Live Biotherapeutics-derived Short-Chain Fatty Acids as Potent Class I HDAC Inhibitors

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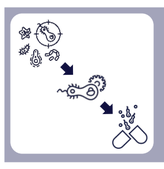
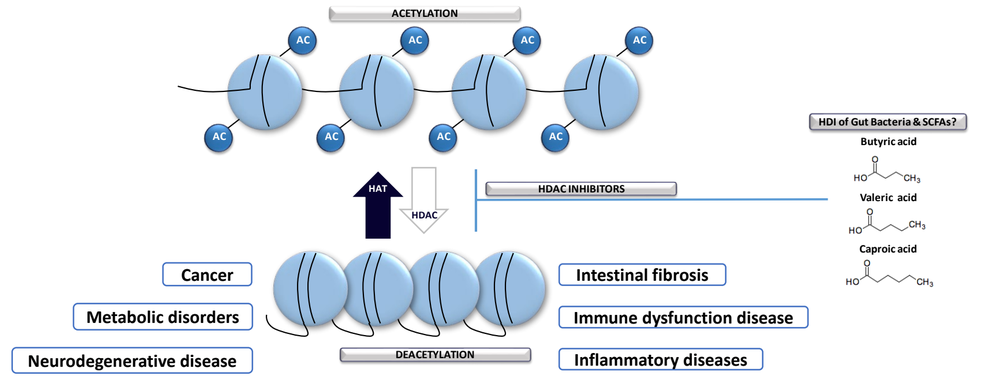


4D pharma is a pharmaceutical company focussed on developing Live Biotherapeutic products (LBPs) from the human gut microbiome. LBPs represent a new class of drugs that contain live organisms for the prevention, treatment or cure of disease. 4D pharma has clinical stage programmes in cancer, asthma, IBS and IBD, and a strong pipeline of pre-clinical programmes in oncology, CNS disease, autoimmunity and inflammation. We have developed a multi-disciplinary functional screening platform, MicroRx®, which enables us to target specific biological functions and select bacterial candidates from our large proprietary culture collection of commensal bacterial isolates from the gut microbiome of healthy human donors.

Screening for histone deacetylase inhibitors

Posttranslational modifications of histones play a pivotal role in the development and progression of various diseases by modulating gene expression, chromatin remodelling, and nuclear architecture. Overexpression of histone deacetylase (HDAC) isoforms has been implicated in a variety of pathologies, such as oncological and inflammatory conditions as well as cardiovascular and neurodegenerative diseases. As such, HDAC inhibitors have been identified as therapeutic targets.

The gut microbiota is known to modulate host physiology, particularly immunology, and microbial metabolites have been associated with host epigenetic mechanisms. HDAC inhibition (HDI) by gut commensals has been attributed to the short chain fatty acid (SCFA) butyrate. However, the potent and diverse metabolic reservoir provided by the gut microbiota and its role in host physiology warrants further investigation in a variety of diseases.



Results

Screening gut isolates for HDAC activity analyses

The initial screening of the cell-free supernatants (CFS) of 79 bacterial strains for total HDAC inhibitory effects on HT-29 whole cells resulted in the identification of potential HDAC inhibiting bacterial strains (**Fig 1**).

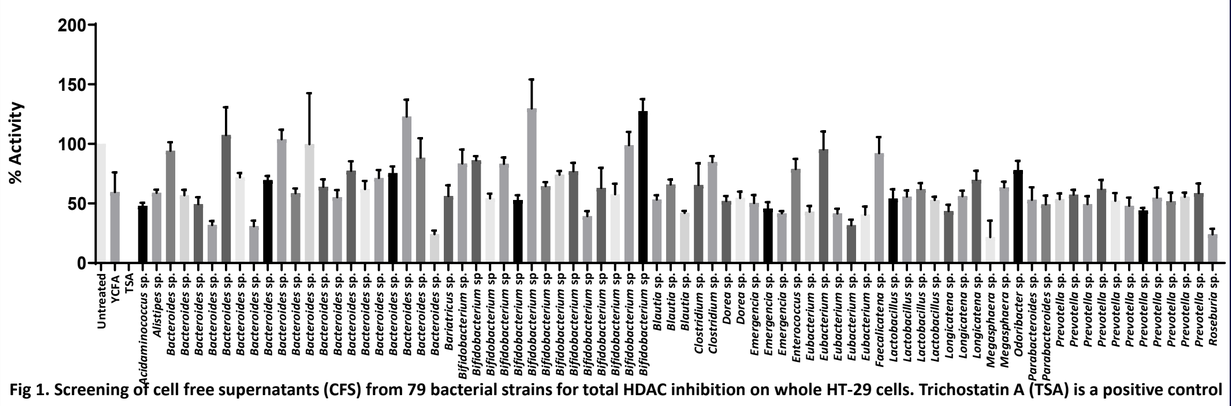


Fig 1. Screening of cell free supernatants (CFS) from 79 bacterial strains for total HDAC inhibition on whole HT-29 cells. Trichostatin A (TSA) is a positive control

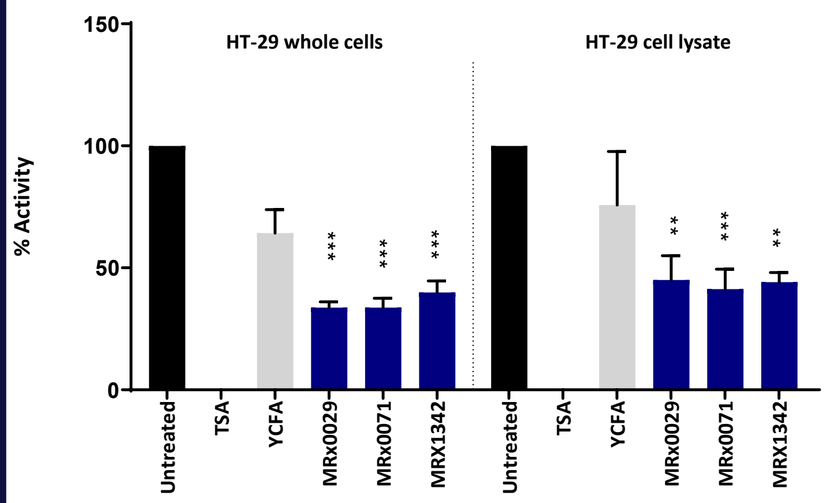


Fig 2. CFS of three selected bacterial strains tested for HDAC inhibition on HT-29 whole cell and HT-29 cell lysates. Trichostatin A (TSA) is used as a positive control. Significances tested against YCFA ** (p<0.005) * (P<0.001).**

Three strains with strong HDAC inhibitory effect were identified as ***Megasphaera massiliensis*** **MRx0029**, ***Roseburia intestinalis*** **MRx0071**, and ***Bariatricus massiliensis*** **MRx1342**. CFS of these selected strains were tested again to confirm their HDAC inhibition in HT-29 whole cells and on HT-29 cell lysate to confirm that the HDAC inhibition was not a result of the treatment of the cells prior to nuclear protein extraction.

The results in **Fig 2** show a similar HDAC inhibition of the supernatants on HT-29 cell lysates as compared to HT-29 whole cells.

SCFAs quantification of bacterial supernatants

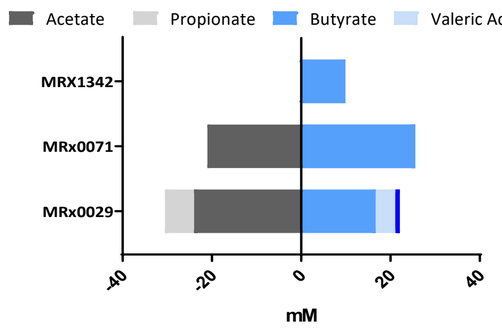


Fig 3. SCFAs profile of three candidate strains

All three bacterial strains, MRx0029, MRx0071 and MRx1342 produced butyrate (**Fig 3**).

The most potent butyrate producer MRx0071 (25.6 mM) closely matches MRx0029 (butyrate 16.7 mM, valeric acid 4.4 mM) with regard to HDI. This suggests a cumulative effect of butyrate and molecules other than SCFA produced by MRx0029 being involved in the observed HDI. (**Fig 2**)

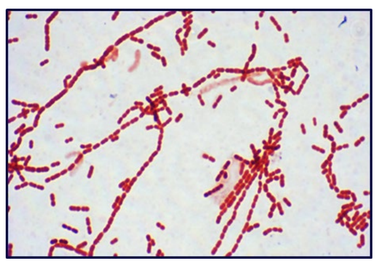


Fig 4. Gram staining of *M. massiliensis* MRx0029

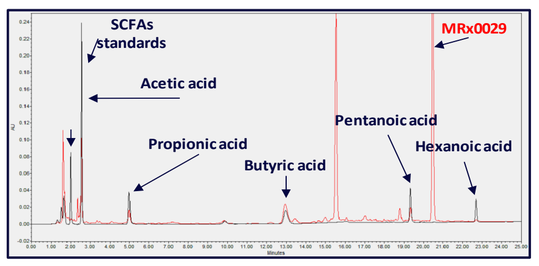


Fig 5. HPLC SCFAs profile of *M. massiliensis* MRx0029

MRx0029 (**Fig 4**), the strain whose supernatant showed the strongest HDI, was the only strain which produced valeric acid and hexanoic acid (1.0 mM) (**Fig 5**).

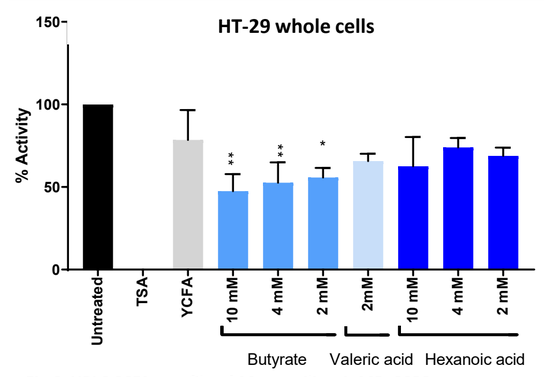


Fig 6. HPLC SCFAs profile of *M. massiliensis* MRx0029

To investigate what SCFAs were the responsible for the total HDAC inhibition, different concentrations of sodium butyrate, valeric acid and hexanoic acid were tested for HDI on whole HT-29 cells.

The results (**Fig 6**) show a significant (P<0.05) inhibition of HDAC activity by sodium butyrate on HT-29 whole cells, while hexanoic acid did not have a significant effect with any of the tested concentrations. Valeric acid concentrations above 2 mM were toxic to the HT-29 cells, thus HDI could not be measured.

HDAC inhibition by *M. massiliensis* MRx0029 is transferable to a microbiota model system (SimMi)

MRx0029 shifted the SCFA profile of a consortium of 17 bacteria (SimMi) towards higher butyrate concentrations (18.5 and 13.2 mM), and lower acetate (47 and 55 mM) and propionate (5.6 and 7 mM) concentrations than SimMi without MRx0029 (**Fig 7**).

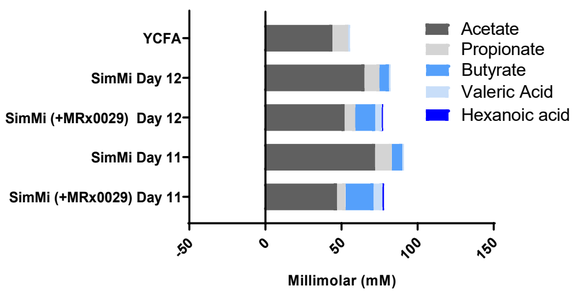


Fig 7. SCFA and MCFA concentrations of SimMi consortia (+/- MRx0029) on day 11 and 12 of continuous culture

The results from the HDAC activity assay demonstrate that the SimMi consortium + *M. massiliensis* MRx0029 exhibited a more potent total HDAC inhibition than the standard consortium on whole HT-29 cells (p<0.001) and on HT-29 cell lysate (p<0.05) (**Fig 8**). This demonstrates the physiologically relevant potential of *M. massiliensis* MRx0029, as a butyrate and valeric acid producing bacteria, to stimulate HDAC inhibition within an established bacterial community.

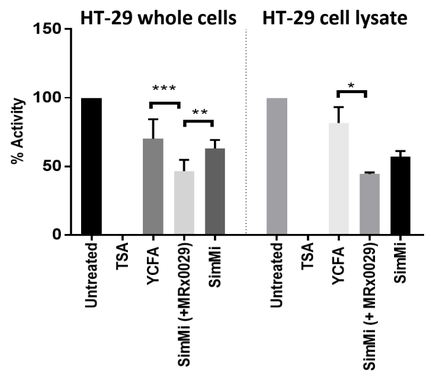


Fig 8. HDAC inhibition of CFS, obtained SimMi (+/- MRx0029) on whole HT-29 cells and on HT-29 cell lysate. TSA is used as a negative control. Significances tested against YCFA * (p<0.05) ** (p<0.005) * (P<0.001)**

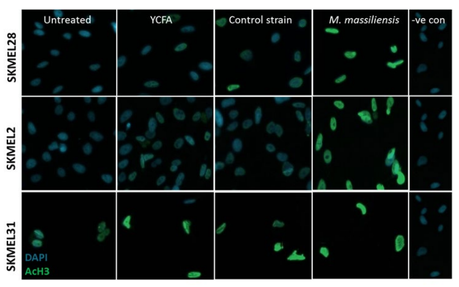


Fig 9. MRx0029 acetylates H3 in melanoma cell lines

To explore the application of HDAC inhibitory bacteria in a disease-relevant setting, assays were carried out for Class I and Class II HDACs using the supernatants of MRx0029, MRx0071, MRx1342, SimMi consortium +/- MRx0029. MRx0029 acetylates histones H3 and H4 (data not shown) in melanoma and CRC (data not shown) cell lines. This was accompanied by reduced clonogenic growth (**Fig 9**).

Correlation of SCFAs profile with specific HDI activity

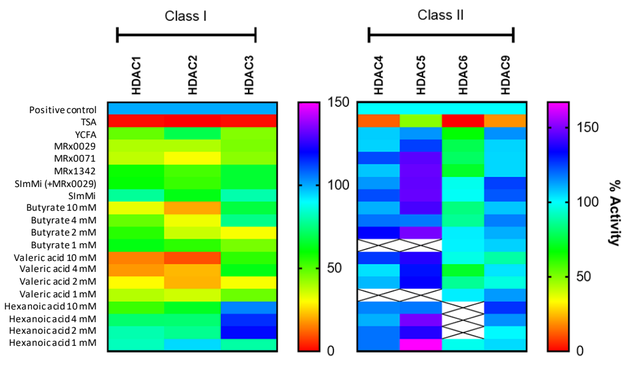


Fig 10. Inhibition of different isoforms of Class I and Class II HDACs by CFS of MRx0029, MRx0071, MRx1342, SimMi (+/- *M. massiliensis* MRx0029) and dilutions of SCFAs butyrate, valeric acid and MCFA hexanoic acid.

The Class I isoforms HDAC2 and HDAC3 were inhibited by the CFS and SCFAs tested (**Fig 10**).

Within the Class I HDACs the strongest effects were measured for the HDAC2 isoform, where CFS from all three candidate strains as well as SimMi with and without *M. massiliensis* MRx0029 resulted in a significant reduction of HDAC2 activity (F**ig 11B**).

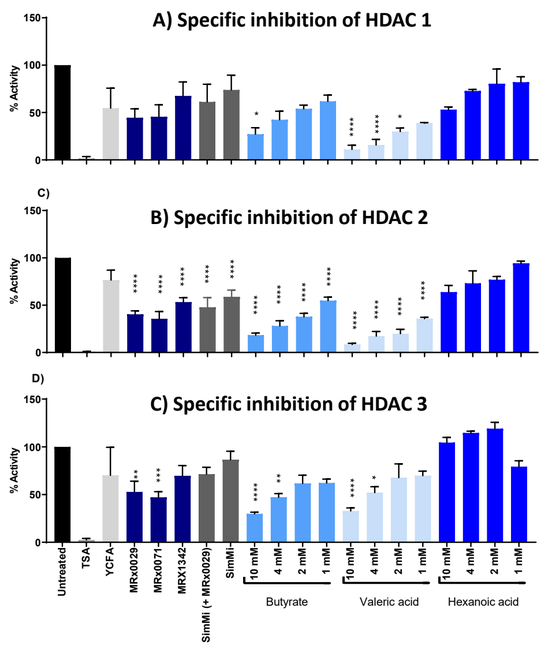


Fig 11. Specific inhibition of HDACs by CFS. TSA is used as a negative control. Significances tested against YCFA * (p<0.05) ** (p<0.005) * (P<0.001) **** (p<0.0001).**

The inhibitory effect of SimMi + MRx0029 was stronger than the core consortium alone. Sodium butyrate and valeric acid inhibited HDAC2 at all concentrations tested, while hexanoic acid did not show any significant inhibitory effect. HDAC3 was significantly inhibited by MRx0029 and MRx0071, and only by the higher concentrations of sodium butyrate and valeric acid tested (10 mM and 4 mM) (**Fig 11C**). HDAC1 was inhibited by butyrate and valeric acid (**Fig 11A**).

Key findings

HDAC inhibitory properties of cell-free supernatants (CFS) derived from a panel of phylogenetically diverse human gut commensals were correlated to their SCFA profiles.

We identified three bacteria as the most potent total HDAC inhibitors, which produced valerate and/or butyrate and specifically inhibited Class I HDACs.

The gut microbiota can influence HDAC activity via a plethora of microbial-derived metabolites. We here show that single bacterial strains from the human gut microbiota have potential as novel HDI-based therapeutics for diseases including oncology and neurodegenerative conditions.

REFERENCE: Yuille, Samantha, et al. "Human gut bacteria as potent Class I histone deacetylase inhibitors in vitro through production of butyric acid and valeric acid." PloS one 13.7 (2018): e0201073

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