Study of human allergic milk whey protein from different mammalian species using computational method

Shikha Jaiprakash Dixit¹, Appu Kuttan KK² & Kiran Singh¹*  

¹Department of Chemical Engineering and Biotechnology, Maulana Azad National Institute of Technology, Bhopal 462051, Madhya Pradesh, India; ²Maulana Azad National Institute of Technology, Bhopal; Kiran Singh – Email: Researchnm.kiran@gmail.com; *Corresponding author

Received October 15, 2012; Accepted October 17, 2012; Published October 31, 2012

Abstract: Nowadays, safety and quality assessment of food used for human consumption have to consider by its possible contribution to the maintenance or improvement of the consumer’s health. Milk is an important food with many nutrients. Cow milk is an important source of energy, protein, vitamins and minerals for the growing child as well as adults. But, numerous cow milk proteins have been implicated in allergic responses and most of these have been shown to contain multiple allergic epitopes. The present study disclosed best alternatives to cow milk, which are not allergic and as good as cow milk in nutritional value. The in silico analysis of casein (alpha s1, alpha s2, beta and kappa) and beta-lactoglobulin, unveils that sheep milk is a more suitable alternate to cow milk for allergic infants and buffalo milk for allergic adult humans.

Keywords: Casein, beta-lactoglobulin, Multiple Sequence Alignment, Neighbour joining method

Background: Milk is a white liquid produced by the mammary glands of mammals and is an important food with many nutrients. The cow (Bos taurus), domestic goat (Capra hircus), Domestic sheep (Ovis aries) and Water buffalo (Bubalus bubalis) plays important role as milk producers [1]. The best nutritional option for newborn infants is mother’s milk; however some infants may not be exclusively breast fed during the first month of life. In that case, another substitute must be provided for cow milk. This substitution results in an allergic disease known as cow milk protein allergy (CMPA) in 2-6% of children [2]. Nowadays, most common alternatives are soy and extensively hydrolyzed formulae. However, there is evidence that 10-20% of children allergic to cow milk do not tolerate soy derivatives [3-5], and some cases of high immunological reaction to extensively hydrolyzed formulae have been reported [6-8]. Allergies to cow milk are often broadly classified into immunoglobulin E (IgE)-mediated allergy and non-IgE-mediated allergy [9]. Cow’s milk contain more than 25 different proteins, but only the whey proteins alpha-lactalbumin, beta-lactoglobulin, BSA and lactoferrin as well as 4 caseins, have been identified as allergens [10]. The casein fraction is composed of alpha s1, alpha s2, beta and kappa-casein, of which alpha-s1 casein seems to be a major allergen according to IgE and T-cell recognition data [11-14].

In developed countries there is increasing interest in goat milk and its derivatives, the quality of which is considered of special importance in the light of current tendencies favoring healthy eating. In particular, the composition of goat milk is said to have certain advantages over that of cow milk, and thus former is preferable for some consumers [15]. In one case Scientific and clinical studies also suggest that infants and children who are sensitive to cow milk based product often thrive better when goat milk based product is substituted [16]. In order french extensive clinical studies with children allergic to cow milk, the treatment with goat milk produced positive results in 93% of the children and was recommended as a valuable aid in child nutrition because of less allergenicity and better digestibility than cow milk [17]. But in some cases goat milk also proved to be allergenic and intolerant for infants [18]. In some childrens...
and adult humans, water buffalo milk show more tolerance than cow milk. Repeat skin testing was performed at age 2 years of children and continued to be positive for cow milk but children found to be negative for skin prick tested to water buffalo milk based yogurt [19]. On these bases, selection of suitable milk for consumption is an important target for nutritionist and pediatricians. Therefore, the present study leads to evaluate the suitability of buffalo and sheep milk for nutrition to the people and children allergic to cow milk by study of amino acid composition, secondary structure, CLUSTALW2 analysis and phylogenetic relationship of Cow, Goat, Sheep and Buffalo milk whey proteins.

**Methodology:**

**Dataset**

Search for some milk whey protein was made in GenBank and EMBL databases. Obtained protein sequences filtration was done to remove any redundant sequences from the population and cross-checked by UniProtKB data base. The sequences are available at NCBI (http://www.ncbi.nlm.nih.gov/protein) with accession no. as given in **Table 1** (see supplementary material). The dataset consists of casein protein (α-s1, α-s2, β and κ) and β-lactoglobulin.

**Sequence analysis**

The ProtParam tool of ExPASy (http://www.expasy.ch/) was used to analyze amino acid and atomic composition [20]. Further isoelectric point, Extinction coefficient and hydropathicity was observed and compared between four species. Secondary structure predictions was done using SABLE (http://www.sable.cchmc.org) of obtained sequence in dataset.

**Multiple Sequence Alignment**

CLUSTALW2 at European Bioinformatics Institute (www.ebi.ac.uk/tools/msa/clustalw2/) was used for protein multiple sequence alignment (MSA) using standard parameters.

**Phylogeny tree construction**

The Phylip tool (http://bioweb.pasteur.fr/phylogeny/intro.en.html) was used to construct phylogenetic trees from aligned sequences. Computation of distance was done by program ProtDist, which constructs distance matrices by a process called “Bootstrapping”. A rooted tree was plotted using NJ plot software package [21] and distance matrix was calculated between each selected species [22].

**Discussion:**

**Sequence analysis**

Number of amino acid residue in selected milk whey protein in each species given in **Table 2** (see supplementary material). These proteins also share considerable similarity in their percentage content of amino acids as given in **Table 3-7** (see supplementary material). There was considerable similarity in predicted protein secondary structure of *Capra hircus* and *Ovis aries* as shown in **Figure 1** (A-T). At the same time there were some similarities as well as some differences in predicted protein secondary structure of *Bos Taurus* and *Bubalus bubalis* as shown in **Figure 1** (A-T).

![Figure 1: Predicted secondary structure in different species.](image-url)
Multiple sequence Alignment
The MSA of protein sequences obtained from CLUSTALW2 including the family of candidate protein sequences, show the maximum pairwise similarity in beta-casein group (95%) between goat and sheep. On the other hand minimum pair wise similarity shown in kappa casein (85%) between cow - goat and cow - sheep. The most common and generally more accessible approach to protein function prediction is ‘inheritance through homology’- that is, the knowledge that proteins with similar sequences frequently carry out same function [23]. For a majority of proteins it is already possible to predict their approximate function with resonable accuracy based on their evolutionary relationship or sequence similarity to proteins with known functions [24-26]. Since the whey protein from goat and sheep share a great similarity this supports functional similarity between goat and sheep milk. At the same time buffalo shows great similarity with cow at sequence level but some dissimilarities at secondary structure level makes it less allergenic. The reason behind this may be absence of allergenic epitopes but it should be analyzed further in vitro.

Phylogenetic tree construction
The result of phylogenetic tree analysis shown that for casein and beta-lactoglobulin each subgroup is divided in to two subgroups containing two species (Bos taurus, Bubalus bubalis) in one group and two species (Capra hircus, Ovis aries) in another group is shown in Figure 2 (A-E). Distance matrix for each protein groups in between four different species was calculated as given in Table 8-12 (see Supplementary material). Calculated distance matrix evaluates close relationship between goat-sheep and cow-buffalo.
Conclusion:
In case of cow milk allergic infants, since milk whey proteins of sheep and goat are almost identical, hence sheep milk is suitable and acceptable substitute of goat milk. In cow milk allergic childrens and adult humans buffalo milk is a good substitute of cow milk without any change in nutritional quality.

Acknowledgement:
I acknowledge Department of Chemical Engineering and Biotechnology, MANIT, Bhopal for support and encouragement.

References:
[1] Colin P & Groves, Bulletin of zoological nomenclatures. 1997 52: 137
[2] El-Agamy EL, Small Rum Res. 2007 68: 64
[3] Businco L et al. J Pediatr. 1992 121: S21 [PMID: 1447630]
[4] Maldonado J et al. Early Hum Dev. 1998 53: S23 [PMID: 10102652]
[5] Zeiger RS et al. J Pediatr. 1999 134: 614 [PMID: 1022898]
[6] Businco L et al. Ann Allergy. 1989 62: 333 [PMID: 2705659]
[7] Sampson HA et al. Pediatrics. 1992 90: 463 [PMID: 1518709]
[8] deBoissieu D & Dupont C, J Pediatr. 2002 141: 271 [PMID: 12183726]
[9] Hil DJ et al. Environ ToxicolPharmacol. 1997 4: 101 [PMID: 21781807]
[10] Wal JM, Allergy. 1998 53: 1013 [PMID: 9860234]
[11] Chatchatee P et al. J Allergy Clin Immunol. 2001 107: 379 [PMID: 11174208]
[12] Spuergin P et al. Allergy. 1996 51: 306 [PMID: 8836334]
[13] Ruiter B et al. ClinExp Allergy. 2006 36: 303 [PMID: 16499640]
[14] Ruiter B et al. Int Arch Allergy Immunol. 2007 143: 119 [PMID: 17228168]
[15] SanzSampelayo MR et al. Small Ruminant Research. 2002 43: 141
[16] Gupta K et al. BMC Bioinformatics. 2005 6: 105 [PMID: 15850477]
[17] Doerks T et al. Nucleic Acids Res. 2004 32: 6321 [PMID: 15576358]
[18] Haenlein GFW. Small Ruminant Research. 2004 51: 155
[19] William J & Sheikhhan, Ann Allergy Asthma Immunol. 2009 22: 121 [PMID: PMC3218082]
[20] Tariq ahmad Masoodi, Bioinformation. 2010 4: 430 [PMID: PMC2951635]
[21] Saitou N & Nei M, Mol Bio E. 1987 4: 406 [PMID: 3447015]
[22] Pareek & Arora, IJARPB. 2012 3: 326
[23] Lee D et al. Nat Rev Mol Cell Biol. 2007 8: 995 [PMID: 18037900]
[24] Whisstock JC & Lesk AM, Q Rev Biophys. 2003 36: 307 [PMID: 15029827]
[25] Dobson PD et al. Curr Med Chem. 2004 11: 2135 [PMID: 15279553]
[26] Restani P, Journal of Pediatric Gastroenterology and Nutrition. 2004 39: 323 [PMID: 15484147]

License statement: This is an open-access article, which permits unrestricted use, distribution, and reproduction in any medium, for non-commercial purposes, provided the original author and source are credited

Citation: Dixit et al, Bioinformation 8(21):1035-1041 (2012)
Supplementary material:

Table 1: Selected proteins for comparison analysis

| Milk whey protein | Bos Taurus | Capra hircus | Ovis aries | Bubalus bubalis |
|-------------------|------------|--------------|------------|----------------|
| α-s1 casein       | P02662     | P18626       | P04653     | O62823         |
| α-s2 casein       | P02663     | P33049       | P04654     | E9NZN2         |
| β casein          | P02666     | P33048       | P11839     | Q9TSI0         |
| K casein          | P02668     | P02670       | P02669     | P11840         |
| β-lactoglobulin   | P02754     | P02756       | P67976     | C3W955         |

Table 2: No of amino acid in selected proteins

| Milk whey protein | Bos Taurus | Capra hircus | Ovis aries | Bubalus bubalis |
|-------------------|------------|--------------|------------|----------------|
| α-s1 casein       | 214        | 214          | 214        | 214            |
| α-s2 casein       | 222        | 223          | 223        | 222            |
| β casein          | 224        | 222          | 222        | 224            |
| K casein          | 190        | 192          | 192        | 190            |
| β-lactoglobulin   | 178        | 180          | 180        | 180            |

Table 3 & 4: Amino acid composition of casein alpha s1 and s2 protein

| Alpha s1 | Bos Taurus | Capra hircus | Ovis aries | Bubalus bubalis |
|----------|------------|--------------|------------|----------------|
| Ala      | 5.0%       | 5.4%         | 5.4%       | 5.0%           |
| Arg      | 2.7%       | 3.1%         | 3.1%       | 2.3%           |
| Asn      | 6.3%       | 5.8%         | 5.4%       | 5.9%           |
| Asp      | 1.8%       | 2.2%         | 2.7%       | 2.3%           |
| Cys      | 1.4%       | 1.3%         | 1.3%       | 1.4%           |
| Glu      | 7.2%       | 13.2%        | 11.2%      | 10.4%          |
| Gly      | 0.9%       | 0.9%         | 0.9%       | 0.9%           |
| His      | 1.4%       | 2.2%         | 2.2%       | 2.3%           |
| Ile      | 5.4%       | 5.8%         | 5.4%       | 5.4%           |
| Leu      | 7.2%       | 5.8%         | 6.3%       | 6.8%           |
| Lys      | 11.3%      | 11.2%        | 11.2%      | 10.8%          |
| Met      | 2.3%       | 2.2%         | 2.2%       | 2.3%           |
| Phe      | 4.1%       | 4.5%         | 4.0%       | 4.1%           |
| Pro      | 4.5%       | 5.8%         | 5.4%       | 4.5%           |
| Ser      | 7.7%       | 6.3%         | 6.7%       | 7.7%           |
| Thr      | 7.2%       | 6.7%         | 6.7%       | 7.7%           |
| Tyr      | 5.4%       | 5.4%         | 5.4%       | 5.9%           |
| Val      | 6.8%       | 5.4%         | 5.8%       | 6.3%           |

Table 5 & 6: Amino acid composition of casein beta and kappa protein

| Beta-lactoglobulin | Bos Taurus | Capra hircus | Ovis aries | Bubalus bubalis |
|--------------------|------------|--------------|------------|----------------|
| Ala                | 10.7%      | 11.1%        | 11.1%      | 11.1%          |
| Arg                | 1.7%       | 1.7%         | 1.7%       | 1.7%           |
| Asn                | 2.8%       | 3.3%         | 3.9%       | 3.9%           |
| Asp                | 5.6%       | 4.4%         | 4.4%       | 5.6%           |
| Cys                | 3.9%       | 3.9%         | 3.9%       | 3.9%           |
| Glu                | 5.6%       | 5.6%         | 5.6%       | 5.6%           |
| Gly                | 9.0%       | 8.3%         | 8.3%       | 8.9%           |
| His                | 1.1%       | 1.1%         | 1.7%       | 1.1%           |
| Ile                | 5.6%       | 6.1%         | 5.6%       | 5.6%           |
| Leu                | 15.2%      | 15.0%        | 15.0%      | 15.0%          |
| Lys                | 9.0%       | 9.4%         | 8.9%       | 8.9%           |
| Met                | 2.8%       | 2.8%         | 2.8%       | 2.8%           |
Table 7: Amino acid composition of casein Beta-lactoglobulin protein

| Beta-actoglobulin          | Bos Taurus | Capra hircus | Ovis aries | Bubalus bubalis |
|----------------------------|------------|--------------|------------|-----------------|
| Ala                        | 10.7%      | 11.1%        | 11.1%      | 11.1%           |
| Arg                        | 1.7%       | 1.7%         | 1.7%       | 1.7%            |
| Asn                        | 2.8%       | 3.3%         | 3.9%       | 2.8%            |
| Asp                        | 5.6%       | 4.4%         | 4.4%       | 5.6%            |
| Cys                        | 3.9%       | 3.9%         | 3.9%       | 3.9%            |
| Glu                        | 9.0%       | 8.3%         | 8.3%       | 9.9%            |
| Gly                        | 2.8%       | 3.9%         | 3.9%       | 3.3%            |
| His                        | 1.1%       | 1.1%         | 1.7%       | 1.1%            |
| Ile                        | 5.6%       | 6.1%         | 5.6%       | 5.6%            |
| Leu                        | 15.2%      | 15.0%        | 15.0%      | 15.0%           |
| Lys                        | 9.0%       | 9.4%         | 8.9%       | 9.9%            |
| Met                        | 2.8%       | 2.8%         | 2.8%       | 2.8%            |
| Phe                        | 2.2%       | 2.2%         | 2.2%       | 2.2%            |
| Pro                        | 4.5%       | 4.4%         | 4.4%       | 4.4%            |
| Ser                        | 3.9%       | 3.3%         | 3.3%       | 3.9%            |
| Thr                        | 5.1%       | 4.4%         | 4.4%       | 4.4%            |
| Trp                        | 1.1%       | 1.1%         | 1.1%       | 1.1%            |
| Tyr                        | 2.2%       | 2.2%         | 1.7%       | 2.2%            |
| Val                        | 5.1%       | 5.6%         | 6.1%       | 5.6%            |

Table 8: Distance matrix of four species in casein alpha s1

| Casein alpha s1 | Bos Taurus | Capra hircus | Ovis aries | Bubalus bubalis |
|-----------------|------------|--------------|------------|-----------------|
| Bos Taurus      | 0          | 0.161        | 0.151      | 0.035           |
| Capra hircus    | 0.161      | 0            | 0.034      | 0.168           |
| Ovis aries      | 0.151      | 0.034        | 0          | 0.158           |
| Bubalus bubalis | 0.035      | 0.168        | 0.158      | 0               |

Table 9: Distance matrix of four species in casein alpha s2

| Casein alpha s2 | Bos Taurus | Capra hircus | Ovis aries | Bubalus bubalis |
|-----------------|------------|--------------|------------|-----------------|
| Bos Taurus      | 0          | 0.138        | 0.125      | 0.023           |
| Capra hircus    | 0.138      | 0            | 0.013      | 0.115           |
| Ovis aries      | 0.125      | 0.013        | 0          | 0.102           |
| Bubalus bubalis | 0.023      | 0.115        | 0.102      | 0               |

Table 10: Distance matrix of four species in casein Beta Casein Kappa

| Casein Beta     | Bos Taurus | Capra hircus | Ovis aries | Bubalus bubalis |
|-----------------|------------|--------------|------------|-----------------|
| Bos Taurus      | 0          | 0.103        | 0.089      | 0.014           |
| Capra hircus    | 0.103      | 0            | 0.014      | 0.089           |
| Ovis aries      | 0.089      | 0.014        | 0          | 0.075           |
| Bubalus bubalis | 0.014      | 0.089        | 0.075      | 0               |

Table 11: Distance matrix of four species in casein Kappa

| Casein Kappa    | Bos Taurus | Capra hircus | Ovis aries | Bubalus bubalis |
|-----------------|------------|--------------|------------|-----------------|
| Bos Taurus      | 0          | 0.174        | 0.138      | 0.081           |
| Capra hircus    | 0.174      | 0            | 0.036      | 0.165           |
| Ovis aries      | 0.138      | 0.036        | 0          | 0.129           |
| Bubalus bubalis | 0.081      | 0.165        | 0.129      | 0               |
Table 12: Distance matrix of four species in casein Casein Beta-lactoglobulin

| Casein Beta-lactoglobulin | Bos Taurus | Capra hircus | Ovis aries | Bubalus bubalis |
|---------------------------|------------|--------------|------------|----------------|
| **Bos Taurus**            | 0          | 0.070        | 0.093      | 0.032          |
| **Capra hircus**          | 0.070      | 0            | 0.023      | 0.038          |
| **Ovis aries**            | 0.093      | 0.023        | 0          | 0.061          |
| **Bubalus bubalis**       | 0.032      | 0.038        | 0.061      | 0              |