A study on microbial mucin utilizers from healthy Indian adult human faeces

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Introduction

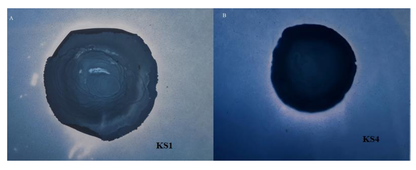
- The human body is home to more than trillions of complex microorganisms and more than 1000 bacterial species are known to reside in the gastrointestinal (GI) tract with weight up to 3 kg.
- A layer of miscellaneous hydrogel biopolymer called mucus is present at the interface between the epithelium surfaces and their extracorporeal environment (10 L daily). This barrier considered as the front line of defence, plays a vital role in keeping the obnoxious microbes, microbial products, and toxins aside to shield the epithelial layer. It also possesses humectant properties, acts as an immune regulator, and serves as a home to indigenous bacterial flora. Mucus, a heterogeneous molecule, composed of a wide array of intricate components, including water (90-95%), mucin monomers (1-5%), proteins, mineral salts, and lipids.
- The key structural and functional element responsible for the formation of mucus gel is mucin. Mucin is constituted of O-glycans, namely N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc), fucose, mannose, and galactose (Gal) residues, connected to threonine or serine residues via O-glycosidic. These elaborate oligosaccharide side chains originating from the protein backbone serve as adhesion site for microorganisms (primarily bacteria), and therefore a natural reservoir for the commensal microbiome, which coevolved in a mutual relationship.
- Indeed, the bacterial population renders the complex mucin glycans breakdown into simpler ones, producing beneficial metabolites that play a significant role in the host's immune system, regulation of certain genes, and metabolism. The host, in turn, provides an appropriate environment to these microbes to colonize and flourish. Therefore, mucin degrading bacteria seem vital to the host as the former play a significant role in gut homeostasis. Several authors have outlined the presence of such bacteria in various organ systems, e.g., *Bacteroides fragilis* in the human colon, *Bifidobacterium longum*, *B. bifidum* in the vaginal system, *Mobiluncus mulieri* in the reproductive tract, *R. torques*, *B. thetaiotaomicron*, *R. gnavus*, *A. muciniphila*, and *A. mucolyticum* in the gastrointestinal tract and *P. aeruginosa, E. coli, Staphylococcus aureus, S. epidermidis* appeared in lungs epithelial cell.
- It is speculated that only 0.9% of fecal flora participates in the degradation of mucin molecules, and to date, fewer than 100 bacterial strains with this capability have been characterized. Understanding the specific bacterial population responsible for mucin degradation remains in its early stages, given that a substantial portion of the microbiota residing in mucin remains uncultured and poorly understood. Therefore, in order to elucidate the contributions of bacterial species and their associated enzymes in mucus degradation, this study aims to isolate and identify a bacterial strain from the human gut that utilizes mucin and evaluates various in-vitro parameters pertaining to gastrointestinal conditions.

Results

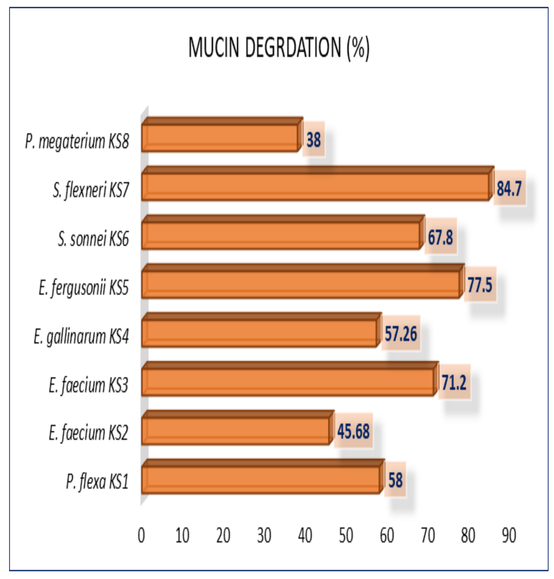
Selection of Mucin utilizers

Selection	Number of isolates
Samples	30
Total colonies obtained via enrichment process	1610
Randomly picked colonies	260
Selected based on Amido assay	47
Selected based on Quantitative assay	8

Amido black assay



Mucin degradation percentage



Identified Mucolytic strains

Sr. No	Strains	Accession no.
1	*Priestia flexa KS1*	MZ618950
2	*Enterococcus faecium KS2*	MZ683214
3	*Enterococcus faecium KS3*	OM281295
4	*Enterococcus gallinarum KS4*	OM281298
5	*Escherichia fergusonii KS5*	OM281299
6	*Shigella sonnei KS6*	OM281300
7	*Shigella flexneri KS7*	OM281759
8	*Priestia megaterium KS8*	OM304331

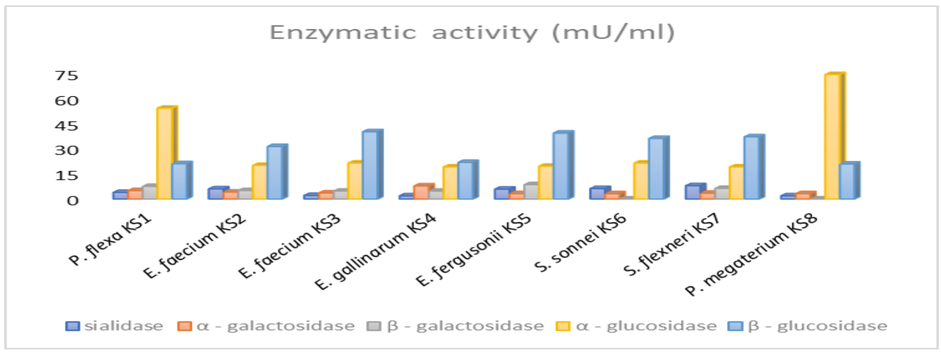
Virulence attributes

Strains	Hemolytic activity
P. flexa KS1	γ
E. faecium KS2	β
E. faecium KS3	α
E. gallinarum KS4	γ
E. fergusonii KS5	β
S. sonnei KS6	β
S. flexneri KS7	β
P. megaterium KS8	γ

Strains	Gelatinase
P. flexa KS1	NZ
E. faecium KS2	Z
E. faecium KS3	NZ
E. gallinarum KS4	NZ
E. fergusonii KS5	Z
S. sonnei KS6	Z
S. flexneri KS7	Z
P. megaterium KS8	Z

Z: zone; NZ: no zone

Enzymatic activity of isolates



Methodology

Collection of fecal sample of healthy adults (18-60 years; not under medication in last 3 months) from various regions of Haryana, India.

Preliminary screening of mucin degrading bacteria by enrichment technique followed by amido black assay

Quantification of Mucin degradation by decrease in Carbohydrate and Protein concentration

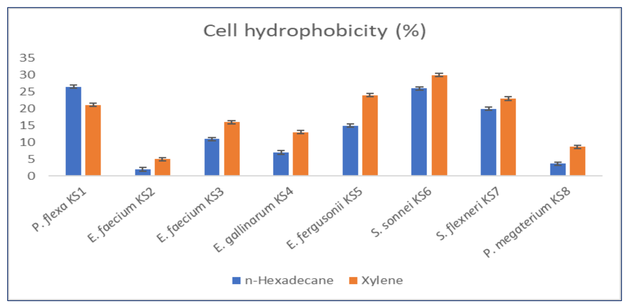
Molecular characterization of isolates by genomic DNA isolation, 16S rRNA amplification and sanger sequencing

Enzymatic profile (neuraminidase,α-galactosidase, β-galactosidase, α-glucosidase, and β-glucosidase) of mucolytic isolates was analysed

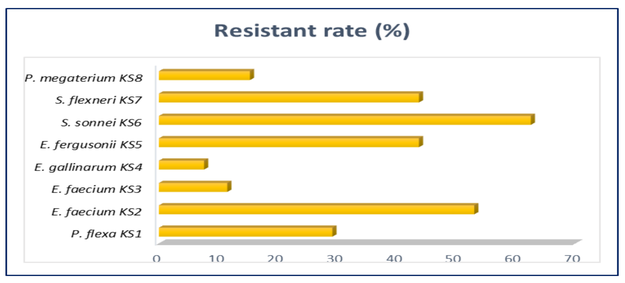
Cell hydrophobicity and antibiotic resistance was evaluated; and isolates were selected based on safety assessment

Survival under stimulated gastrointestinal condition (acidic pH and high bile salt) was studied

Cell hydrophobicity percentage



Antibiotic profiling of strains



$$\text{Resistant rate} = \frac{\text{Isolate resistant to No. of antibiotic} \times 100}{\text{Total No. of antibiotics}}$$

Survival under simulated gastrointestinal conditions

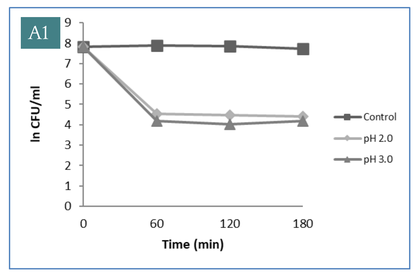


Figure A1 : Low pH tolerance of *P. flexa* KS1

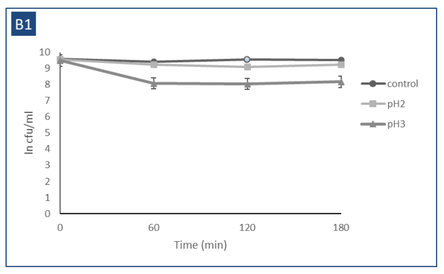


Figure B1 : Low pH tolerance of *E. gallinarum* KS4

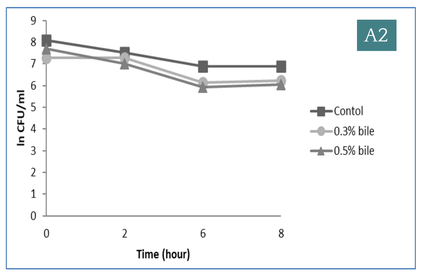


Figure A2 : Bile salt tolerance of *P. flexa* KS1

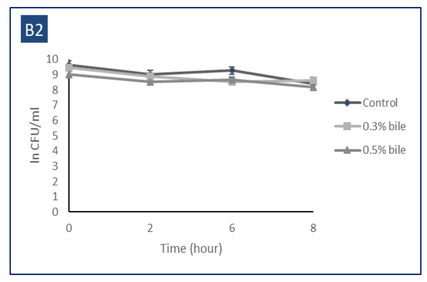


Figure B2 : Bile Salt tolerance of *E. gallinarum* KS4

Conclusion

The present study, affirmed the isolation of eight new strains, from the human fecal samples, that exhibited mucin degradation ability. The mucolytic isolates possess repository of glycosidase enzyme viz sialidase, α- galactosidase, β-galactosidase, α- glucosidase and β-glucosidase, that might act cooperatively on densely decorated mucin oligosaccharide side chains. Cell hydrophobicity results exhibited strains show low hydrophobicity towards non-polar solvents. The safety parameters suggests two strains *P. flexa* KS1 and *E. gallinarum* KS4 avirulent whereas gastrointestinal stability of these culture indicate adaption in the gut environment. Further research should be focused on the identification of certain microbial species that may act as biomarkers for diagnostic and prognostic purposes. The disease progression due to the alteration of mucus structure by microbial activities will provide new insights into understanding some complex diseases such as IBD.

References

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Acknowledgments

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