. Mabesoone, J. Mayer, L. Paoli, S. Sunagawa, A.R. Uria, T. Wakimoto, J. Piel (**2023**). Animal-associated marine Acidobacteria with a rich natural product repertoire. ***Chem*** *(Cell Press)* 9 (12), 3696-3713.

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　1件／うち国際共著　2件／うちオープンアクセス　1件）

１．著者名 Haedar Jabal Rahmat、Uria Agustinus Robert、Lallo Subehan、Dibwe Dya Fita、Wakimoto Toshiyuki	４．巻 20
２．論文標題 New Theonellapeptolides from Indonesian Marine Sponge Theonella swinhoei as Anti-Austerity Agents	５．発行年 2022年
３．雑誌名 Marine Drugs (MDPI)	６．最初と最後の頁 661～661
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/md20110661	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Rumengan Inneke F. M.、Roring Vera I. Y.、Haedar Jabal R.、Siby Mayse S.、Luntungan Aldian H.、Kolondam Beivy J.、Uria Agustinus R.、Wakimoto Toshiyuki	４．巻 84
２．論文標題 Ascidian-associated photosymbionts from Manado, Indonesia: secondary metabolites, bioactivity simulation, and biosynthetic insight	５．発行年 2021年
３．雑誌名 Symbiosis	６．最初と最後の頁 71～82
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/s13199-021-00766-4	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Leopold-Messer Stefan、Chepkirui Clara、Mabesoone Mathijs F.J.、Meyer Joshua、Paoli Lucas、Sunagawa Shinichi、Uria Agustinus R.、Wakimoto Toshiyuki、Piel Joern	４．巻 9
２．論文標題 Animal-associated marine Acidobacteria with a rich natural-product repertoire	５．発行年 2023年
３．雑誌名 Chem (Cell Press)	６．最初と最後の頁 3696～3713
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.chempr.2023.11.003	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計4件（うち招待講演　4件／うち国際学会　0件）

１．発表者名 A.R. Uria
２．発表標題 Potential of biosynthetic pathways and biocatalysts from marine metagenomes for the smart eco-bioproduction of highly valuable compounds
３．学会等名 SemNasHas XII (National Seminar), Faculty of Agriculture, Gadjah Mada University (UGM)　2022/10（招待講演）
４．発表年 2022年

１．発表者名
A.R. Uria, T. Wakimoto

２．発表標題
Marine microbial natural products with unusual building blocks: discovery of their biosynthetic machineries as a basis for drug development

３．学会等名
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４．発表年
2021年

１．発表者名
A.R. Uria

２．発表標題
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International Virtual Course on Marine Natural Product at ITB Bandung（招待講演）

４．発表年
2021年

１．発表者名
A.R. Uria

２．発表標題
Sustainable use of biodiversity for drug discovery: accessing biosynthetic genes in microbial symbionts of marine sponges

３．学会等名
2nd Wallacea International Webinar for Biotechnology & Conservation, Sam Ratulangi University（招待講演）

４．発表年
2021年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関