Immunogen strain of *Chlamydophila psittaci* for biotechnology of human biopeptide vaccine production

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Introduction: Genus *Chlamydophila*, species: *Chlamydophila psittaci*.

Chlamydia psittaci, is pathogen obligate intocellular, small coccoid gram negative parasitic bacteria. Could be stained by Romanovski-Giemza: elementar bodies are purple color agains the background of the blue cytoplasm of the cell. Reticular bodies staining to blue colour.Staining by Stamp method includes fuxine of Pfeiffer fuchsine. Elementar bodies staining red, reticular bodies staining blue. Machiavello method of staining: carbolic basic fuchsine and methylene blue. Elementar bodies in red colour, reticular bodies staining blue. Main stage of life cycle of *Chlamydophila psittaci* are elementar bodyes small 0.2-0.4 micrometer in size, round or spherical shape. They are infective and insures survival in the extracellular environment and to infect new, heathy cells. Retucar bodyes a bit larger 0.8-1.5 micrometers in size spheric formations, they grow from elementary bodyes inside cells. They are devoid of infectivity and undergoing division ensure the reproduction of *Chlamydophila psittaci*. Intermediate stage between elementary and reticular

bodies. General protein of external membrane of *Chlamydophila psittaci* included in cell walls of reticular and elementar bodyes.

Sunstainable general, total immunity not formed. Only possibly for formation of local immunity for short time in the place of intracellular parasitic infection. Chlamydia has group and genus-spescific antigen, lipopolysaccharide complex (LPS) which is located on the inner membrane of *Chlamydophila psittaci*, elementar bodyes. LPS it is thermo-stable endotoxine. *Chlamydophila psittaci* also produce thermo-labile exotoxin which able to bocks macrophage degranulation after phagocytosis therefore phagocytosis can not be completed for the destruction of *Chlamydophila psittaci*. Life cycle of *Chlamydophila psittaci* includes 48-72 hours.

Elementar bodyes of *Chlamydophila psittaci* which did not appeared in the host cells are not sensitive to antibiotics. Reticular bodyes appeared into host cells and actively reproducing are sensitive to antibiotics. Infective beginning for humans are elementary bodies of *Chlamydophila psittaci*.

Material and Methods: Strain of *Chlamydophila psittaci* isolated in 1992 year from men who working in the bird farm and constantly contacted in ducks. *Chlamydophila psittaci* isolated from sampe of conjunctivitis of the eyes and deposited at Culture Collection USRCB – Ch009.

Cultivation of *Chlamydophila psittaci* included cultivation in cell culture of FLECH-13 received for research from Research Institiute of Grippe, St.Peterburg. Cell culture McCoy received for the research from Culture Collection of Research Institute Cytology, St.Petersburg.

For cultivation of cell culture used liquid media Eagles!s minimal essential meduum (MEM) with double amount of aminoacids and witamins. Bovine serum 10% for cell culture cultivation. Glutamine 300 mgs for 500 mls of liquid medium and gentamicine 10 mgs for 500 mls of liquid media. Before to start of cell cultivation added for ajusment pH 0,1 N HEPES.

To select of *Chlamydophila psittaci* strain for a mutation and receiving new properties such as immunogenity and non tocicity we used ultraviolet light. The

ultraviolet lamp DRT-240 produced in Lisma, Ukraine. The lamp is discharged, contain mercury inside, arc, tubular, high pressure lamp. Power – 240 Watt, lamp length – 180 mms, lamp buld diameter – 19 mms. The ultraviolet lamp irradiated to growing *Chlamydophila psittaci* in glass culture lask with volume 25 mls. Irradiation made from the distance between ultraviolet lamp and glass flask where growing *Chlamydophila psittaci* in cell culture for the 20 cms. Irradiation continued during 15 minutes.

Biopeptide N-Formil-L-Methionine, produced by Sigma Chemical Company (USA) used to increase of immunogenity at *Chlamydophila psittaci*. Laboratory mice 18 and laboratory rabbits 12 used to check titers of antibodies before and three weeks after vaccination.

Results and Discussion: Ultra-violet rays able to damage DNA in the big distance from the place of exposure and leads to mutations. Ultra-violet rays interfere connections to transport RNA interact with amino acids and interfere with the synthesis of new molecules. That is why to get new mutations we are using ultra-violet rays.

Received results after ultra-violet rays confirm us *Chlamydophila psittaci* loses infectivity during futher five passages in the cell cultures. It mean that toxicity of *Chlamydophila psittaci* strain almost is zero and could be used for vaccine preparation.

As a *Chlamydophila psittaci* are not very immunogen we decided to increase immunogenity adding biopeptide of aminoacid methyonine. For this purpose we processed *Chlamydophila psittaci* biomass with biopeptide N-Formil-L-Methionine, produced by Sigma Chemical Company (USA).

Testing in laboratory mice and laboratory rabbits we received high antigenic and immunogen effect. Strain not toxic and not reactogenic, harmless culure of *Chlamydophila psittaci* received after mutation from radiation of ultra-violet rays. This is very desirable effect of mutation of strain *Chlamydophila psittaci*.

Table 1

Indicator	Immunogenity (titer)	Toxicity	Reactogenity
Strain withot UV irradiation			
Laboratory mice	23±0.14	Toxic	Reactogen
Laboratory rabbits	28±0.21	Toxic	Reactogen
Strain after UV irradiation			
Laboratory mice	52±0.20	Not toxic	Not reactogen
Laboratory rabbits	64±0.24	Not toxic	Not reactogen

Laboratory mice and rabbits got intacutaneous injections twice with interval two weeks.

It checked titers of blood before injection of vaccine and three weeks after last injected of conjugated vaccine. See result in table 1. Results of table confirm that prepared conjugated vaccine is effective, not toxic, not reactogenic compare with native strain of *Chlamydophila psittaci* without ultra-violet rays.

Conjugated vaccine could be for further used for a people which are alredy infected and suffering with *Chlamydiosis*. Body needs in this case to be desensitized to Chlamydial antigen. For this purposes will be great to use conjugated Chlamydial vaccine in amount which will be able to neutralize Chlamydial infection.

Conclusions: It was isolated strain of *Chlamydophila psittaci* perspective for immunogen vaccine preparation.

To improve properties of strain it irradiated by ultra-violet rays to get necessary mutations.

To increase immunogenity of strain it was conjugated *Chlamydophila psittaci* strain.

Conjugated vaccine checked after vaccination of titres antobodies to laboratory mice and laboratory rabbits. It confirmed efficiency of conjugated vaccine.

Further research needed to use this vaccine for desenstisation of Chlamydia antigene during treatment infected body.

Keywords: Desensitization, biopeptide, ultra-violet rays, *Chlamydophila psittaci*.