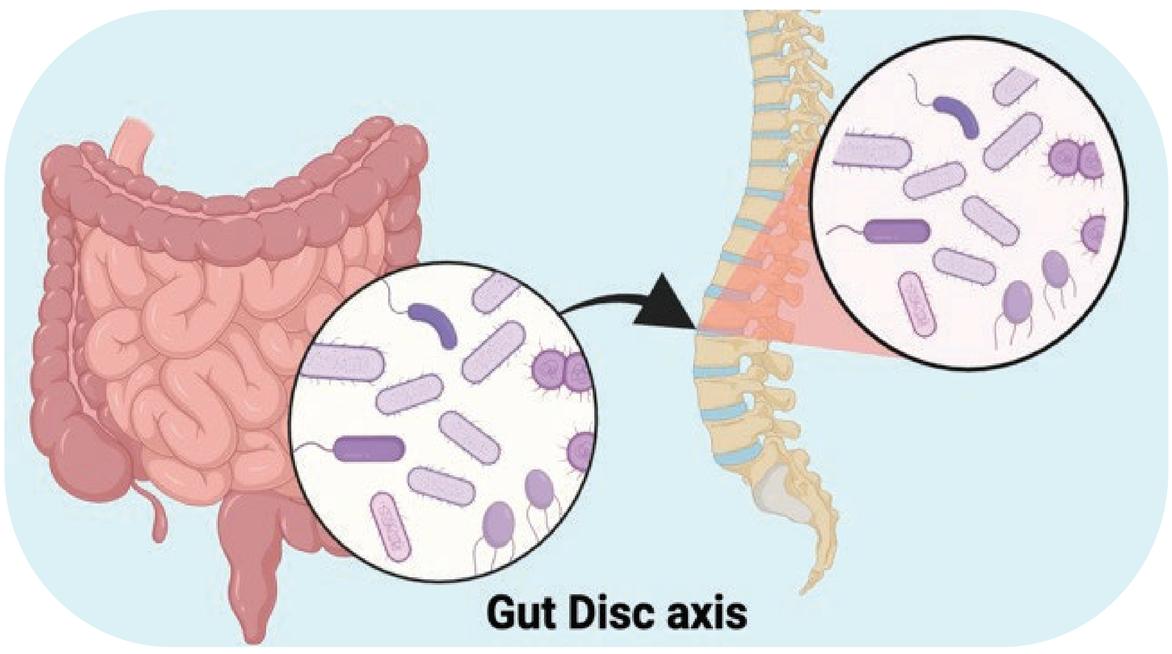
Detection of microbial DNA in gut and intervertebral discs of germ free and specific pathogen free mice

Ashish Kumar[1,2], Rosemary Abdoul Ahad[3], Nora Brigit Joseph[3], Junhua Wang[3], Ziad Al Nabhani[3], Alessandro Bertolo[4], Benjamin Gantenbein[1,4]

[1]Tissue Engineering for Orthopaedics and Mechanobiology, Bone and Joint Program, Department for BioMedical Research (DBMR) of the Medical Faculty, University of Bern, Bern, Switzerland. ,[2]Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern, Switzerland , [3]Department of Visceral Surgery and Medicine Inselspital Bern University Hospital, Department for BioMedical Research DBMR Faculty of Medicine, University of Bern, Bern, Switzerland, [4]Swiss Paraplegic Research, Nottwil, Switzerland, [4]Department of Orthopaedic Surgery and Traumatology, Inselspital, Bern University Hospital, Medical Faculty, University of Bern, Bern, Switzerland

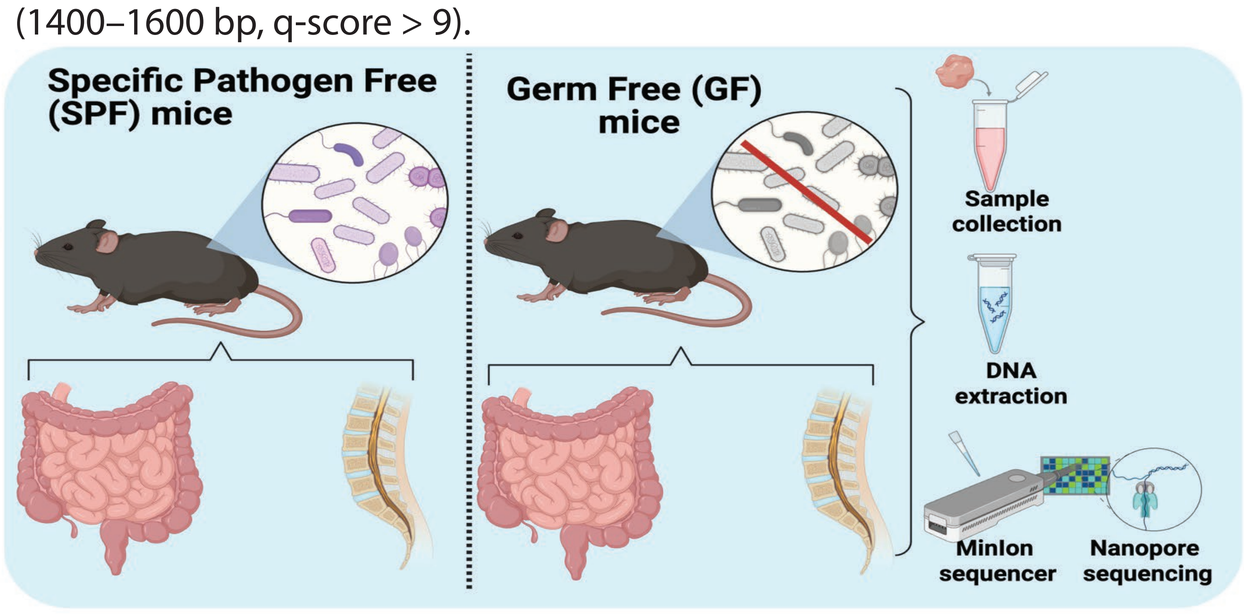
Introduction

1. Low back pain (LBP) is a major global burden of disability, often caused by intervertebral disc (IVD) degeneration (IDD)
2. Bacterial infiltration into the disc tissue could contribute to IDD and LBP, and several studies have identified *Cutibacterium acnes* in pathological discs
3. Recent research has suggested a role of the microbiome in the 'gut-spine axis' concept.
4. **The aim** of this study is to sequence DNA and identify bacteria from intervertebral disc and gut samples in both control and germ-free mice



Materials & Methods

1. C57BL6J mice (n = 3) under SPF or GF conditions. DNA concentrations were measured with Qubit.
2. DNA was extracted using the QIAamp UCP Pathogen Mini Kit from IVD and gut tissues of mice from both groups.
3. A defined bacterial mixture (ZymoBIOMICS Microbial Community Standard) served as a positive control, and an internal spike-in control (ZymoBIOMICS Spike-in Control I) was added before 16S rRNA gene amplification for absolute quantification
4. Sequencing was performed using nanopore technology on a MinION device, followed by base calling with Dorado and filtering with Nanofilt (1400–1600 bp, q-score > 9).



Results

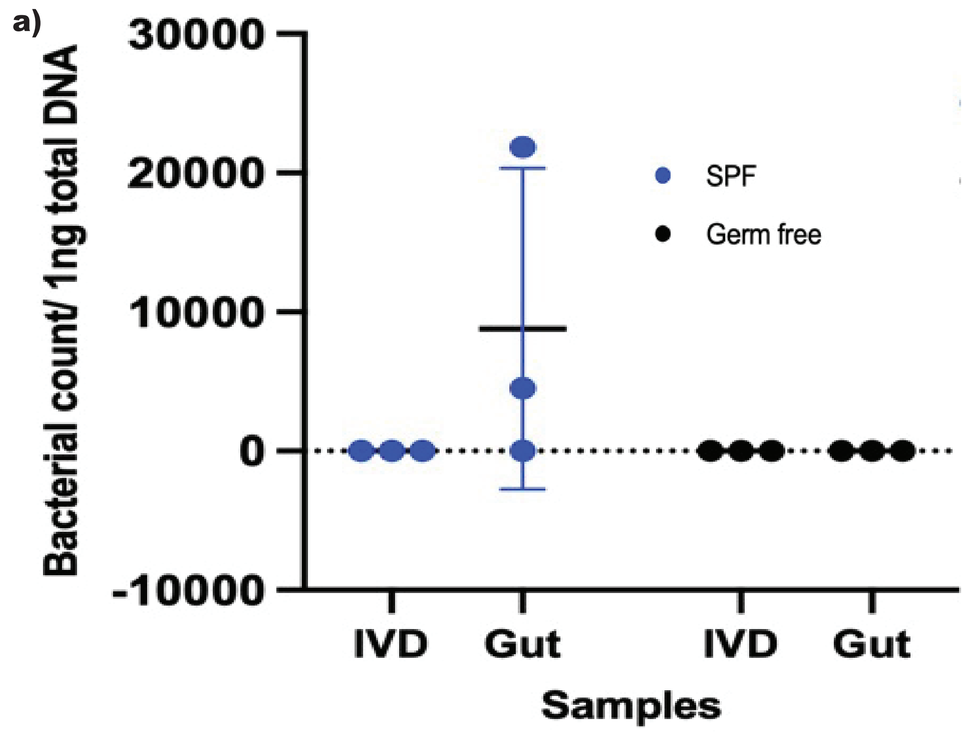


Figure 1: Comparsion of bacterial load in intervertebral disc (IVD) and gut sample from specific pathogen free (SPF) and Germ free (GF) mice. Bacterial counts were quantified as the number of bacteria per 1ng of total DNA in each sample. Bacteria were only identified in the gut of SPF mice, while both IVDs and all germ free samples displayed negligible to undetectable bacterial loads. Each data point represents an individaul biological replicate, horizontal bars indicate group mean± standard deviation

a)

	Expected	Measured
Bacillus subtilis	17.4%	17.7%
Limosilactobacillus fermentum	18.4%	18.5%
Staphylococcus aureus	15.5%	11.9%
Listeria monocytogenes	14.1%	10.8%
Escherichia coli	10.1%	12.5%
Salmonella enterica	10.4%	14.1%
Enterococcus faecalis	9.9%	13.1%
Pseudomonas aeruginosa	4.2%	0.8%
Shigella flexneri	0.0%	0.8%

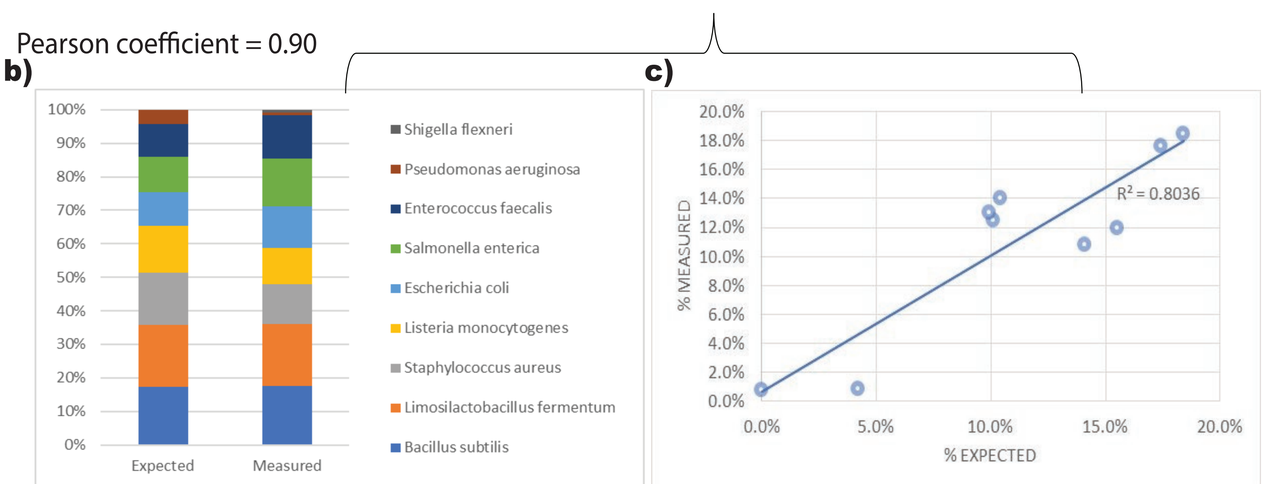


Figure 2: Validation of microbial community profiling using a defined Microbial Community Standard. **Fig 2a**: Table summarizing expected versus measured relative abundance of nine bacterial taxa in standard mix, **Fig 2b**: Stacked bar chart comparing expected and measured proportions of each bacterial species, **Fig2c**: Linear regression analysis demonstrating the correlation between measured and expected percentage abundance for each bacterial species in standard(R^2=0.8036). Each point represents an individual bacterial taxon

DISCUSSION

- These findings indicate that SPF mice may be unsuitable for studying a native IVD microbiome, as discs lacked detectable bacterial DNA. The study also confirmed the GF status of GF mice.
- Future work will include faecal samples to further understand the gut-disc axis and the potential relationship between gut microbes and IVD health.

Conclusion

- No bacteria were detected in the intervertebral disc samples. If bacterial DNA was present, it was likely to be a false positive. In contrast, bacterial DNA was identified in the gut samples of control/SPF mice, although it was only present in two out of three samples

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Contact

benjamin.gantenbein@unibe.ch
ashish.kumar@unibe.ch