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Qualitative Identification of *Roseburia hominis* in Faeces Samples Obtained from Patients with Irritable Bowel Syndrome and Healthy Individuals †

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Abstract: Various products coded by genes recognized in microbiota are involved in many biochemical pathways in thehuman body. Bacteria composition in the gastrointestinal tract may be an important aspect of selected diseases' pathogenesis, including irritable bowel syndrome (IBS). Traditional research methods based on classical microbiology, using selective media for bacterial growth, have proven to be ineffective. The use of genetic methods allows the identification of unidentified microbiota, including anaerobes. *Roseburia hominis* is a flagellated gut anaerobic commensal bacterium, producing short-fatty acids. The knowledge about the microbial components of the intestinal ecosystem is still very limited, including *Roseburia hominis*. This study aimed to identify *Roseburia hominis* in the faeces samples obtained from IBS patients and healthy individuals, using PCR techniques. The differences between studied groups were observed, and it was concluded that *R. hominis* may play a role in IBS etiology.

Keywords: gut microbiome; gut microbiota; *Roseburia hominis*; IBS

1. Introduction

All the microorganisms inhabiting a particular human body region or organ are collectively called a microbiome. The human body encompasses several different microbiomes that include specific populations of microorganisms [1]. Currently, intensive research is underway on the

microbiomes and their influence on human health. The knowledge about microbiomes is still very limited, including the intestinal microbiome [2].

Nowadays, the gut microbiome is one of the great interests of researchers because of its potential. Bacteria composition in the gastrointestinal tract may be an essential aspect of the pathogenesis of selected diseases. Gut microbial imbalance (dysbiosis) may lead to various diseases, including irritable bowel syndrome (IBS) [3]. As human microbiomes are abundant in unculturable bacteria, and traditional research methods based on classical microbiology have proved ineffective, it is necessary to characterize in detail their composition to further evaluate the function of particular microbiota [4]. *Roseburia hominis* is a flagellated gut anaerobic commensal bacterium, producing short-fatty acids. This property is essential in gut motility, immunity maintenance, and anti-inflammatory properties. There are reports suggesting that *Roseburia* spp. may play a role in IBS's pathogenesis [5]. The knowledge about the microbial components of the intestinal ecosystem is still very limited, including *Roseburia hominis*.

2. Methods

The study protocol was approved by the Institutional Review Board at Poznan University of Medical Sciences. All individuals provided informed consent after the possible consequences of the study were explained, in accordance with the Declaration of Helsinki. The aim of the study was to identify *Roseburia hominis* in the faeces samples using PCR techniques. The study was conducted on samples obtained from IBS patients (women, n = 70, and men, n = 50) and individuals without any intestinal symptoms (women, n = 28, and men, n = 23). After bacterial DNA extraction using the spin-column method (*ZymoBIOMICS DNA Miniprep Kit*, Zymo Research, USA), DNA concentrations were measured using DeNovix Spectrophotometer (DeNovix, USA) and stored in −20 ± 2 °C for further analyses.

Qualitative identification of *Roseburia hominis*, based on the amplification of *RHOM_14625* and *RHOM_14635* gene fragments, was performed. PCR products were purified using an ExoSAP-IT for PCR product Clean-Up (Affymetrix, Santa Clara, CA, USA), and the specificity was confirmed by Sanger sequencing (sequence reading was performed at Genomed, Poland).

Then, a statistical analysis of the obtained data using the Chi-square test was conducted.

3. Results and Discussion

The human digestive tract, especially its distal segment, is colonized by numerous bacteria that create an intricate community called the gut microbiome. Its presence is crucial for maintaining health by preventing gut colonization by pathogens, producing nutrients, and maintaining the integrity of intestinal mucosa [6]. Nowadays, the human gut microbiota is under research to better understand the vast influence it has on the human body. Some species likely play an essential role in the gut microbiome, especially in some diseases' pathogenesis.

R. hominis are a relatively newly recognized probiotic bacteria species [7], are the most proficient butyrate producers [8], and are considered as the most mobile species in the gut microbiome [9]. These bacteria occur predominantly in the colon. *R. hominis* have the ability to penetrate the mucus layer and stick to the surface of host intestinal epithelial cells, which promotes the probiotic potential of these gut bacteria [10].

Roseburia spp. may play a role in gut diseases. *Roseburia* spp. was observed to be reduced in the gut in individuals affected by inflammatory bowel diseases (IBD) [11,12]. Machiels et al. indicated that the lack of *R. hominis* in the gut microbiome among patients with colitis ulcerosa (UC) was found [13]. What is more, Chassard et al. hypothesized that IBS symptoms were correlated with *Roseburia* spp. dysbiosis [14].

In our study, the assessed *RHOM_14625* gene fragments of *R. hominis* were recognized in samples derived from 9 (13%) female and 21 (42%) male IBS patients, and in 15 (54%) and 7 (30%) female and male control individuals, respectively. The difference in the presence of the evaluated gene between healthy individuals and IBS patients was statistically significant, and the p-value was 0.0001 (Figure 1). Considering the presence of the second evaluated gene fragment of *RHOM_14635*

in *R. hominis* fragment, the PCR-amplified fragment was detected in 35 (50%) and 33 (66%) samples obtained from female and male patients, respectively, and in 18 (64%) and 7 (30%) samples from female and male control individuals. The difference in the analyzed gene distribution was statistically significant (p-value = 0.02) (Figure 2). Previously, Rigsbee et al. reported that *Roseburia* spp. abundance was the same among healthy children as well as in diarrhoea-predominant IBS children in stool samples [15]. However, Chassard et al. reported in detail that *Roseburia* spp. was reduced among patients with the IBS-constipation subtype compared to healthy individuals [12]. What is interesting is that, after IBS treatment, *Roseburia* spp. abundance in the gut microbiome was found to be comparable to healthy individuals [16,17]. These and other scientific reports suggest the considerable complexity of bacterial composition and function in various diseases, including IBS.

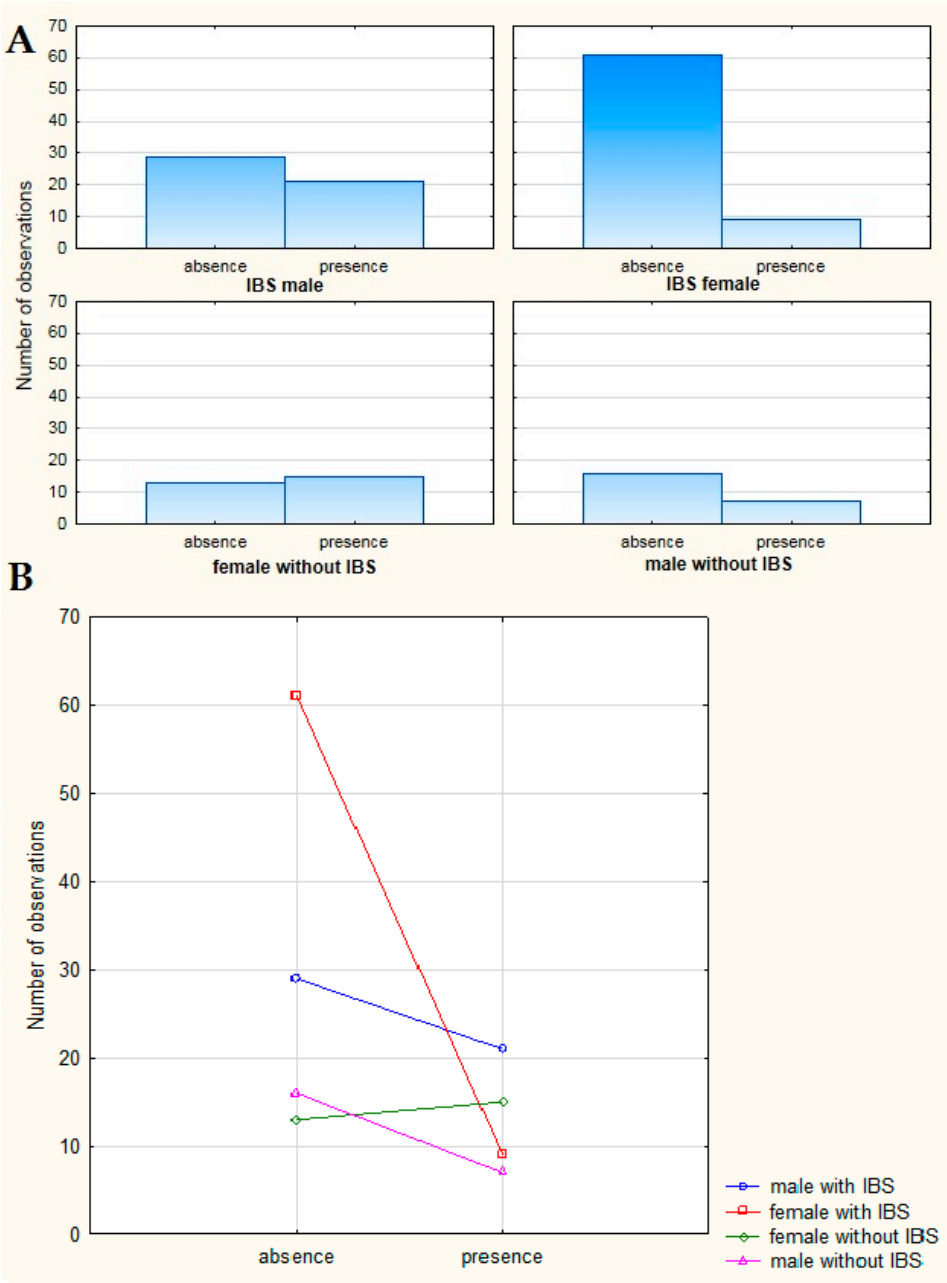


Figure 1. (**A**) Histograms representing an occurrence of the *RHOM_14625* gene fragment among the studied groups. (**B**) Interaction plot shows the difference between the frequencies of the *RHOM_14625* gene fragment occurrence in the studied groups. The association is seen between: (i) females with IBS and males with IBS; (ii) females with IBS and females without IBS; (iii) females with IBS and males without IBS.

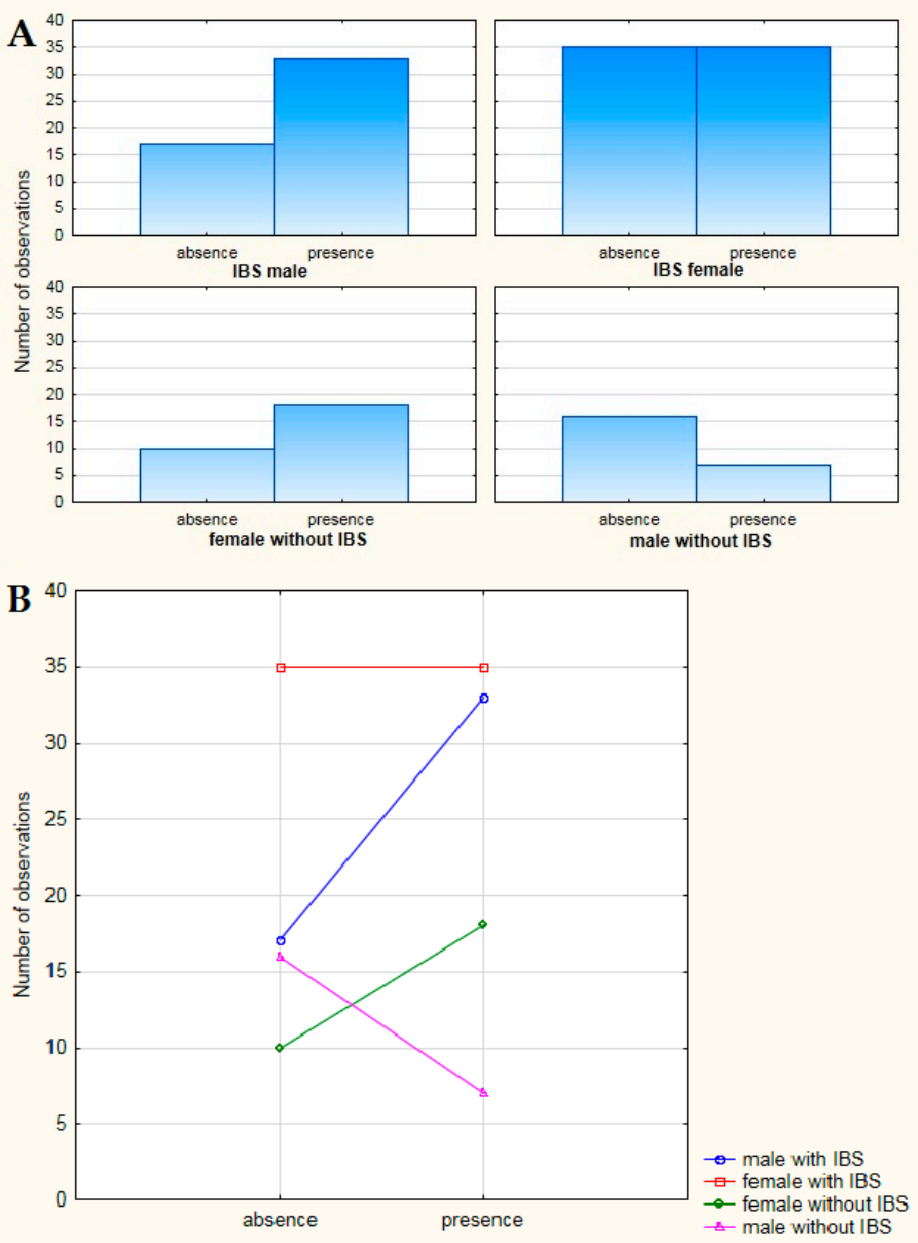


Figure 2. (**A**) Histograms representing an occurrence of the *RHOM_14635* gene fragment among the studied groups. (**B**) Interaction plot shows the difference between the frequencies of the *RHOM_14635* gene fragment occurrence in the studied groups. The interaction is seen between females without IBS and males with IBS.

4. Conclusions

Further molecular studies are necessary to evaluate the role of *Roseburia hominis* in the intestinal microbiome in IBS patients. Taking into consideration the obtained results, it can be assumed that *R. hominis* might play a role in the assessed microbiome in IBS aetiology.

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Conflicts of Interest: The authors declare no conflict of interest.

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