

Disease Area Specific –Human Microbiome



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सी.एस.आई.आर - सूक्ष्मजीव प्रौद्योगिकी संस्थान
विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार
CSIR - Institute of Microbial Technology
Ministry of Science & Technology, Govt. of India

ABOUT DIRECTOR'S PROFILE RESEARCH ACHIEVEMENTS



Home / Research / Research Areas

Research Areas

Research Areas [Skype](#)

Disease Area Units

Antimicrobial Research

Virology

Microbiome


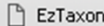



Biotherapeutics and Metabolics



CSIR-IMTECH

Welcome to CSIR-IMTech - E X

→ ↻ <https://www.imtech.res.in/focus-area/microbiome> ☆

Apps  IMTECH  EzTaxon  BLAST  @Gov.in  Post Independent India

Focus Areas

- **Disease Area Units** -
 - Antimicrobial Research
 - Virology
 - **Microbiome**
 - Biotherapeutics and Metabolics
- **Technology Area Units** +
- **Enabling Area Units** +

Focused Research Areas : Microbiome

Current research activities in the unit

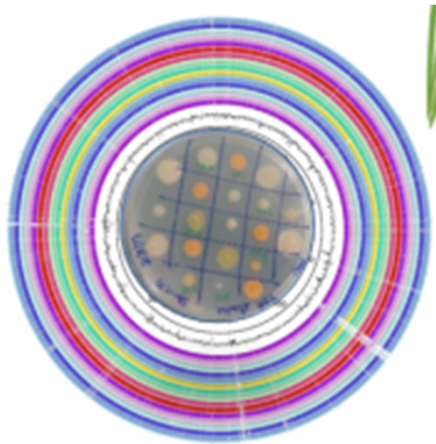
Human microbiomes of healthy and diseased individuals carry diverse strains and species of microbes (Eckburg et.al, Science 2005). Recently, a unique species of Staphylococcus lugdunensis has been identified from Skin that produces a novel antibiotic that can kill pathogenic species like S. aureus (Zipper et.al. Nature, 2016). Consequently, signifying the existence of microbes in the human gut that has enough potential to prevent us from diseases. Keeping in view of the above facts. gut, lung and skin samples will be collected from TB, IBD and acne patients, their house hold contacts and healthy subjects. Bacterial consortia will be generated, which will mainly comprise of Clostridium spp., Enterobacteriaceae, lactic acid bacteria, etc. Further, their efficacy will be tested in the experimental model of TB, IBD and acne. The major objective of the current activities is towards MiCure or Microbiome Based Consortia as Therapeutics.

Past research activities / Achievements

IMTECH was the nodal laboratory of XII Five Year CSIR Network Project (2012-2017) titled "Man as a Superorganism: the Human Microbiome (HUM)". This project was one of the first dedicated programs on Human Microbiome in the country and led to large number of high quality publications in this field related to immunology, microbiology, genomics, and evolutionary aspects of human microbiome of Indian origin.

AIM

EXPLOITING NATIVE MICROBIOTA OF
HEALTHY INDIVIDUALS AS
BIOTHERAPUTICS



HIGH-THROUGHPUT CULTUROMICS
& GENOMICS OF HUMAN MICROBIOTA

Disease Specific Area – Human Microbiome



PRODUCT

Microbial Cocktail
(genomic/evolutionary potential,
antimicrobial, immuno-
modulatory potential
and animal testing of native
oral/gut/skin microbiota)



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Disease Area Specific –Human Microbiome

MiCURE - Microbiome based Consortium as therapeutics for Sepsis, Inflammatory Bowel Disease, Tuberculosis and Acne



Dr. Manoj Raje
Adhesion, invasion assay and electron microscopy



Dr. SR Chaudhuri
Sample collection and characterization of E coli from Gut



Dr. Rashmi Kumar
Immunology and IBD



Dr. Prabhu Patil
Preparing mega-genomic resource & microbial consortium of Indian isolates



Dr. Venkata Ramana
Isolation and identification of anaerobic microbes



Dr. Anil Kumar Pinnaka
Probiotics from Yak

12 Scientist
★ 4 from MTCC)



Dr. Krishnamurthi
Isolation, identification & culturing of aerobic and facultative microbes. Metagenomic sequencing of sample



Dr. Suresh
Isolation and identification of anaerobic microbes for IBD and help in other samples



Dr. Ashwani
Understanding the role of lung microbiome in TB susceptibility using mice model.



Dr. G. Rajamohan
Shotgun metagenomic & functional metagenomics



Dr. Sachin
Animal model development for IBD & Acne



Dr. Neeraj Khatri
Animal Model development for Sepsis



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Dr. Ashwani
group

HIGH-IMPACT FACTOR PUBLICATION IN MICROBIOME FROM INDIA

Singh *et al. Microbiome* (2017) 5:61
DOI 10.1186/s40168-017-0277-3


Microbiome

RESEARCH

Open Access



The development of lower respiratory tract microbiome in mice

Nisha Singh¹, Asheema Vats¹, Aditi Sharma¹, Amit Arora^{1,2,3*} and Ashwani Kumar^{1*} 

Abstract

Background: Although culture-independent methods have paved the way for characterization of the lung microbiome, the dynamic changes in the lung microbiome from neonatal stage to adult age have not been investigated.

Results: In this study, we tracked changes in composition and diversity of the lung microbiome in C57BL/6N mice, starting from 1-week-old neonates to 8-week-old mice. Towards this, the lungs were sterilely excised from mice of different ages from 1 to 8 weeks. High-throughput DNA sequencing of the 16S rRNA gene followed by composition and diversity analysis was utilized to decipher the microbiome in these samples. Microbiome




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ORIGINAL RESEARCH ARTICLE

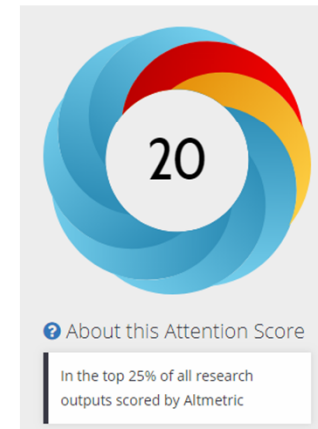
Front. Microbiol., 27 February 2018 | <https://doi.org/10.3389/fmicb.2018.00333>

Phylogenomic Based Comparative Studies on Indian and American Commensal *Staphylococcus epidermidis* Isolates

 Shikha Sharma[†],  Vasvi Chaudhry[†],  Sanjeet Kumar and  Prabhu B. Patil^{*}

Bacterial Genomics and Evolution Laboratory, CSIR-Institute of Microbial Technology, Chandigarh, India

Staphylococcus epidermidis is a prominent commensal member of human skin microbiome and an emerging nosocomial pathogen, making it a good model organism to provide genomic insights, correlating its transition between commensalism and



28 SEP isolates from different body sites of healthy individuals of India

Commensal SEP



Genomic relatedness : ANI tree

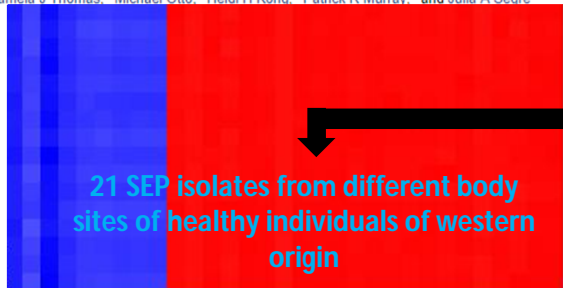


Genome Biol. 2012; 13(7): R64.
Published online 2012 Jul 25. doi: 10.1186/gb-2012-13-7-r64

PMCID: PMC4053731
PMID: 22830599

Staphylococcus epidermidis pan-genome sequence analysis reveals diversity of skin commensal and hospital infection-associated isolates

Sean Conlan,^{#1} Lilia A Mijares,^{#1,2,3} NISC Comparative Sequencing Program,⁴ Jesse Becker,⁴ Robert W Blakesley,⁴ Gerard G Bouffard,⁴ Shellise Brooks,⁴ Holly Coleman,⁴ Jyoti Gupta,⁴ Natalie Gurson,⁴ Morgan Park,⁴ Brian Schmidt,⁴ Pamela J Thomas,⁴ Michael Otto,⁵ Heidi H Kong,⁶ Patrick R Murray,² and Julia A Segre^{#1}



Phylogenomic aspect

Geography ??

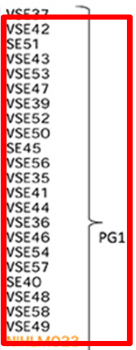
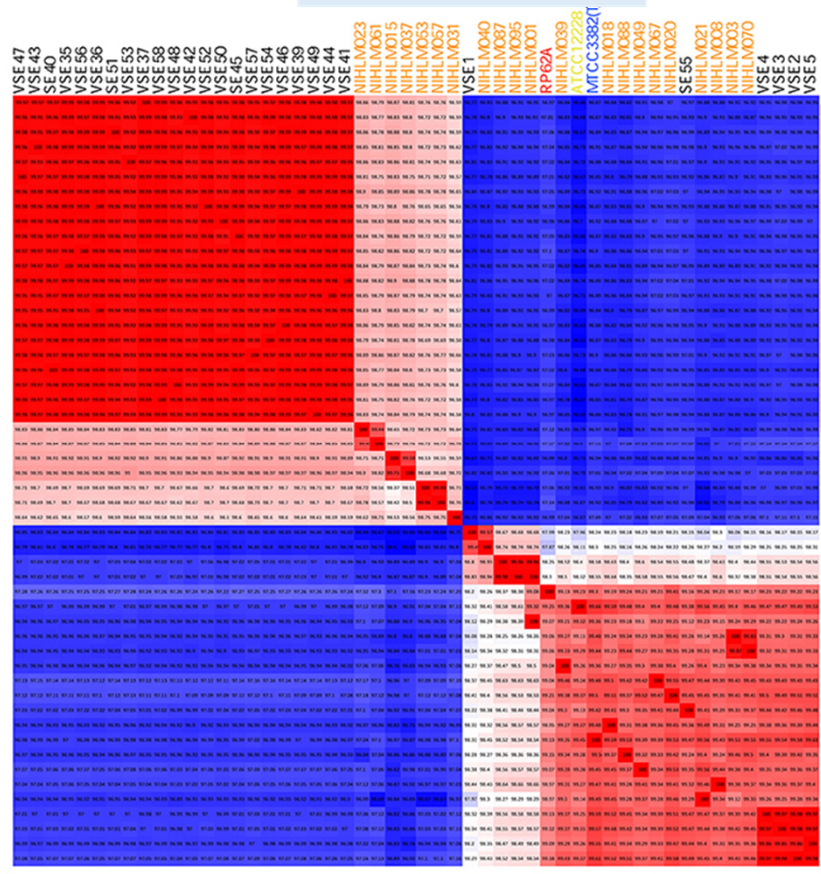
Phylogeographic aspect

	Isolate	Site and Source	Size (Mb)	GC (%)	CDS	Accession Number
1	VSE35	Retroauricular crease, F1	2.5	32.3	2330	MLXN00000000
2	VSE57	Retroauricular crease, F1	2.4	32	2258	MLXL00000000
3	VSE46	Retroauricular crease, F1	2.4	32	2272	MLXE00000000
4	VSE43	Anetecubital Fossa, F1	2.4	31.9	2581	MLXD00000000
5	VSE52	Anetecubital Fossa, F1	2.4	32	2256	MLXI00000000
6	VSE37	Retroauricular crease, F2	2.4	31.9	2268	MLWZ00000000
7	VSE42	Retroauricular crease, F2	2.4	32	2253	MLXC00000000
8	VSE44	Retroauricular crease, F2	2.4	32	2331	MNAB00000000
9	VSE58	Retroauricular crease, F2	2.4	31.9	2271	MLXM00000000
10	VSE47	Retroauricular crease, F2	2.4	32	2249	MLXF00000000
11	SE55	Retroauricular crease, F2	2.4	32	2304	LUBV00000000
12	VSE50	Anetecubital Fossa, F2	2.39	32	2241	MNBF00000000
13	VSE54	Anetecubital Fossa, F2	2.4	31.9	2255	MLXK00000000
14	VSE39	Nares, F2	2.4	31.9	2264	MLXA00000000
15	SE40	Nares, F2	2.48	31.9	2279	LUBM00000000
16	VSE41	Nares, F2	2.4	32	2256	MLXB00000000
17	VSE48	Nares, F2	2.3	32	2178	MLXG00000000
18	VSE49	Nares, F2	2.4	32	2256	MLXH00000000
19	SE51	Retroauricular crease, M1	2.47	31.9	2275	LUBR00000000
20	SE45	Retroauricular crease, M1	2.48	31.9	2272	LUBH00000000
21	VSE56	Retroauricular crease, M1	2.4	31.9	2302	LUBG00000000
22	VSE53	Nares, M1	2.4	32	2282	MLXJ00000000
23	VSE1	Opisthenar hand, F3	2.54	31.9	2315	LGVM00000000
24	VSE2	Opisthenar hand, F3	2.42	32.1	2242	LGVI00000000
25	VSE3	Opisthenar hand, F3	2.42	32.1	2258	LGVI00000000
26	VSE4	Opisthenar hand, F3	2.42	32.1	2263	LGVK00000000
27	VSE5	Opisthenar hand, F3	2.48	32	2300	LGVL00000000
28	VSE36	Nare, F1	2.45	32.5	2411	MSYS00000000

Phylogeographic Analysis

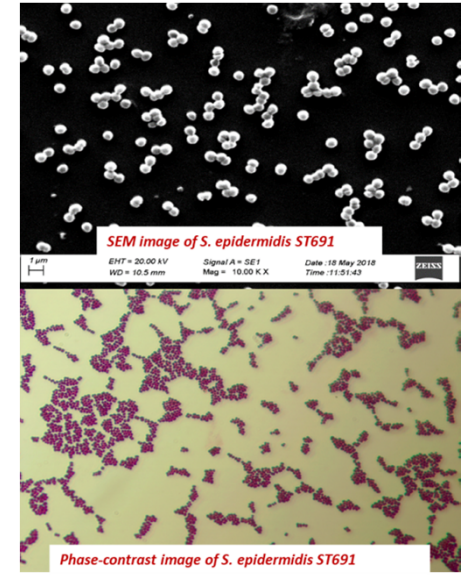


ANI tree



MLST
Clonal

New ST : 691

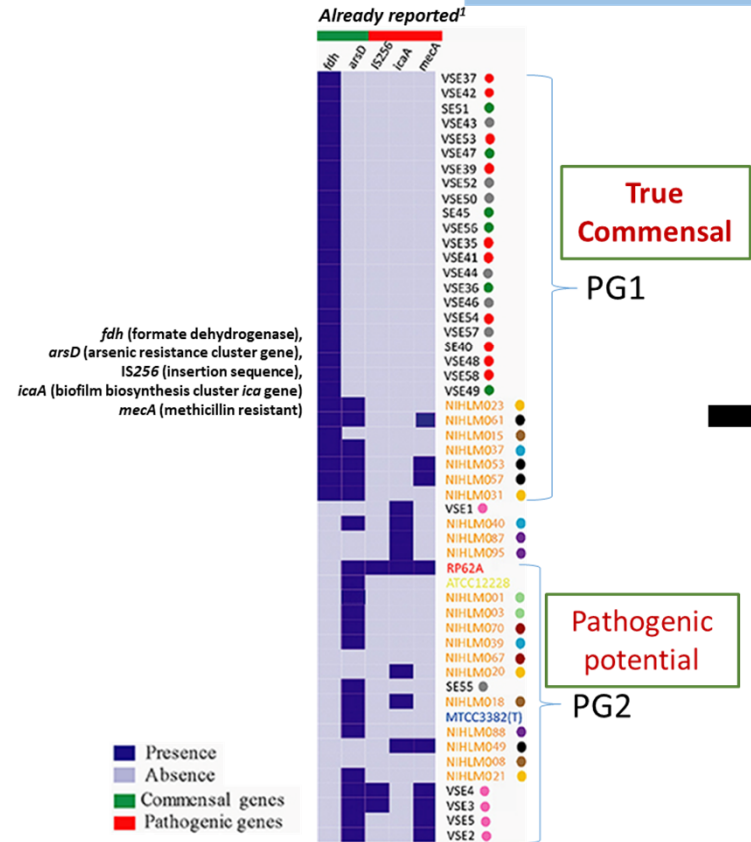


Pathogenic strain
Commensal strain

- Geography independent, 2 lineages PG1 and PG2
- Pathogenic as well as commensal strain in PG2
- A transitional phase

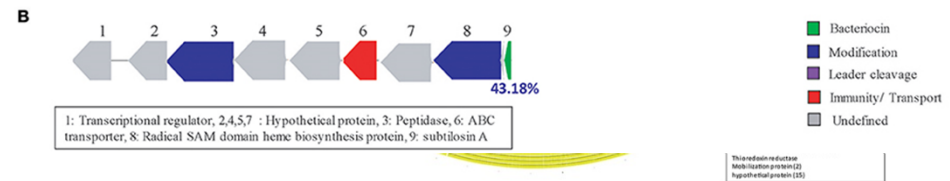
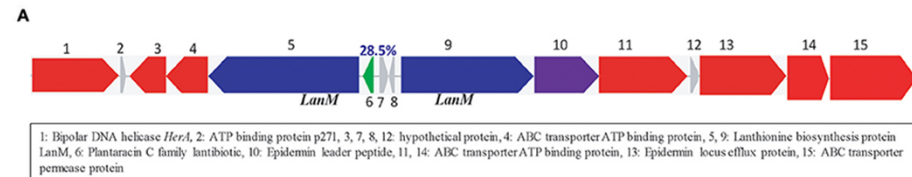
Phylogeographic Analysis

Marker Mining



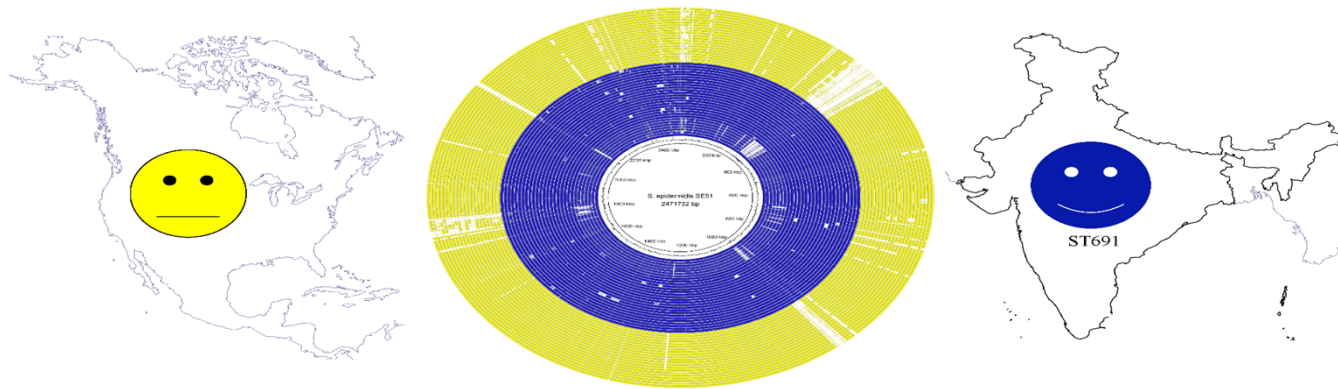
Lantipeptide

Cluster	Type	BlastP of putative bacteriocin peptide	Secondary metabolite prediction	%GC (cluster)	Amino Acids	ORFs in cluster	Sequence
Region A	Lantipeptide	100 % similar to Plantaracin C family antibiotic of <i>Bacillus subtilis</i>	18% Epilancin 15X biosynthetic gene cluster of <i>Staphylococcus epidermidis</i>	27.14	49	15	MKKKLELLYSAGNLIK ELEDGELDS YISGQSEIRKKKKSYSQKTGNDGKNC TVTWECSICPTHICWC
Region B	Sactipeptide	100% identity Subtilosin A family bacteriocin of <i>Staphylococcus</i>	25% Subtilosin A biosynthetic gene cluster of <i>Staphylococcus epidermidis</i>	25.41	33	10	MEQGV MVS NKGCSACAVGAACLAD GPIPDFEVAGITGTFGMAS



Comparative analysis

FIRST LARGE-SCALE PHYLOGEOGRAPHIC STUDY OF A HUMAN COMMENSAL BACTERIUM



A NOVEL CLONE OF COMMENSAL *S. epidermidis* on the Skin of Healthy Individuals from India

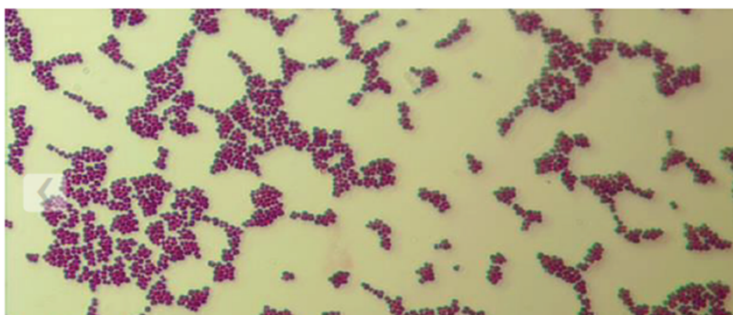


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CONCLUSION

1. New **ST691** is **predominant in Indian** individuals
2. ST691 isolate harbor a novel putative **antimicrobial peptide**
3. SEP isolates divide themselves in **2 lineages** independent of **individual, body site and geographical location**
4. *fdh* (formate dehydrogenase) is a putative **marker for commensalism** of SEP isolates
5. Absence of *fdh* gene along with presence of other pathogenic markers (*icaA*, *mecA*, IS256), can be used to distinguish between a true commensal or a strain with pathogenic potential





Smart probiotics from the skin of Indians

© Sharma, S. et al.

Latest research

Most read

Smart probiotics from the skin of Indians
in *Cell & molecular biology*



Who are the future leading women in science?

The Nature Research Awards are open for nominations...



NOMINATE YOUR INSPIRATION

RESEARCH HIGHLIGHTS

doi:10.1038/nindia.2018.45 Published online 20 April 2018

Smart probiotics from the skin of Indians

By sequencing and comparing the genomes of different strains of a skin bacterium from Indians and North Americans, geneticists have discovered a novel microbial strain that carries unique gene clusters that encode antimicrobial proteins¹. This bacterium could potentially be used to develop smart probiotics and cosmetic supplements made of health-promoting live bacteria.

Hundreds of friendly bacterial species thrive on human skin, nourishing and protecting the host. Although there have been reports of new species of skin-inhabiting bacteria, variation within different strains of same species across diverse geographic locations has yet to be identified.

Researchers from the CSIR-Institute of Microbial Technology in Chandigarh, India, led by Prabhu B Pali, compared the whole genome sequence of different strains of the skin bacterium known as *Staphylococcus epidermidis* from healthy individuals from India and North America.

The Indians have been found to harbour a novel strain that is absent on the skin of healthy Americans. After sequencing its genome, two gene clusters containing genes encoding at least two antimicrobial proteins were detected only in the Indian strains.

Besides protecting the host against disease-causing microbes, the antimicrobials might have played roles in helping the novel strain to adapt to harsh conditions such as humidity, high temperature and intense sunlight in India.



The Research team (clockwise from top left): Shilpa Sharma, Vasvi Chaudhry, Prabhu Pali, Sanjeet Kumar.





Lactobacillus plantarum + *Fructo-oligosaccharide*

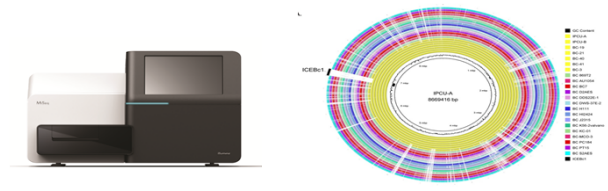
40% reduction in sepsis and lower respiratory track infections

Can be increased?

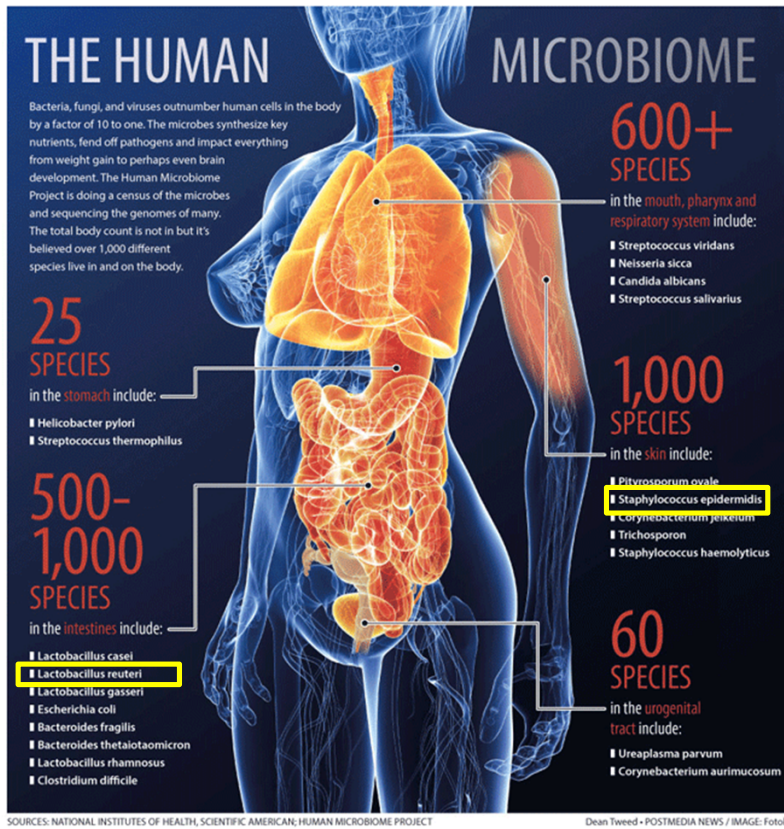
Indian strain rather than US strain

Population genomics and selection of right strain

Exploiting novel probiotic strains and species available at **MTCC**



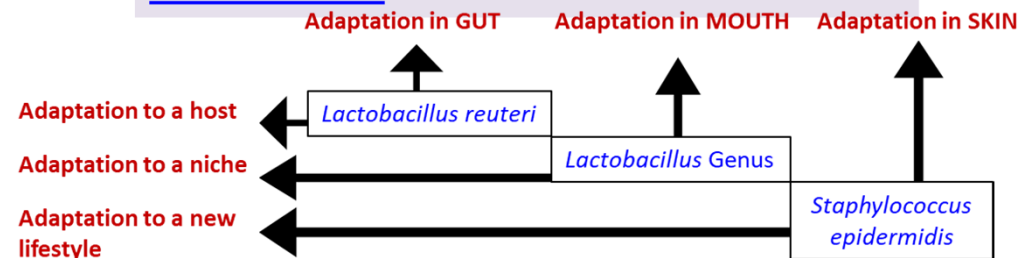
In-depth genomic studies at IMTECH revealed that sepsis isolates and commensal isolates from India are unique from rest of world



Human Microbiome

~30 trillion ~100 trillion microbes

Evolution trends of bacteria in human microbiome



Understanding of Evolution and Adaptation Process



Differences in human gut as compared to other hosts for colonization of gut

human stomach lacks forestomach completely as well as squamous stratified epithelia for adhesion

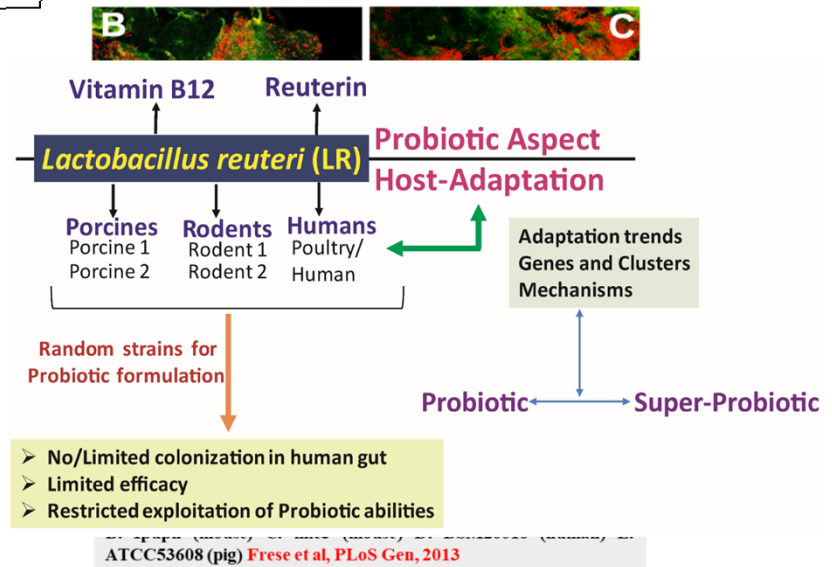
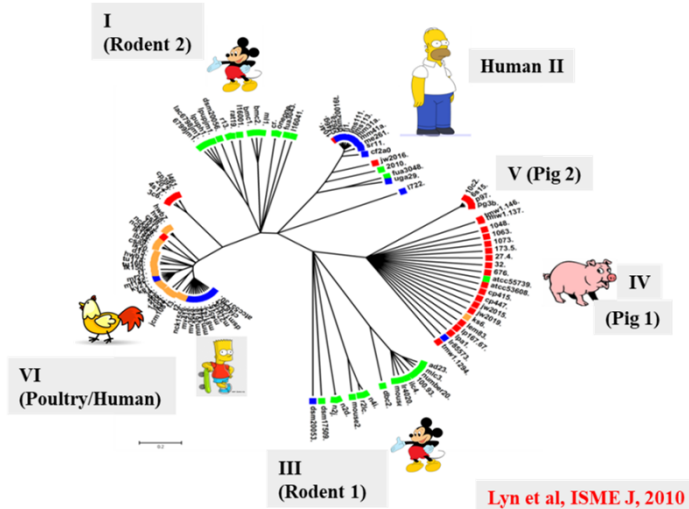
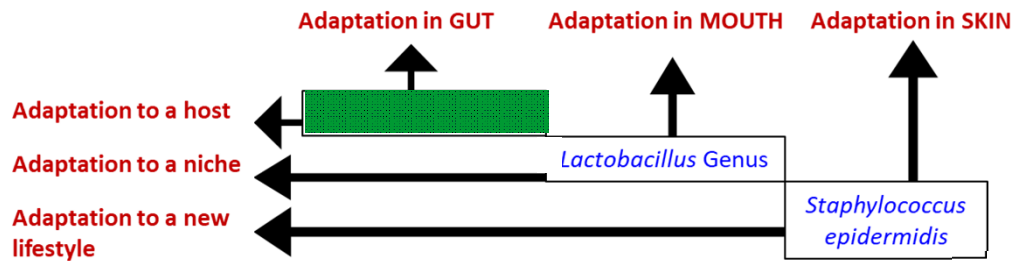
In humans, nutrients are absorbed in small intestine and are in small supply in colon, further complicating the adhesion of bacteria (Walter et al., 2010)

Human intestinal lining is thickly lined with mucus leaving little space for colonization of *L. reuteri*.

What is important for being a successful human commensal?

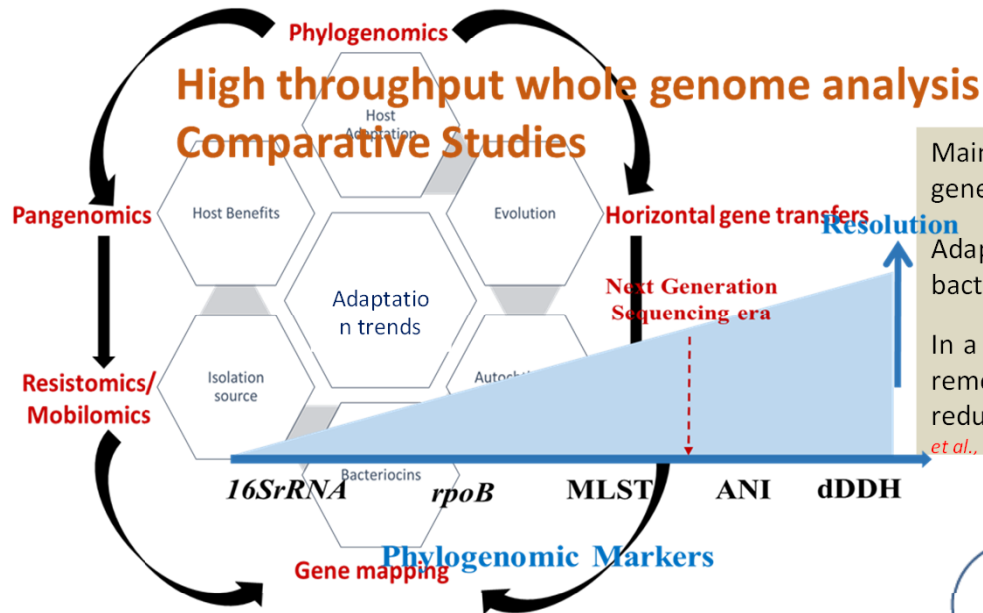
To adhere or not to adhere!





Understanding Host Adaptation

PROBIOGENOMICS



Mechanisms and processes considered to understand host adaptation

Role of Genome Dynamics

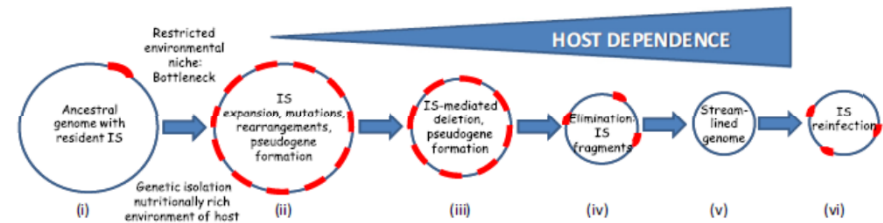
Next Generation Sequencing

Maintains plasticity of genome and is source of antimicrobial resistance genes, gene clusters etc.

Adaptation and evolution of commensal, pathogenic and environmental bacteria (Darmon and Leach, 2014).

In a specific niche, IS expansion disrupts genes, ultimately leading to their removal from the genome, leading to genome streamlining and genome reduction. e.g. *Wigglesworthia* in the tsetse fly (Bordenstein & Reznikoff, 2005; Moya et al., 2008).

Average Nucleotide identity (ANI) >95% : Same species
 Digital DNA-DNA hybridization (dDDH) >70% : New species



Patricia et al., 2014



Candidate clusters

Cluster	Function	Human	Poultry/ Human	Rodent	Pig	IMTECH outlier isolate
<i>pdu-cbi-cob-hem</i>	Reuterin, Vitamin B12	-	-	-	+	+
Urease	Surviving in acidic conditions	-	-	+	Rare	+
SecA2-SecY2	Facilitates export of adhesins	-	-	+	+	+
SPS1/SPS2	Helps in biofilm formation	-	Rare	+	+	+
Levansucrase	Helps in biofilm formation	-	+	+	+	+

Human lineage:

No gene for biofilm formation

-- Has lost most of the genes

-- *pdu-cbi-cob-hem* cluster is required

-- reuterin production is required

Rodent lineage:

Role of these clusters: Numerous biofilm formation genes are required

-- No function of *pdu-cbi-cob-hem* cluster

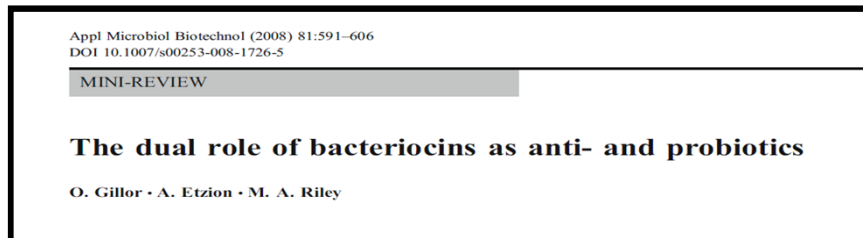
Novel Species, *Lactobacillus* spp. M31

? LR

- LR (Pigs)
- LR (Rodents)
- LR (Humans)
- LR (Poultry/Humans)

Probiotic strains - properties

- Vitamin absorption, Production of vitamin B and K, Recycling of food, Absorption of minerals, Fermentation – Production of short chain fatty acids (acetate, butyrate, propionate etc.) ..
- Production of antimicrobial substances such as bacteriocins
- Absence of genes encoding hemolysin and enterotoxins



Class	Property	Bacteriocins
I	Lantibiotic; small peptides containing the unusual amino acid Lan, MeLan and dehydrated amino acids	Nisin A, Nisin Q, Nisin Z, Lacticin 481, Lactocin S
II	Small and heat-stable Non-Lan-containing a: antilisterial activity peptides with YNGV consensus sequence b: two-peptide bacteriocins c: other bacteriocins	Pediocin PA-1, Sakacin P Enterocin A, Lactococcin Q, ABP-118, Durancin EF Lacticin Q
III	Large (>30kDa) heat-labile proteins	Helveticin J, Enterolysin A Millericin B
IV	Complex bacteriocins carrying lipid or carbohydrate moieties	Unknown
V	Circular peptides	Enterocin AS-48 Gassericin A



Suresh Korpole, MTCC ,IMTECH



- Singh PK, Sharma V, Patil PB, Korpole S. Identification, purification and characterization of laterosporulin, a novel bacteriocin produced by *Brevibacillus* sp. strain GI-9. *PLoS One*. 2012 Mar 5;7(3):e31498
- Baindara P, Chaudhry V, Mittal G, Liao LM, Matos CO, Khatri N, Franco OL, Patil PB, Korpole S. Characterization of the antimicrobial peptide penisin, a class Ia novel lantibiotic from *Paenibacillus* sp. strain A3. *Antimicrobial agents and chemotherapy*. 2016 Jan 1;60(1):580-91.
- Baindara P, Singh N, Ranjan M, Nallabelli N, Chaudhry V, Pathania GL, Sharma N, Kumar A, Patil PB, Korpole S. Laterosporulin10: A novel defensin like class IId bacteriocin from *brevibacillus* sp. strain SKDU10 with inhibitory activity against microbial pathogens. *Microbiology*. 2016 Aug 1;162(8):1286-99.
- Baindara P, Gautam A, Raghava GP, Korpole S. Anticancer properties of a defensin like class IId bacteriocin Laterosporulin10. *Scientific reports*. 2017 Apr 19;7:46541.

Identification of strains producing antimicrobial activity isolated from fecal sample of Rajasthan (Jaipur)

S. No.	Strain Designation	Identified as	% Similarity
1	RJAA.1(b)	<i>Enterococcus faecalis</i>	100%
2*	RJ AA.1	<i>Lactococcus gravieae</i>	99.72%
3*	RJPB2.1	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	100%
4	RJRCA(1)	<i>Enterococcus hirae</i>	99.93%
5*	RJAA.1(a)	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	99.86%
6	RJ10 ⁰ cream (b)	<i>Enterococcus faecium</i>	99.86%
7*	RJRCA 10 ⁰	<i>Clostridium butyricum</i>	99.64%
8	RJBFA 10 ⁻²	<i>Shigella flexneri</i>	99.78%
9	RJAA 10 ⁰	<i>Enterococcus durans</i>	99.88%
10	RJBFA 10 ⁻¹	<i>Enterococcus fergusonii</i>	99.93%

Identification of isolates based on 16S rRNA gene sequence analysis

*GRAS strains

Identification of isolates obtained from fecal sample (Chandigarh)

S.No.	Strain	Identified as	% similarity (16s rRNA)
1	CR1	<i>Lactobacillus delbrueckii</i> subsp. <i>indicus</i>	99.08
2	CR6	<i>Lactobacillus fermentum</i>	99.23
3	CA4	<i>Lactobacillus delbrueckii</i>	100
4	CHMRS1	<i>Lactobacillus fermentum</i>	99.52
5	BFACB1	<i>Lactobacillus delbrueckii</i>	99.58
6	CHMRS14	<i>Lactobacillus delbrueckii</i> subsp. <i>indicus</i>	99.31
7	CHB1	<i>Lactobacillus delbrueckii</i>	100
8	CA2	<i>Lactobacillus delbrueckii</i>	99.78
9	CB4	<i>Lactobacillus delbrueckii</i>	99.28

Antimicrobial activity of strains 48 h activity profile:

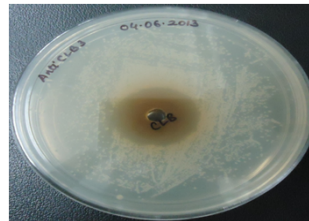
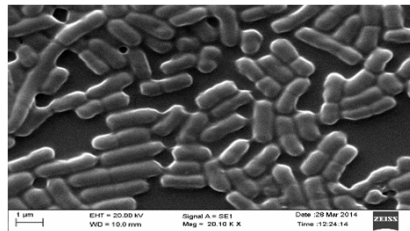
Test strains	CR1 (mm)	CR6 (mm)	CA4 (mm)	CH MRS 1 (mm)	BFA CB1 (mm)	CH MRS 14 (mm)	CHB1 (mm)	CA2 (mm)	CB4 (mm)
<i>Citrobacter</i>	15	20	21	22	23	20	-	-	23
<i>Bacillus</i>	14	16	18	16	18	-	-	-	13
<i>S.aureus</i>	16	20	22	22	20	18	-	-	TE
<i>Listeria</i>	14	16	15	15	14	13	12	-	13
<i>Candida</i>	-	-	-	-	-	-	-	-	-
<i>Vibrio</i>	30	>30	>30	>30	>30	26	20	12	>30
<i>Proteus</i>	>25	>25	>25	>25	>25	25	19	-	>25
<i>Micrococcus</i>	21	>25	>25	>25	>25	>25	>25	>25	22
<i>E.coli</i>	15	24	17	16	18	16	-	-	15
<i>Pseudomonas</i>	19	21	22	21	19	19	TE	-	19



Antimicrobial peptide production by *Clostridium* spp. from Rajasthan and Chandigarh

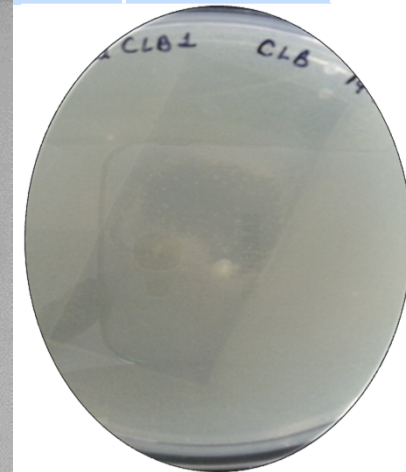
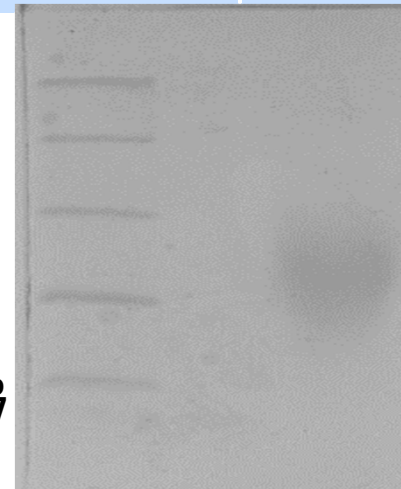
16S rRNA gene sequence similarity of the strain clb with closely related species

S No	Phylogenetic close relative	Strain	Described by	16S rRNA	% similarity
1	Clostridium butyricum	ATCC 19398(T)	Prazmowski 1880	AB075768	99.64
2	Clostridium diolis	DSM 5431(T)	Biebl and Spröer 2003	AJ458418	97.97



<i>Vibrio cholerae</i>	16 mm
<i>Pseudomonas aeruginosa</i>	12 mm
<i>Bacillus tequilensis</i>	12 mm
<i>Leuconostoc mesenteroides</i>	11 mm

kDa
40.0
25.0
15.0
10.0
4.6
1.7

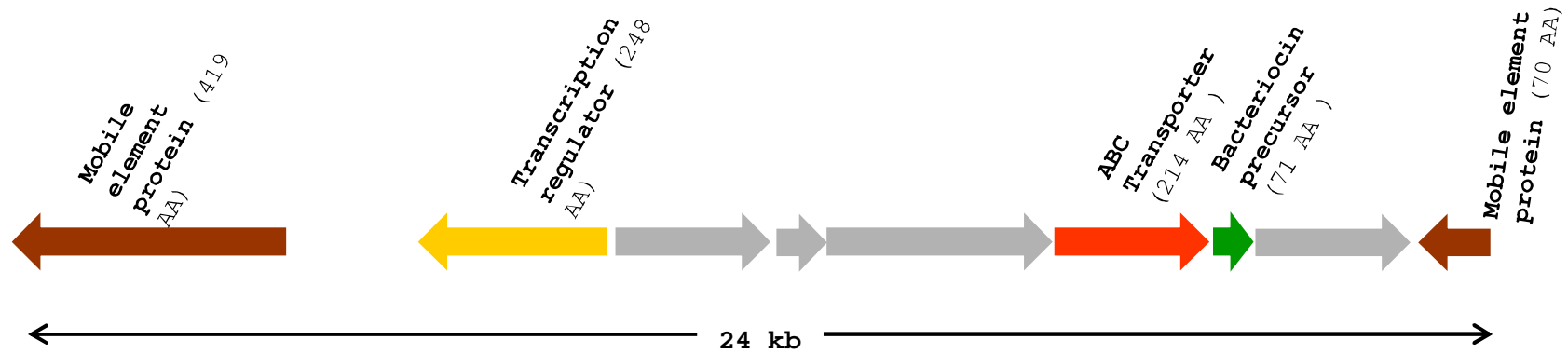


16% SDS-PAGE analysis and in-gel activity assay of antimicrobial peptide

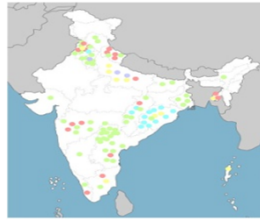


CSIR-IMTECH

Arrangement of genes involved in biosynthesis of antimicrobial peptide produced by *C. butyricum* stain clb

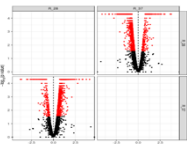


Whole GENOMIC STUDIES population studies PIPELINE (IMTECH published **first** such study from Asia and one of the first in the world)



HOSPITAL ACQUIRED INFECTIONS and OUTBREAKS of MDR strains in NICUs (IMTECH study pinpointed an outbreak to surface cap of antibiotic vial)

HIGH-THROUGHPUT MICROBIAL GENOMICS (CAPABILITIES)

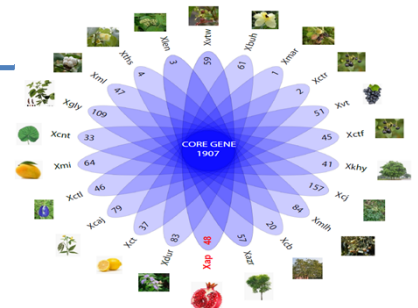


TRANSCRIPTOME SEQUENCING PIPELINE (**First** bacterial RNA seq data submitted from India)

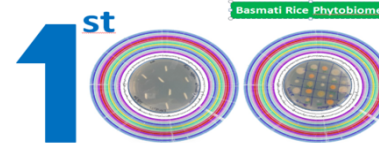


MiSeq from ILLUMINA – Since 2013 (**REVOLUTION ON A DESKTOP**)

UNIQUE MARKERS FOR EPIDEMIOLOGY AND AS DRUG TARGETS



Genomic resource of taxonomic, reference, medically important and probiotic strains (**First such resource of a multidrug resistant bacterium - highly accessed**)



MICROBIOME RESOURCES (IMTECH published **first** such study from Asia and one of the first in the world)

ECO-EVO STUDIES for Microbial Cocktail

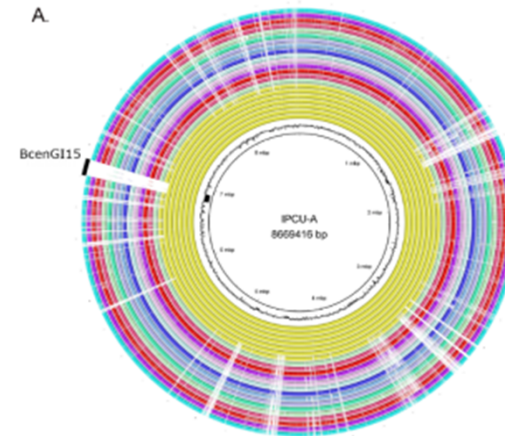
(ECOLOGICAL AND EVOLUTIONARY STUDIES)



HUMAN DIVERSITY
DIVERSITY IN FOOD HABITS
DIVERSITY IN CULTURE
TROPICAL CLIMATE



INDEPTH GENOME BASED EVOLUTIONARY STUDIES
(at the level of genus, species, strains and clones)



GENERIC, NOVEL and UNIQUE PROBIOTICS
for Consortia



THANK YOU!