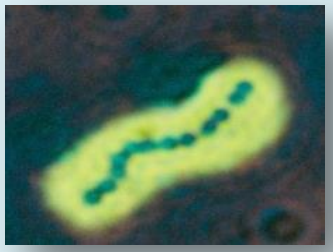
Emerging Trends in Nutrition for Health and Scope for Innovations-Way Forward" (EMTREND)



2. ILSI INDIA YOUNG SCIENTIST AWARD

IMPROVING PUBLIC HEALTH – FOOD SAFETY, NUTRITION & WELLBEING

Dr. Pradip Behare, Senior Scientist
Dairy Microbiology Division, ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana

ORCID ID: https://orcid.org/0000-0002-0403-2398

RESEARCHGATE:
https://www.researchgate.net/profile/Pradip_Behare

October 12, 2022

RESEARCH FOCUS

Key Genera
- *Lactococcus*
- *Lactobacillus*
- *Streptococcus*

Lactic Acid Bacteria (LAB) and Functional Food

Key Genera
- *Pediococcus*
- *Leuconostocs*
- *Propionibacterium*

Bio-prospecting Native Strains

- Isolation & genomic characterization
  - ✓ Technological attributes
  - ✓ Functional attributes
- Proteomics of LAB
  - ✓ Gut adaptability/stress tolerance mechanism

Exploring Bio-molecules Production

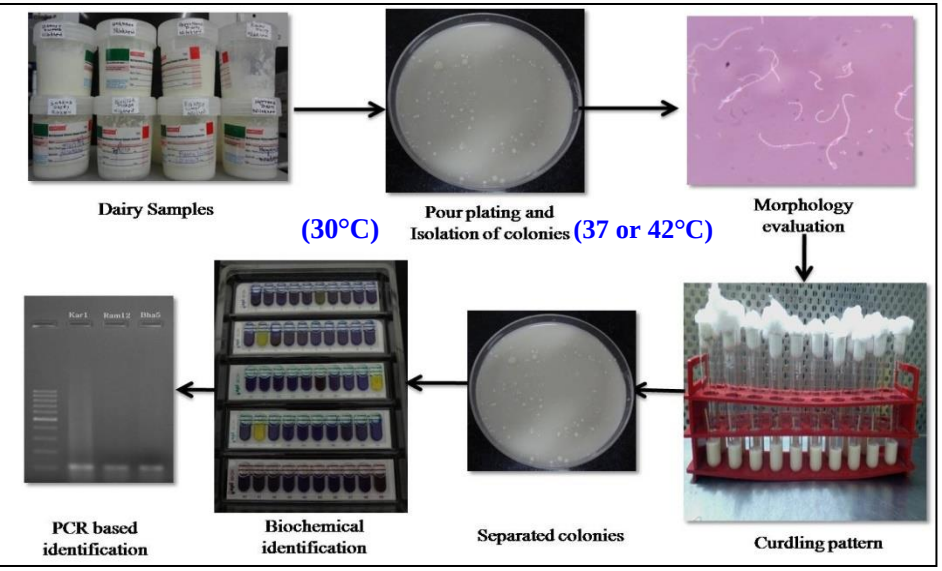
- Exopolysaccharides
  - ✓ Textural & Health properties
  - ✓ Micronutrient bioavailability
- Vitamins (B_{12})
- Bioactive peptides
  - ✓ Immunomodulation
- Low-calorie sugar (Mannitol)
- Phenyllactic acid
  - ✓ Bio-preservative

Developing Functional Milk Products

- Low-fat Dahi, Lassi, Shrikhand
- Sour Dahi
- Misti Dahi
- Greek-Style Yoghurt
- Vitamin B_{12} bio-enriched Soy curd
- Extended shelf-life Paneer

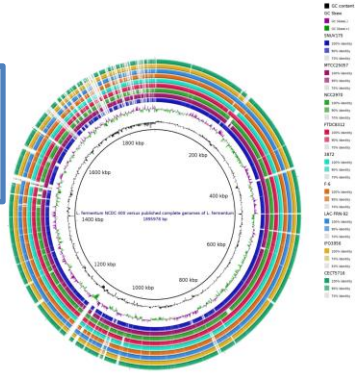
Basic research → **Strategic research** → **Technology development** → **Commercialization** → **Improving Human Health**

Bio-prospecting Native LAB Strains from Dairy and Non-Dairy Sources- Technological to Functional Characterization



Whole Genome Sequencing: 06	16S ribosomal RNA gene, partial sequence: 15

Identification of industrially important genes



Submission to NCBI for end-users

- LAB species (*Lactococcus*, *Lactobacillus*, *Streptococcus*, *and Leuconostocs* genus) have been well characterized.
  - ✓ Acidification, flavour & textural Profile (Firm & Soft)
  - ✓ Other specific attributes (Sugar Tolerance, Bacteriocin, GABA, Trehalose etc)
- Deposition of potential strains to National Repository **"National Collection of Dairy Cultures"** (NCDC).

Significance of Research Work

- ***Over 150 LAB strains are made available for end users through National Collection of Dairy Cultures (NCDC), NDRI, Karnal, Haryana.***
- ***In-depth genomic information on technological and functional features of the strains***

Probiotic Potential of Indigenous LAB Strains- *In vitro* and *in vivo*

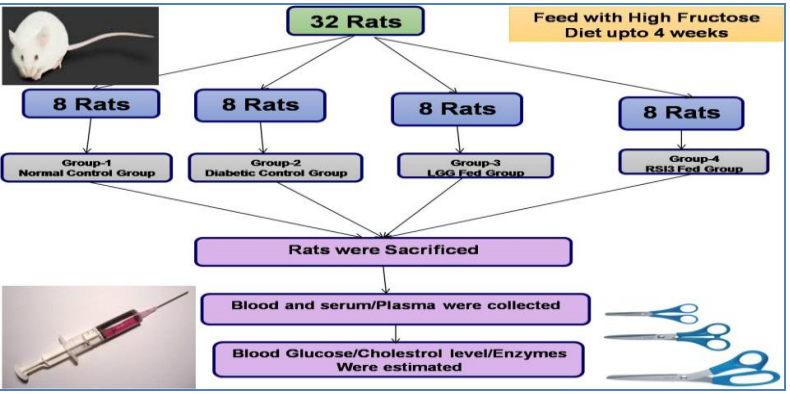
Cont…

Dairy and non-dairy origin strains
***L. rhamnosus* NCDC 610**
***L. fermentum* NCDC400**
***L. fermentum* NCDC605**

In vitro properties

- Bile tolerance: 1.5% for 6 h
- Acid tolerance: pH 2.5 for 3 h
- Cell surface hydrophobicity: 11.3%

L. rhamnosus NCDC 610



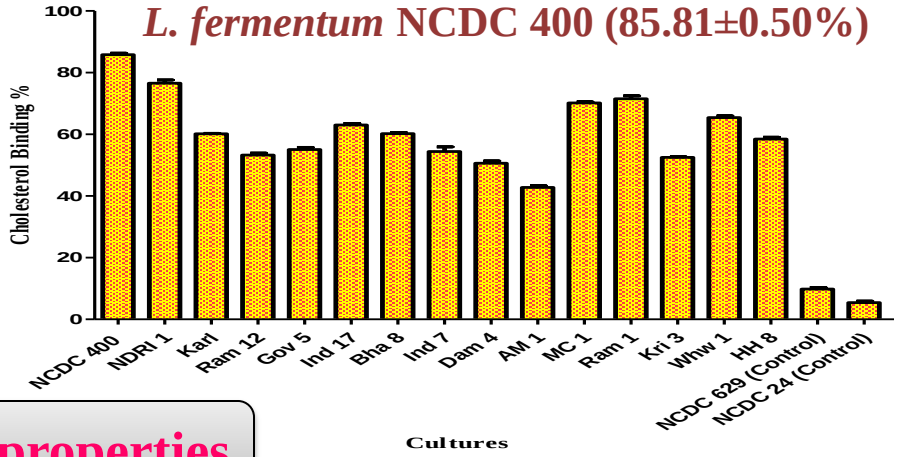
Biochemical measures in rats fed with different experimental diets along with control normal diet for 4 weeks and 10 weeks

Week	Variables	Group - 1	Group - 2	Group - 3	Group -4
4	FBG (mg/dL)	87.48±1.85aA	331.60±30.60aB	299.18±44.75aB	333.75±27.26aB
10	FBG (mg/dL)	88.83±1.00aA	391.00±37.59bB	256.40±38.00bC	223.08±42.53bC
4	Hb (%)	15.01±0.54aA	7.72±0.08aB	8.12±0.16aB	7.17±0.14aB
10	Hb (%)	14.11±0.52aA	5.94±0.25bB	14.53±0.39bA	14.45±0.20bA

Kadam, 2013; Minz, 2011

LRNCDC610 has shown anti-diabetic activity

L. fermentum NCDC 400 (85.81±0.50%)



In vivo properties

LFNCDC400 shown greater cholesterol lowering properties compared to non-probiotic strain in animal model

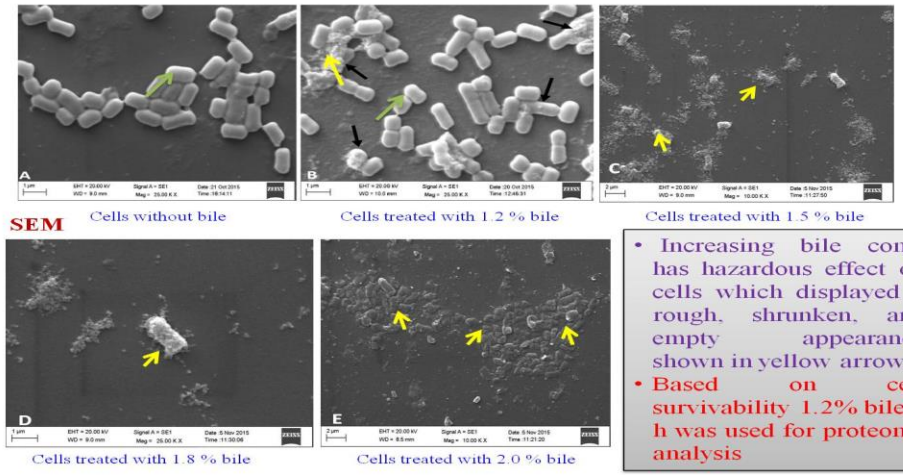
Gawande, K., Kolhekar, M……**Behare, P. V.** 2021, Food Hydrocolloides for Health

Significance of Probiotic Research

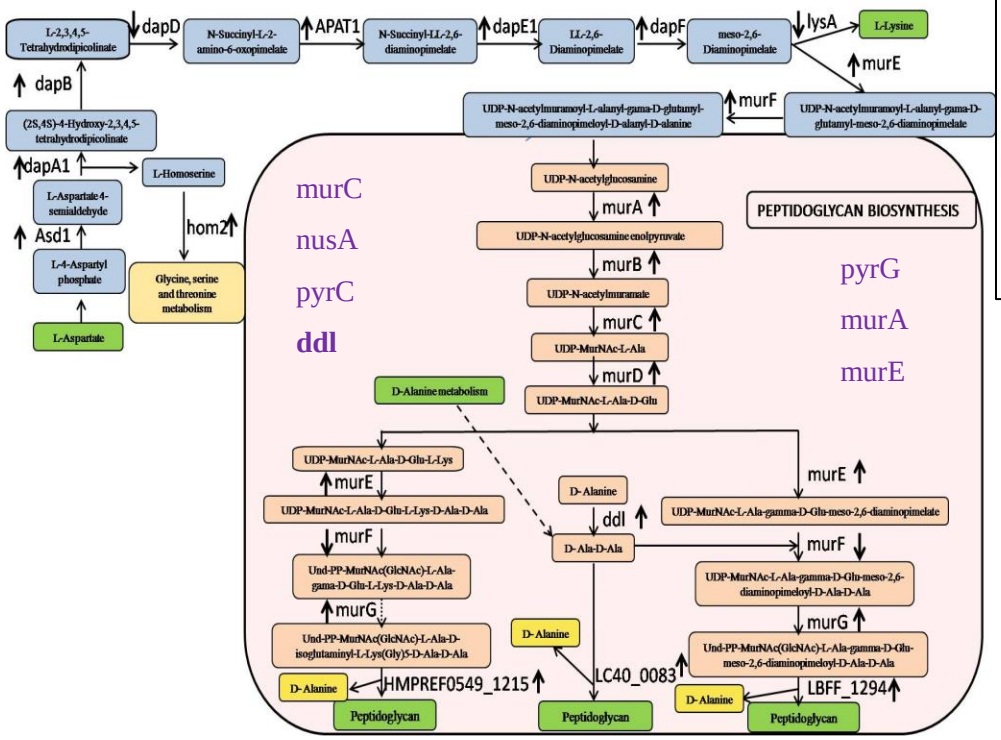
- ***Two indigenous probiotic strains for human health applications***
  - ✓ ***Indigenous probiotic L. rhamnosus NCDC 610***
  - ✓ ***Indigenous probiotic L. fermentum NCDC 400***

Evaluation of Bile Stress Tolerance Process in *L. fermentum* Strains by Proteome Analysis

Cont…

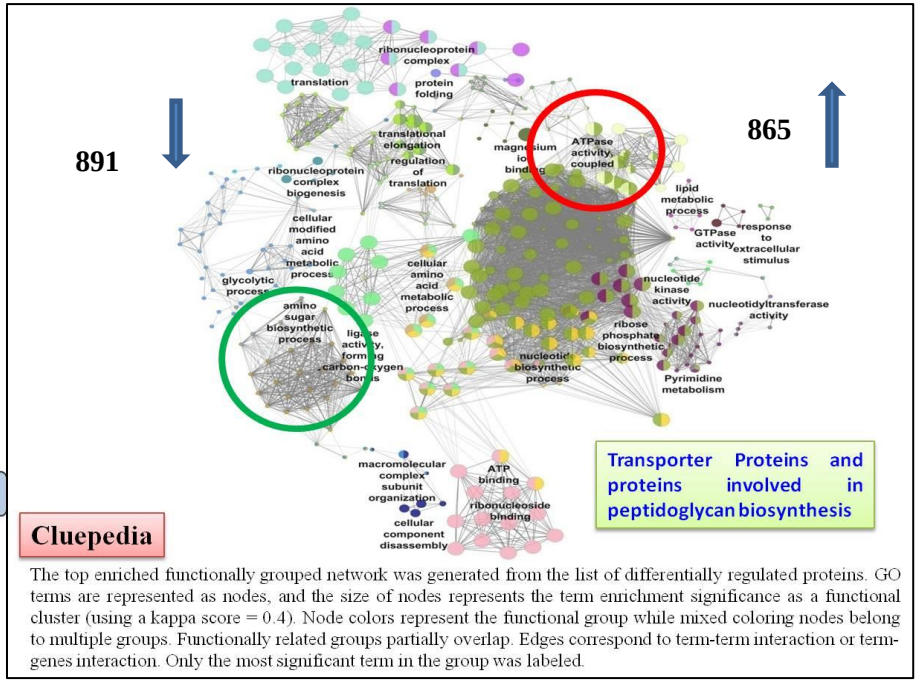


- Increasing bile conc. has hazardous effect on cells which displayed a rough, shrunken, and empty appearance shown in yellow arrows
- Based on cell survivability 1.2% bile/6 h was used for proteome analysis



Identified peptidoglycan remodeling pathway for bile tolerance

Identified bile responsive key proteins in indigenous probiotic by proteomic techniques



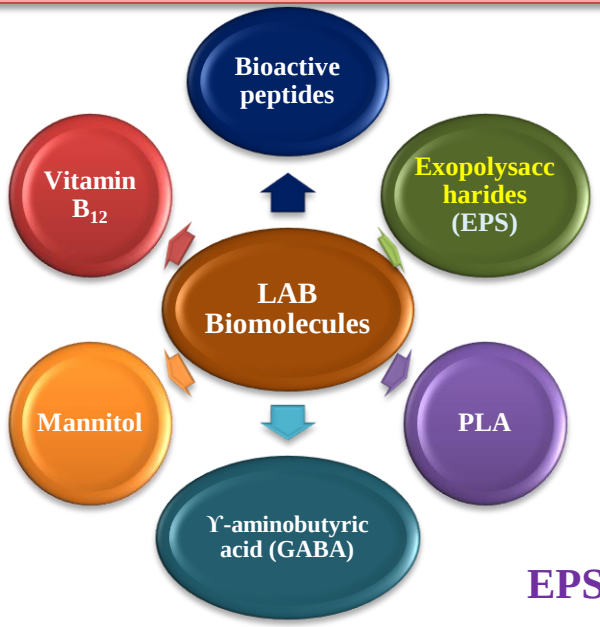
The top enriched functionally grouped network was generated from the list of differentially regulated proteins. GO terms are represented as nodes, and the size of nodes represents the term enrichment significance as a functional cluster (using a kappa score = 0.4). Node colors represent the functional group while mixed coloring nodes belong to multiple groups. Functionally related groups partially overlap. Edges correspond to term-term interaction or term-genes interaction. Only the most significant term in the group was labeled.

Kaur, G, ….. Mohanty, A. K, **Behare P. V. et al.** Journal of Proteomics 2017a; Pragya, ….**Behare** P. V. **etal.** Journal of Proteomics 2017 b

Significance of Proteomic Research

- ***First report from India on probiotic proteome and bile stress tolerance process in indigenous probiotic strain.***
- ***Enhanced our understanding of stress responsive changes and gut adaptability by the probiotic strain***

Exploring LAB Producing Bio-molecules- Technological to Nutritional Perspective



EPS cultures- 28

EPS producing LAB- Texturizing agent

EPSAM1, EPS399, EPSRam12, EPSWhw1, EPSGrv3 (High molecular weight > 10^4 Da and stiffer chains in the linkages) for set and stirred FMP

- ✓Greater intrinsic viscosities/WHC
- ✓Shear thinning behavior
- ✓Sensory properties

Significance of the Study

- *LAB derived EPS as biothickening molecule*
- *EPS producing strains with technological properties are categorized*

Exopolysaccharides (EPS) producing LAB

L. rhamnosus Kar1 derived "EPSKar1"

Iron balance studies (Experimental period -20 days)

Duration	1st week			2nd week			3rd week		
Groups → / Parameter ↓	Only $FeSO_4$	EPS-Fe 25 mg/Kg BW	EPS-Fe 50 mg/Kg BW	Only $FeSO_4$	EPS-Fe 25 mg/Kg BW	EPS-Fe 50 mg/Kg BW	Only $FeSO_4$	EPS-Fe 25 mg/Kg BW	EPS-Fe 50 mg/Kg BW
Iron intake (mg/day)	11.00	11.00	22.00	11.00	11.00	22.00	11.00	11.00	22.00
Faecal excretion (mg/day)	6.81±0.15	6.14±0.06	11.00±0.52	6.92±0.06	6.16±0.04	10.93±0.21	6.83±0.20	6.19±0.13	10.76±0.11
Urinary excretion (mg/day)	0.05±0.002	0.04±0.002	0.04±0.004	0.05±0.002	0.03±0.006	0.03±0.008	0.06±0.004	0.03±0.01	0.03±0.01
Apparent digestibility coefficient (%)	$38.06±1.38^a$	$44.12±0.59^b$	$50±2.40^c$	$37.03±0.57^a$	$44±0.37^b$	$50.30±0.99^c$	$37.87±1.83^a$	$43.72±1.20^b$	$51.06±0.52^c$
Iron balance (mg/day)	$4.24±0.15^a$	$4.90±0.06^a$	$11.04±0.53^b$	$4.12±0.06^a$	$4.87±0.04^b$	$11.10±0.21^c$	$4.22±0.19^a$	$4.89±0.14^b$	$11.27±0.11^c$
% Retention/Intake	$38.58±1.39^a$	$44.55±0.56^b$	$50.21±2.41^b$	$37.52±0.60^a$	$44.34±0.43^b$	$50.47±0.99^c$	$38.43±1.81^a$	$44.08±1.29^b$	$51.24±0.53^c$

The faecal and urinary iron excretion for the anaemic control group was 0.05±0.00 and 0.02±0.00 mg throughout the experimental period.

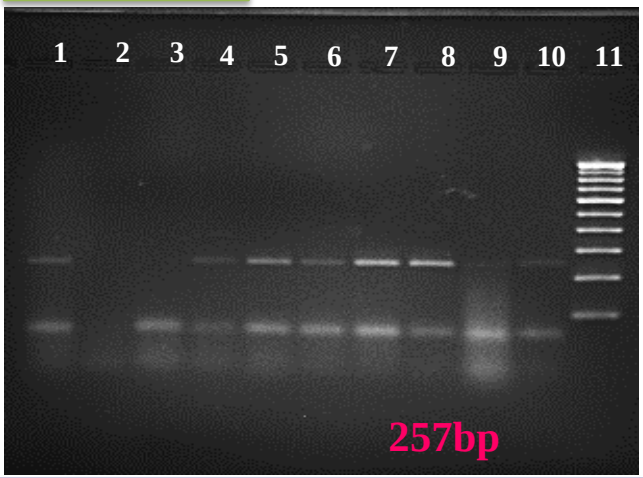
Significance of the Study

- *The EPSKar1-iron complex showed exhibited anti-anaemic effect in anaemia induced rats.*
- *EPSKar1-iron can be used to develop iron fortified food product.*

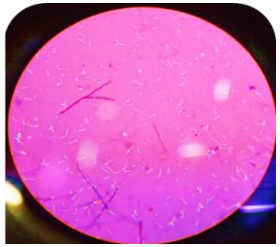
Vitamin B_{12} Production by *Lactobacillus reuteri* NCDC 958/F2

Cont…

cbiK gene



Screening of cbiK gene for Vitamin B_{12} production by lactobacilli. Lane 1- *L. reuteri* NCDC 958; Lane 2- *L. plantarum* CDR2; Lane 3- *L. plantarum* NCDC 697; Lane 4- *L fermentum* NCDC 400; Lane 5- *L. plantarum* HD48; Lane 6- *L. plantarum* HD51; Lane 7- *L. rhamnosus* NCDC 610; Lane 8- *L. fermentum* NCDC 701; Lane 9- *L. plantarum* NCDC 691 and Lane 10- *L. salvarius* NCDC 696



Vit. B_{12} bio-fortified food

- **Prevalence of vitamin B_{12} deficiency is 47% in north Indian population.** (Singla et al., 2019)
- RDA of Vitamin B_{12} is 2.4 µg/day
- Intracellular by NCDC 958 was 1.948 ± 53 and 2.6334 ± 40 µg/mL respectively.

- **Vitamin B_{12} produced by *L. reuteri* F2 showed amelioration of the Vitamin B12 deficiency in Wistar rats**

LAB strain	Intracellular Vitamin B_{12} (µg/mL)	Extracellular Vitamin B_{12} (µg/mL)
***L. reuteri* NCDC 958**	**1.948 ± 53**	**2.6334 ± 40**
L. fermentum NCDC 400	1.160 ± 49	2.762 ± 27.5

Significance of the Study

- **Native *L. reuteri* NCDC 958 endowed with greater vitamin B_{12} producing capabilities**
- ***Vitamin B_{12} biofortified Soy food was developed and would be useful to tackle B_{12} deficiency in Indian population***

LAB Fermentation Derived Bioactive Peptides

In vitro immunomodulatory activity of custom synthesized peptides derived from *L. rhamnosus* NCDC24 fermented milk

Based on the structure-activity relationship four peptides from casein were selected and custom synthesized

- **AGWNIPM and YLGYLEQLLR** showed the highest antioxidative ability (ABTS scavenging assay)
- These peptides have increased the anti-inflammatory cytokine and reduced the proinflammatory cytokine (IL-6, IL-1β, and TNF-α) in LPS-stimulated mouse macrophages .

Srivastava, U., Basavaprabhu, H. N. ……**Behare, P. V.** 2022 **Peptides** vol. 155, September 2022, 170843

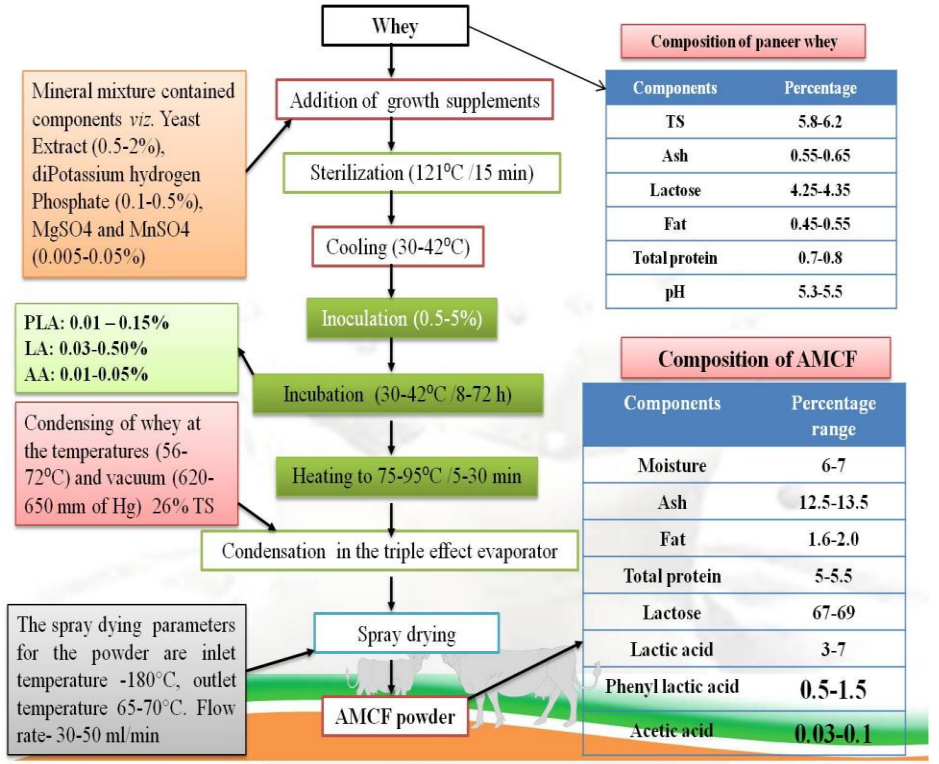
Significance of the Study

- *Antioxidative and immunomodulatory peptides for nutraceutical application*

LAB Fermentation Derived Phenyllactic Acid

Cont…

L. plantarum NCDC 769- PLA production



Composition of paneer whey

Components	Percentage
TS	5.8-6.2
Ash	0.55-0.65
Lactose	4.25-4.35
Fat	0.45-0.55
Total protein	0.7-0.8
pH	5.3-5.5

Composition of AMCF

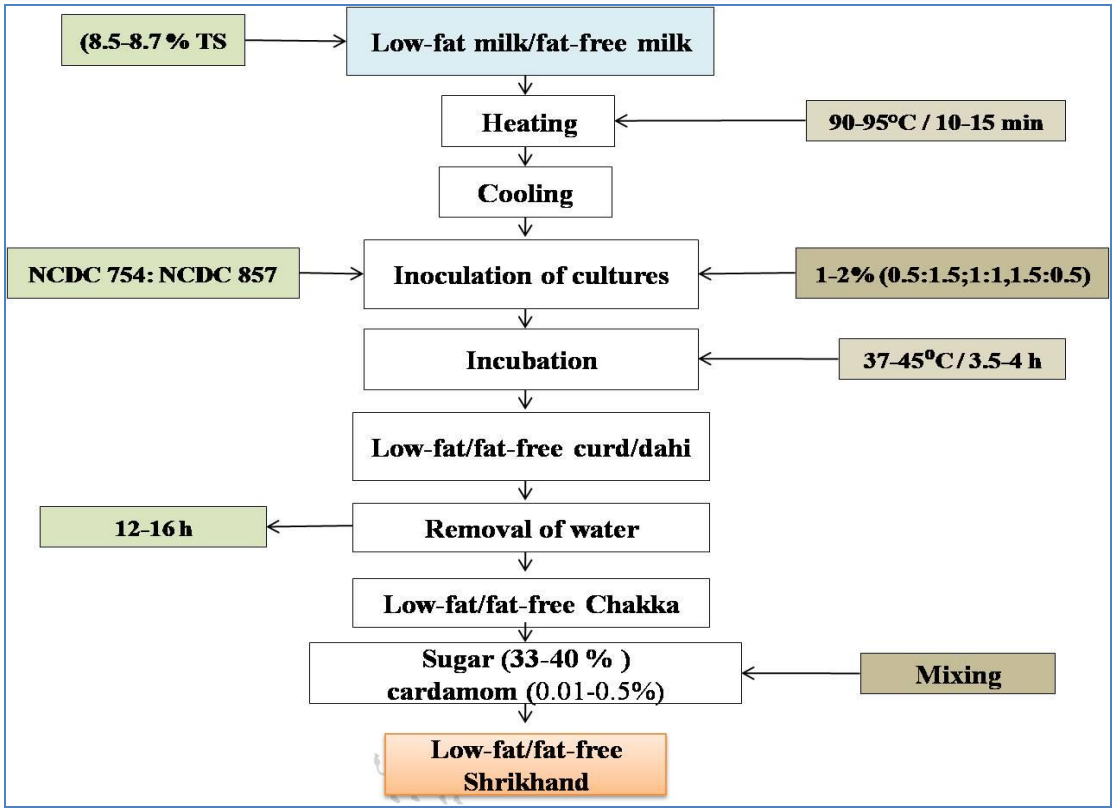
Components	Percentage range
Moisture	6-7
Ash	12.5-13.5
Fat	1.6-2.0
Total protein	5-5.5
Lactose	67-69
Lactic acid	3-7
Phenyl lactic acid	0.5-1.5
Acetic acid	0.03-0.1

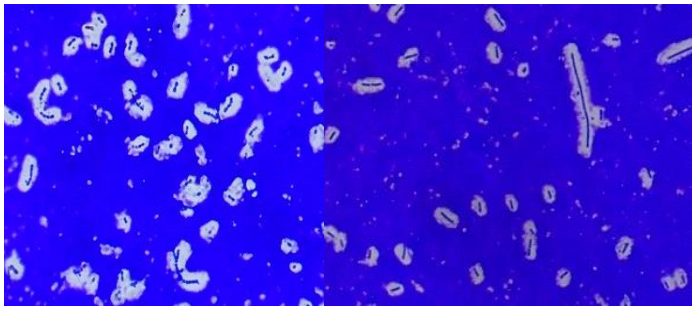
Patent Application No: 202111007461

Significance of the Study

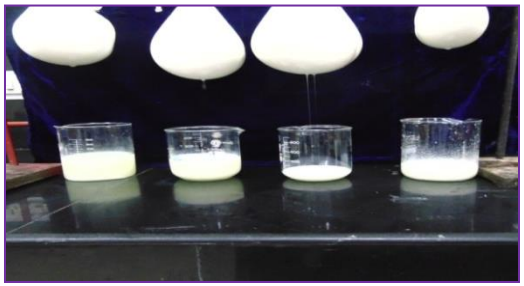
- *Phenyllactic acid rich AMCF formulation can be used as coagulant and biopreservative agent for Paneer*

Development of Functional Fermented Milk Products





MTCC25192 + MTCC25193



Patent Granted- 388158

Low-fat Shrikhand

Low-fat Dahi

Low-fat Lassi

Other FMP

- **Misti Doi/Dahi**
- **Sour Dahi**
- **Greek-Style Yoghurt**

Significance of the Study

- *LAB based technologies have been developed or patented and are available for transfer to stakeholders*
- *Use of these technologies either improve quality, health or increases 5-7% yield*

Impact of Research Contribution and Technologies Commercialization

Name of technologies	Company Name	Brand Name	Production (Kg/Month) approx.	Sale (Rs./Month) approx.	Status
Technology of Sour Dahi using prolific acidifying lactic cultures (19.03.2019)	**Firm 1**	-	-	-	Under trial at industry
Exopolysaccharides producing cultures for preparation of low-fat dahi (11.02.2016)	**Firm 2**	X	60 (DVS powder)	9,00,000	Available in the market
Bioprocess for direct vat set misti dahi culture (11.02.2016)	**Firm 2**	Y	10 (DVS powder)	1,50,000	Available in the market
Sugar tolerating lactic cultures for preparation of Misti Doi" (01.11.2014)	**Firm 3**	Z	9000	1,269,000	Available in the market

(as on 31.12.2020)

***Note:** The information on production and sale of product/DVS cultures is obtained from owner of the plant or Director/managers of the plant, production/sale of Misthi *Dahi*.
Calculation of Sale of products per month: Sale of DVS culture: 40-50 paisa/Lit of milk (15000 Rs/Kg); **Sale of Mishti *Dahi***: 12 Rs./85 gm of Mishti Dahi (i.e. 141 Rs./kg/day)

Enhanced scientific knowledge, developed and commercialized health promoting dairy food technologies, promoted entrepreneurs for manufacture of value added products and created employment opportunities for the younger population.

ONGOING RESEARCH WORK

- The role of phosphorylated proteins in the probiotic features of *L. fermentum* NCDC400
- Formulation of shelf stable thermophilic liquid starter blends
- Focus on eliminating micronutrient deficiency
  - ✓ Assessing EPSKar1-iron complex fortification in different food matrices
  - ✓ Characterization of zinc-binding EPS
- Development of vitamin B12 bio-enriched stirred fermented milk

FUTURE PLANS

- Development of Breed-Specific Lactic Dairy Starter Culture Blends (Gir and Sahiwal Breed)
- Investigating the effect of LAB-derived EPS on the modulation of the gut microbiota
- Exploring LAB strains
  - ✓ ϒ-aminobutyric acid production (GABA)
  - ✓ Vitamin K production
  - ✓ Mannitol production
- Efficacy of EPSKar1-iron complex-fortified food in anaemic subjects

TFSL/NCDC Lab Team

Dr. Pradip V. Behare
Senior Scientist & In-charge NCDC

Mr. Saurabh Kadyan
Scientist

Mrs. Yogita Sharma
Technical Officer

Ms. Harshita Naithani
Young Professional-II

Mr. Jasbir Singh
Young Professional-I

Mr. Rajbir
Lab attendant

Mr. Satish Kumar
Lab attendant

Mrs. Manorama Kumari
PhD scholar

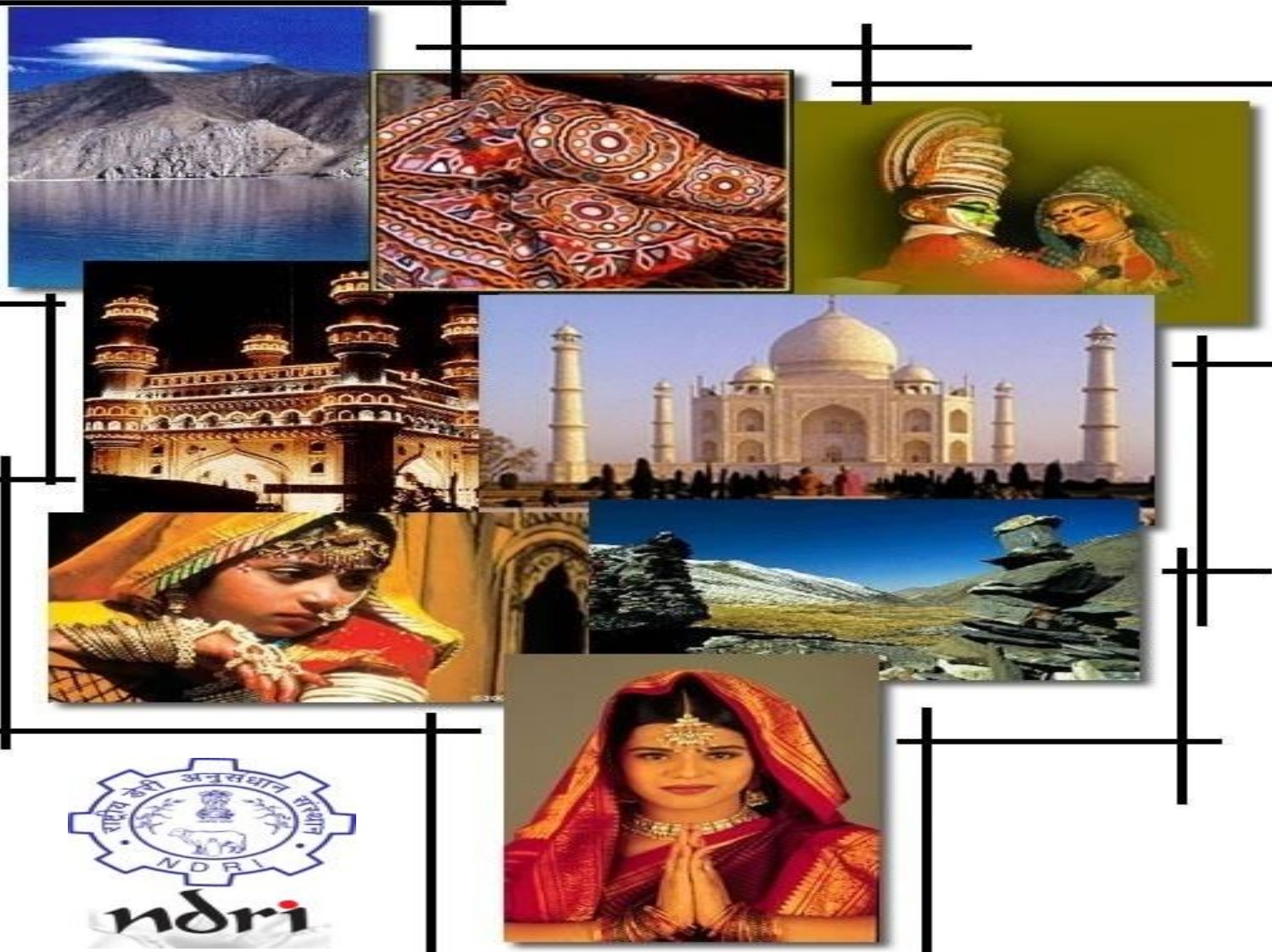
Ms. Vaishali Dasriya
PhD scholar

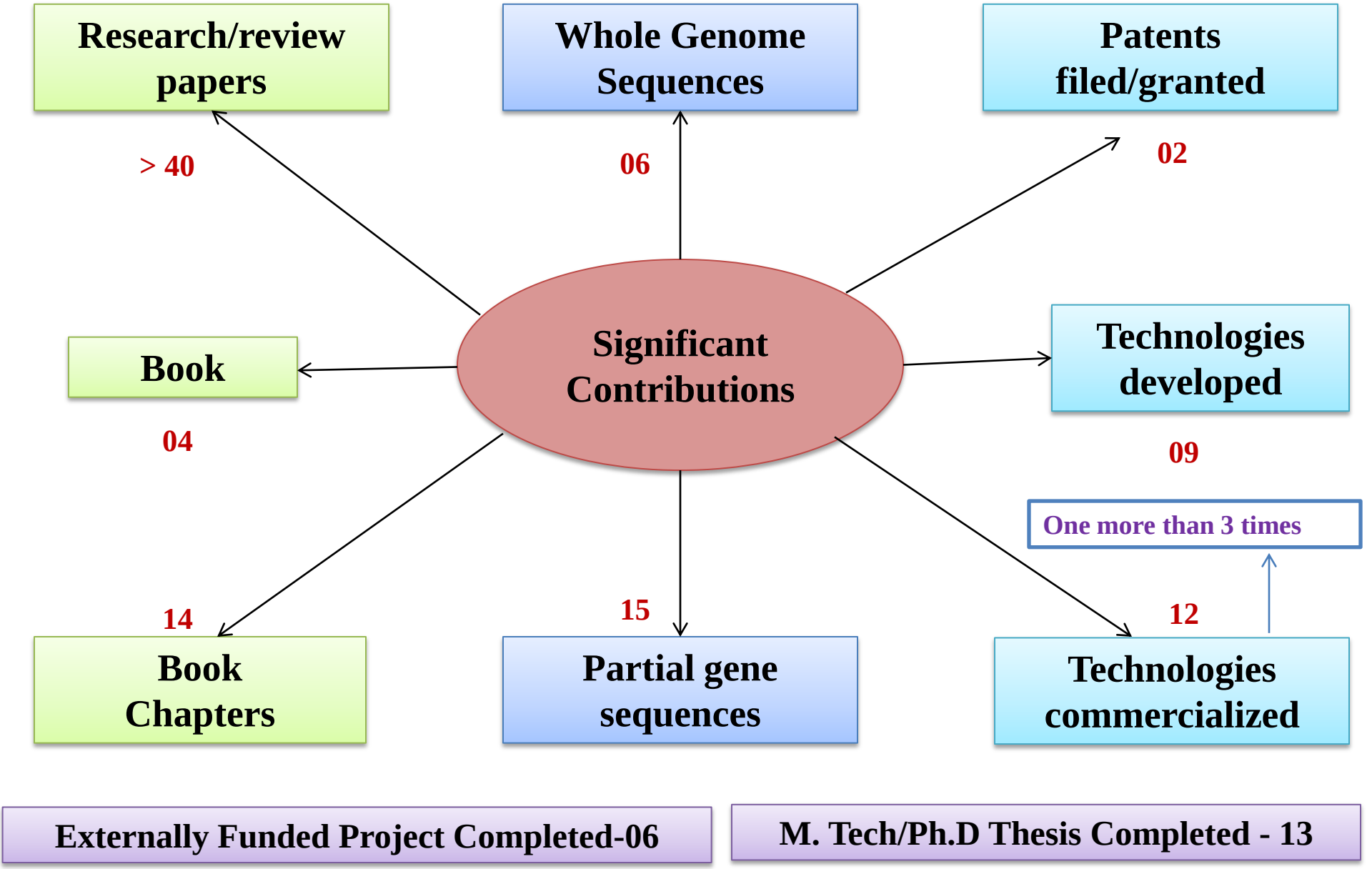
Mr. Basavaprabhu H.N.
PhD scholar

Ms. Sonia Ranveer
PhD scholar

Ms. Nishu Saini
PhD scholar

Thank You





Externally Funded Project Completed-06

M. Tech/Ph.D Thesis Completed - 13