

Status of Biotechnical Faculty, University of Ljubljana: Coordinator

Title of the project: **The development of enzyme formulation for disinfecting prion-contaminated areas.**

Coordinator: Prof.dr. Nataša Poklar Ulrich

ARRS code: L7-8277 (C)

General information on financing

Duration: 1.5.2017 - 30.04.2019

Range of financing: 1,58 FTE

Participating research organizations

ARRS code	Research organization	Status
0481	Biotechnical Faculty University of Ljubljana	Faculty (coordinator)
0406	Veterinary Faculty, University of Ljubljana	Faculty
0787	Center of Excellence for Integrall Approaches in Chemistry and Biology of Proteins	Private research institution
/	Borer Chemie AG Borer	Private company

Membership of the project team

Name	ARRS code	Research area	Position
Avbelj Martina	28299	Biotechnology / Microbe biotechnology	Researcher
Bahun Miha	39095	Biochemistry and molecular biology	Junior researcher
Čadež Neža	18332	Biotechnology / Microbe biotechnology	Researcher
Juntes Polona	04630	Veterinarian medicine / Animal pathology and epizootiology	Researcher
Karničar Katarina	32503	Biotechnology / Microbe biotechnology	Researcher
Lindner Vilma	50382		Technical associate
Mubi Irena	50383		Technical associate
Osojnik Črnivec Ilja Gasan	29336	Chemistry / Physical chemistry	Researcher
Paš Maja	15618	Biotechnology / Microbe biotechnology	Researcher
Petković Hrvoje	13542	Biotechnology / Recombinant DNA technology	Researcher
Poklar Ulrich Nataša	10873	Chemistry / Physical chemistry	Head
Prislan Iztok	23417	Chemistry / Physical chemistry	Researcher
Ropret Sandra	35360	Biotechnology / Microbe biotechnology	Researcher
Šnajder Marko	30763	Biochemistry and molecular biology	Researcher
Taler - Verčič Ajda	29544	Biochemistry and molecular biology	Researcher
Turk Dušan	04988	Biochemistry and molecular biology	Researcher
Zabavnik Piano Jelka	08506	Veterinarian medicine / Morphology, physiology and animal reproduction	Researcher

## Abstract

Transmissible spongiform encephalopathy (TSE) forms a family of neurodegenerative diseases of animal and humans. The emergence of a variant form of human Creutzfeldt-Jakob disease (CJD), as a result of the bovine spongiform encephalopathy (BSE) epidemic in the United Kingdom, has increased the profile of TSE as they have become a serious risk to public health and health policy worldwide. Furthermore, standard sterilisation methods do not eliminate the risk of TSEs, and although there are physical and chemical disinfection methods that can greatly reduce TSE infectivity, many of these are not appropriate for sensitive medical equipment, and can be harmful for medicine personnel.

There are a few alkaline and enzymatic detergents that can reduce the prion infectivity, and the optimal practice proposed includes an enzyme detergent-like formulation that can be used across a broad range of temperatures and pH conditions. In 2011, we demonstrated *in-vitro* enzymatic degradation of protein aggregates by pernisine, including for infective prions (PrP<sup>Sc</sup>) from different origins (i.e., mouse, bovine, deer, human) *in vitro*. Pernisine is a thermostable serine protease from *Aeropyrum pernix* that has a potential usage in various industries, from the cleaning industry to medical fields, where medium to high temperatures or harsh conditions are encountered (e.g., with denaturants, reductants, detergents).

Heterologous expression *Streptomyces* spp. as heterologous expression are well known to secrete such products into the medium. The recent development of rapid and easy transformation methods, good fermentation properties, and robust protein export for secretion makes *Streptomyces* spp. a more attractive tool for heterologous expression of proteins. In 2014, we have described and protected method for overexpression of pernisine in heterologous bacterial host (Šnajder, Mihelič et al. 2015).

Despite the decline of in classical BSE cases, prions still represent a serious health problem. In the recent years several articles and patents have been published that have been described the proteolytic degradation of TSE prions. There thus remains a pressing need for new, efficient, environmentally friendly ways of inactivation, elimination and/or degradation of prion-infected medical waste and surfaces. Recommended chemical decontamination treatments for infectious prions have been, despite being effective, not used due to medical equipment damage.

The main aim is to develop enzyme preparations that will be as effective as decontaminants of PrP<sup>Sc</sup> (i.e., to reduce prion infectivity by >4.5 log units) for contaminated surfaces and surgical equipment. To incorporate this main aim, we can divide the project into the following four objectives:

**(O1)** Construction of improved *Streptomyces* sp. strain using gene engineering, random mutagenesis and process development to increase further the yield of recombinant pernisine production by applying a novel expression system.

**(O2)** Biophysical and biochemical characterisation of recombinant pernisine in solution in the presence of different solution conditions used for the final formulation of any effective products.

**(O3)** New product formulation for decontamination of PrP<sup>Sc</sup> or other deposited protein decontamination. The versatility of pernisine to degrade different substrates will be exploited. Novel formulations will decrease the risk of TSE transmission.

**(O4)** Design of a platform for implementation of other industrially important proteins. Using the same methods will allow other biotechnological perspective enzymes to become useful for industry.

## Phase of the project and its realization

We are concluding the third phase of the project and we are entering the last one.

## Bibliographical references

We are finishing article and will be ready to submit till the end of the year. Within the framework of the project, we presented the relevant findings of research at the following conferences:

BAHUN, Miha, ŠNAJDER, Marko, HARTMAN, Kevin, HABIČ, Anamarija, POKLAR ULRIH, Nataša. Calcium induced folding of pernisine, a thermostable serine protease from extremophilic archaeon *Aeropyrum pernix*. V: SZÜTS, Dávis (ur.), BUDAY, László (ur.). *FEBS3+ Conference : [from molecules to living systems] : final programme & book of abstracts : 2-5 September 2018, Siófok, Hungary*. Veszprém: OOK-Press. 2018, str. 125, P3-04. [COBISS.SI-ID [4944248](#)]

ŠNAJDER, Marko, BAHUN, Miha, KRANJC, Luka, PETKOVIĆ, Hrvoje, JUNTES, Polona, POKLAR ULRIH, Nataša. Production and application of thermostable protease pernisine from *Aeropyrum pernix* K1. V: SZÜTS, Dávis (ur.), BUDAY, László (ur.). *FEBS3+ Conference : [from molecules to living systems] : final programme & book of abstracts : 2-5 September 2018, Siófok, Hungary*. Veszprém: OOK-Press. 2018, str. 209, P9-07. [COBISS.SI-ID [4943992](#)]

JUNTES, Polona, ŠNAJDER, Marko, BAHUN, Miha, POKLAR ULRIH, Nataša. Enzymatic degradation of bovine cellular and pathological form of prion proteins and potential use for decontamination. V: *Joint ESVP and ECVP Congress, September 5th - 8th 2018, Cluj-Napoca, Romania*, ESVP and ECVP congress, September 5th-8th 2018, Cluj. Cluj: University of Agricultural sciences and veterinary medicine. 2018, str. 229. <https://www.ecvpath.org/joint-esvp-and-ecvp-congress-2018-final-program-abstract-book/>. [COBISS.SI-ID [4664698](#)]

ŠNAJDER, Marko, BAHUN, Miha, KRANJC, Luka, PETKOVIĆ, Hrvoje, POKLAR ULRIH, Nataša. Construction and production of active pernisine in *Streptomyces rimosus*. V: GORIČAR, Katja (ur.), HUDLER, Petra (ur.). *Book of abstracts = Zbornik povzetkov, 12th Meeting of the Slovenian Biochemical Society with International Participation = 12. srečanje Slovenskega biokemijskega društva z mednarodno udeležbo, Bled 20 - 23 september 2017*. Ljubljana: Slovenian Biochemical Society. 2017, str. 98, PI-24. [COBISS.SI-ID [4817272](#)]

CARRILLO RINCON, Andres Felipe, MAGDEVSKA, Vasilka, KRANJC, Luka, ŠNAJDER, Marko, BAHUN, Miha, PAŠ, Maja, PETKOVIĆ, Hrvoje, POKLAR ULRIH, Nataša. Heterologna produkcija aktivnega pernizina v bakteriji *Streptomyces rimosus*. V: VODOVNIK, Maša (ur.), KUŠAR, Darja (ur.), MARINŠEK-LOGAR, Romana (ur.). *Knjiga povzetkov, 7. kongres Slovenskega mikrobiološkega društva, 20.-22. september 2017, Bled, Slovenija*. Ljubljana: Slovensko mikrobiološko društvo. 2017, str. 127, Po54. [COBISS.SI-ID [4829816](#)]