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Casein Denaturation Through Probiotic Microbes L.Delbruekii Sub Sp Bulgaricus and L.Acidophilus And Streptococcus Thermophilus In Yogurt

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Abstract: Casein is a family of related phosphoproteins. Denaturation of casein was observed with the selected probiotic microorganisms, which can produce an enzyme dipeptidyl peptidase-4. Dipeptidyl peptidase-4 enzyme is the enzyme which helps in the denaturation of casein through probiotic microbes L. delbruekii sub sp bulgaricus and L. acidophilus. Probiotics' potential health and nutritional benefits have boosted the demand for functional probiotic foods. The efficacy of probiotics depends on providing a specific number of viable cells for their consumption. In the study enzyme secretion plays the main factor to show the viability of probiotic microorganisms to denaturate casein protein. HPLC-High-Performance Liquid Chromatography was conducted to observe the enzyme secretion. It was observed that the strain Lactobacillus delbruekii sub sp. bulgaricus had produced the dipeptidyl peptidase-4 enzyme at a higher amount of 174.841sum with a retention time of 14.27. The second probiotic strain Lactobacillus acidophilus resulted by producing enzyme Dipeptidyl peptidase-4 at 163.786 sums with a retention time of 13.209, The third strain and general probiotic strain Streptococcus thermophilus resulted dipeptidyl peptidase-4 enzyme production was not observed; hence denaturation of the milk protein was not resulted, due to the absence of the enzyme production.

Introduction:

Casein: Casein plays an important role in human nutrition. Casein micelles are composed of α_{s1},α_{s2},β and κ-casein proteins. Casein contains a high number of proline amino acids which hinder the formation of common secondary structural motifs of proteins. In some people, casein allergy or casein intolerance is observed which can be expressed as milk protein intolerance. It is an abnormal immune response by the body to the protein consumed by the human being (Sanctuary et al,2018). There areno disulphide bridges; as a result, it has relatively little tertiary structure, is hydrophobic, and is not soluble in water. It is found in milk as a suspension of particles called casein micelles (Dalgleish DG,1998).

Casein Intolerance: If casein protein cannot be handled by our body, then it is considered a milk allergy or casein intolerance, whereas casein consumption triggers an allergic reaction with symptoms such as a skin rash or hives, swollen lips, mouth, or tongue; and runny nose. When it is not treated immediately it can become a life-threatening allergic reaction. Casein allergies are generally diagnosed early in life and suddenly appear in adulthood (Robinson, 2002). A small fraction of the population is allergic to casein, casein intolerance is also known as “milk protein intolerance”. It is experienced when a body cannot break down the proteins of casein. In general,

casein intolerance ranges from 0.25 to 4.9% in young children (Monica Ramakrishnan et al 2020).

Probiotic Microorganisms: Probiotics play a key role in a variety of medical conditions, including diarrhoea, gastroenteritis, irritable bowel syndrome, inflammatory intestinal disease, hepatic diseases, immune function, child allergies, failure-to-thrive, hyperlipidemia, Helicobacter pylori infections, etc. These help in reducing the health risk factors of the above-mentioned diseases (Diaz et al., 2020). They are widely used for treating allergic diseases like dermatitis, allergic rhinitis, etc., vaginosis and related infections and even for the prevention of tooth issues. They are found effective in many gastrointestinal disorders. Probiotics are considered an encouraging and hopeful alternative to antibiotics because they can reduce the number of unsafe bacteria (Bustamante et al., 2020).

Casein Denaturation with Dipeptidyl Peptidase 4(DPP4) enzyme: Dipeptidyl peptidase 4 is a form of the enzyme, which is found in the Gastrointestinal tract of humans, released by Lactobacillus Microorganisms. A few selected Lactobacillus microorganisms can produce Dipeptidyl peptidase 4 enzymes through their metabolic activity, which helps our Gastrointestinal tract digest casein by producing a peptide (Kameoka et al,2006).Some oral protease enzymes recommended for casein or gluten digestion have only been able to provide low enzymatic activity due to stomach acidity in some people (Zhang et al., 2015).

Material, Methods and Results:

The cow milk was procured and then yoghurt was prepared by using three different probiotic bacteria L. delbruekii sub spbulgaricus,L. acidophilusand Streptococcus thermophilus. The probiotic bacteria were procured fromthe culture centre of ICAR-National Dairy Research Institute (NDRI)- Karnal, India. The probiotic bacterial strains were used in a 2% culture with a 1:1 ratio in milk for the yoghurt preparation. The Pure culture strains which were procured from the culture centre are represented by the code Lactobacillus delbruekii subsp bulgaricus–NCDC 009, Lactobacillus acidophilus - NCDC 14, Streptococcus thermophiles- NCDC

Storage of Cultures and yoghurt preparation: The probiotic bacterial species were used in a 2% culture with a 1:1 ratio in milk for the yoghurt preparation. Specific Probiotic microorganism strains (Lactobacillus acidophilus, Lactobacillus delbruekii sub sp. bulgaricus, Lactobacillus helveticus and Streptococcus thermophilus) were selected which produce the Dipeptidyl Peptidase -4 enzyme during their metabolism. The optimized probiotic cultures and other probiotic bacteria isolates were kept in (MRS) De Man, Rogosa and Sharpe broth with 20% glycerol (v/v) at - 20℃. The cultures were rehydrated in newly sterilized MRS broth. On agar plates of (MRS) De Man, Rogosa and Sharpe fresh colonies were streaked further and picked. MRS agar plates were used to sustain the cultures.

Effect of enzyme and probiotic microorganisms on milk: The enzyme Dipeptidyl peptidase-4(DPP-4) activity cleaves important hormones and peptides, which affects the intestine. According to certain investigations, human DPP-4 homologs have been found in commensal bacteria, such as Lactobacillus and Prevotella. The gut microbiota may encode a DPP-4-like function that could be a novel way to control host metabolism and protein digestion. And behaviour. at the same time, the probiotic microorganism helps in the digestion of food and keeps the bad bacteria out from getting control inside the body leading to getting sick. They help the cells lining your stomach to fight against harmful bacteria that enter in your blood after eating. Probiotic Lactobacillus strains offer many health benefits, such as improving cholesterol levels, less risk for cardiovascular disease, and immune system support. The specified gathered probiotic species will help people in Lactose intolerant or Dairy intolerant to digest lactose through their metabolic process by producing the enzyme Dipeptidyl peptidase-4 in the milk sample when they are inoculated in the milk. Dipeptidyl peptidases (DPPs) such as DPP4, alpha (FAP), DPP2, and DPP8, cleave N- terminal dipeptides from peptides with proline at the final step help in controlling different metabolic disorders inside the body (Lacroix & Li-Chan, 2012). The mentioned enzymes are members of the serine proteases based on similarities in three-dimensional structure, catalytic residues arrangement of the (Ser, Asp, His), and the initial series around their catalytic residues (Gly-X-Ser-X X-Gly where X is any amino acid) .

High-Performance Liquid Chromatography (HPLC) (Markovitch et al., 2020):
The technique used for the separation of components in a liquid mixture is high-performance liquid chromatography. HPLC Can differentiate and recognize each compound based on how quickly it moves across the column. The component flows through the column with the mobile phase at a higher rate the stronger their affinity (such as the van der Waals force) is among them. The retention time (tR), dead time (t0) and peak height (h) were tabulated based on the result obtained (Table 4.3 pg. no-51). The components in the sample will be analyzed both qualitative and quantitative using these results.

Table 3.2-The Protocol followed in the process is as given below: Column	**ZORBAX Eclipse Plus C18, 3.5m, 4.6 X 100 mm**
Column flow Stop time Solvent program	1.0 mL/min 22 min Gradient
Mobile Phase A Mobile Phase B	5% acetic acid in water Acetonitrile: Methanol (1 1, v/v)

In Lactobacillus delbruekii sub sp. bulgaricus HPLC results showed the maximum DPP-4 quantity with the retention time and the area were 14.27 & 174.84 respectively. In Lactobacillus acidophilus the retention time and the area were 13.20 & 163.78 respectively, hence the quantity of DPP-4 was less than the L. delbruekii sub sp. bulgaricus. Minimum retention time 14.27 and area 31.44 were reported in L. helveticus which was the minimum amongst all the selected

bacterial strains. Whereas in S. thermophilus retention time and area are shown in Maximum Denaturation of casein protein (10kda, 19kda-two fragments) was observed in L. delbruekii. sub sp. bulgaricus as it consists of a maximum amount of DPP-4. Denaturation of casein protein followed by L. acidophilus (10kda, 19kda-two fragments) and L. helveticus (28kda). No denaturation was reported in control and S. thermophilus because of a lack of DPP-4. The enzyme production through different Probiotic strains was analyzed through high–performance liquid Chromatography (HPLC). The study suggested by Saranya et al (2020) has similar results, which stated that the milk proteins were known for their high nutritional properties and had a wide range of antimicrobial, mineral-binding and anti-lipidemic properties. The results with little variation in the case in isolating enzyme retention time through HPLC with different casein fragments. The yoghurt sample is injected into the HPLC column to observe the enzyme produced by the different probiotic strains with retention time and area of the peak obtained.

S. No.	Name of the strain	Retention Time	Area	Identified Compound
1.	Lactobacillus delbruekii sub sp. Bulgaricus	14.27	174.841	DPP-4
2.	Lactobacillus acidophilus	13.209	163.786	DPP-4
3.	Lactobacillus helveticus	14.27	31.448	DPP-4
4.	Streptococcus thermophilus	Nil	Nil	DPP-4

Table: Enzyme Effect or production observed through HPLC in each different probiotic Strain

S.No.	Name of the strain	Retention Time	Area	Identified Compound
1.	Lactobacillus delbruekii sub sp. Bulgaricus	14.27	174.841	DPP-4
2.	Lactobacillus acidophilus	13.209	163.786	DPP-4
3.	Lactobacillus helveticus	14.27	31.448	DPP-4
4.	Streptococcus thermophilus	Nil	Nil	DPP-4

Protein Quantification: The quantification of protein is followed after the estimation of enzyme production from each different probiotic strain. SDS-PAGE analysis was conducted to observe the molecular bands of denatured casein protein with individual strains. Javanovic, (2007) results with a similar variation in the bands were observed.

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Table - Casein-denatured molecular bands were analyzed using the SDS-PAGE method

S.No.	Different Microbes Inoculated Milk (Yogurt) with 1.5 Fat%	Denatured Casein Protein molecular weight Observed
1.	Control	80kDa
2	Lactobacillus delbruekii sub sp. bulgaricus	10kDa,19kDa-Two fragments
3.	Lactobacillus acidophilus	10kDa,19kDa-Two fragments
4.	Lactobacillus helveticus	28kDa
5.	Streptococcus thermophilus	80kDa

The table explains that various kinds of molecular bands were observed with different probiotic bacterial strains utilised in the study. The probiotic strains are Lactobacillus helveticusand Lactobacillus. delbruekii sub sp. bulgaricus, and Lactobacillus acidophilus,were selected, considering their capability to generate the enzyme Dipeptidyl peptidase -4 during their metabolism. The enzyme further was broken down into smaller fragments as example of ketones, acids, aldehydes, terpenes, esters, aromatic hydrocarbons, alcohols, and aliphatic hydrocarbons. General strain Streptococcus thermophilus is used in the research to observe the difference between DPP-4 enzyme-producing microorganisms. According to the evaluation of the SDS-PAGE results greater part of the consequence of the soluble co-aggregates is the result of the disulphide interactions. Following the 2-mercaptoethanol use, the co-aggregate bands with the highest intensivity have disappeared completely. Fractions of different fragments were observed from different probiotic strains used in the study. The control strain has not produced any of the degraded fragments of the molecular band, as it cannot produce the DPP-4 enzyme. Whereas the first probiotic strain Lactobacillus delbruekii sub sp. bulgaricus produced two fragments with molecular weights of 10kDa & 19kDa. The second strain Lactobacillus acidophilus /resulted in two fragments similar to Lactobacillus delbruekii sub sp. bulgaricus, 10kDa and 19kDa respectively. The third probiotic strain Lactobacillus helveticus produced a molecular band at 28kDa, and the fourth final strain Streptococcus thermophilus showed no signs of any degradation band as it cannot be able to produce the enzyme Dipeptidyl peptidase – 4.

References:

1. Afoakwa, E. O., Paterson, A., Fowler, M., & Ryan, A. (2008). Flavor formation and character in cocoa and chocolate: A critical review. Critical Reviews in Food Science and Nutrition, 48(9), 840-857. https://doi.org/10.1080/10408390701719272

2. Ahmad, I., Khalique, A., Shahid, M. Q., Ahid Rashid, A., Faiz, F., Ikram, M. A., Ahmed, S., Imran, M., Khan, M. A., Nadeem, M., Afzal, M. I., Umer, M., Kaleem, I., Shahbaz, M., & Rasool, B. (2020). Studying the influence of Apple peel Polyphenol extract fortification on the characteristics of probiotic yoghurt. Plants, 9(1), 77. https://doi.org/10.3390/plants9010077

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 4, 2022

3. Anema, S. G. (2021). Heat-induced changes in caseins and casein micelles, including interactions with denatured whey proteins. International Dairy Journal, 122, 105136. https://doi.org/10.1016/j.idairyj.2021.105136
4. Anema, S. G., & Li, Y. (2003). Association of denatured whey proteins with casein micelles in heated reconstituted skim milk and its effect on casein micelle size. Journal of Dairy Research, 70(1), 73-83. https://doi.org/10.1017/s0022029902005903
5. Ashraf, A., Mudgil, P., Palakkott, A., Iratni, R., Gan, C., Maqsood, S., & Ayoub, M. A. (2021). Molecular basis of the anti-diabetic properties of camel milk through profiling of its bioactive peptides on dipeptidyl peptidase IV (DPP-IV) and insulin receptor activity. Journal of Dairy Science, 104(1), 61-77. https://doi.org/10.3168/jds.2020-18627
6. Auestad, N., & Layman, D. K. (2021). Dairy bioactive proteins and peptides: A narrative review. Nutrition Reviews, 79(Supplement_2), 36-47. https://doi.org/10.1093/nutrit/nuab097
7. Bolaji, O., Apotiola, Z., Ojo, T., Akoro, S., & Ogunsola, A. (2019). Drying kinetics and impact on the volatile compounds of ogi using response surface modelling. Food Research, 4(2), 474-481. https://doi.org/10.26656/fr.2017.4(2).287
8. Borse, B. B., Rao, L. J., Ramalakshmi, K., & Raghavan, B. (2007). Chemical composition of volatiles from coconut sap (neera) and effect of processing. Food Chemistry, 101(3), 877-880. https://doi.org/10.1016/j.foodchem.2006.02.026
9. Bull, M., Plummer, S., Marchesi, J., &Mahenthiralingam, E. (2013). The life history of Lactobacillus acidophilus as a probiotic: A tale of revisionary taxonomy, misidentification and commercial success. FEMS Microbiology Letters, 349(2), 77-87. https://doi.org/10.1111/1574-6968.12293
10. Bustamante, M., Oomah, B. D., Oliveira, W. P., Burgos-Díaz, C., Rubilar, M., & Shene, C. (2019). Probiotics and prebiotics potential for the care of skin, female urogenital tract, and respiratory tract. Folia Microbiologica, 65(2), 245-264. https://doi.org/10.1007/s12223-019-00759-3
11. Chapman, C. M., Gibson, G. R., & Rowland, I. (2011). Health benefits of probiotics: Are mixtures more effective than single strains? European Journal of Nutrition, 50(1), 1-17. https://doi.org/10.1007/s00394-010-0166-z
12. Cheng, H. (2010). Volatile flavor compounds in yogurt: A review. Critical Reviews in Food Science and Nutrition, 50(10), 938-950. https://doi.org/10.1080/10408390903044081
13. Dabaj, F. K., Lasekan, O., Manap, M. Y., & Ling, F. H. (2020). Evaluation of the volatilomic potentials of the Lactobacillus casei 431 and Lactobacillus acidophilus la-5 in fermented milk. CyTA - Journal of Food, 18(1), 291-300. https://doi.org/10.1080/19476337.2020.1741688
14. Dalgleish, D. (1998). Casein micelles as colloids: Surface structures and stabilities. Journal of Dairy Science, 81(11), 3013-3018. https://doi.org/10.3168/jds.s0022-0302(98)75865-5
15. Hao, P., Zheng, H., Yu, Y., Ding, G., Gu, W., Chen, S., Yu, Z., Ren, S., Oda, M., Konno, T., Wang, S., Li, X., Ji, Z., & Zhao, G. (2011). Complete sequencing and pan-genomic analysis of

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 UGC CARE Listed (Group -I) Journal Volume 11, Iss 4, 2022

lactobacillus delbrueckii subsp. bulgaricus reveal its genetic basis for industrial yogurt production. PLoS ONE, 6(1), e15964. https://doi.org/10.1371/journal.pone.0015964

16. Hoque, M.Z., Akter, F., Hossain, K.M., Rahman, M.S.M., Billah, M.M. and Islam, K.M.D. 2010. Isolation, identification and analysis of probiotic properties of lactobacillus spp. from selective regional yoghurts.World J. Dairy Food Sci. **5**, 39-46.

17. Hoque, M.Z., Akter, F., Hossain, K.M., Rahman, M.S.M., Billah, M.M. and Islam, K.M.D. 2010. Isolation, identification and analysis of probiotic properties of lactobacillus spp. from selective regional yoghurts.World J. Dairy Food Sci. **5**, 39-46.