Executive summary (not exceeding 500 words)

IIT Kharagpur has been working for the last 5 years in exploring new healthcare molecules with chemical structure of homo- or hetero oligosaccharides (arabinogalactan, arabinoxylan, glucomannan, thelebolan etc from various bioresources; including grain crops; mostly beta linked carbohydrates) Our investigation so far has revealed the that novel prebiotic, bifidogenic, immunomodulatory and anti-hyperglycemic molecules are abundant in common grain crops (rice, wheat, barley, oat, ragi, bajra, jawar etc) but their effects are masked by predominant polysaccharides. Thelebolan as novel prebiotic molecule from a fungus has already been characterized and a process for making oligosaccharides from it has been IPR protected (999/KOL/2013). About six oligosaccharides molecules have been isolated from grain crops and their final chemical structure determination is in progress. Biological activities of the purified isolates have been observed. Oligosaccharides from grain crops as new chemical entities have rarely been investigated.

This project aims at laboratory scale purification and characterization of these new pure molecules (in gram levels), scale up of the oligosaccharide-rich fractions (kg level) by acid/enzymatic hydrolysis, formulation for their prebiotic, bifidogenic, immunomodulatory and anti-hyperglycemic roles by cell-cell/cell-molecule interactions by live cell imaging, and evaluate activity in vivo (in rat model). Lastly, nutraceuticals and value-added food items will be produced in pilot scale (in 10-50 kg per batch) in collaboration with specific industries. the formulations and products will be tested for their prebiotic roles in supporting the growth of psychobiotic bacterial cultures in germ-free mice to prove their exclusive efficacies. Such products do not exist in Indian market and are very vital for healthcare of children, mother and elderly persons. very few industries in Europe and USA has just started developing such products.
This is a collaborative project of M/s Ultra Seedtech (India) Pvt Ltd, Hyderabad (USPL)- IIT Kharagpur (IITKGP). The project envisages pilot scale production of prebiotic molecules and nutraceutical formulation in Phase-I (first 2 years) and pilot scale product development in M/s USPL (e.g., for biscuits, drinks and health drink, phyto-pharma, nutraceutical industries) in Phase-II (3rd and 4th years).
In the first 2 years, the estimated budget in Rs. 120 lakhs (M/s USPL will contribute Rs. 15 lakhs in cash and Rs. 15 lakhs in kinds), and in the last 2 years the estimated budget is about Rs 280 lakhs (M/s USPL will contribute Rs. 35 lakhs in cash for specific equipment and Rs. 35 lakhs in kinds for market survey, collaborative experimentation with target industries, consutant engagement for quality assurance and regulatory clearance etc). Joint IPR protection will be done for the molecules, formulations and the processes to be developed.
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Background and motivation (not exceeding 500 words)

Consumer demands in the field of food production have changed considerably in recent years. Consumers now believe that foods contribute directly to their health. Today foods are not are far from satisfying hunger and to prevent nutrition-related diseases and improve physical and mental well-being of the people In this regard, functional foods play an outstanding role. The increasing demand on such functional foods/nutraceuticals foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for improved quality of their later years, In India there are specific challenges in using and modifying food ingredients for tackling children malnutrition, improving their immunity, taking care of mothers' health, etc as routing healthcare items. Emerging evidences suggest that proper consumption of dietary oligosaccharide can be useful for managing diabetes, neuro-degeneration and in curing important diseases like autism.
The β- linked glucans are the predominant form found in fungi and plant cell walls. Fungal derived β (1→3) D- glucan has been reported to modulate various aspects of immunity. Ganoderma lucidum have been known as traditional remedy in treatment of different human diseases, such as hepatitis, hypertension, hyperglycemia, chronic bronchitis, bronchial asthma, cancer and others. Research in recent years confirms the medicinal efficacy and efforts to identify such bioactive molecules are in progress. Complex polysaccharides and oligosaccharides have been projected to be prebiotic molecules. These are bifidogenic molecules supporting selective growth of probiotic flora and inhibiting enteric pathogens. Such molecules are immunomodulators and are major contributors of tertiary functions in food as dietary fiber originated ingredients.

Most commonly they occur as cellulose in plants, the bran and cereal grains, and the cell wall of certain fungus, mushroom and bacteria. Oat is a rich source of the water-soluble fibre (1, 3/1, 4) β-glucan, and its effects on health have been extensively studied for the last 30 years. Oat β-glucans are the only dietary fiber currently recognized by the European Food Safety Authority (EFSA) to be able to reduce a disease risk.
This project is envisaged from a collaborative theme with an industry (M/s USPL) of making value-added healthcare products and functional foods from carbohydrates of grain crops but with molecules with specific chemistry as stated in the description here.
India is major producer of several grain crops (barley, oat, ragi, bajra, jawar, colored rice etc) that are useful source of such oligosaccharides, available in million tonnes. Food and nutraceuticals industries have started using multi-grain ingredients to provide additional healthcare benefits. But none of the product in market has fully scientific formulation that clearly mentions the constituent oligosaccharides and their benefits. Adding this element in the product will not only give healthcare benefits to consumers but will improve prospect of exporting such products to global market.
Investigations by Davis & Milner (2009) and others are exciting in understanding the enormous importance of prebiotic molecules in supporting Bifidobacteria in eliciting commensal immunomodulation and prebiotic diet design for predictive healthcare. The global nutraceutical market is estimated to be USD 85 billion.

Project outcomes (please list specific objectives): *The project should address a specific need of the industry/industries and there should be clear expected outcomes from the project. It is expected that joint patents will result from this project.*

1. Isolation of new prebiotics oligosaccharide molecules and their complete structure elucidation (work is already in advanced stage in the PI's laboratory and remaining work will be completed during the tenure of the project)
2. Scale up of purification to get gram quantity molecules per batch for proper testing
3. Scale up further to get kg quantity of oligosaccharide rich fractions
4. Formulation of nutraceuticals
5. Pilot scale product manufacturing (e,g., biscuit; ready to serve drink etc)

Scope (not exceeding 1500 words): *The scope should clearly lay out the contributions of the academic partner and the industry partner.*

I. Role of industry M/s USPL-
A. Raw material supply
B. Market survey
C. Pilot scale production in Phase-II
D. Liaison with food/Pharma/nutraceutical industries for the market feed back during pilot scale production
II. Role of IIT Kharagpur
A. Complete structural elucidation of concerned oligosaccharide molecules and its digestion product (oligosaccharide) followed by biological activity screening, leading to healthcare product development in collaboration with industries.
B. In-vivo anti-hyperglycemic activity screening of oligosaccharides.
C. Studies focusing on the pre-clinical tests of oligosaccharides and their effect on gut health immunily linked to colon cancer having commercial implications.
D. IPR protection of the molecules/processes/formulations.
A few typical experiments to be performed for testing and evaluation-

Degradation of grain polysaccharides (GP) into oligosaccharide
GP will be digested following the method of Kumara and Ojima (2010). GP (5mg/mL) in 10 mM sodium phosphate buffer (pH 6-10) was degraded into oligosaccharide fragments by 0.01 U/mL of β- (1→3) D-Glucanase (Sigma) at 4°C-70°C for 0-12h. In alternative way, GP (5mg/mL) will be treated with 0.5 N HCL for 12 h to make oligosaccharides.
Chemical analysis
Chemical and physical property of GP and its digested product was carried out using GC-MS/MS, HPLC, FTIR, 1-D NMR (13 C, 1H) , 2D NMR (COSY, NOESY, HSQC, HMBC), Viscometer etc.
Toxicity evaluation
The presence of endotoxins oligosaccharide will be determined by FDA approved LAL assay (E-TOXATETM kits, Sigma).Endotoxin if present will be removed by passing through Polymyxin B Sepharose matrix.

Anti-hyperglycemic effect screening of oligosaccharides
Induction of diabetes mellitus
Diabetes mellitus will be induced in healthy male Wislar albino rats by a single intraperitoneal injection of freshly prepared streptozotocin (STZ; in 0.1 M citrate buffer pH 4.5) at a dose of 55 mg/kg body weight. After three days of STZ injection, animals with fasting blood glucose above 400 mg/dL be included in the study. After three days of injection, diabetic rats will be fed with oligosaccharides.
Recording of body weight, blood glucose, and cholesterol
The animals will berandomly assigned into following groups and be given the following treatments:
Group I: Normal control with citrate buffer
Group II: Placebo control (water)
Group III: Normal rats with oligosaccharides (200mg/Kg body weight)
Group IV: Normal rats with oligosaccharides (100mg/Kg body weight)
Group V: Normal rats with oligosaccharide (200mg/Kg body weight)
Group VI: Normal rats with oligosaccharide (100mg/Kg body weight)
Group VII: Diabetic control with water
Group VIII: Diabetic negative control without glidenclamide
Group IX: Diabetic positive control with glibenclamide
Group X: Diabetic rat with oligosaccharides (200mg/Kg body weight)
Group XI: Diabetic rat with oligosaccharides (100mg/Kg body weight)
Group XII: Diabetic rat with oligosaccharide (200mg/Kg body weight)
Group XIII: Diabetic rat with oligosaccharide (100mg/Kg body weight)
Effect of EPS and oligosaccharide on growth of gut microflora
Tested strain
Bifidobacterium longum and Lactobacillus brevis
Testing for prebiotic activity of oligosaccharide
The oligosaccharides will be tested in comparison to FOS, GOS, Inulin
Histology and tumor weight
Colon tumor/ cancerous cell mass will be taken from mice treated with or without oligosaccharides after the treatment period and Section of colon inner and outer parts slides were prepared. Finally, the sections were stained with hematoxyline-eosinand observed under microscope. Tumor weight also measured in treated group in comparison to untreated control.