A. Zholdasbekova et al /J. Pharm. Sci. & Res. Vol. 10(1), 2018, 162-163

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Method for producing attenuated Salmonella strain

A. Zholdasbekova, K.B. Biyashev, B.K. Biyashev, D.A. Sarybaeva, K.T. Zhumanov
Kazakh National Agrarian University, Republic of Kazakhstan, 050010, Almaty, Abay ave., 8

Abstract.
Successes of bacterial genetics and molecular biology have recently made it possible to design safe, immunogenic, effective, labeled vaccine strains.
Keywords: salmonella**,** attenuated strains, live vaccines, reversion.

INTRODUCTION.

The problem of salmonellosis in animals is becoming increasingly important. This is caused by a wide circulation of numerous serovars of salmonella in nature, polyfactor determination of the virulence factors of pathogens, and variety of routes of administration into the body of animals and humans. Damage caused by this disease includes not only death of the animal. Recovered animals also become bacterial carriers for a long period of time as well as permanent sources of contamination of the environment. Products of animal origin (meat, milk, eggs) obtained from salmonella carriers in case of insufficient thermal treatment can cause foodborne toxic infections in humans. Thus, the fight against foodborne diseases is a very urgent daily task for veterinarians and healthcare providers [1,2].

The issues of prevention of salmonellosis most often come down to vaccination as the most traditional and universal method.

In case of salmonellosis in animals, various variants of dead vaccines have been studied. However, practice has shown that dead vaccines don't create a sufficiently intense immunity in young animals, and most researchers [3,4,5] point to the potential and advantage of live vaccines for the prevention of salmonella infections in animals.

Live vaccines are biological preparations from genetically altered forms (mutants) of pathogens of infectious diseases suspended or dried in appropriate protective media. From genetic point of view mutants allow us to define them as forms that underwent genotypic changes as a result of which they lost the ability to induce in the susceptible organism irretrievable pathological changes that previously had caused the disease. At the same time they retained in their genetic constitution determinants on their ability to cause specific immunological changes and rearrangements. In accordance with the transformed genome, these mutants have also changed their phenotypic areas.

MATERIALS AND METHODS

The attenuated Salmonelladublin 31 strain obtained in the laboratory of antibacterial biotechnology at the Kazakh National Agrarian University was used in the work. Salmonelladublin 31 strain was deposited in the Collection of Microorganisms of the Republican State Enterprise "Research Institute for Biological Safety Problems" of the Ministry of Education and Science of the Republic of Kazakhstan (RSE RIBSP CM MES RK).

RESULTS

The purpose of our study was to obtain a strain of Salmonella that had stable biological properties, moderate reactogenicity and residual virulence, harmlessness, high immunogenicity, epizootical safety and genetic markers to distinguish it from naturally occurring strain.

Salmonella dublin 31 strain was obtained by selection of the original virulent strain Salmonelladublin 76. An original Salmonelladublin 76 strain virulent for mice (LD_{50} = 100 bacteria) was inoculated on plates of Hottinger agar in concentration of 10^{10} CFU (colony-forming unit). Agar plates contained Nut mutagen in concentration of 100 µg/mL, and Rif mutagen in concentration of 50 µg/mL. Among the grown clones, we selected mutants which acquired resistance to Nal in concentration of 400-500 µg/mL, and Rif in concentration of 100-200 µg/mL. The obtained mutants with the desired phenotype were inoculated 3 times on a selective medium with mutagens.

Virulence of the isolated clones was studied in experiments on mice following their intraperitoneal infection. Nal^R 500 and Rif^R 100 mutants were characterized by decrease in virulence on 6-7 orders as opposed to Nal-resistant mutants that were also resistant to high concentrations of Rif (Nal^R 700 and Rif^R 200 phenotype). An attenuated clone No. 31 (LD_{50} = 10^7 bacteria) was selected, which was a donor strain in transduction experiments involving P22 bacteriophage. It was shown that transmission of each of the mutations which determined mutant phenotype No. 31 to the initial virulent strain No. 76 led to decrease in its virulence. Salmonelladublin 31 strain was selected among transductants that simultaneously acquired mutations that caused resistance to Nal^R and Rif^R.

Salmonelladublin 31 strain is characterized by the following features.

Morphological signs.

Cells of the strain are straight, rod-shaped (1.5-1.7) * (2.4-5.6) µm, mobile, gram-negative, do not form spores.

Cultural properties.

Bacteria of the strain when growing on Hottinger agar, Difco nutrient broth form smooth, round, shiny, semi-translucent colonies of gray color with a flat edge of 2 mm in 24 hours, in Endo medium they form round colorless semi-translucent colonies with a flat edge of 2 mm in 24 hours. When cultivated in liquid media (Difco broth), bacterial strains form homogeneous turbidity in 18 hours.

Physiological and biochemical signs.

The range of growth temperatures is 37-39 °C; an optimal temperature is 37 °C. An optimal pH is 6.8-7.5. Glucose, maltose, galactose, mannitol, sorbitol are used as a source of carbon. Lysine has ornithine decarboxylase activity, it does not have urease activity, does not form hydrogen sulphide and indole.

Antigenic structure.

Typical for salmonella of birds 0-1,9,12; H-g, p.
Sensitive to bacteriophage P22.

Resistance to mutagens.

It is resistant to Nal^R in concentration of 100 µg/mL, and Rif^R in concentration of 100 µg/mL.

Residual virulence.

In case of intraperitoneal infection of white mongrel mice $LgLD_{50}$ = 7.0±0.3 (according to the method of Reed and Mench with the mean error calculated by Pizzi formula).

Stability of the residual virulence.

10x passages of S.dublin 31 strain through the body of white mice reveals preservation of the initial level of residual virulence and resistance stability of Nal^R and Rif^R markers. All subcultures isolated from the mice organism after each passage have

approximately the same indices of residual virulence $LgLD_{50}$ = 0.7 ± 0.3.

Stability of attenuation and resistance markers to Nal^R and Rif^R in the strain S.dublin 31 was determined by the treatment of the strain with nitrosoguanidine mutagen. The study of these properties in 10 clones of the selected strain after such treatment revealed their retention at the same level as in the untreated culture ($LgLD_{50}$ -7.0 ± 0.3; minimal suppressive concentration is Nal^R 100 μg/mL and Rif^R 100 μg/mL).

Immunogenicity for white mongrel mice.

The strain has a significant immunogenicity for mice protecting 100% of the animals on the background of immunization with a dose of 10^5 CFU, 95% of white mice – on immunization with a dose of 10^4 CFU.

Genetic analysis of the safety of the strain as a live vaccine.

Genetic study of Salmonelladublin 31 strain conducted using transducing phage P22 revealed that the phenotype was determined by the presence of two independent mutations - in the Rif^R gene and in a gene that determined Nal^R resistance. It had been established that mutations controlling the resistance to Rif^R and Nal^R led to the damage of ribosomal proteins (S_{13}, S_{16}) and thereby influenced the correctness of reading of genetic information. As a result, we can see a decrease in the virulent properties, i.e. attenuation. Thus, attenuation is associated with violation of the translation of genes encoding the synthesis of important pathogenicity factors of the bacterium or genes, the products of which are important in life of the bacterium.

The study of the virulence of the resulting transductants in case of intraperitoneal infection of white mice revealed a decrease in its clones. There were 2 mutations causing resistance to Nal^R and Rif^R, otherwise each mutation could be transduced separately. The presence of two mutations in S. dublin 31 strain, each of which can reduce virulent properties, is a convincing proof of the stability and safety of Salmonelladublin 31 vaccine strain.

In addition, we transduced third mutation to S.dublin 31 strain, which reported high sensitivity to surface active substances, including dodecyl sulfate and sodium deoxycholate. This mutation, designated as Hst (highsensibility), does not affect attenuation and immunogenicity of the strain, but limits the time of bacterial survival in the host's intestine and in the environment. Potential vaccine strains with Hst mutation are not able to stay in the environment for a long time, and therefore they can be considered as "environmentally friendly" live vaccines characterized by a limited ability to form an infectious chain (i.e. incapable of epidemic or epizootic distribution).

Differentiation of the vaccine strain from cultures of natural origin.

Salmonelladublin 31 strain is differentiated from naturally occurring cultures due to resistance to Nal^R , Rif^R and high sensitivity to surface active substances. The presence of genetic markers to three mutagens allows to differentiate vaccine strains from the field ones for 16-20 hours in lab conditions by using simple nutrient media in case of suspected salmonellosis or when salmonella strains are isolated in products of animal origin.

Thus, Salmonelladublin 31 strain meets all the requirements for vaccine strains: it has stable biological properties, moderate reactogenicity and residual virulence, high immunogenicity for mice and chickens, is epizootically safe for use, and has three genetic markers to distinguish it from a naturally occurring strain. The presence in Salmonelladublin 31 strain of three mutations with known mechanisms of action serves as convincing genetic evidence of the stability and safety of attenuated Salmonelladublin 31 strain. Theoretical frequency of reverse mutation for all markers at the same time is approximately 10^{-21}, which is virtually impossible.

CONCLUSION

The purpose of Salmonelladublin 31 strain is to be used for the production of vaccines against salmonellosis in birds, cattle, sheep, pigs and birds.

Salmonelladublin 31 strain was deposited in the Collection of Microorganisms of the Republican State Enterprise "Scientific Research Institute for Biological Safety" of the Ministry of Education and Science of the Republic of Kazakhstan (RSE RIBSP CM MES RK) - collection number M-42-15/D. The patent "Salmonelladublin 31 strain used for the production of live vaccine against bovine salmonella" No. 32022 dated March 31, 2017 was obtained for the current strain.

References

1. Mayer M., Gessler K. and Weiss H, E. Infection chains and infection cycles of salmonella in the broiler and turkey production.Tierarztl.Umsch.,1984; 39(7): 538 – 548.
2. Biyashev K.B. et al. Salmonellezy zhivotnykh i mery borby [Salmonellosis of animals and treatment measures]. Alma-Ata: Kainar, 1991, pp. 42.
3. WHO. Fighting salmonellosis: the role of veterinary medicine and food hygiene. Report of the WHO Expert Committee. Geneva: WHO, 1991, pp. 83.
4. Linde K., Beer J. and Randhagen B. Patent of the Russian Federation No. 2177804 C2. Live salmonella vaccine. Published on 10/01/2002
5. Charles D.Ya., Chetfild N.S. and Fairventer F.N. Sposob polucheniia attenuirovannogo shtamma bakterii Salmonella i vaktsyna [The method for producing an attenuated strain of Salmonella bacteria and vaccine]. Patent the Russian Federation No. 2126447 C1. Published on February 20, 2002.