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Proteolysis of goat milk caseins with trypsin and antibacterial activity of the derived products

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Significance. Proteolytic cleavage of caseins with trypsin released various products, including peptides affecting cardiovascular system (antithrombotic and hypotensive agents), neural functions (antagonist and agonists of opioid receptors), digestive tract (casophosphopeptide, glucomacropeptide), immune status (modulators, antimicrobials). For instance, opioid peptides, like β -casomorphine-7, casoxyn d, casoxyn A interact with gastrointestinal receptors, influencing gut peristalsis; peptides displaying angiotensive action (α_{s1} -casein (f 23-34)), casokinines reduce blood pressure; peptides derived from β -casein (f 17-21) inhibit microbial growth; k-casein fragments block thrombocyte aggregation (f 106–112, 113–116) and fibrinogen binding with the thrombocyte surface (f 106–116); peptides stemming from β -casein (f 87-91) and k-casein (f 26-28) cause antidiabetic effect. Hydrolysates based on casein enhance production of antibodies, promote resistance to *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Candida albicans* cultures colonizing oral mucosa of healthy humans.

Peptides liberated from casein show antimicrobial activity against many gram-positive and gram-negative bacteria. For example, peptides from α -casein inhibit growth of pathogenic species *Bacillus cereus* and *Escherichia coli* and concurrently stimulate development of probiotic culture *Lactobacillus acidophilus*. A_{s2} -casein (f 183–207) also demonstrate antagonistic action towards pathogen *Cronobacter sakazakii*.

Commercial product BioPURE-GMP containing glycomacropeptide originating from k-casein possesses antimicrobial potential. Likewise the effective caries preventive formulas – Recaldent, CoC tooth mousse, M1 paste, Trident extra care incorporate phosphopeptides of casein hydrolysates.

Aim of study – production of bioactive peptides by casein proteolysis with trypsin; investigation of the peptide antibacterial activity.

Materials and methods. Enzymatic hydrolysis, electrophoresis in polyacrylamide gel, densitometry, ultrafiltration, freeze-drying, high performance liquid chromatography, plate technique to evaluate antibiotic resistance. Gram-positive bacterial cultures of genera *Amycolatopsis*, *Bacillus*, *Corynebacterium*, *Geobacillus*, *Micrococcus*, *Nocardia*, *Oerskovia*, *Sporosarcina*, *Streptomyces* and gram-negative bacteria of genera *Acinetobacter*, *Alcaligenes*, *Escherichia*, *Enterobacter*, *Enterococcus*, *Pseudomonas*, *Serratia* deposited at Belarusian collection of non-pathogenic microorganisms were used as model objects to test antibacterial activity of the peptides.

Results and conclusions. The conditions were optimized for enzymatic hydrolysis of caseins with trypsin, allowing to achieve the maximum yield of total peptide fraction within minimal time. Protein electrophoresis in polyacrylamide gel confirmed full completion of hydrolytic process by 30 min. The selected 46 bacterial test cultures were examined for sensitivity to hydrolysates derived from caseins. The resulting clarification zones served as indicators of the activity of the obtained peptide samples. It was found that different extent of biological activity was expressed by peptides with regard to 60 % of tested bacterial strains. The diameter of growth inhibition zone varied in the range 9–26 mm. Strains *Alcaligenes faecalis* BIM B–79 and *Bacillus mycoides* BIM B–179 were distinguished by the lowest value of growth suppression zone – 9–10 mm. The moderate sensitivity to peptide impact was recorded for strain *Escherichia coli* BIM B–399 – 15–16 mm. Strains *Bacillus sphaericus* BIM B–396 and *Geobacillus stearothermophilus* BIM B–415 exhibited the peak susceptibility, with growth inhibition zones reaching 24–26 mm in diameter. Further research will be focused on peptide fractionation, aiming to elaborate biopreparations to be applied in veterinary practice, food processing and cosmetic formulas.