Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2023i1.568
*Biol. Clin. Sci. Res. J., Volume, **2023**: 568*
Original Research Article

DIVERSITY OF β-GLUCURONIDASE AMONG THE MICROBIOME OF HEALTHY INDIVIDUALS AND PCOS PATIENTS

MUCCEE F[*1], RAZZAQ F[2], IQBAL R[3], RAFIQUE F[4], AMJAD A[5], NASIR QUA[6]

[1]*School of Biochemistry and Biotechnology, University of Punjab, Lahore 52254, Pakistan*
[2]*Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan*
[3]*Department of Zoology, Government College University Lahore, Pakistan*
[4]*Faculty of Allied Health Sciences (FAHS), Superior University, Raiwind Road, Lahore, Pakistan*
[5]*Department of Botany, University of the Punjab, Lahore, Pakistan*
[6]*Department of Life Sciences, School of Science, University of Management and Technology, Lahore, Pakistan*
**Corresponding author email address:* fatima.sbb@pu.edu.pk

(Received, 30th September 2023, Revised 24th November 2023, Published 26th December 2023)

Abstract: *PCOS is a heterogeneous disorder caused by reproductive and immuno-metabolic abnormalities. It is accompanied by dysbiosis of the human gut microbial community. Bacterial enzyme β-glucuronidase (GUSB) performs deglucuronidation of conjugated estrogen, resulting in abnormal conc. of this hormone in females and PCOS incidence. Present study was initiated to characterize the GUSB enzyme in bacteria associated with the gut of healthy and PCOS individuals. Enzyme sequences from ten bacteria were retrieved from the UniProt database and characterized by CELLO, Protparam, SOPMA, AlphaFold, and HDOCK tools. Analysis revealed the localization of enzymes in periplasm and cytoplasm in most bacteria and, in addition to this, the outer membrane only in B. intestinalis. PCOS-associated enzymes were alkaline, with high thermostability and in-vitro stability compared to healthy gut bacterial enzymes. Secondary (2D) and tertiary (3D) structures were comparable in enzymes of both these groups. The affinity of GUSB was higher for catechin in PCOS-associated bacteria than bacteria found in a healthy gut. Hence, catechin can be an effective ligand for inhibiting the GUSB enzyme in PCOS patients.*

Keywords: Polycystic Ovary Syndrome, β-Glucuronidase, *Faecalibacterium*, Configuration, Sub-Cellular Localization

Introduction

Polycystic ovary syndrome (PCOS) is an endocrinopathy in reproductively mature women. Infertility, polycystic ovaries, irregular menses, follicle development arrest, hyperinsulinemia, and hirsutism are the hallmarks of this disease (Khomami et al., 2015; Zehra and Khursheed, 2018). Globally, its incidence rate has been reported to be 4-21% (Zhang et al., 2019). It is a multifactorial trait contributed by environmental, epigenetic, and polygenic factors. Insulin resistance and obesity may aggravate this disease (Chaudhuri, 2023; Kicińska et al., 2023). In addition to these factors, enzymes and metabolites of bacteria associated with the human gastrointestinal tract (GIT) might also trigger PCOS (Parker et al., 2022).

The Microecosystem of the human intestine comprises of 100 trillion bacteria belonging to 1000-1500 species (Gill et al., 2006). Human transcriptomic analysis revealed 4 x 10^6 mRNAs belonging to these gut microbes (Sasaki, 2005). This large number of transcripts clearly indicates the possible role of these gut microbes in human metabolism, immunity, nutrition, and physiological functions (Zhao et al., 2020). Under normal circumstances, the human GIT is mostly occupied by *Bifidobacterium bifidum* W23, *B. lactis* W51, *Lactobacillus brevis* W63, *L. lactis* W58 and *L. casei* W56 (Siddiqui et al., 2022). This composition is not always constant. Instead, it changes in response to antibiotic treatment and different pathological conditions called dysbiosis (Rueb et al., 2021). Hence, these microbes might serve as biomarkers for the diseases. PCOS is also always accompanied by dysbiosis. In most of the literature, *Bacteroidaceae* and *Faecalibacterium prausnitzii* have been reported to increase in number in PCOS patients gut (Chu et al., 2020; Guo et al., 2022; Huang et al., 2022; Liang et al., 2021). *Bacteroides vulgatus* and *B. fragilis* were also found to be the most abundant (Lindheim et al., 2017; Qi et al., 2019). Other reported bacteria abundantly found in PCOS patient's gut include bacteria belonging to *Bacteroidaceae*, *Lactobacillaceae*, *Lachnospiraceae*, *Erypelotrichidae*, *Clostridiaceae*, *orphyromonadaceae* and *Ruminococcaceae* (Liu et al., 2017).

The β-glucuronidase (GUSB) enzyme is encoded by uidA bacterial gene which catalyzes the hydrolysis of S- and O-glycosidic moieties (Awolade et al., 2020; Muccee et al., 2022). In human, the neurotransmitters, bilirubin, and hormones like estrogens are conjugated in the liver by glucuronidation, which alters the polarity of these molecules. Thus helping in their excretion from the body (Thackray, 2019). However, β-glucuronidase, also known as GIT microbiome encoded GUS enzyme (GUSOME), tends to deconjugate these molecules, especially estrogens in the intestine. This deglucuronidation results in activated estrogens, i.e., aglycone estradiol and aglycone estrone. Both these forms enter the blood circulation via mucosa and cause PCOS-associated estrogen dominance (Flores et al., 2012). This GUSB enzyme from gut microbes can be used as a diagnostic and prognostic biomarker, as its concentration has been reported to be higher in PCOS patients than in healthy individuals (Patel et al., 2023).

The GUSOME is expressed not only in probiotics but also in bacteria inhabiting the PCOS patients. Considering the

[Citation: Muccee, F., Razzaq, F., Iqbal, R., Rafique, F., Amjad, A., Nasir, Q.U.A. (2023). Diversity of β-glucuronidase among the microbiome of healthy individuals and PCOS patients. *Biol. Clin. Sci. Res. J.*, **2023***: 568.* doi: https://doi.org/10.54112/bcsrj.v2023i1.568]

role of GUSB in PCOS, we have initiated a present research project targeting this enzyme in PCOS and healthy human gut associated bacteria. This study might help us identifying the GUSB characteristics that can be recommended as a prognostic and diagnostic biomarker for PCOS.

Methodology

To analyze the variation in GUSB enzyme among the bacteria of healthy individuals and PCOS patients, five probiotics and five PCOS patients gastrointestinal tract (GIT) associated bacteria were selected.

Retrieving the GUSB sequences from the Uniprot database

Sequences of GUSB from the bacteria *Lacticaseibacillus rhamnosus*, *L. casei*, *Bifidobacterium breve*, *Bifidobacterium longum* subsp. *infantis*, *B. longum* subsp. *longum*, *Bacteroides vulgatus*, *B. intestinalis*, and *Faecalibacterium prausnitzii* were retrieved from the UniProt database (https://www.uniprot.org, accessed Sep. 2023). Bacterial names, accession numbers, and sequences of GUSB are shown in Supplementary Data Figure 1.

Determining the sub-cellular localization of GUSB using the CELLO tool

To determine the variation in enzyme sub-cellular location between bacteria from healthy individuals and PCOS patients, the sub-cellular localization predictor CELLO tool (http://cello.life.nctu.edu.tw, accessed in Sep. 2023) was consulted. The tool predicted the localization scores in five locations, i.e., periplasm, cytoplasm, outer membrane, extracellular, and inner membrane.

Prediction of physicochemical properties of GUSB using ProtParam

To comprehend the physicochemical properties of GUSB in the present study bacteria, the Expasy ProtParam tool (https://web.expasy.org/protparam/, accessed in Sep. 2023) was employed. Tool predicted the enzyme's molecular weight, isoelectric point (pI), aliphatic index, instability index, and grand average of pathogenicity (GRAVY).

Predicting the secondary structure using the SOPMA tool

The secondary configuration (2D) of GUSB was predicted in terms of α-helix, extended strand, β-turn and random coil using SOPMA secondary structure prediction method (https://npsapbil.ibcp.fr/cgibin/npsa_automat.pl?page=/NPSA/npsa_sopma.html, accessed in Sep. 2023).

Assessment of three-dimensional structure using AlphaFold

To compare the level of folding of GUSB, the AlphaFold protein structure database (https://alphafold.ebi.ac.uk/, accessed in Sep. 2023) was consulted, and 3D structures were retrieved.

Phylogeny prediction using Phylogeny.fr

Analysis of the evolutionary relationship between the present study bacterial GUSB enzymes, a phylogenetic tree was constructed using Phylogeny.fr (www.phylogeny.fr/phylogeny.cgi, accessed in Sep. 2023). The advanced mode, which comprised of four steps, was selected for this task. i.e., multiple MUSCLE alignments, alignment curation via Gblocks, phylogenetic tree construction by PhyML, and tree visualization by TreeDyn. The bootstrapping procedure with 100 bootstraps was selected as statistical branch support along with the default setting of the substitution model.

Docking analysis of GUSB with flavonoid inhibitor (catechin)

An HDOCK server was used to determine the tendency of GUSB binding with flavonoid inhibitor catechin. This server is a hybrid algorithm of template-based modeling and ab initio free docking (Available at hdock.phys.hust.edu.cn, accessed in Sep. 2023). The structure of catechin in sdf format was downloaded and converted into pdb format using OPENBABEL, a chemical file format converter (www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html, accessed in Sep. 2023).

Results

Sub-cellular localization

The sub-cellular location assessment revealed that some of the PCOS patients associated bacterial GUSB was localized in the outer membrane (*B. intestinalis*) and extracellular (*F. prausnitzii* C) in addition to cytoplasm and periplasm like healthy individual's bacterial enzyme. In the case of *F. prausnitzii* B, the enzyme was only localized in the periplasm, while in *B. breve*, *B. longum* subsp. *infantis* and *B. intestinalis*, the location was cytoplasmic. *L. rhamnosus*, *L. casei*, *B. longum* subsp. *longum*, *B. vulgatus*, *F. prausnitzii* A, and *F. prausnitzii* C, the enzyme was present in both the periplasm and cytoplasm (Table 1).

Table 1: Prediction of sub-cellular localization of β-glucuronidase (GUSB) in present study bacteria

Bacterium	Periplasm	Cytoplasm	Outer membrane	Extracellular	Inner membrane
Healthy individuals					
L. rhamnosus	2.440	1.402	0.789	0.311	0.058
L. casei	2.378	1.608	0.638	0.324	0.052
B. breve	0.273	3.248	0.641	0.788	0.050
B. longum subsp. infantis	0.248	2.994	0.801	0.912	0.045
B. longum subsp. longum	2.735	1.870	0.041	0.315	0.038
PCOS individuals					
B. vulgatus	1.832	1.205	0.928	0.656	0.379
B. intestinalis	0.688	2.310	1.130	0.557	0.316
***F. prausnitzii* A**	2.574	1.680	0.314	0.344	0.087
***F. prausnitzii* B**	3.691	0.894	0.139	0.218	0.059
***F. prausnitzii* C**	1.276	2.011	0.273	1.147	0.293

[Citation: Muccee, F., Razzaq, F., Iqbal, R., Rafique, F., Amjad, A., Nasir, Q.U.A. (2023). Diversity of β-glucuronidase among the microbiome of healthy individuals and PCOS patients. *Biol. Clin. Sci. Res. J.*, **2023***: 568.* doi: https://doi.org/10.54112/bcsrj.v2023i1.568]

Physicochemical properties

Analysis of physicochemical properties revealed significant variation in molecular weight between the healthy individuals and PCOS individuals' gut bacteria i., e., ranging between 22421.28 to 66842.30 and 67535.00 to 105870.59, respectively. The pI was highest in *B. vulgatus* (8.46) followed by *F. prausnitzii* A (6.27) and *B. intestinalis* (6.23). In the remaining bacteria, approximately similar values were observed (4.98 to 5.47). The highest aliphatic index value was 79.89 in (*B. intestinalis*), and the lowest was 55.98 in *F. prausnitzii* C. In the rest of the cases, it ranged between 70.66 and 75.98. Maximum and minimum values of the instability index were found to be 39.59 and 27.89, respectively. *B. intestinalis* and *F. prausnitzii* B exhibited the smallest values for GRAVY i. e., -0.373 and -0.369, respectively, while the most enormous value was observed in *F. prausnitzii* C (-0.606) (Table 2).

Table 2: Prediction of physicochemical properties of GUSB using the ProtParam tool

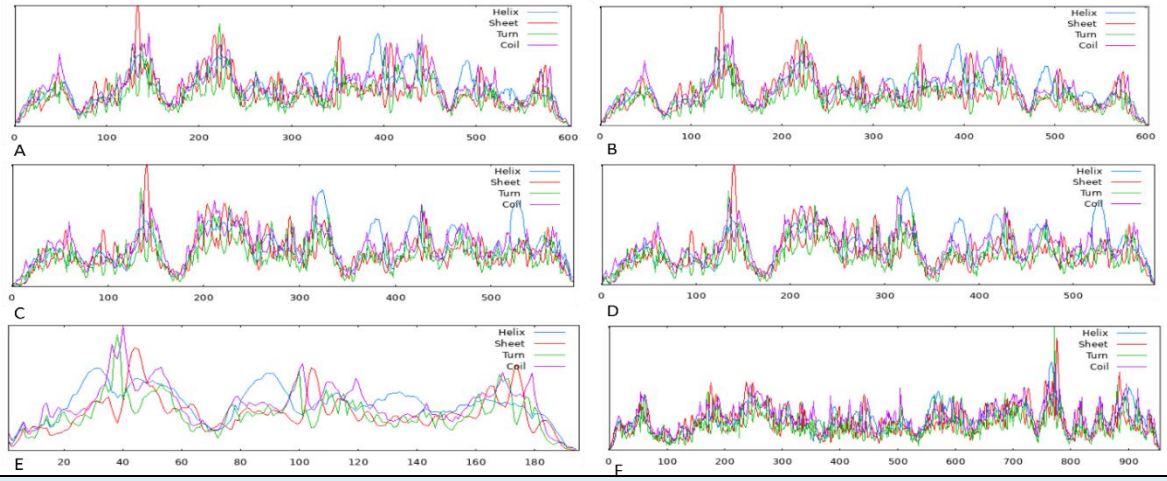
Bacteria	Mol. wt.	pI	Aliphatic index	Instability index	GRAVY
Healthy individuals					
L. rhamnosus	68627.97	5.35	71.01	30.50	-0.502
L. casei	68842.30	5.45	70.66	34.77	-0.509
B. breve	66618.37	4.98	72.66	31.49	-0.482
B. longum subsp. infantis	66562.31	5.00	72.49	30.62	-0.481
B. longum subsp. longum	22421.28	5.00	59.13	31.91	-0.499
PCOS individuals					
B. vulgatus	109987.51	8.46	73.70	29.72	-0.490
B. intestinalis	105870.59	6.23	79.89	27.89	-0.373
F. prausnitzii **A**	72425.93	6.27	75.98	32.34	-0.414
F. prausnitzii **B**	67535.00	5.34	74.03	35.75	-0.369
F. prausnitzii **C**	14397.28	5.47	55.98	39.59	-0.606

Secondary structure

Maximum deviation was observed between *B. longum* subsp. *longum* and *F. prausnitzii* C in terms of α-helix, extended strand, β-turn, and random coil. The α-helix values were observed to be highest in *F. prausnitzii* C (48.36) and *B. longum* subsp. *longum* (42.05), followed by values ranging between 22.22 and 27.42 for all the remaining bacteria. The extended strand, β-turn, and random coil values were observed to be 10.66 to 28.48, 3.59 to 7.98, and 33.61 to 47.27, respectively (Table 3).

Table 3: Prediction of the 2D configuration of GUSB in present study bacteria using the SOPMA tool

Bacterium	α-helix (%)	Extended strand (%)	β-turn (%)	Random coil (%)
Healthy individuals				
L. rhamnosus	26.53	23.88	7.63	41.96
L. casei	26.20	23.05	6.30	44.44
B. breve	23.04	23.89	7.17	45.90
B. longum* subsp. *infantis	23.04	22.35	7.34	47.27
B. longum* subsp. *longum	42.05	15.38	3.59	38.97
PCOS individuals				
B. vulgatus	24.40	21.36	7.54	46.70
B. intestinalis	26.42	22.27	7.75	43.56
F. prausnitzii **A**	22.22	28.48	7.98	41.31
F. prausnitzii **B**	27.42	22.74	6.69	43.14
F. prausnitzii **C**	48.36	10.66	7.38	33.61



[Citation: Muccee, F., Razzaq, F., Iqbal, R., Rafique, F., Amjad, A., Nasir, Q.U.A. (2023). Diversity of β-glucuronidase among the microbiome of healthy individuals and PCOS patients. *Biol. Clin. Sci. Res. J.*, **2023***: 568*. doi: https://doi.org/10.54112/bcsrj.v2023i1.568]

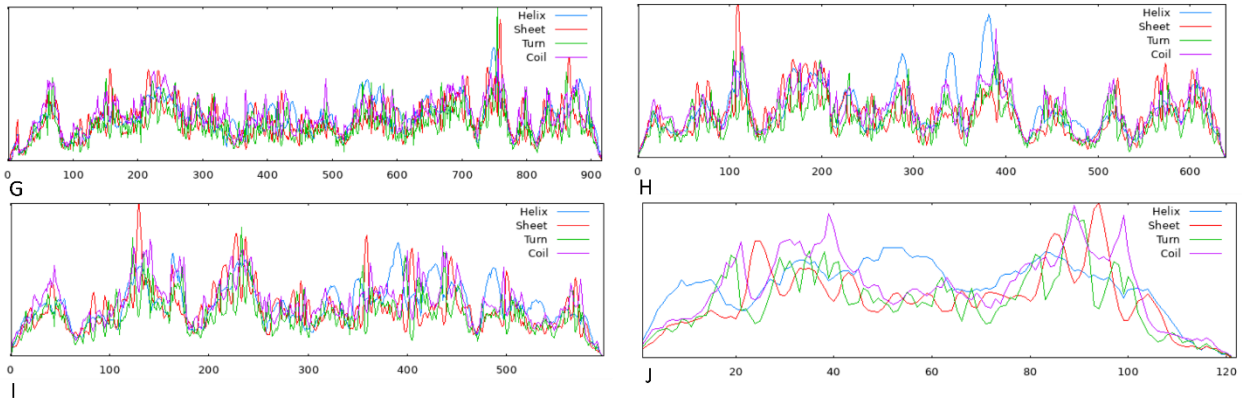


Figure 1: Assessment of secondary structure (2D) of β-glucuronidase in present study bacteria using SOPMA tool

The simplest structure was observed in F. prausnitzii C, followed by B. longum subsp. longum. Bacteria found in gut of PCOS patients exhibited highly complex folding pattern of β-glucuronidase compared to healthy individuals' bacteria (Figure 2).

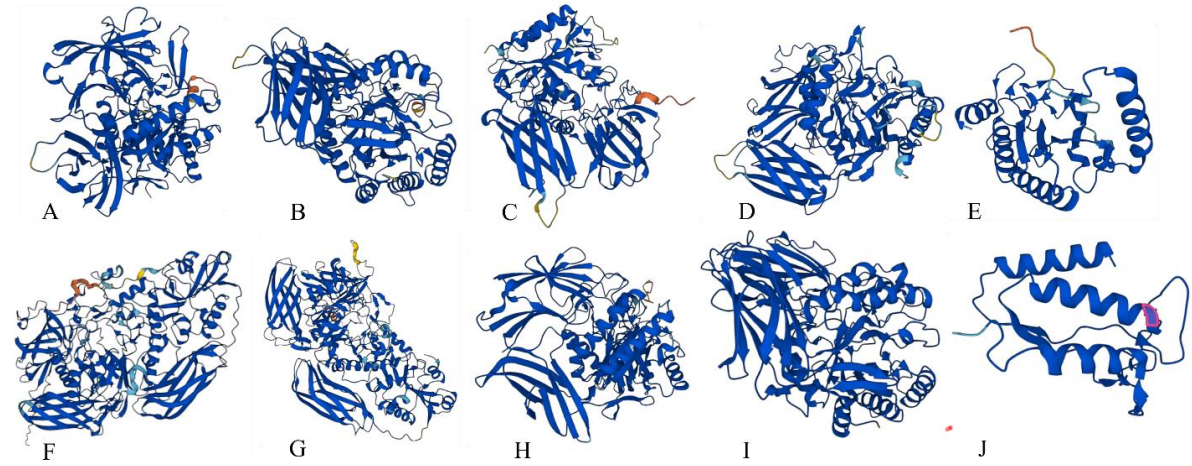


Figure 2: Prediction of three dimensional (3D) configuration in GUSB in present study bacteria using AlphaFold database A: *L. rhamnosus*, B: *L. casei*, C: *B. breve*, D: *B. longum* subsp. *infantis*, E: *B. longum* subsp. *longum*, F: *B. vulgatus*, G: *B. intestinalis*, H: *F. prausnitzii* A, I: *F. prausnitzii* B, J: *F. prausnitzii* C

Phylogeny

The phylogenetic relationship between the present study bacteria with reference to the GUSB enzyme revealed that the *L. rhamnosus* and *L. casei* were closely related because they shared the same clade. Fork of these bacteria also originated from the same point as that of *B. longum* subsp. They were long, representing their closeness compared to the rest of the bacterial enzyme. The enzymes of *B. breve* and *B. longum* subsp. *infantis* is also shared with *B. vulgatus*. All three strains of *F. prausnitzii* showed divergence during the evolution of GUSB (Figure 3).

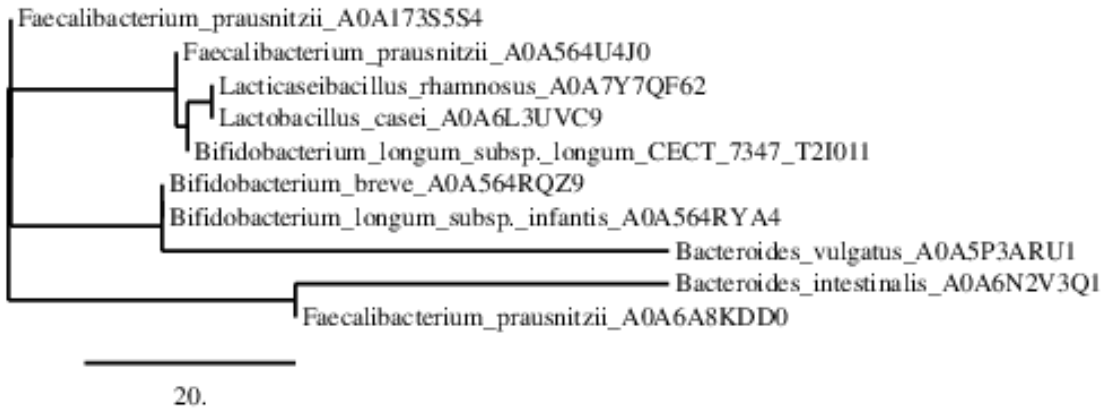


Figure 3: Phylogenetic tree constructed concerning β-glucuronidase to interpret the relation between the bacteria documented in the present study

[Citation: Muccee, F., Razzaq, F., Iqbal, R., Rafique, F., Amjad, A., Nasir, Q.U.A. (2023). Diversity of β-glucuronidase among the microbiome of healthy individuals and PCOS patients. *Biol. Clin. Sci. Res. J.*, **2023***: 568*. doi: https://doi.org/10.54112/bcsrj.v2023i1.568]

Binding affinity of GUSB with catechin inhibitor

The docking analysis of GUSB with flavonoid ligand catechin revealed the highest affinity for ligand in all the PCOS-associated bacteria (docking score = -203.65 to -216.54) except in *F. prausnitzii* A0A564U4J0 (score = -182.48) (Figure 4, Table 4).

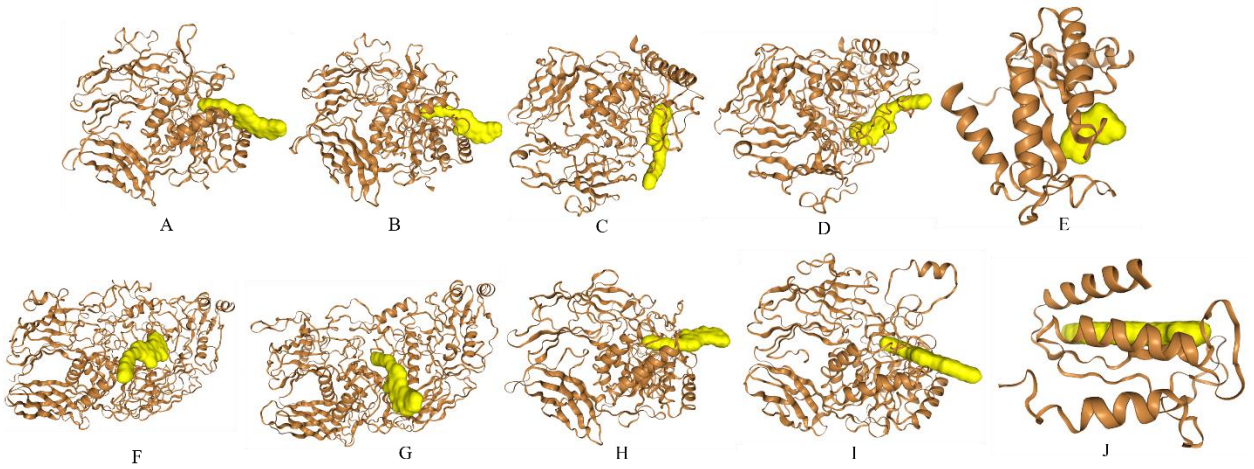


Figure 4: Assessment of binding affinity of GUSB enzyme documented in the present study with catechin
A: *L. rhamnosus*, B: *L. casei*, C: *B. breve*, D: *B. longum* subsp. *infantis*, E: *B. longum* subsp. *longum*, F: *B. vulgatus*, G: *B. intestinalis*, H: *F. prausnitzii* A, I: *F. prausnitzii* B, J: *F. prausnitzii* C

Table 4: Docking score, confidence score, and ligand rmsd predicted for GUSB enzymes documented in the present study, using HDOCK server

Bacterium	Docking score	Confidence score	Ligand rmsd (Å)
Healthy individuals			
L. rhamnosus	-180.03	0.6458	27.05
L. casei	-175.75	0.6260	18.84
B. breve	-155.10	0.5255	20.49
B. longum subsp. infantis	-197.30	0.7203	30.61
B. longum subsp. longum	-147.51	0.4876	27.59
PCOS individuals			
B. vulgatus	-203.65	0.7452	30.21
B. intestinalis	-216.54	0.7910	22.16
***F. prausnitzii* A**	-205.79	0.7532	29.93
***F. prausnitzii* B**	-215.37	0.7871	12.29
***F. prausnitzii* C**	-182.48	0.6569	33.90

Discussion

The present study deals with characterizing the GUSB enzyme from bacteria living in the gut of healthy and PCOS-infected individuals. Analysis revealed diversity in this enzyme, which might be due to antibiotics, diet, and pathological conditions (Faith et al., 2011). Several studies are reported in the literature which characterized this bacterium in human gut microbes like *Ruminococcus gnavus, Streptococcus equi, Staphylococcus pasteuri, Lactobacillus and Enterococcus* (Beaud et al., 2005; Cheng et al., 2015; Krahulec and Krahulcová, 2007; Krahulec et al., 2010; Mroczynska and Libudzisz, 2010; Wei et al., 2018). One study has also reported the in silico characterization of this enzyme and analyzed diversity in breast cancer patients with GIT bacteria (Muccee et al., 2022). However, no one has ever studied the diversity of GUSB among the bacteria of healthy and PCOS-affected individuals.

The 3D configuration of three different forms of GUSB from *F. prausnitzii* exhibited complex folding in two cases, i.e.. A0A173S5S4 and A0A6A8KDD0 are in accordance with this enzyme's previously reported crystal structure. However, in the case of A0A564U4J0, the predicted structure is inconsistent with previous literature (Pellock et al., 2019).

Molecular weight was higher in B. vulgatus, B. intestinalis, and *F. prausnitzii* A than in healthy individuals' bacteria, i.e., 109987.51, 105870.59, and 72425.93. However, in the GUSB variant (A0A6A8KDD0), the molecular weight (67535.00) was comparable with the enzymes of healthy individual microbes. GUSB was acidic in healthy individual bacteria, while in PCOS patients, it was alkaline in *B. vulgatus*, *B. intestinalis*, and *F. prausnitzii* A (Righetti, 2004). The aliphatic index was comparatively higher in enzymes of PCOS-associated bacteria except *F. prausnitzii C*, revealing their higher thermostability (Pack and Yoo, 2004). Instability index values below 40 indicate their in-

vitro stability (Gamage et al., 2019). The highest and lowest stabilities were observed in B. intestinalis and *F. prausnitzii* (A0A6A8KDD0) enzymes. GRAVY assessment predicted the non-polar nature of GUSB in all the cases (Babnigg and Joachimiak, 2010).

Flavonoid inhibitor catechin was selected to perform a docking analysis of GUSB (Awolade et al., 2020). High affinity for catechin in most PCOS bacteria associated with GUSB shows that this molecule can effectively inhibit this enzyme from working, thus preventing the pathological effects caused by its activity.

Conclusion

As per the diversity of GUSB enzymes, it seems complicated to manipulate this enzyme to prevent PCOS-associated metabolic dysregulation. However, the structure and other properties predicted in the present study might help design personalized treatment of PCOS.

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.
Ethics approval and consent to participate
Approved by the department Concerned.
Consent for publication
Approved
Funding
Not applicable

Conflict of interest

The authors declared absence of conflict of interest.

Author Contribution

FATIMA MUCCEE
Conception of Study, Development of Research Methodology Design, Study Design,, Review of manuscript, final approval of manuscript
Coordination of collaborative efforts.
FATIMA RAZZAQ
Coordination of collaborative efforts.
RIFFAT IQBAL
Manuscript revisions, critical input.
Coordination of collaborative efforts.
FAZILAT RAFIQUE
Data acquisition, analysis.
AMINA AMJAD
Data entry and Data analysis, drafting article
QURAT-UL-AIN NASIR
Data acquisition, analysis.
Coordination of collaborative efforts.

References

Awolade, P., Cele, N., Kerru, N., Gummidi, L., Oluwakemi, E., and Singh, P. (2020). Therapeutic significance of β-glucuronidase activity and its inhibitors: A review. *European journal of medicinal chemistry* **187**, 111921.

Babnigg, G., and Joachimiak, A. (2010). Predicting protein crystallization propensity from protein sequence. *Journal of structural and functional genomics* **11**, 71-80.

Beaud, D., Tailliez, P., and Anba-Mondoloni, J. (2005). Genetic characterization of the β-glucuronidase enzyme from a human intestinal bacterium, *Ruminococcus gnavus*. *Microbiology* **151**, 2323-2330.

Chaudhuri, A. (2023). Polycystic ovary syndrome: Causes, symptoms, pathophysiology, and remedies. *Obesity Medicine*, 100480.

Cheng, T. C., Chuang, K. H., Roffler, S. R., Cheng, K. W., Leu, Y. L., Chuang, C. H., Huang, C. C., Kao, C. H., Hsieh, Y. C., and Chang, L. S. (2015). Discovery of specific inhibitors for intestinal *E. coli* β-glucuronidase through in silico virtual screening. *The Scientific World Journal* **2015**.

Chu, W., Han, Q., Xu, J., Wang, J., Sun, Y., Li, W., Chen, Z. J., and Du, Y. (2020). Metagenomic analysis identified microbiome alterations and pathological association between intestinal microbiota and polycystic ovary syndrome. *Fertility and Sterility* **113**, 1286-1298. e4.

Faith, J. J., McNulty, N. P., Rey, F. E., and Gordon, J. I. (2011). Predicting a human gut microbiota's response to diet in gnotobiotic mice. *Science* **333**, 101-104.

Flores, R., Shi, J., Fuhrman, B., Xu, X., Veenstra, T. D., Gail, M. H., Gajer, P., Ravel, J., and Goedert, J. J. (2012). Fecal microbial determinants of fecal and systemic estrogens and estrogen metabolites: a cross-sectional study. *Journal of translational medicine* **10**, 1-11.

Gamage, D. G., Gunaratne, A., Periyannan, G. R., and Russell, T. G. (2019). Applicability of instability index for in vitro protein stability prediction. *Protein and peptide letters* **26**, 339-347.

Gill, S. R., Pop, M., DeBoy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., Gordon, J. I., Relman, D. A., Fraser-Liggett, C. M., and Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *science* **312**, 1355-1359.

Guo, J., Shao, J., Yang, Y., Niu, X., Liao, J., Zhao, Q., Wang, D., Li, S., and Hu, J. (2022). Gut microbiota in patients with polycystic ovary syndrome: a systematic review. *Reproductive Sciences*, 1-15.

Huang, J., Su, C., Zhang, X., Dai, R., Jiang, L., Zhang, T., Sun, Y., and Zhu, Z. (2022). Alteration of gut microbiota in polycystic ovary syndrome patients and the correlated clinical parameters: a cross-sectional analysis study from Chinese women.

Khomami, M. B., Tehrani, F. R., Hashemi, S., Farahmand, M., and Azizi, F. (2015). Of PCOS symptoms, hirsutism has the most significant impact on the quality of life of Iranian women. *PLoS One* **10**, e0123608.

Kicińska, A. M., Maksym, R. B., Zabielska-Kaczorowska, M. A., Stachowska, A., and Babińska, A. (2023). Immunological and metabolic causes of infertility in polycystic ovary syndrome. *Biomedicines* **11**, 1567.

Krahulec, J., and Krahulcová, J. (2007). Characterization of the new β-glucuronidase from *Streptococcus equi* subsp. *zooepidemicus*. *Applied microbiology and biotechnology* **74**, 1016-1022.

Krahulec, J., Szemes, T., and Krahulcová, J. (2010). Bioinformatics characterization of potential new beta-glucuronidase from *Streptococcus equi* subsp. *zooepidemicus*. *Molecular biotechnology* **44**, 232-241.

Liang, Z., Di, N., Li, L., and D., Y. (2021). Gut microbiota alterations reveal potential gut–brain axis changes in

polycystic ovary syndrome. *Journal of Endocrinological Investigation*, 1-11.

Lindheim, L., Bashir, M., Münzker, J., Trummer, C., Zachhuber, V., Leber, B., Horvath, A., Pieber, T. R., Gorkiewicz, G., and Stadlbauer, V. (2017). Alterations in gut microbiome composition and barrier function are associated with reproductive and metabolic defects in women with polycystic ovary syndrome (PCOS): a pilot study. *PloS one* **12**, e0168390.

Liu, R., Zhang, C., Shi, Y., Zhang, F., Li, L., Wang, X., Ling, Y., Fu, H., Dong, W., and Shen, J. (2017). Dysbiosis of gut microbiota associated with clinical parameters in polycystic ovary syndrome. *Frontiers in Microbiology* **8**, 324.

Mroczynska, M., and Libudzisz, Z. (2010). Beta-glucuronidase and beta-glucosidase activity of *Lactobacillus* and *Enterococcus* isolated from human feces. *Pol J Microbiol* **59**, 265-269.

Muccee, F., Ghazanfar, S., Ajmal, W., and Al-Zahrani, M. (2022). In-silico characterization of estrogen reactivating β-glucuronidase enzyme in git associated microbiota of normal human and breast cancer patients. *Genes* **13**, 1545.

Pack, S. P., and Yoo, Y. J. (2004). Protein thermostability: structure-based difference of amino acid between thermophilic and mesophilic proteins. *Journal of Biotechnology* **111**, 269-277.

Parker, J., O'Brien, C., and Hawrelak, J. (2022). A narrative review of the role of gastrointestinal dysbiosis in the pathogenesis of polycystic ovary syndrome. *Obstetrics & gynecology science* **65**, 14-28.

Patel, J., Chaudhary, H., Rajput, K., Parekh, B., and Joshi, R. (2023). Assessment of gut microbial β-glucuronidase and β-glucosidase activity in women with polycystic ovary syndrome. *Scientific Reports* **13**, 11967.

Pellock, S. J., Walton, W. G., Ervin, S. M., Torres-Rivera, D., Creekmore, B. C., Bergan, G., Dunn, Z. D., Li, B., Tripathy, A., and Redinbo, M. R. (2019). Discovery and characterization of FMN-binding β-glucuronidases in the human gut microbiome. *Journal of molecular biology* **431**, 970-980.

Qi, X., Yun, C., Sun, L., Xia, J., Wu, Q., Wang, Y., Wang, L., Zhang, Y., Liang, X., and Wang, L. (2019). Gut microbiota–bile acid–interleukin-22 axis orchestrates polycystic ovary syndrome. *Nature medicine* **25**, 1225-1233.

Righetti, P. G. (2004). Determination of the isoelectric point of proteins by capillary isoelectric focusing. *Journal of chromatography A* **1037**, 491-499.

Rueb, A. M., Tsakmaklis, A., Gräfe, S. K., Simon, M. C., Vehreschild, M. J. G. T., and Wuethrich, I. (2021). Biomarkers of human gut microbiota diversity and dysbiosis. *Biomarkers in Medicine* **15**, 139-150.

Sasaki, T. (2005). The map-based sequence of the rice genome. *Nature* **436**, 793-800.

Siddiqui, R., Makhlouf, Z., Alharbi, A. M., Alfahemi, H., and Khan, N. A. (2022). The gut microbiome and female health. *Biology* **11**, 1683.

Thackray, V. G. (2019). Sex, microbes, and polycystic ovary syndrome. *Trends in Endocrinology & Metabolism* **30**, 54-65.

Wei, B., Wang, P. P., Yan, Z. X., and Yan, R. (2018). Characteristics and molecular determinants of a highly selective and efficient glycyrrhizin-hydrolyzing β-glucuronidase from *Staphylococcus pasteuri* 3I10. *Applied microbiology and biotechnology* **102**, 9193-9205.

Zehra, B., and Khursheed, A. A. (2018). Polycystic ovarian syndrome: symptoms, treatment and diagnosis: a review. *Journal of Pharmacognosy and Phytochemistry* **7**, 875-880.

Zhang, J., Sun, Z., Jiang, S., Bai, X., Ma, C., Peng, Q., Chen, K., Chang, H., Fang, T., and Zhang, H. (2019). Probiotic *Bifidobacterium lactis* V9 regulates the secretion of sex hormones in polycystic ovary syndrome patients through the gut-brain axis. *Msystems* **4**, 10.1128/msystems. 00017-19.

Zhao, X., Jiang, Y., Xi, H., Chen, L., and Feng, X. (2020). Exploration of the relationship between gut microbiota and polycystic ovary syndrome (PCOS): a review. *Geburtshilfe und Frauenheilkunde* **80**, 161-171.

[Citation: Muccee, F., Razzaq, F., Iqbal, R., Rafique, F., Amjad, A., Nasir, Q.U.A. (2023). Diversity of β-glucuronidase among the microbiome of healthy individuals and PCOS patients. *Biol. Clin. Sci. Res. J.*, **2023***: 568.* doi: https://doi.org/10.54112/bcsrj.v2023i1.568]