



Microbial diversity within the human stomach by culturing and culture-independent methods

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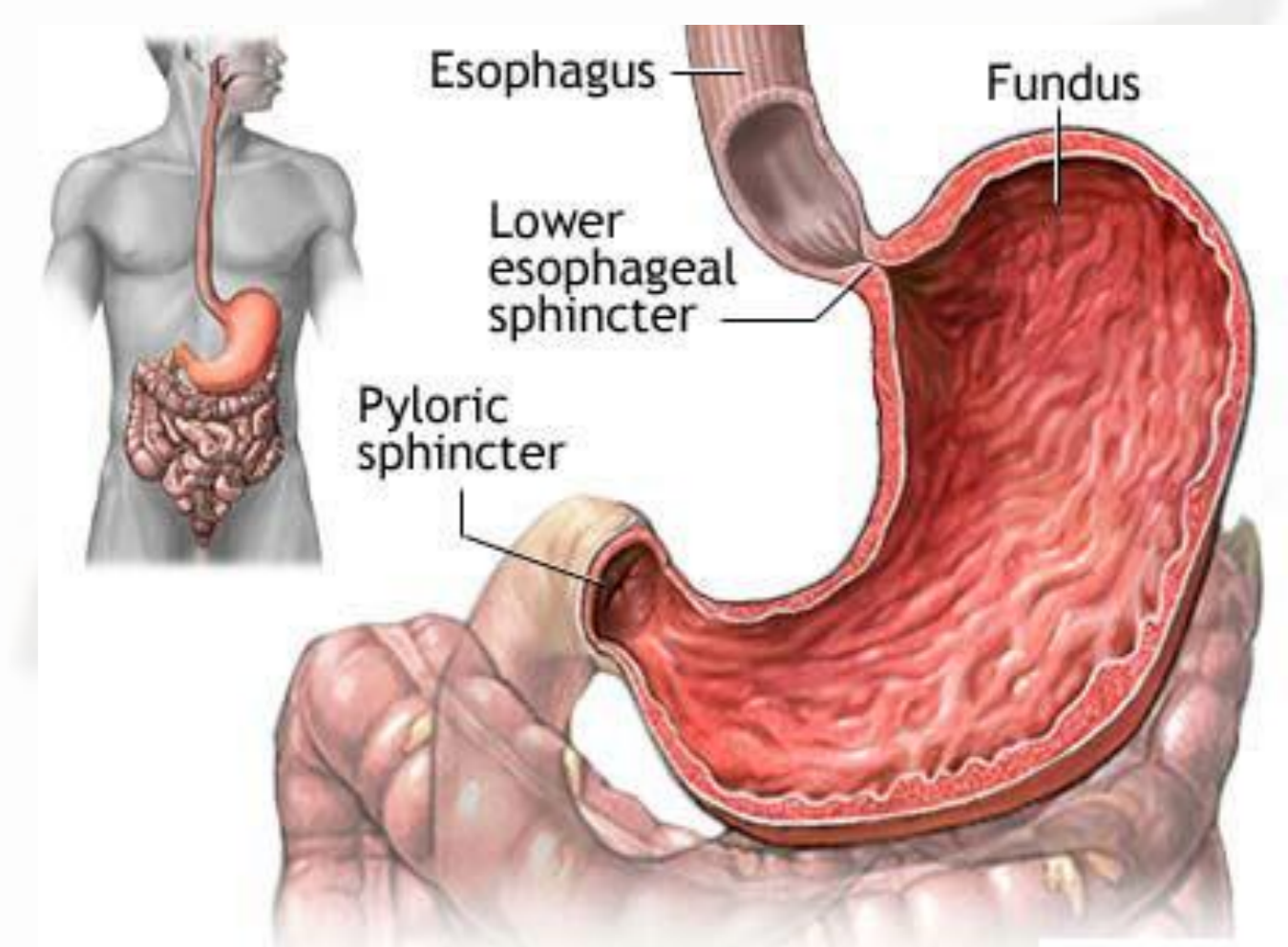
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INTRODUCTION

The human stomach has been considered a sterile environment until recently, due to the high level of chlorhydric acid and the presence of digestive enzymes. Therefore, it was socking the identification of the pathogen *Helicobacter pylori* in this ecosystem. This opened the conception to the existence of a microbial community adapted to this ecological niche. In the last years, culture-independent techniques, such as DGGE, construction and analysis of 16S rRNA gene libraries and next generation sequencing technologies, have broadened the bacterial diversity of the human stomach.

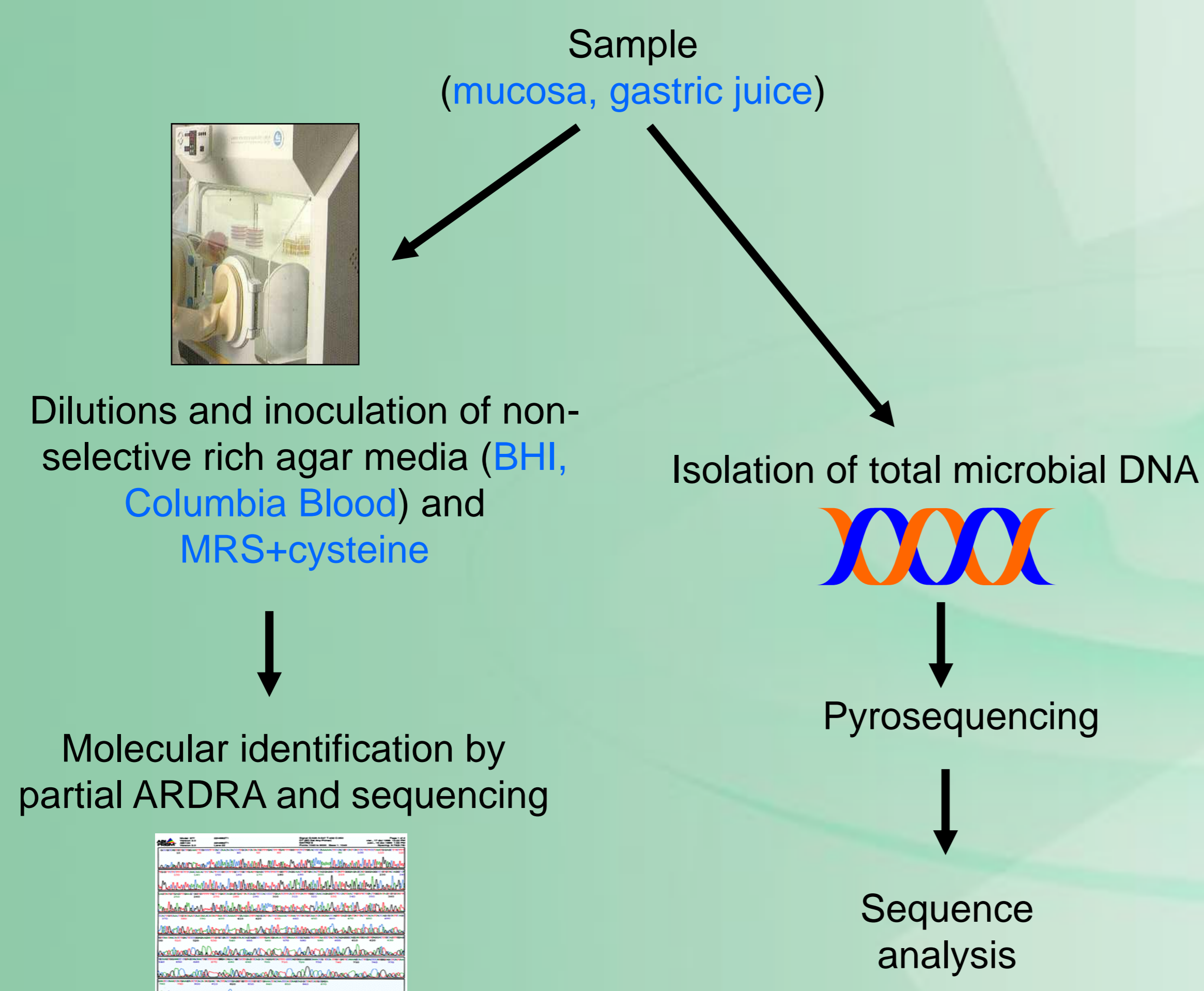
OBJECTIVES

This study was aimed to evaluate the microbial diversity of stomach samples (mucosa and gastric juice) from healthy humans by culturing and the phylogenetic metagenomic approach of pyrosequencing. The conventional culturing method would allow the subsequent identification, typing and characterization of strains with appropriate properties of probiosis to be used as organ-specific probiotics.



METHODOLOGY

Figure 1. General flow chart of the methodology follow in this study.



2. BACTERIAL DIVERSITY OF THE GASTRIC MUCOSA BY PYROSEQUENCING

Total microbial DNA was purified from four mucosa samples and subjected to sequential nested PCR amplifications with 16S rDNA universal bacterial primers; amplicons were then pyrosequenced. A total of 15,659 high-quality, partial 16S rDNA reads larger than 200 nt were obtained (Figure 2). Sequence analysis grouped the reads into 59 families, 69 genera (Figure 3), and more than 300 OTUs (defined at a 97% of sequence identity). As in the cultures, notable differences in microbial numbers and types were observed between samples from different individuals (Figure 3, Figure 4). However, the most abundant reads belonged in all four cases to *Streptococcus*, *Propionibacterium* and *Lactobacillus* species. Comparison of the stomach microbiota to that present in other parts of the human gastrointestinal tract showed distinctive microbial communities (Figure 5), that may be adapted to this harsh niche.

RESULTS

1. CULTURING ANALYSIS OF HUMAN STOMACH SAMPLES

Twelve mucosa and their corresponding gastric juice samples were subjected to microbial analysis by culturing. Cultivable microorganisms were recovered from all but three samples. Counts ranged from 10^2 and 10^4 cfu/g or ml. Representative isolates of all morphotypes from the different agar plates were identified by molecular methods. Cultures were assigned to 16 bacterial species (Table 1). *Propionibacterium acnes*, *Lactobacillus gasseri*, and *Staphylococcus epidermidis* showed to be the dominant species, although inter-individual differences were noted.

Table 1. Cultivable microorganisms from mucosa and gastric juice samples

SAMPLE	IDENTIFIED MICROORGANISMS			Total
	Lactobacilli	Propionibacteria	Other	
M1	-	<i>P. acnes</i> (6)	<i>Br. paraconglomeratum</i> (2) <i>S. saprophyticus</i> (3) <i>A. viridans</i> (2)	16
M2	-	<i>P. acnes</i> (6) <i>P. granulosum</i> (1)	<i>S. epidermidis</i> (3)	10
M3	<i>L. reuteri</i> (4) <i>L. gasseri</i> * (2)	<i>P. acnes</i> (2)	<i>S. pasteurii</i> (1) <i>S. epidermidis</i> (2)	11
M4	-	-	-	-
M5	<i>L. gasseri</i> (2) <i>L. vaginalis</i> (2)	<i>P. acnes</i> (2)	-	6
M6	-	<i>P. acnes</i> (11)	-	11
M7	<i>L. paracasei</i> * (1) <i>L. fermentum</i> * (2)	<i>P. acnes</i> (6)	<i>St. salivarius</i> * (1) <i>S. epidermidis</i> (1)	11
M8	-	<i>P. acnes</i> * (4)	<i>S. epidermidis</i> * (1)	5
M9	-	-	-	-
M10	<i>L. gasseri</i> * (2)	<i>P. acnes</i> (1)	-	3
M11	-	-	<i>E. coli</i> (3) <i>Clostridium</i> sp. (3)	6
M12	<i>L. gasseri</i> * (3) <i>L. vaginalis</i> * (1)	-	<i>St. salivarius</i> (2) <i>P. pentosaceus</i> (1)	7
Total	19	42	25	86

*, Isolated from gastric juice

Figure 2. Rarefaction curves of 16S rRNA sequences from the four stomach mucosa samples at a 97% of similarity.

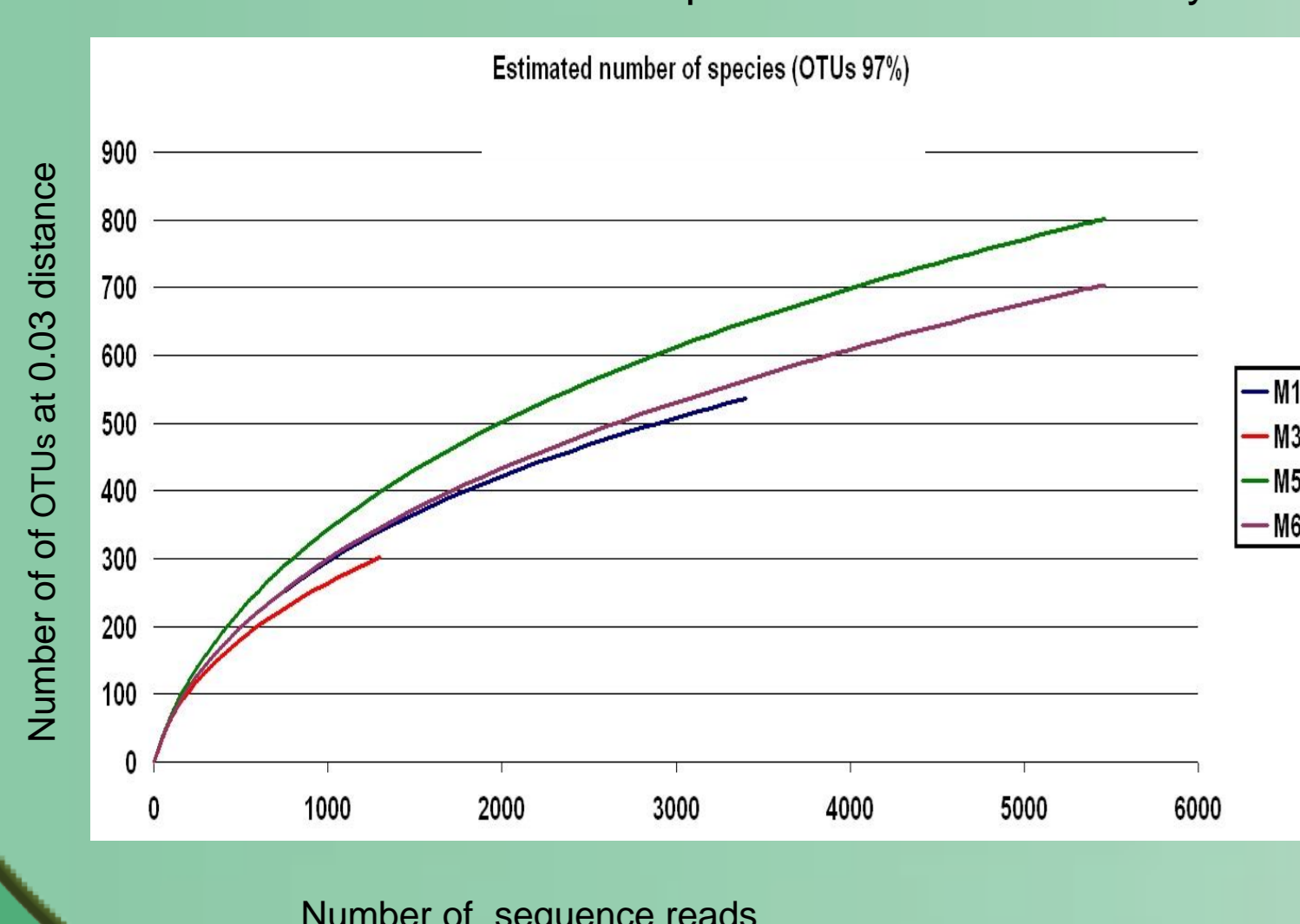


Figure 3. Genera composition in the four mucosa samples by pyrosequencing.

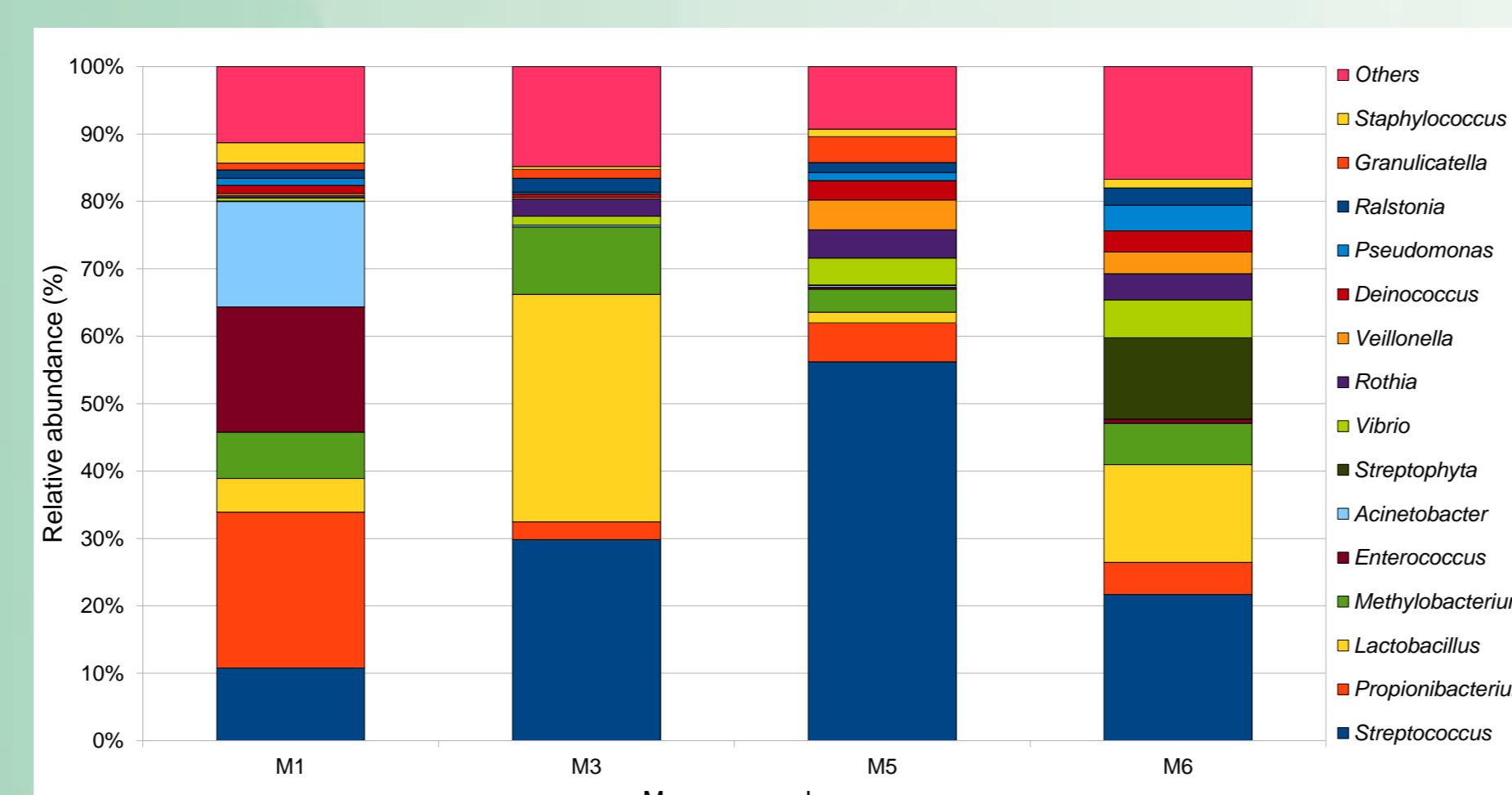


Figure 4. Venn diagram showing specific and common genera in the mucosa samples

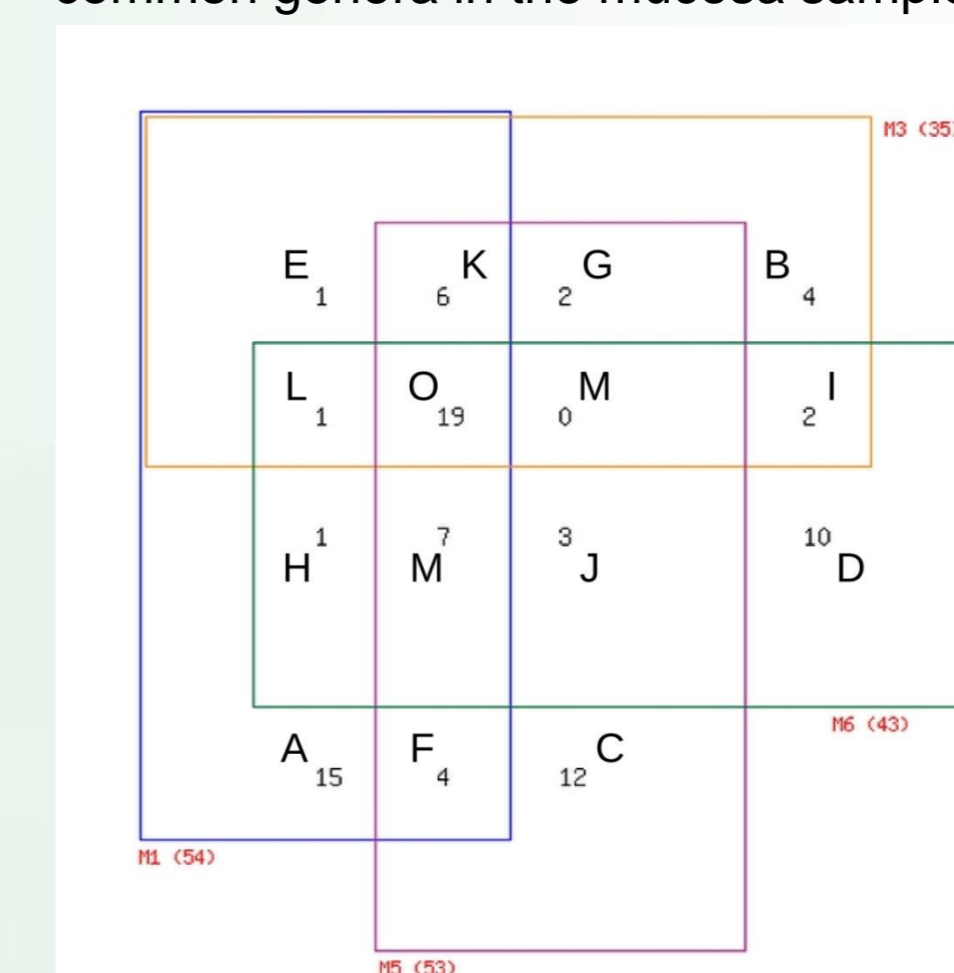
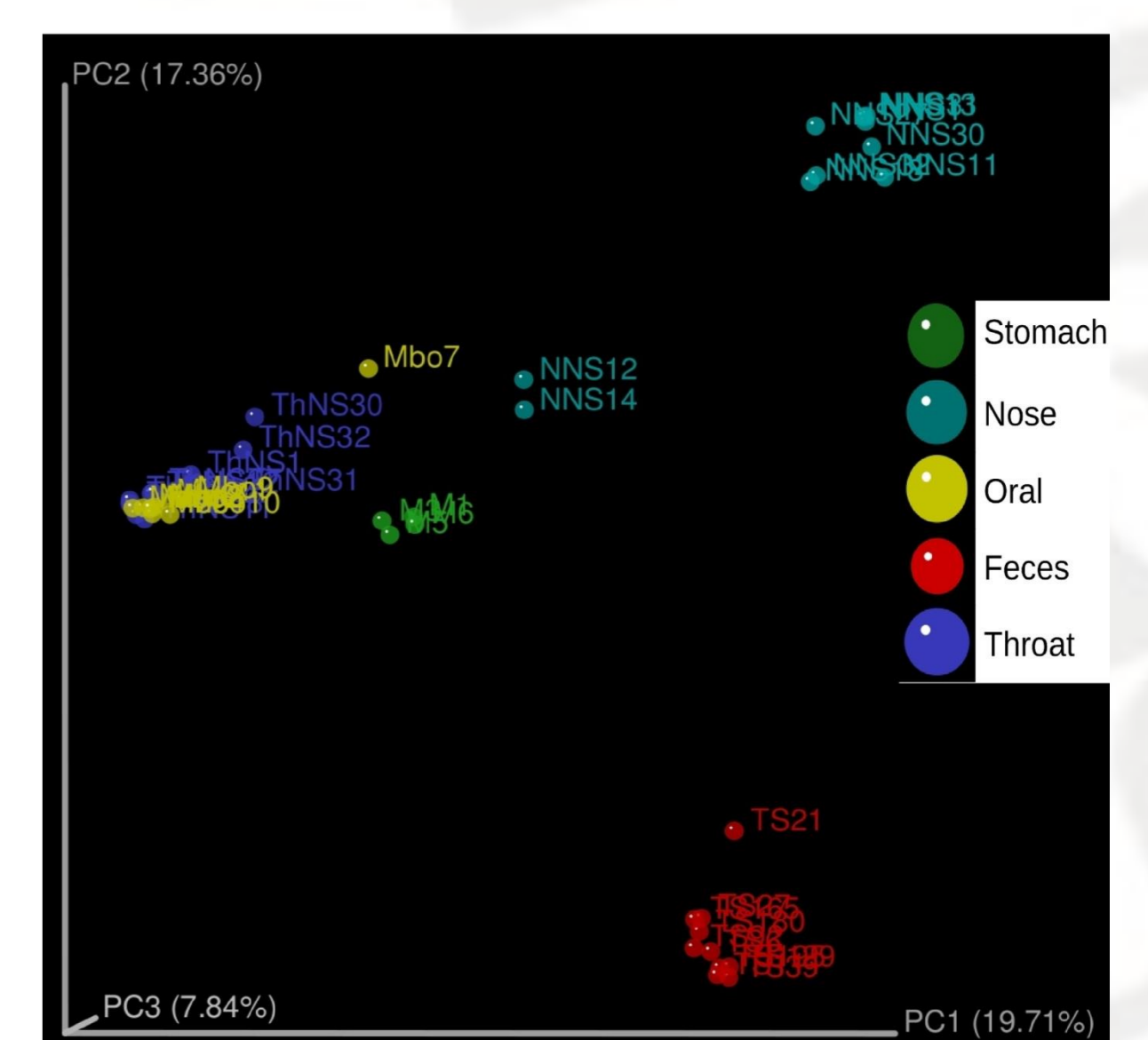


Figure 5. Scatterplot showing comparison of bacterial composition among samples from stomach, nose, mouth and throat.



CONCLUSIONS

- 1.- High inter-individual diversity in numbers and/or types of bacterial species were shown in the different stomach samples by both culturing and culture-independent methods.
- 2.- An extremely-rich, indigenous gastric microbiota, different from those of other parts of the gastrointestinal tract, was revealed in this study using pyrosequencing.
- 3.- By either the two methods, dominant bacteria were shown to belong to the genera *Streptococcus*, *Propionibacterium* and *Lactobacillus*, suggesting these organisms may play a key role in the microbial homeostasis of this organ.