Harvesting the riches of earth through bioprospecting and biotechnological exploitation of genomic data.

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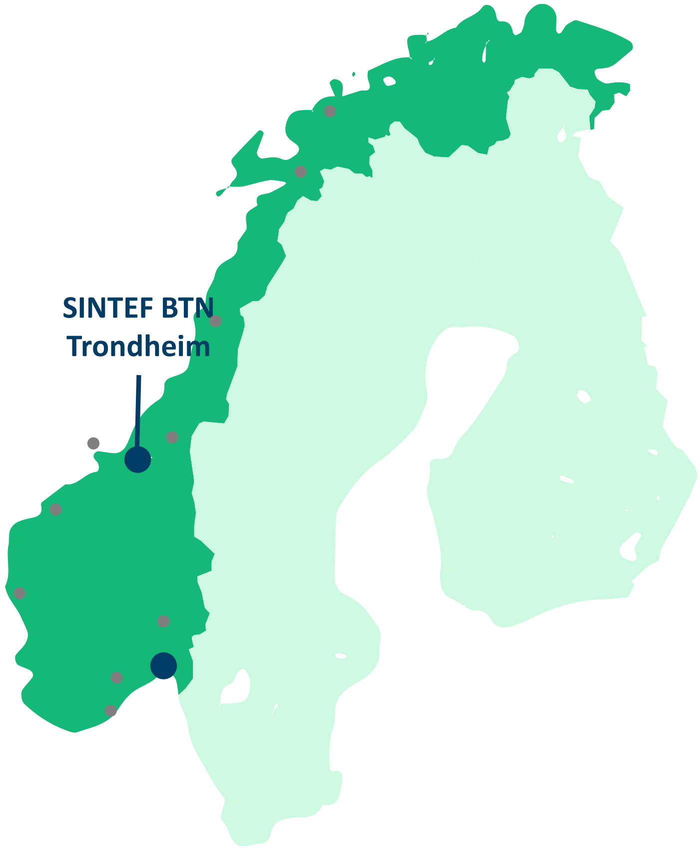
SINTEF Industry

Outline

- Who are we and what do we do
- Genomic data in an industrial setting:
  - Organism-focus vs. gene/product-focus
- Bioprospecting
- Examples



Biotechnology and Nanomedicine covers all the steps from bioprospecting to bioprocess development and analytics

Molecular Biology Strain development → High throughput screening → Laboratory- and pilot scale fermentation → Mass Spectrometry laboratory

DNA-sequencing (Illumina, ONT), ddPCR, Robot for DNA-prep, new lab with all necessary instrumentation

4 robotic systems for High throughput screening. Robot for colony picking. Fluorescence-activated cell sorting (FACS).

MP2-labs microreactor system, 40 laboratory scale fermenters, 50- and 300-L pilot-scale fermenters. Small and pilot scale DSP-equipment

26 instruments (GC-MS, GC-MS/MS, LC-SQ, LC-QQQ, LC-Q-TOF, FT-ICR, FFF, ICP-MS/MS, rapid fire)

Applied within: Pharmaceuticals, vaccines, biomaterials, enzymes, food, feed chemicals, and energy

Organisms of relevance: **fungi** (pharmaceuticals, enzymes, production hosts), **thraustochytrids** (n3-fatty acids; food/feed), **kelp/seaweed** (biomaterials, food, feed), microbiota/symbionts in interaction with **sponges** or **microalgae** (pharmaceuticals)

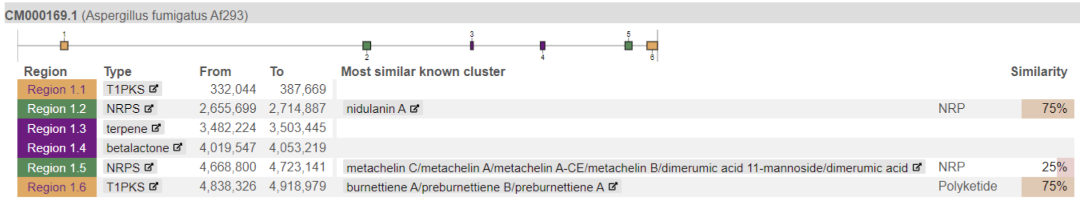
Biotechnological exploitation of sequencing data

Organism focus

- Understanding the biology of industrially important organisms and their microbiota
  - (Bacteria)
  - Yeasts and fungi
  - Microalgae
  - Thraustochytrids (protists)
  - Sponges (and their symbionts)
  - Seaweed
  - Plants
- Use as direct producers or host for recombinant expression of eukaryote genes/gene clusters
  - Yeasts and filamentous fungi
  - Plants (e.g., tobacco, cereals, legumes, vegetables)
  - Microalgae

Gene/product focus

- Bioprospecting
  - Enzymes for the synthesis or degradation of biomaterials and petrochemical materials
    - Bioremediation of pollutants
    - Biodegradation
    - Enzymes for biorefinery purposes
  - Cells and gene clusters to produce
    - Pharmaceuticals and bioactive compounds
    - Polysaccharides
    - Pesticides, herbicides, …



CM000169.1 (Aspergillus fumigatus Af293)

Region	Type	From	To	Most similar known cluster		Similarity
Region 1.1	T1PKS	332,044	387,669			
Region 1.2	NRPS	2,655,699	2,714,887	nidulanin A	NRP	75%
Region 1.3	terpene	3,482,224	3,503,445			
Region 1.4	betalactone	4,019,547	4,053,219			
Region 1.5	NRPS	4,668,800	4,723,141	metachelin C/metachelin A/metachelin A-CE/metachelin B/dimerumic acid 11-mannoside/dimerumic acid	NRP	25%
Region 1.6	T1PKS	4,838,326	4,918,979	burnettiene A/preburnettiene B/preburnettiene A	Polyketide	75%

https://fungismash.secondarymetabolites.org/upload/fungal-example/index.html

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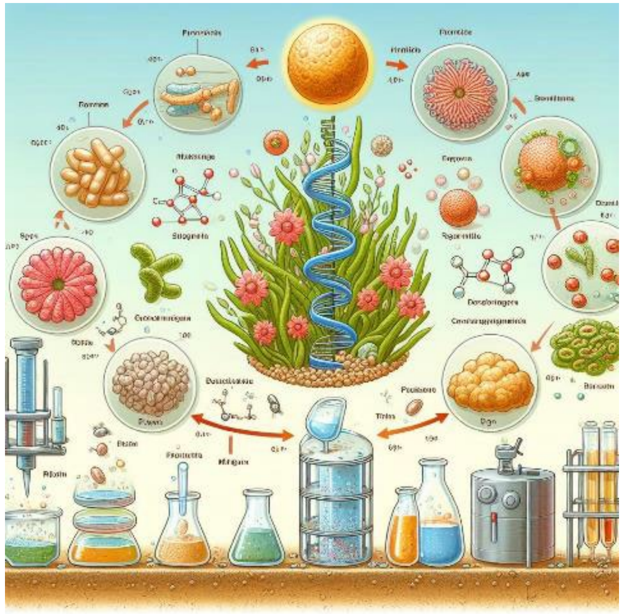
Bioprospecting

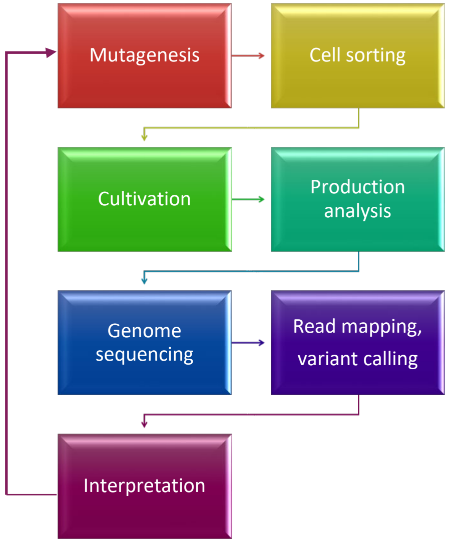
- The discovery and commercialization of products based on biological resources. This includes e.g., microorganisms, algae, plants, and animals, and useful genetic and biochemical properties of these organisms.
- Not a modern phenomenon
  - Medicinal use of plants for thousands of years (no chemical knowledge)
  - Morphine from poppy commercialized in 1827
  - Penicillin discovered in 1928
- Speed of discovery accelerated through genomic bioprospecting
- Wide range of relevant targets
  - Pharmaceuticals, nutraceuticals, veterinary medicines
  - Enzymes
  - Vaccine targets
  - Cosmetics/personal care products
  - Fertilizers, pesticides, and herbicides
  - Bioremediation and Biosensors



Bioprospecting according to Microsoft Copilot

Fungi

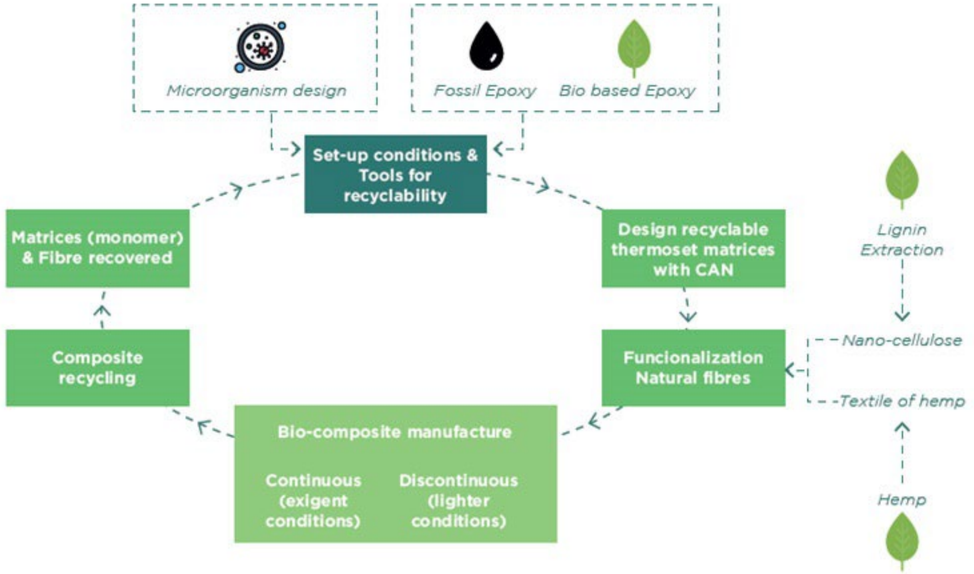
- Excellent sources for pharmaceuticals *
  - Novel compounds
  - Improved production strains and processes
  - Strain development regimes uses genome sequencing to gain insight into the biosynthesis of the compound and by-products
- Example: New API process for Leo Pharma
  - Leo Pharma (DK) collaborated with SINTEF for development of a new production strain and a suitable process for production of the antibiotic Fusidic acid.
  - SINTEF contributed with competence in strain development, molecular biology, and bioprocess development
  - The work relied heavily on SINTEFs competence, efficient labs and infrastructure for High Throughput Screening and fermentation
  - Financed by Leo Pharma



* https://doi.org/10.3390/antibiotics12081250

Fungi

- Heterologous expression host
- Source for important industrial enzymes §
  - Laccases & peroxidases
  - Cellulases & xylanases
  - Lipases etc.
- The ESTELLA project is generating bio-based epoxy composites, with increased biodegradability and recyclability
- SINTEF role: find microorganisms and enzymes that can aid the biodegradation/recycling of the materials.
  - Enriching for microorganisms that can grow utilizing the materials as sole carbon source
  - Mining for novel enzymes, esp. laccases, peroxidases, and epoxide hydrolases (typically found in fungi) that can degrade the materials
- Enzyme discovery pipelines established in SEP Agree

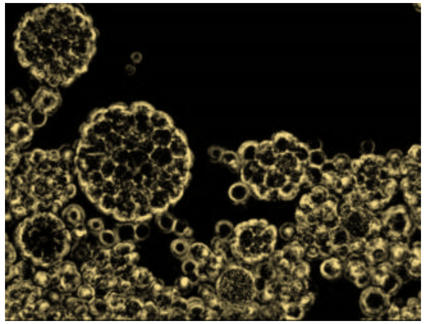


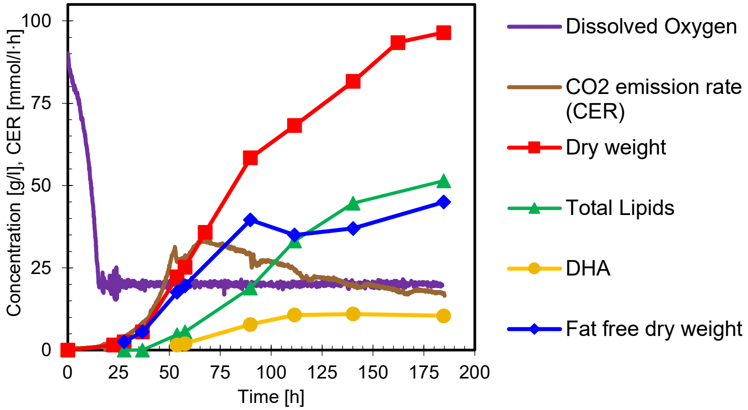
ESTELLA

§ https://www.mdpi.com/2309-608X/8/1/23

Thraustochytrids

- Heterotrophic protists, widely distributes in marine ecosystems
- Studied at SINTEF/NTNU for 20+ years
- Important source for n-3 fatty acids (DHA and EPA), important for fish farming; squalene (vaccine adjutant), and carotenoids like astaxanthin
- *Aurantiochytrium* spp. T66 and S61 sequenced (Liu et al. 2016*, unpublished)
- Accumulates fatty acids during N-starvation
- Global transcriptome analysis showed that N-starvation mainly affects the Fatty acid Synthase and less pronounced the PUFA-synthase in *Aurantiochytrium* sp. T66 (Heggeset et al 2019#).
- Recombinant expression of a Δ12-desaturase-like enzyme from *Aurantiochytrium* sp. T66 into *A. limacinum* SR21 revealed that this gene encodes a Δ9-desaturase accepting C16:0 as a substrate (Rau et al 2021§)



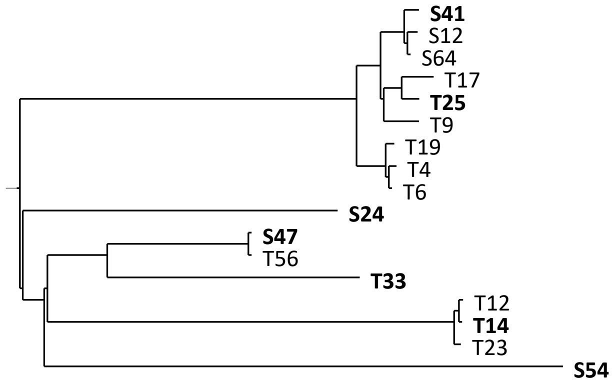


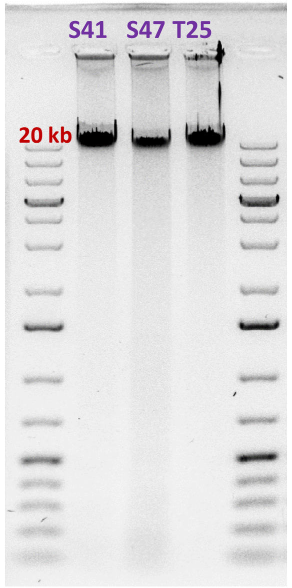
Thraustoeng, Auromega

* https://doi.org/10.1016/j.gdata.2016.04.013
# https://doi.org/10.1038/s41598-019-55902-4
§ https://doi.org/10.1007/s00253-021-11425-5

Thraustochytrids

- Strain collection with 87 isolates from water, sediments, and seaweed samples from 17 locations in Norway (and 10+ isolates also from other locations in Europe)
- A subset classified by 18S rDNA sequencing
- Genomic DNA extracted from 7 isolates, 3 selected for whole genome Nanopore sequencing at NMBU as part of EBP-Nor
  - *Thraustochytrium* spp. S41 and T25
  - Thraustochytriidae sp. S47
- In the internal SINTEF project SUSFF acetate produced by CO_2-fermenting bacteria is used as sole C-source for cultivation of thraustochytrids. Insight into the genomics and biology of the organism is of high importance when adapting growth media and setting up the cultivation





EARTH BIOGENOME NOR PROJECT

SUSFF Sustainable Food and Feed

SINTEF

Kelp and brown seaweed

- Producer of important biomaterials like alginate and fucoidan;
- *Alaria esculenta* (bladderlocks) and *Saccharina latissima* (sugar kelp) cultivated in Norway
- Alginate studied at SINTEF and NTNU since the 1950s
- From bacterial studies, alginate shown to be synthesized as polymannuronic acid, then mannuronan C5 epimerases (MC5E) introduces guluronic acid in the polymer
- Biosynthesis pathways for alginate in brown algae unravelled by genome sequencing of *S. japonica* (Chi et al 2018*) and *Ectocarpus siliculosius* (Fischl et al 2016[£])
  - Different families of MC5E, differentially expressed, implying specific roles in the plant life cycle
- Genomes of important species like *A. esculenta*[#], *S. latissima*[+], and Macrocystis pyrifera (giant kelp)[§] recently released, the quality and availability of annotation varies
- Genomic data provides increased insight into the biology of the organism and may affect the decision making with respect to e.g., where and when and how to inoculate and harvest; how to limit fouling, etc.

Juvenile sugar kelp cultivated on twine (Photo: SINTEF Ocean)

Norwegian Seaweed Biorefinery Platform

SFI Industriell bioteknologi Industrial biotechnology

* https://doi.org/10.1007/s00294-017-0733-4
£ https://doi.org/10.1093/glycob/cww040
https://doi.org/10.1111/mec.16714
+ https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_034768055.1/
§ https://doi.org/10.1016/j.dib.2022.108068

Sponges (Porifera)

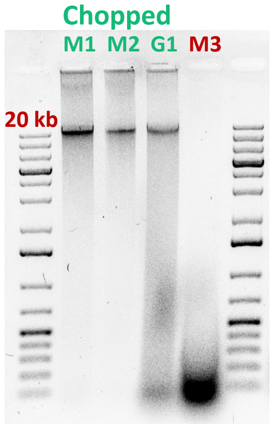
- Lives in symbiosis with a large number of microorganisms[£]
  - microbes responsible for the degradation of organic matter[$]
  - ~50% (i.e., 2659) of new marine natural products reported from invertebrates in the 2010s were sponge associated[#] (likely produced by the symbionts)
- MARBLES sampled sponges in the Trondheim fjord, for bioprospecting of the symbiont microbiomes
- Materials shared with EBP-Nor for sponge whole genome sequencing
- Extraction of HMW DNA is difficult; several kits and approaches tested
  - Chopping rather than cryo-grinding gave the best results
  - *P. ventilabrum* targeted by others (published by ERGA/ A. Riesgo Jan 2024)*
  - *Antho dichotoma* excluded as DNA extraction failed
  - *G. barretti* – promising, but then published by Steffen et al. Aug. 2023[§]
  - *M. lingua* – promising

Phakellia ventilabrum

Geodia barretti

Antho dichotoma

Mycale lingua



EARTH BIOGENOME NOR PROJECT

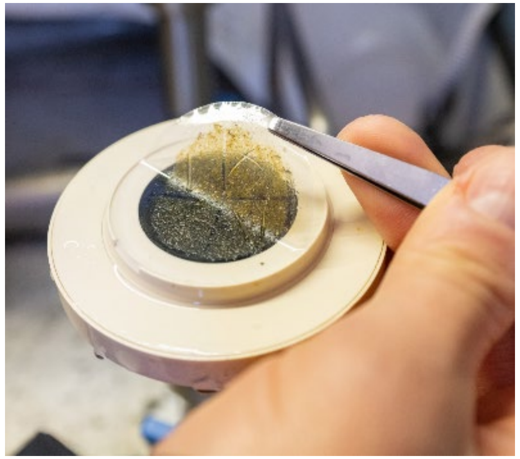
MARBLES

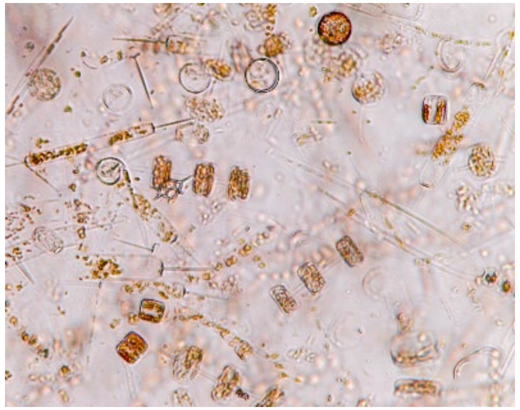
[£] https://doi.org/10.1038/ncomms11870
[$] https://doi.org/10.1038/s41396-020-0706-3
[#] https://doi.org/10.3390/md20060389
*https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_963921505.1/
[§] https://doi.org/10.1093/g3journal/jkad192

Exploring the ocean

- AtlantECO aims to develop and apply a novel, unifying framework that provides knowledge-based resources for a better understanding and management of the Atlantic Ocean and its ecosystem services.
- Scientific pillars: microbiomes; plastics and plastisphere; seascape and conductivity
- Relevant activities:
  - Bioprospecting for bioproducts like e.g., pharmaceuticals or enzymes able to digest plastics
  - Study the impact of offshore industry (oil, gas, diamond mining) on marine microbiomes
  - Development of genetic sensors for harmful microorganisms (e.g. algae)

AtlantECO
Atlantic Ecosystems Assessment, Forecasting & Sustainability





Photos: Tara Ocean Foundation

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RCN #227005 **ThraustoENG**

RCN #269432 **AurOmega**

https://www.digitallifenorway.org/projects/auromega/

RCN #294946 Norwegian Seaweed Biorefinery Platform

https://www.sintef.no/projectweb/seaweedplatform/

RCN # 309558 SFI Industriell bioteknologi Industrial biotechnology

https://sfi-ib.com/

RCN #326819 EARTH BIOGENOME NOR PROJECT

https://www.ebpnor.org/

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AtlantECO Atlantic Ecosystems Assessment, Forecasting & Sustainability

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https://www.atlanteco.eu/

MARBLES

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https://marblesproject.eu/

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Technology for a better society

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