

Laboratory of Marine Environmental Microbiology

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This laboratory is doing researches on microalgal productions of ω -3 fatty acids, carotenoids and third-generation biofuels by genetic engineering. ω -3 Fatty acids and carotenoids have physiological functions and are used for dietary supplements. We are also searching novel and useful fungi from marine environments. In addition, we have studied ecophysiology and evolution of 'earth-eating' microorganisms inhabiting various extreme marine environments such as deep-sea hydrothermal fields.

ω -3 Fatty acids, carotenoids and biofuel production using microalgae

Chlorella spp. produce ω -3 fatty acids and *Dunaliella* spp. produce β -carotene known as a vitamin A pre-cursor. We are doing researches on molecular biology of these microalgae to produce useful compounds.



Dunaliella salina

Marine fungi and methanogens

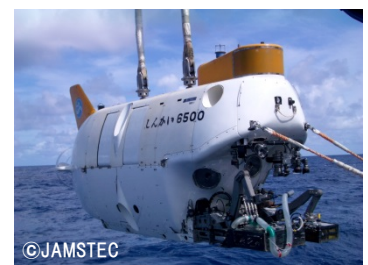
Fungi producing large amount of enzymes are widely used for fermentation industries. We are conducting research on screening of novel marine fungi. We are also studying ecological roles of methanogens in aquatic environments.



Marine fungus with melon flavor

Microbial ecophysiology and evolution in extreme marine environments.

Rich microbial ecosystems exist in deep-sea and oceanic sediments, and even in rock deep inside Earth's crust, where not long ago it was thought that life could not exist. We have studied ecophysiology and evolution of microorganisms inhabiting extreme marine environments.



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Key words

Microalgae, ω -3 Fatty acid, Carotenoid, Biofuel, Fungi, Yeast, Methanogen, Deep-sea vents, Symbiosis, Extremophiles

Research Achievements

2017

- Overexpression of DnaJ-like chaperone enhances carotenoid synthesis in *Chlamydomonas reinhardtii*. Morikawa T, Uruguchi Y, Sanda S, Nakagawa S, Sawayama S. Appl Biochem Biotechnol, (2017) doi: 10.1007/s12010-017-2521-5.
- Effect of nitrogen concentration on growth, lipid production and fatty acid profiles of the marine diatom *Phaeodactylum tricorutum*. Yodsuwan N, Sawayama S, Sirisansaneeyakul S. Agric Nat Res, 51, 190-197 (2017)
- Cultivation mutualism between a deep-sea vent galatheid crab and its chemosynthetic epibionts. Watsuji T-o, Tsubaki R, Chen C, Nagai Y, Nakagawa S, Yamamoto M, Nishiura D, Toyofuku T, Takai K. Deep Sea Res I, (2017) doi:10.1016/j.dsr.2017.04.012
- Isolation and characterization of novel polymorphic microsatellite loci for the deep-sea hydrothermal vent limpet, *Lepetodrilus nux*, and the vent-associated squat lobster, *Shinkaia crosnieri*. Nakajima Y, Shinzato C, Khalturina M, Nakamura M, Watanabe H, Nakagawa S, Satoh N, Mitarai S. Mar Biodiv, (2017) doi:10.1007/s12526-017-0704-5
- Microbiota in the coelomic fluid of two common coastal starfish species and characterization of an abundant Helicobacter-related taxon. Nakagawa S, Saito H, Tame A, Hirai M, Yamaguchi H, Sunata T, Aida M, Muto H, Sawayama S, Takaki Y. Sci Rep, 7, 8764 (2017)
- Lebetimonas natsushimae* sp. nov., a novel strictly anaerobic, moderately thermophilic chemoautotroph isolated from a deep-sea hydrothermal vent polychaete nest in the Mid-Okinawa Trough. Nagata R, Takaki Y, Tame A, Nunoura T, Muto H, Mino S, Sawayama S, Nakagawa S. Syst Appl Microbiol, 40, 352-56 (2017)
- A simple and efficient RNA extraction method from deep-sea hydrothermal vent chimney structures. Muto H, Takaki Y, Hirai M, Mino S, Sawayama S, Takai K, Nakagawa S. Microbes Environ, in press (2017)
- Endemicity of the cosmopolitan mesophilic chemolithoautotroph *Sulfurimonas* at deep-sea hydrothermal vents. Mino S, Nakagawa S, Makita H, Toki T, Miyazaki J, Sievert SM, Polz MF, Inagaki F, Godfroy A, Kato S, Watanabe H, Nunoura T, Nakamura K, Imachi H, Watsuji TO, Kojima S, Takai K, Sawabe T. ISME J, 11, 909-19 (2017)

2016

- Production of β -xylosidase from *Trichoderma asperellum* KIF125 and its application in efficient hydrolysis of pretreated rice straw with fungal cellulase. Inoue H, Kitao C, Yano S, Sawayama S. World J Microbiol Biotechnol, 32(11), 1-10 (2016)
- Identification and characterization of D-arabinose reductase and D-arabinose transporters from *Pichia stipitis*. Watanabe S, Utsumi Y, Sawayama S, Watanabe Y. Biosci Biotechnol Biochem, 80, 2151-2158 (2016)
- Iron requirements of *Heterosigma akashiwo* (Raphidophyceae), *Heterocapsa circularisquama* (Dinophyceae) and two common centric diatoms. Fukuzaki K; Yoshioka T; Sawayama S, Imai I. Bull Fish Sci Hokkaido Univ, 66, 121-128 (2016)
- Discovery of a Novel Alginate Lyase from *Nitratiruptor* sp. SB155-2 Thriving at Deep-sea Hydrothermal Vents and Identification of the Residues Responsible for its Heat Stability. Inoue A, Anraku M, Nakagawa S, Ojima T. J Biol Chem, 291, 15551-63 (2016)

2015

- Melon aroma-producing yeast isolated from coastal marine sediment in Maizuru Bay, Japan. Sutani A, Ueno M, Nakagawa S, Sawayama S. Fish Sci, 81, 929-936 (2015)
- Effect of β -Mannanase and β -Mannosidase supplementation on the total hydrolysis of softwood polysaccharides by the *Talaromyces cellulolyticus* cellulase system. Inoue H, Yano S, Sawayama S. Appl Biochem Biotechnol, 176, 1673-1686 (2015)
- Draft genome sequence of *Talaromyces cellulolyticus* strain Y-94, a source of lignocellulosic biomass-degrading enzymes. Fujii T, Koike H, Sawayama S, Yano S, Inoue H. Genome Announc, 3(1) e00014-15 (2015)
- Effects of Hemagglutination Activity in the Serum of a Deep-Sea Vent Endemic Crab, *Shinkaia crosnieri*, on Non-Symbiotic and Symbiotic Bacteria. Fujiyoshi S, Tateno H, Watsuji T, Yamaguchi H, Fukushima D, Mino S, Sugimura M, Sawabe T, Takai K, Sawayama S, Nakagawa S. Microbes Environ, 30, 228-34 (2015)
- Identification of a gene cluster responsible for hydrogen evolution in *Vibrio tritonius* strain AM2 with transcriptional analyses. Matsumura Y, Al-saari H, Mino S, Nakagawa S, Maruyama F, Ogura Y, Hayashi T, Kurokawa K, Sawabe T, Sawabe T. International Journal of Hydrogen Energy, 40, 9137-46 (2015)
- Takai K, Nakagawa S, Nunoura T. Comparative Investigation of Microbial Communities Associated with Hydrothermal Activities in the Okinawa Trough. Subseafloor Biosphere Linked to Hydrothermal Systems: TAIGA Concept2015. p. 421-35.

Research Achievements

2014

- RNAi knockdown of potent sugar sensor in cellulase-producing fungus *Acremonium cellulolyticus*. Asada, S., Watanabe S, Fujii, T, Inoue, H, Ishikawa, K, Sawayama, S. *Appl Biochem Biotechnol*, 172, 3009-3015 (2014)
- Fluorescent characteristics of dissolved organic matter produced by bloom-forming coastal phytoplankton. Fukuzaki, K, Imai, I, Fukushima, K, Ishii, K, Sawayama, S, Yoshioka, T. *J Plankton Res*, 36, 685-694 (2014).
- Breeding of a xylose-fermenting hybrid strain by mating genetically engineered haploid strains derived from industrial *Saccharomyces cerevisiae*. Inoue H, Hashimoto S, Matsushika A, Watanabe S, Sawayama S. *J Ind Microbiol Biotechnol* 41, 1773-1781 (2014)
- Identification and characterization of core cellulolytic enzymes from *Talaromyces cellulolyticus* (formerly *Acremonium cellulolyticus*) critical for hydrolysis of lignocellulosic biomass. Inoue H, Decker SR, Taylor LE 2nd, Yano S, Sawayama S. *Biotechnol Biofuels*, 7, 151-936 (2014)
- Bench-scale bioethanol production from eucalyptus by high solid saccharification and glucose/xylose fermentation method. Fujii, T, Murakami, K, Endo, T, Fujimoto, S, Minowa, T, Matsushika, A, Yano, S, Sawayama, S. *Bioproc Biosyst Eng*, 37, 749-754 (2014)
- Allying with armored snails: the complete genome of gammaproteobacterial endosymbiont. Nakagawa, S, Shimamura, S, Takaki, Y, Suzuki, Y, Murakami, SI, Watanabe, T, Fujiyoshi, S, Mino, S, Sawabe, T, Maeda, T, Makita, H, Nemoto, S, Nishimura, SI, Watanabe, H, Watsuji, TO, Takai, K. *ISME J*, 8, 40-51 (2014)
- Family *Nautiliaceae*: The Genera *Caminibacter*, *Lebetimonas*, and *Nautilia*. In *The Prokaryotes*, Nakagawa, S, Takai, K. *The Prokaryotes*, pp 393-399 (2014)
- Deep-sea *Rhodococcus* sp. BS-15, lacking the phytopathogenic *fas* genes, produces a novel glucotriose lipid biosurfactant. Konishi M, Nishi S, Fukuoka T, Kitamoto D, Watsuji TO, Nagano Y, Yabuki A, Nakagawa S, Hatada Y, Horiuchi J. *Mar Biotechnol (NY)*, 16, 484-93 (2014)
- Enhanced hydrogen production by a newly described heterotrophic marine bacterium, *Vibrio tritonius* strain AM2, using seaweed as the feedstock. Matsumura Y, Sato K, Al-saari N, Nakagawa S, Sawabe T. *International Journal of Hydrogen Energy*, 39, 7270-77 (2014)
- Sulfurovum aggregans* sp. nov., a hydrogen-oxidizing, thiosulfate-reducing chemolithoautotroph within the Epsilonproteobacteria isolated from a deep-sea hydrothermal vent chimney, and an emended description of the genus *Sulfurovum*. Mino S, Kudo H, Arai T, Sawabe T, Takai K, Nakagawa S. *Int J Syst Evol Microbiol*, 64, 3195-201 (2014)

2013

- High-coverage gene expression profiling analysis of the cellulase-producing fungus *Acremonium cellulolyticus* cultured using different carbon sources. Hideno, A, Inoue, H, Yano, S, Tsukahara, K, Fujii, T, Murakami, K, Yunokawa, H, Sawayama, S. *Appl Microbiol Biotechnol*, 97 (12), 5483-5492 (2013)
- Characterization of a recombinant flocculent *Saccharomyces cerevisiae* strain that co-ferments glucose and Xylose: I. Influence of the ratio of glucose/xylose on ethanol production. Matsushika, A, Sawayama, S. *Appl Biochem Biotechnol*, 169, 712-721 (2013)
- Development of genetic transformation and heterologous expression system in carboxydrotrophic thermophilic acetogen *Moorella thermoacetica*. Kita, A, Iwasaki, Y, Sakai, S, Okuto, S, Takaoka, K, Suzuki, T, Yano, S, Sawayama, S, Tajima, T, Kato, J, Nishio, N, Murakami, K, Nakashimada, Y. *J Biosci Bioeng*, 115 (4), 347-352 (2013)
- High connectivity of animal populations in deep-sea hydrothermal vent fields in the central Indian ridge relevant to its geological setting. Beedessee G, Watanabe, H, Ogura, T, Nemoto, S, Yahagi, T, Nakagawa, S, Nakamura, K, Takai, K, Koonjul, M, Marie, DEP. *PLoS One*, 8, e81570 (2013)
- Biogeography of *Persephonella* in deep-sea hydrothermal vents of the Western Pacific. Mino, S, Makita, H, Toki, T, Miyazaki, J, Kato, S, Watanabe, H, Imachi, H, Watsuji, TO, Nunoura, T, Kojima, S, Sawabe, T, Takai, K, Nakagawa, S. *Front Microbiol*, 4, 107 (2013)

2012

- Isolation of uracil auxotrophs of the fungus *Acremonium cellulolyticus* and the development of a transformation system with the *PyrF* Gene. Fujii, T, Iwata, K, Murakami, K, Yano, S, Sawayama, S. *Biosci Biotechnol Biochem*, 76, 245-249 (2012)
- Combination of hot compressed water treatment and wet disk milling for high sugar recovery yield in enzymatic hydrolysis of rice straw. Hidenno, A, Inoue, H, Yanagida, T, Tsukahara, K, Endo, T, Sawayama, S. *Biores Technol*, 104, 743-748 (2012)
- Characterization of non-oxidative transaldolase and transketolase enzymes in the pentose phosphate pathway with regard to xylose utilization by recombinant *Saccharomyces cerevisiae*. Matsushika, A, Goshima, T, Fujii, T, Inoue, H, Sawayama, S, Yano S. *Enzyme Microbial Technol*, 51 (2), 16-25 (2012)
- Characterization of a recombinant flocculent *Saccharomyces cerevisiae* strain that co-ferments glucose and xylose: II. Influence of pH and acetic acid on ethanol production. Matsushika, A, Sawayama, S. *Appl Biochem Biotechnol*, 168, 2094-2104 (2012)
- Effects of hydrogen sulfide on the bacterial communities on the surface of galatheid crab, *Shinkaia crosnieri*, and in bacterial mat, by using rearing tanks. Konishi, M, Watsuji, T, Nakagawa, S, Hatada Y, Takai, K, Toyofuku, T. *Microbes Environ*, 28, 25-32 (2012)
- Thiofractor thiocaminus* gen. nov., sp. nov., a novel hydrogen-oxidizing, sulfur-reducing epsilonproteobacterium isolated from a deep-sea hydrothermal vent chimney in the Nikko Seamount field of the northern Mariana Arc. Makita, H, Nakagawa, S, Miyazaki, M, Nakamura, KI, Inagaki, F, Takai, K. *Arch Microbiol*, 194, 785-794 (2012)
- Microbial communities associated with Holothurians: presence of unique bacteria in the coelomic fluid. Enomoto, M, Nakagawa, S, Sawabe, T. *Microbes Environ*, 27, 300-305 (2012)
- Thermotomaculum hydrothermale* gen. nov., sp. nov., a novel heterotrophic thermophile within the phylum Acidobacteria from a deep-sea hydrothermal vent chimney in the Southern Okinawa Trough. Izumi, H, Nunoura, T, Miyazaki, M, Mino, S, Toki, T, Takai, K, Sako, Y, Sawabe, T, Nakagawa, S. *Extremophiles*, 16, 245-253 (2012)

2011

- Production and characterization of cellulases and hemicellulases by *Acremonium cellulolyticus* using rice straw subjected to various pretreatments as the carbon source. Hidenno, A, Inoue, H, Tsukahara, K, Yano, S, Fang, X, Endo, T, Sawayama, S. *Enzyme Microbial Technol*. 48, 162-168 (2011)
- Introduction of dehydration process into mechanochemical pretreatment for bioethanol production. Yanagida, T., Fujimoto, S, Bespyatko, LY, Tsukahara, K, Hidenno, A, Sawayama, S, Minowa, T. *石油学会誌*, 54, 215-221 (2011)
- Ethanol production from xylo-oligosaccharides by xylose-fermenting *Saccharomyces cerevisiae* expressing β -xylosidase gene. Fujii, T, Yu, G, Matsushika, A, Kurita, A, Yano, S., Murakami, K, Sawayama, S. *Biosci Biotechnol Biochem* 75, 1140-1146 (2011)
- Enhancement of beta-xylosidase productivity in cellulase producing fungus *Acremonium cellulolyticus*. Kanna, M, Yano, S, Inoue, H, Fujii, T., Sawayama, S. *AMB Express*, 1:15 1-8 (2011)
- Comparative study on a series of recombinant flocculent *Saccharomyces cerevisiae* strains with different expression levels of xylose reductase and xylulokinase. Matsushika, A, Sawayama, S. *Enzyme Microbial Technol*, 48, 466-471 (2011)
- Bioethanol production by *Pichia stipitis* from enzymatic hydrolysates of corncob-based spent mushroom substrate. Oguri, E, Takimura, O, Matsushika, A, Inoue, H, Sawayama, S. *Food Sci Technol Res*17, 267-272 (2011)
- Enhancement on biodegradation and anaerobic digestion efficiency of activated sludge using a dual irradiation process. Yang, Y N, Tsukahara, K, Yang, R, Zhang, Z, Sawayama, S. *Biores Technol*, 102, 10767-10771 (2011)
- Genetic analysis and preservation of chemolithoautotrophic bacteria dominating deep-sea vents. Nakagawa, S. *IFO Res Commun*, 25, 31-39 (2011)
- Hydrothermal fluid geochemistry at the Iheya North field in the mid-Okinawa Trough: Implication for origin of methane in subseafloor fluid circulation systems. Kawagucci, S, Chiba, H, Ishibashi, J, Yamanaka, T, Toki, T, Muramatsu, Y, Ueno, Y, Makabe, A, Inoue, K, Yoshida, N, Nakagawa, S, Nunoura, T, Takai, K, Takahata, N, Sano, Y, Narita, T, Teranishi, G, Obata, H, Gamo, T. *Geochem J*, 45, 109-124 (2011)

2010

- Pretreatment of rice straw by a hot compressed water process for enzymatic hydrolysis. Yu, G, Yano, S, Inoue, H, Inoue, S, Endo, T, Sawayama, S. *Appl Biochem Biotechnol*, 160, 539-551 (2010)
- Cellulase hyperproducing mutants derived from the fungus *Trichoderma reesei* QM9414 produced large amounts of cellulase at the enzymatic and transcriptional levels. Fujii, T, Murakami, K, Sawayama, S, *Biosci Biotechnol Biochem*, 74, 419-422 (2010)
- Evolutionary adaptation of recombinant shochu yeast for improved xylose utilization. Matsushika, A, Oguri, E, Sawayama, S. *J Biosci Bioeng* 110, 102-105 (2010)
- Effect of initial cell concentration on ethanol production by flocculent *Saccharomyces cerevisiae* with xylose-fermenting ability. Matsushika, A, Sawayama, S. *Appl Biochem Biotechnol*, 162, 1952-1960 (2010)
- Cost analysis of ethanol production from rice straw by phosphoric acid-hydrothermal pretreatment and enzymatic hydrolysis method. Yanagida, T, Fujimoto, S, Bespyatko, LY, Inoue, S, Tsukahara, K, Sawayama, S, Minowa, T. *J Jpn Soc Energy Resour*, 31, 15-20 (2010)
- Diversity and function of epibiotic microbial communities on the galatheid crab, *Shinkaia crosnieri*. Watsuji, T, Nakagawa, S, Tsuchida, S, Toki, T, Hirota, A, Tsunogai, U, Takai, K. *Microbes Environ*, 25, 288-294 (2010)
- Archaeoglobus sulfaticallidus* sp. nov., a novel thermophilic and facultatively lithoautotrophic sulfate-reducer isolated from black rust exposed to hot ridge flank crustal fluids. Steinsbu, BO, Thorseth, IH, Nakagawa, S, Inagaki, F, Lever, MA, Engelen, B, Ovreås, L, Pedersen, RB. *Int J Syst Evol Microbiol*, 60, 2745-2752 (2010)
- Molecular characterization of inorganic sulfur-compound metabolism in the deep-sea epsilonproteobacterium *Sulfurovum* sp. NBC37-1. Yamamoto, M, Nakagawa, S, Shimamura, S, Takai, K, Horikoshi, K. *Environ Microbiol*, 12, 1144-1153 (2010)
- Bacterial Lifestyle in a Deep-sea Hydrothermal Vent Chimney Revealed by the Genome Sequence of the Thermophilic Bacterium *Deferribacter desulfuricans* SSM1. Takaki, Y, Shimamura, S, Nakagawa, S, Fukuhara, Y, Horikawa, H, Ankai, A, Harada, T, Hosoyama, A, Oguchi, A, Fukui, S, Fujita, N, Takami, H, Takai, K. *DNA Res*, 17, 123-137 (2010)

2009

- Strain Improvement of *Acremonium cellulolyticus* for cellulase production by mutation. Fang, X, Yano S, Inoue, H, Sawayama, S. *J Biosci Bioeng*, 107, 256-261 (2009).
- Bioethanol production performance of five recombinant strains of laboratory and industrial xylose-fermenting *Saccharomyces cerevisiae*. Matsushika, A, Inoue, H, Murakami, K, Takimura, O, Sawayama, S. *Biores Technol* 100, 2392-2398 (2009).
- Wet disk milling pretreatment without sulfuric acid for enzymatic hydrolysis of rice straw. Hideno, A., Inoue, H, Tsukahara, K, Fujimoto, S, Minowa, T, Inoue, S, Endo, T, Sawayama, S. *Biores Technol*, 100, 2706-2711 (2009).
- Efficient bioethanol production by recombinant flocculent *Saccharomyces cerevisiae* with genome-integrated NADP⁺-dependent xylitol dehydrogenase gene. Matsushika, A, Inoue, H, Watanabe, S, Kodaki, T, Makino, K, Sawayama, S. *Appl Environ Microbiol*, 75, 3818-3822 (2009).
- Enzymatic hydrolyzing performance of *Acremonium cellulolyticus* and *Trichoderma reesei* against three lignocellulosic materials. Fujii, T, Fang X, Inoue, H, Murakami, K, Sawayama, S. *Biotechnol. Biofuels*, 2:24 (2009).
- Potential of ethanol production from major agricultural residues in Southeast Asia. Yano S., Inoue H., Tanapongpipat S., Fujimoto, S., Minowa, T, Sawayama, S, Imou, K, Yokoyama, S. *Int. J. Energy*, 10, 209-214 (2009).
- Ethanol production potential from oil palm empty fruit bunches in Southeast Asian countries considering xylose utilization. Yano, S, Murakami, K, Sawayama, S, Imou, K, Yokoyama, S. *Jpn Inst Energy*, 88, 923-926 (2009).
- Optimization of illumination time for the production of methane using carbon felt fluidized bed bioreactor in thermophilic anaerobic digestion. Yang, Y N, Tsukahara, K, Zhang, Z, Sugiura, N, Sawayama, S. *Biochem Eng J*, 44, 131-135 (2009)
- Geographical genetic structure and phylogeography of the *Sargassum horneri/filicinum* complex in Japan, based on the mitochondria cox 3 haplotype. Uwai, S, Kogame, K, Yoshida, G., Kawai, H, Ajisaka, T. *Mar Biol*, 156, 901-911 (2009)
- Isolation and physiological characterization of two novel, piezophilic, thermophilic chemolithoautotrophs from a deep-sea hydrothermal vent chimney. Takai, K, Miyazaki, M, Hirayama, H, Nakagawa, S, Querellou, J, Godfroy, A. *Environ Microbiol*, 11, 1983-1997 (2009)
- Nonpathogenic Epsilonproteobacteria. Nakagawa, S, Takaki, Y. *Encyc Life Sci*, a0021895 (2009)