PROJECTS

INSTITUTIONS

Cyanobacteria as a source for bone anabolic compounds

Joana T. Rosa[1] | Graciliana Lopes[2,3] | Vitor Vasconcelos[2,3] | M. Leonor Cancela[1,4] |Vincent Laizé[1]

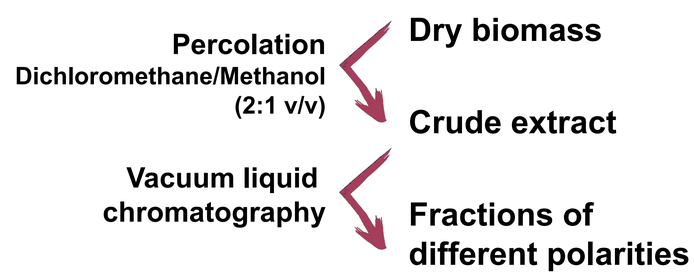
[1]CCMAR | [2]CiiMAR | [3]FCUP | [4]DCBM

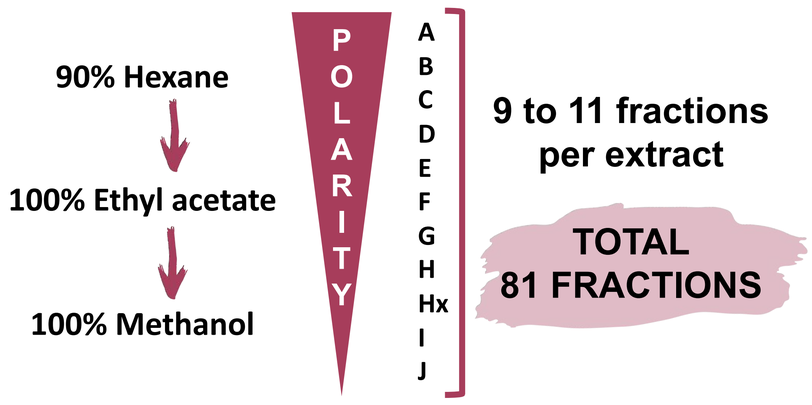
State of the art

Bone is a dynamic tissue under constant remodelling throughout life and any dysfunction in this tightly controlled physiological process often results in severe skeletal disorders, osteoporosis (OP) being the most common. Current therapeutics for OP are limited and have issues related to costs, efficacy and long-term use. It is thus critical to continue searching for new treatments. Recently, the presence of osteoactive compounds were reported in extracts from several marine organisms with the ability to regulate bone homeostasis. This not only sets a new paradigm for bone therapeutics research but also highlights the importance of screening marine resources to identify compounds with bone anabolic potential toward the development of new medicines.

Cyanobacteria: growth, extraction and fractionation

8 cyanobacteria strains were isolated and individually cultured. At the exponential phase, cells were harvested by centrifugation and subsequently freeze-dried. Dry biomass was subject to extraction and fractionation.



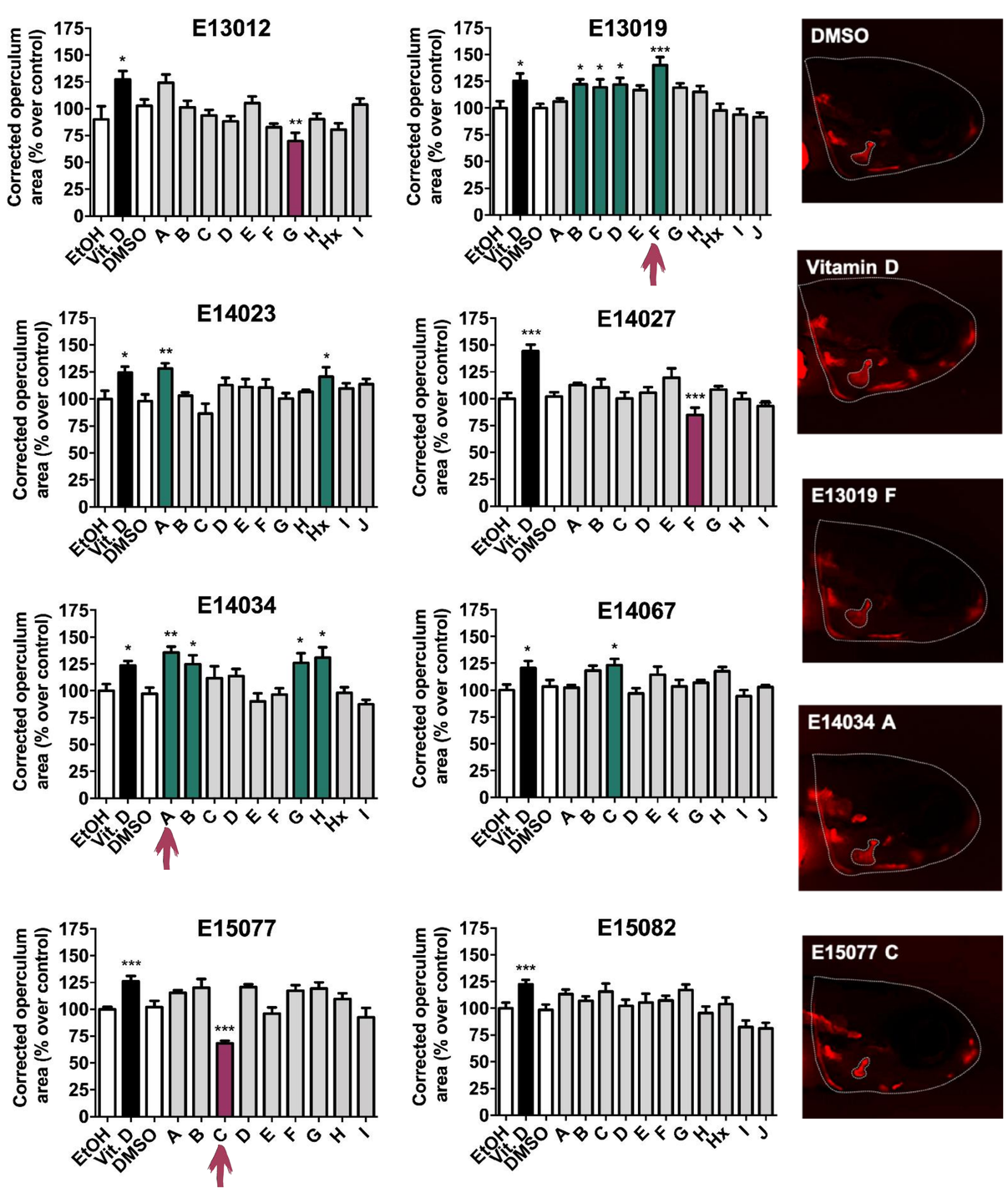


Screening pipeline using fish systems

Fish, in particular zebrafish (ZF) shares with humans a large number of disease-associated targets and drug metabolism pathways, including bone-related[1]. ZF offers fast and cost-effective genetic manipulation when compared to rodent models, and the complex tissues interactions required to model diseases such as OP together with a higher screening throughput. The osteogenic and mineralogenic potential of cyanobacteria was assessed using zebrafish *in vivo* systems and fish *in vitro* cell system.

1st round: potential to increase bone growth

Tool: ZF operculum screening system[2]
[Fraction]: 10 ng/µL
Duration: 3 to 6 dpf (n≥7)
End point: morphometric analysis of alizarin red S stained operculum



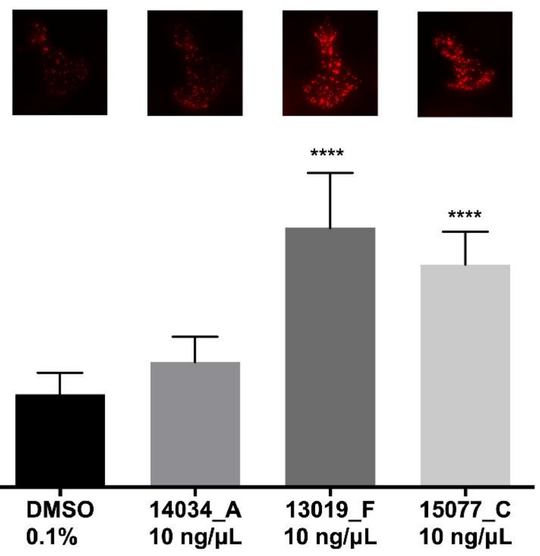
Fraction selection criteria was based on the potential to increase operculum size and/or the intensity of the staining.

3 FRACTIONS SELECTED FOR THE NEXT ROUNDS

2nd round: potential to induce osteoblast maturation

Tool: *Tg*(*Ola.sp7:mCherry*)[3] ZF line
[Fraction]: 10 ng/µL
Duration: 3 to 10 dpf (n≥7)
End point: quantification *of mcherry* fluorescence in the opercular bone

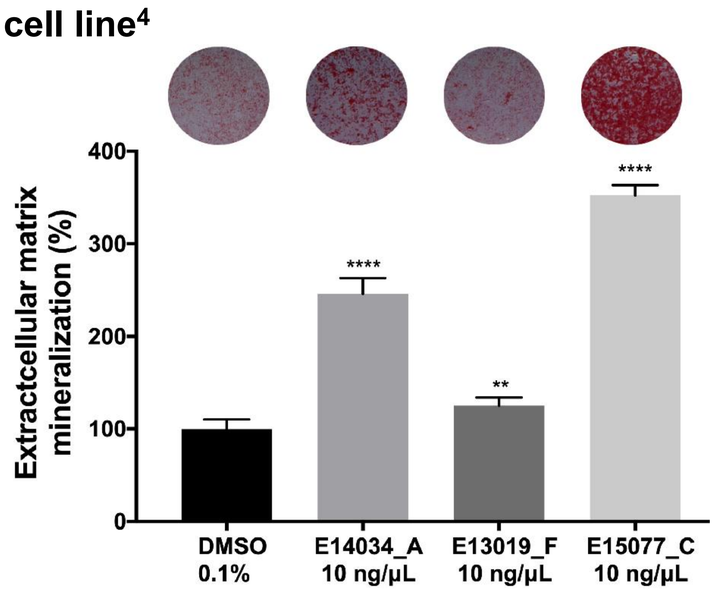
E15077_C & E13019_F INDUCE OSTEOBLAST MATURATION



3rd round: potential to induce matrix mineralization

Tool: Mineralogenic VSa13 cell line[4]
[Fraction]: 10 ng/µL
Duration: 15 days (6 rep.)
End point: quantification of mineral deposition by alizarin red S staining

ALL FRACTIONS INCREASE MINERAL DEPOSITION BUT TO DIFFERENT EXTENTS



Fish based screening systems allowed the identification of 3 cyanobacteria fractions with osteogenic potential that will be further tested in OP fish models followed by validation in mammalian models

Acknowledgments

This work was co-financed by OSTEOMAR (MAR-02.01.01-FEAMP-0057) and BLUEHUMAN (EAPA_151/2016) projects and by the Portuguese Science and Technology Foundation (FCT) through grant UID/Multi/04326/2019 to the CCMAR.

References

[1] Howe *et al.* Nature (2013)
[2] Tarasco *et al.* Comp. Biochem. Physiol. C - Toxicol. Pharmacol. (2017)
[3] Singh *et al.* Dev. Cell (2012)
[4] Pombinho *et al.* Cell Tissue Res. (2004)