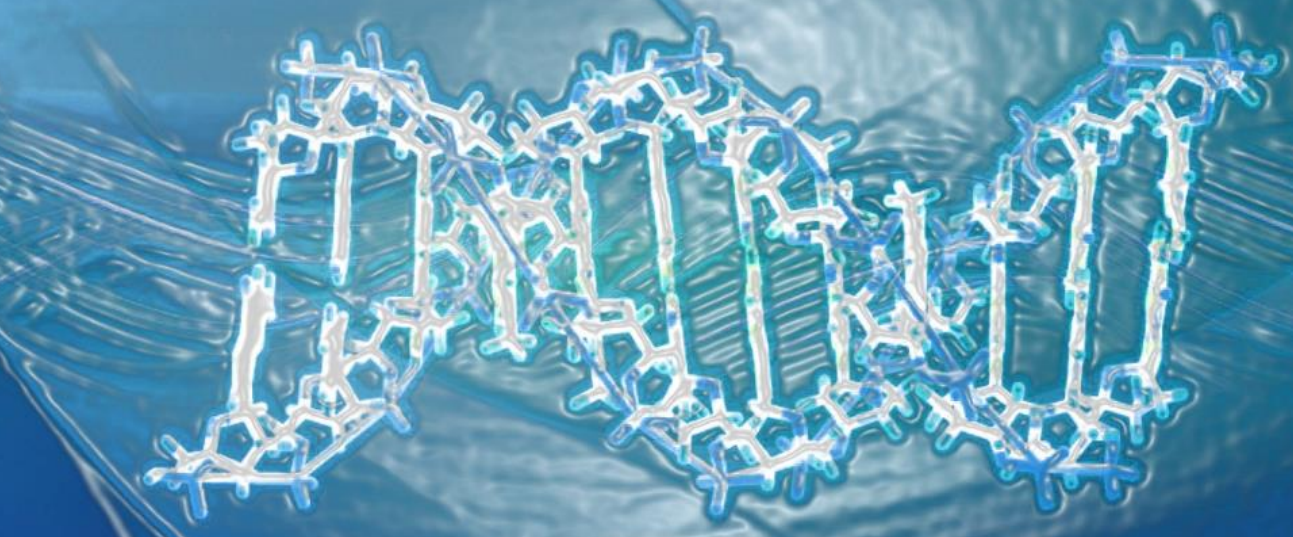
GENETIC SOCIETY SLOVENIA
in collaboration with
SLOVENIAN SOCIETY OF HUMAN GENETICS

5th COLLOQUIUM OF GENETICS

Proceedings



PIRAN

September 23th, 2016

GENETIC SOCIETY OF SLOVENIA

IN COLLABORATION WITH

THE SLOVENIAN SOCIETY OF HUMAN GENETICS

5th COLLOQUIUM OF GENETICS

Proceedings

Marine Biology Station Piran
National Institute of Biology
Piran
September 23rd 2016

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Genetic Society Slovenia in collaboration with
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OPENING LECTURE:	9.10 – 10.00
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Branka Javornik: Studies of hop-Verticillium pathosystem	9.10 – 9.35
Marjanca Starčič Erjavec: Genetic diversity of *Escherichia coli* from gut microbiota	9.35 – 10.00
Biotechnology	**10.00 – 11.30**
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Špela Kos: Gene electrotransfer for DNA vaccination	10.00 – 10.15
Helena Volk: Production of recombinant effector proteins from *Verticillium nonalfalfae*	10.15 – 10.30
Mojca Juteršek: Towards molecular genetics deliniation of *Synechocystis* species	10.30 – 10.45
Ester Stajič: Development of cabbage haploid inducer line with genome editing	10.45 – 11.00
Katja Guček: CRISPR/Cas9 genome editing in rapeseed	11.00 – 11.15
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Urša Lampreht Tratar: Gene electrotransfer of antibiotic-free IL-12 plasmid induces antitumor effect in B16F10 melanomas	12.05 – 12.20
Katja Uršič: Peritumoral gene electrotransfer of IL-12 as adjuvant immunotherapy to intratumoral electrochemotherapy with cisplatin for treatment of murine B16F10 melanoma	12.20 – 12.35
Katarina Žnidar: The effect of gene electrotransfer of blank pDNA on tumor cells' survival and expression of cytosolic DNA sensors	12.35 – 12.50
Vasja Progar: Functional enrichment of differentially expressed genes in hop infected by *V. nonalfalfae*	12.50 – 13.05
Kristina Marton: *Verticillium nonalfalfae* and its candidate secreted effector proteins	13.05 – 13.20
Marija Rogar: Polymorphisms in segregation genes involved in the development of gastric cancer in Slovenian population	13.20 – 13.35
Alja Zottel: Glioblastoma multiforme and nanobodies	13.35 – 13.50
Lunch (self-service bar), poster viewing	**13.50 – 15.00**
Meeting of the Genetic Society Slovenia with announcement of the best lecture and the best poster awards	**15.00 – 16.00**

LECTURES

OPENING LECTURE

STUDIES OF HOP-*VERTICILLIUM* PATHOSYSTEM

Branka Javornik

University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

ABSTRACT

The studies address *Verticillium* resistance in plants, working on the hop-*Verticillium* nonalfalfea (Vna) pathosystem. This work includes two broad aims.

First, to improve hop resistance to Verticillium wilt. This work includes mapping of gene(s) or QTLs for *Verticillium* resistance and the development of molecular markers linked to resistance for application in hop breeding.

The second aim is to improve the management of wilt disease based on an understanding of the mechanisms of host- pathogen interaction and the pathogenicity of Vna. This work has focused on studies of the hop-Vna interaction on proteome and transcriptome levels to search for hop factors implicated in resistance and Vna factors related to virulence. On the pathogen side, Vna has been being further studied by comparison of proteomes and whole genome sequences of Vna isolates with different virulence, and functional analysis of predicted candidate genes.

OPENING LECTURE

GENETIC DIVERSITY OF *Escherichia coli* FROM GUT MICROBIOTA

Marjanca Starčič Erjavec

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ABSTRACT

Mammals have a complex gut microbiota shaped by intestinal anatomy, function and diet[1]. *Escherichia coli* (*E. coli*) is one of the first colonizers of the mammalian gut and afterwards part of the normal gut microbiota. It usually coexists with its host in mutual benefit. Nevertheless, it is known that the intestinal microbiota can also be a reservoir of extraintestinal pathogenic variants of *E. coli* (ExPEC) that can cause disease infections at different extraintestinal anatomic sites (e. g. urinary tract, skin and soft-tissue infections)[2]. Further, it is known that animals (e. g. cats, dogs and birds) can be a potential reservoir of ExPEC[3]. In last years our studies have been focused on the virulence potential for ExPEC among *E. coli* from gut microbiota from different hosts. The lecture will present an overview of the published[4, 5] and recently obtained data.

REFERENCES

1. Stevens CE, Hume ID. The mammalian gastrointestinal tract. In: Comparative physiology of the vertebrate digestive system. 2nd ed. Cambridge University Press, Cambridge, **2004**, 46-92.
2. Leimbach A, Hacker J, Dobrindt U. *E. coli* as an all-rounder: the thin line between commensalism and pathogenicity. *Curr. Top. Microbiol. Immunol.* **2013**, 358, 3-32.
3.Bélanger L, Garenaux A, Harel J, Boulianne M, Nadeau E, Dozois CM. *Escherichia coli* from animal reservoirs as a potential source of human extraintestinal pathogenic *E. coli*. *FEMS Immunol. Med. Microbiol.* **2011**, 62, 1-10.
4. Starčič Erjavec M, Žgur-Bertok D. Virulence potential for extraintestinal infections among commensal *Escherichia* coli isolated from healthy humans – the Trojan horse whitin our gut. *FEMS Microbiol. Lett*. **2015**, 362, 1-9.
5. Vadnov M, Barbič D., Žgur-Bertok D, Starčič Erjavec M. *Escherichia coli* isolated from feces of brown bears (*Ursus arctos*) have a lower prevalence of human extraintestinal pathogenic *E. coli* virulence-associated genes. *Can. J. Vet. Res.* **2016**, In press.

ABSTRACTS and PAPERS of LECTURES

BIOTECHNOLOGY

Špela Kos

Gene electrotransfer for DNA vaccination

Helena Volk

Production of recombinant effector proteins from *Verticillium nonalfalfae*

Mojca Juteršek

Towards molecular genetics deliniation of *Synechocystis* species

Ester Stajič

Development of cabbage haploid inducer line with genome editing

Katja Guček

CRISPR/Cas9 genome editing in rapeseed

Sabina Belc

Distribution analysis and production of fungal actinoporin- and perforin-like proteins

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Abstract

GENE ELECTROTRANSFER FOR DNA VACCINATION

Spela Kos[1], Kevin Vanvarenberg[2], Tanja Dolinsek[1], Maja Cemazar[1, 3], Jure Jelenc[4], Véronique Préat[2], Gregor Sersa[1], Gaëlle Vandermeulen[2]

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ABSTRACT

A number of notable technology advances in DNA vaccination over the past few years have led to the resurgence of this field as a promising treatment modality. Among these advancements are improved physical methods of naked DNA delivery to cells. Of these, gene electrotransfer is considered as a safe and efficient method to deliver the plasmids to target cells or tissues[1]. Among the potential targets, skin is an easy accessible and immunocompetent tissue, which makes it an attractive target for gene electrotransfer[2]. Our study focused on gene electrotransfer of model DNA vaccine coding for the ovalbumin (OVA) protein, delivered into the mouse skin. For this purposes the non-invasive multi-electrode array (MEA) was used[3]. The efficiency of the gene expression and the activation of immune response against delivered antigen were followed. The results demonstrated strong gene expression and an efficient delivery of DNA vaccine. The use of MEA to deliver the ovalbumin plasmid generated a strong immune response, as evidenced by the presence of antibodies in the serum, the IFN-gamma response and the delayed tumor growth when the mice were challenged with B16-OVA cells. The described method of gene electrotransfer by MEA electrode to skin proved as a promising approach to deliver the plasmids coding for the therapeutic molecules. It sets the stage for the further development of electroporation mediated delivery of DNA vaccines against infectious agents, autoimmunity or for cancer therapy.

REFERENCES

1. Lambricht L, Lopes A, Kos S, Sersa G, Preat V, Vandermeulen G. Clinical potential of electroporation for gene therapy and DNA vaccine delivery. *Expert Opinion Drug Deliv*. **2015**, 13, 295-310.
2. Gothelf A, Gehl J. Gene electrotransfer to skin; review of existing literature and clinical perspectives. Curr Gene Ther. **2010**, 10, 287-299.
3. Kos S, Blagus T, Cemazar M, Jelenc J, Sersa G. Utilization of multi-array electrodes for delivery of drugs and genes in the mouse skin. *IFMBE proceedings*. **2015**, 53, 321-324.

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Abstract

PRODUCTION OF RECOMBINANT EFFECTOR PROTEINS FROM *Verticillium nonalfalfae*

Helena Volk [1], Marko Dolinar [2], Branka Javornik [1], Sabina Berne [1]

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[2]Department of Chemistry and Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

ABSTRACT

Verticillium nonalfalfae (*Vna*) is a phytopathogenic fungus causing Verticillium wilt on various plants, including hop (*Humulus lupulus* L.). The fungus enters the plant through the roots and continues to spread into the stem of the plant. To colonise the host plant successfully, *Vna* secrets a repertoire of effector proteins that protect the fungus from the plant immune system or modulate the host plant physiology.

Unravelling how effectors function in the host organism leads to better understanding of the plant-pathogen interactions and may facilitate the discovery of host plant proteins involved in resistance to the pathogen. Recombinant proteins expressed in *Escherichia coli* and *Pichia pastoris* have been used for biochemical characterisation and functional analysis of effectors.

The current study focuses on two effector proteins, *Vna*SSP4.2 and *Vna*CBP8.213, that are highly expressed during infection of hop. The two effectors without signal peptide were cloned to pET32a expression vector and produced in *E. coli*. Various parameters were tested to obtain a high amount of soluble recombinant proteins: bacterial strains Shuffle (optimized for the formation and maintenance of disulphide bonds) and BL21(DE3)pLysS (drives protein production in a reducing environment), optimal expression temperature and concentration of IPTG inducer. Protein expression was examined by Western blot analysis, and the optimal parameters for purification of recombinant protein using Ni-NTA affinity chromatography on FPLC system were chosen.

Our results confirm that the parameters for efficacious recombinant protein production are protein to protein dependent, with bacterial strain and temperature during expression being the most significant factors.

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Article

TOWARDS MOLECULAR GENETIC DELINIATION OF *Synechocystis* SPECIES

Mojca Juteršek, Marina Klemenčič, Marko Dolinar

Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Identification and classification of cyanobacteria have been traditionally based on their morphological features, which often lead to misidentifications and false classifications. To overcome variable morphological criteria, DNA sequencing is becoming the most widely applied molecular method in the identification and cataloguing of cyanobacteria. We focused on the unicellular genus *Synechocystis* that includes the model cyanobacterium *Synechocystis* sp. PCC 6803, broadly used in biotechnology and synthetic biology. Little is known about the distribution of this strain and of its relatives in the environment, which might be important for biosafety assessments in biotechnology and synthetic biology. Two genomic regions, the variable part of the 16S rRNA coding region and the 16S – 23S internal transcribed spacer (ITS), were amplified by PCR from 11 *Synechocystis* members, cloned and sequenced. Comparison of our sequencing data with previously published sequences indicates that the current *Synechocystis* genus is highly heterogeneous, but that the ITS region alone reflects the phylogenetic positioning of strains very well.

INTRODUCTION

Cyanobacteria represent an evolutionary and ecologically important group of photosynthetic microorganisms. Furthermore they are also becoming increasingly studied for their use in biotechnology, e. g. for production of biofuels. This creates the need for use of recombination technology or synthetic biology, resulting in GMOs that pose potential biosafety hazards that need to be identified and minimized. One of such hazards is the possibility of horizontal gene transfer and homologous recombination, two phenomena proven to be significant among prokaryotes[1]. Since the most broadly used cyanobacterial strain in biotechnology and synthetic biology is *Synechocystis* sp. PCC 6803 which is also naturally competent for genetic transformation[2], we wished to know whether there are any close relatives of the strain present in aquatic environments and planned to develop a DNA barcoding approach specifically for these unicellular cyanobacteria.

Most widely used DNA-based method for identification of cyanobacterial species is 16S rRNA gene analysis, using the assumption that individuals of the same species share greater sequence similarity than individuals of different species[3]. Although overall evolution of 16S rRNA gene is rather slow, there are regions that are more variable and enable discrimination of distantly as well as closely related organisms. With its variable length and number, rRNA ITS region has become a popular tool in identification and classification of cyanobacteria[4]. Our goal was therefore to investigate possibilities for eventual ITS-based molecular tool for discrimination between species and strains of the *Synechocystis* genus.

METHODS

Eleven *Synechocystis* strains were obtained from different culture collections (except for *Synechocystis nigrescens* that was obtained from a supplier of teaching consumables), as listed in Table 1.

Table 1: List of analysed strains. SAG - Sammlung von Algenkulturen Göttingen, CCAP - Culture Collection of Algae and Protozoa, CCALA - Culture Collection of Autotrophic Organisms, PCC - Pasteur Culture Collection, Carolina = Carolina Biological Supply Company.

Species	Strain label
Synechocystis aquatilis	SAG 90.79
Synechocystis bourrellyi	CCAP 1480/1
Synechocystis fuscopigmentosa	CCALA 810
Synechocystis limnetica	CCAP 1480/5
Synechocystis minuscula	SAG 258.80
Synechocystis nigrescens	Carolina
Synechocystis pevalekii	SAG 90.79
Synechocystis salina	CCALA 192
Synechocystis sp.	CCAP 1480/4
Synechocystis sp.	PCC 6714
Synechocystis sp.	PCC 6803

Cells from 1 ml of culture were pelleted at 5000 g for 5 min and boiled for 10 min at 95°C. Lysates were used for PCR using Taq polymerase. For amplification of the variable region of 16S rDNA, primers CYA106F (5'-CGGACGGGTGAGTAACGGTGA-3'), CYA781Ra (5'-GACTACTGGGGTATCTAAATCTTATT-3') and CYA781Rb (5'-GACTACAGGGGTATCTAATCCCTTT-3') were used. For amplification of ITS regions we used primers CSIF (5'-GTCACGCCCGAAGTCGTTAC-3') and ULR (5'- CCTCTGTGTGCCTAGGTATC-3'). Reverse primers for 16S rDNA amplification (CYA781Ra in CYA781Rb) were used in equimolar quantities. All used primers are marked on ribosomal RNA operon scheme in Figure 1.

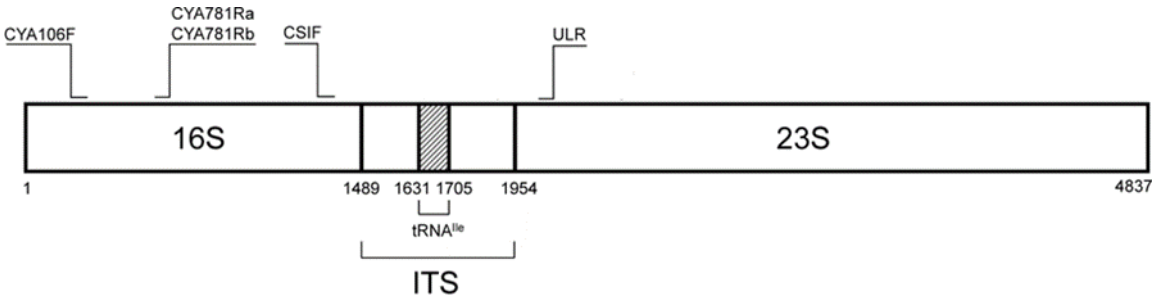

Figure 1: Scheme of partial *rrn* operon with marked primer positions. Nucleotide positions are labelled for reference as deduced from *Synechocystis* sp. PCC 6803 genome.

PCR products were resolved on 1.2% or 1.5% agarose gels and visualized using ethidium bromide. After electrophoresis, PCR products were excised from agarose gels and purified using GeneJet Gel Extraction Kit (Thermo Scientific). Purified products were ligated into pJET1.2 using CloneJET PCR Cloning Kit (Thermo Scientific). After transformation of competent *Escherichia coli* DH5α cells and plating onto selective media, plasmid DNA was isolated from overnight cultures of one to several independent clones using Plasmid MiniPrep Kit (Thermo Scientific). Sequencing was performed by Macrogen Europe.

All the sequences were compared to the non-redundant dataset of the GenBank collection using BLASTN. For other sequence analyses EMBOSS Water and Clustal Omega were used, both at the EMBL-EBI web server. For multiple alignments of 16S sequences we utilized RDP Aligner. We built

neighbourhood-joining tree using MEGA version 6 applying the Jukes-Cantor model. Bootstrap resampling using 500 replicates was performed to test the robustness of the trees.

RESULTS

Agarose gel electrophoresis of ITS amplicons showed great diversity of lengths as can be seen from Figure 2. According to ITS lengths, *Synechocystis* members can be roughly divided into four groups. The shortest regions were found in *S. aquatilis* and *S. fuscopigmentosa* (group A). Most of the analysed representatives belong to the group with lengths similar to PCC 6803 and PCC 6714 (*S. minuscula*, *S. salina*, *S.* sp. CCAP 1480/4). Group C with an intermediate size ITS region was represented by *S. pevalekii* and group D with very long ITS regions by *S. limnetica* and *S. bourrellyi*. These differences allow for a rapid PCR-based discrimination between some of the *Synechocystis* members without sequencing.

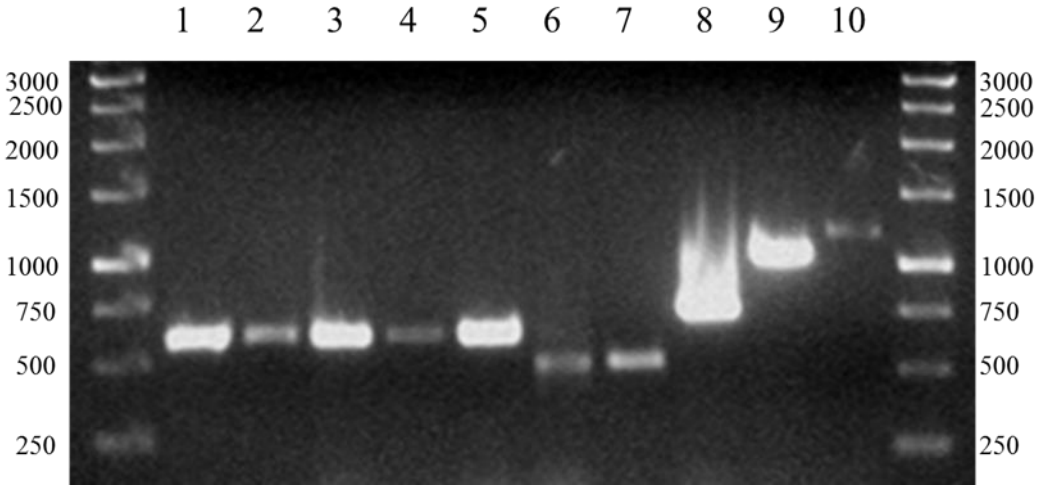

Figure 1: PCR amplification products of ITS regions for the 10 *Synechocystis* strains using CSIF and ULR primers, resolved on 1% agarose gel. (1) *Synechocystis* sp. PCC 6803, (2) *Synechocystis* sp. PCC 6714, (3) *Synechocystis* sp. CCAP 1480/4, (4) *Synechocystis salina* CCALA 192, (5) *Synechocystis minuscula* SAG 258.80, (6) *Synechocystis aquatilis* SAG 90.79, (7) *Synechocystis fuscopigmentosa* CCALA 810, (8) *Synechocystis pevalekii* SAG 91.79, (9) *Synechocystis limnetica* CCAP 1480/5, (10) *Synechocystis bourrellyi* CCAP 1480/1. ITS region of *Synechocystis nigrescens* could not be amplified using CSIF and ULR primers.

Since the observed ITS length heterogeneity exceeded expected differences among species of the same genus, we performed sequencing and BLAST searches. We found that *Synechocystis* members whose ITS lengths differed from the length obtained with PCC 6803, showed higher relatedness to members of different genera. Group B seemed to be related to *Geminocystis* and *Cyanobacterium* genera, group C to *Chamaesiphon* and group D to *Synechococcus*. Results of BLAST search with highest scoring strains and their identity to our sequences are presented in Table 2.

To confirm results obtained with ITS regions, we amplified, sequenced and performed a BLAST search of 16S rRNA gene variable regions and found results supportive of previous findings. With concatenated 16S and ITS sequences we prepared a phylogenetic tree that shows clustering into 4 groups as can be seen in Figure 3.

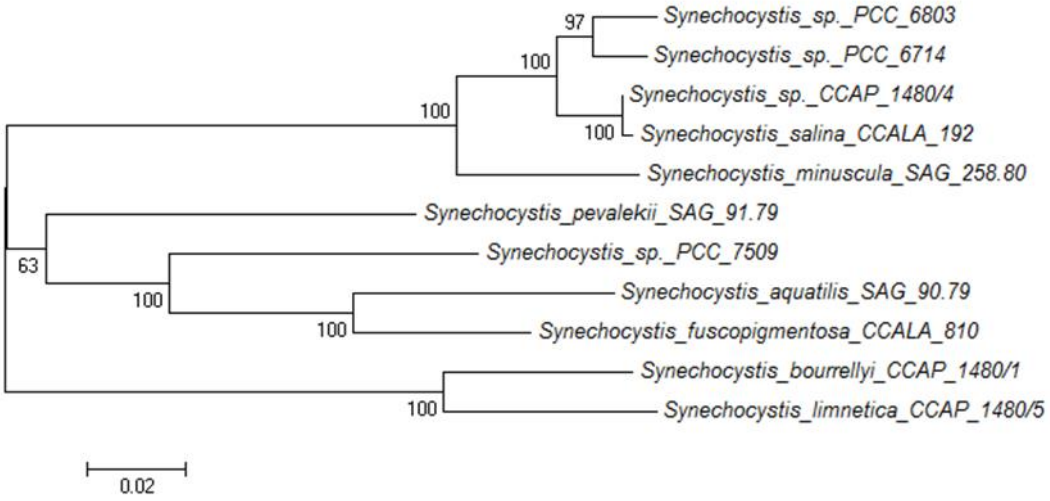

Figure 2: A phylogenetic tree for the analysed members of the *Synechocystis* genus. Tree is based on our sequencing results of the 16S rRNA and ITS regions combined (*S. nigrescens* is not included as its ITS region could not be amplified). For comparison, sequence data for PCC 6714 and PCC 7509 as deposited in GenBank were included.

Table 1: Results of BLAST search with highest scoring strains (GenBank ID numbers of each strain are in brackets) and their identity to our 16S rRNA and ITS sequences. When the highest score was that of the same strain and collection, the second highest score is listed. Results for fully sequenced *S*. sp. PCC 6803 and *S.* sp. PCC 6714 strains are also not shown, since they showed sequences from genomes of both strains as highest scores, as expected. Different results for 16S and ITS for one strain are due to differences in deposited sequences, since for some strains only either 16S or ITS sequence is available. * *S. salina* shows highest identity to *Gloecapsa alpicola* FACHB-400, which has been recently reclassified twice, firstly to *Synechocystis* and then to *Geminocystis* genus.

Species/Strain	Highest BLAST scores			
	16S		ITS	
	Strain and GenBank ID	Identity	Strain and GenBank ID	Identity
S. aquatilis	*Cyanobacterium aponinum* KSU-WH-5 (KT807478.1), lklSCC30 (KM438201.1) and PCC 10605 (CP003947.1)	97.0%	*Cyanobacterium aponinum* PCC 10605 (CP003947.1)	95.0%
S. bourrellyi	*Synechococcus elongatus* CCAP 1479/1B (KM020008.1), *Synechococcus* sp. CCAP 1479/10 (HE975006.1), PCC 7009 (AM709628.1), EW15 (DQ275602.1) and BO8806 (AF317072.1)	99.6%	*Synechococcus* sp. PCC 7009 (AM709628.1)	99.8%
S. fuscopigmentosa	*Geminocystis* sp. NIES-3709 (AP014821.1)	98.9%	*Geminocystis* sp. NIES-3709 (AP014821.1)	96.8%
S. limnetica	*Synechococcus* sp. MA0607K (FJ763779.1)	99.2%	*Prochlorococcus marinus* MIT9313 (BX548175.1)	87.6%
S. minuscula	*Synechocystis salina* LEGE 06155 (HQ832911.1) and *Synechocystis* cf. *salina* LEGE 07073 (HM217083.1)	97.4%	*Gloeothece* sp. PCC 6909 (CCAP 1480/4, (HE975009.1)	80.0%
S. nigrescens	*Synechocystis* sp. SAG 37.92 (KM020010.1)	99.8%	/	/
S. pevalekii	*Chamaesiphon subglobosus* PCC 7430 (AY170472.1)	99.6%	*Chamaesiphon minutus* PCC 6605 (CP003600.1)	90.0%
*S. salina**	*Gloeocapsa alpicola* FACH-400 (JX872524.1) and *Gloeothece* sp. PCC 6909 (HE975009.1)	99.6%	*Gloeothece* sp. PCC 6909 (HE975009.1)	98.8%
S. sp CCAP 1480/4	*Gloeocapsa alpicola* FACHB-400 (JX872524.1)	99.7%	*Synechocystis* sp. PAK12 (EF555570.1)	93.2%

DISCUSSION

Our original goal was to develop DNA barcoding for *Synechocystis* genus. The present analysis showed great genetic heterogeneity the of current *Synechocystis* genus especially in their ITS regions, which is a good basis for development of discrimination methods, either by agarose gel electrophoresis or with use of ITS-specific primers or for developing sequence-based approach.

Also, our research greatly expanded the range of *Synechocystis* members with available sequences which could eventually contribute to a more precise taxonomic delineation of the genus. Namely, variability in 16S rRNA and ITS genomic regions indicates that some current *Synechocystis* members are too genetically different to be assigned to this genus. Taxonomic inconsistencies are not new in Cyanobacteria phylum since cyanobacterial systematics was traditionally based on morphology alone. This turned out to be problematic because of cryptic variability, lack of morphological differences and convergent evolution[5]. Also, cells can change morphology in varying growth conditions or when transferred from natural to laboratory growth conditions[6,7]. Mislabelling of strains in culture collections therefore cannot be excluded as it has also been noted before that more than a half of the strains in culture collections are probably incorrectly identified[8].

Since the introduction of genetic methods to the field of cyanobacterial taxonomy, many systematic reconsiderations were needed, but no extensive research has been done for *Synechocystis* genus so far. Our current results represent a solid basis for taxonomic reconsideration of *Synechocystis* and related cyanobacterial genera.

REFERENCES

1. Achtman, M. and Wagner, M. Microbial diversity and the genetic nature of microbial species. *Nat. Rev. Microbiol.* **2008,** 6, 431-440.

2. Yu, Y., You, L., Liu, D., Hollinshead, W., Tang, Y. J. and Zhang, F. Development of *Synechocystis* sp. PCC 6803 as a phototrophic cell factory. *Marine Drugs* **2013**, *11*, 2894-2916.

3. Eckert, E. M., Coates, R. C., Coci, M. and Callieri, C. Does a barcoding gap exist in prokayotes? Evidences from species delimitation in cyanobacteria. *Life* **2015**, *5*, 50-64.

4. Smit, S., Widmann, J. and Knight, R. Evolutionary rates vary among rRNA structural elements. *Nucl. Acids Res.* **2007**, *35*, 3339-3354.

5. Dvořák, P., Poulíčková, A., Hašler, P., Belli, M., Casamatta, D. A. and Papini, A. Species concepts and speciation factors in cyanobacteria, with connection to the problems of diversity and classification. *Biodiver. Conserv.* **2015**, *24*, 739-757.

6. Castenholz, R. W. and Waterbury, J. B. Oxygenic phototrophic bacteria. Group I. Cyanobacteria. In: Staley, J. T., Bryant, M. P., Pfenning, N. and Holt, J. G (eds) *Bergey's manual of systematic bacteriology*. **1989**. Baltimore: Williams and Wilkins, 1710-1789.

7. Moro, I., Rascio, N., La Rocca, N., Di Bella, M and Andreoli C. *Cyanobacterium aponinum*, a new cyanoprokaryote from the microbial mat of Euganean thermal springs (Padua, Italy). *Algol. Studies* **2007**, *123*, 1-15.

8. Komárek, J. and Anagnostidis, K. Modern approach to the classification system of Cyanophytes 4 – Nostocales. *Algol. Studies* **1989**, *56*, 247-345.

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Abstract

DEVELOPMENT OF CABBAGE HAPLOID INDUCER LINE WITH GENOME EDITING

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ABSTRACT

For plant breeding, induction of haploids is one of the fastest techniques for the production of hybrids because homozygous lines can be made in one generation. One of techniques for production of parental homozygous plants is pollination with inducer lines which can be produced with different point mutations in the centromere-specific histone H3 variant (CENH3)[1]. Because CENH3 is highly conserved, the same point mutations could be generated by genome editing also in cabbage.

Therefore, our aim was to develop a protocol for genome editing of CENH3 gene in cabbage using CRISPR/Cas9 system. For that purpose, three sgRNAs targeting CENH3 in cabbage were designed and expression vectors were constructed that would be introduced in cabbage cells through two different transformation techniques.

We developed a protocol for *Agrobacterium tumefaciens* mediated transformation and tested different transformation parameters, like the type of explant (hypocotyl, leaf, petiole), concentration of selective antibiotic and duration of selection (1- 4 weeks).

For protoplast transformation, a protocol for protoplast isolation and regeneration was first developed using immobilization in calcium alginate layers[2]. We tested the effect of incubation time and source of protoplasts on protoplasts isolation efficiency. For optimization of protoplasts regeneration protocol, protoplasts were cultured at different densities and after formation of calli, they were transferred to medium supplemented with or without different growth regulators.

In the future, we plan to use the developed techniques to first edit cabbage CENH3 gene and later to use them for editing of other targets.

REFERENCES

1. Karimi-Ashtiyani R, Ishii T et al. Point mutation impairs centromeric CENH3 loading and induces haploid plants. *PNAS.* **2015**, 112, 36, 11211-11216.

2. Kielkowska A, Adamus A. An alginate-layer technique for culture of *Brassica oleracea* L. protoplasts. *In vitro Cell.Dev.Biol.-Plant.* **2012**, 48, 265-273.

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Abstract

CRISPR/Cas9 genome editing in rapeseed

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ABSTRACT

Rapeseed (*Brassica napus* L.) is one of the most important crops for oil production and a model organism for microspore embryogenesis studies. The aim of our work was to develop delivery methods for ribonucleoprotein-guided endonucleases (RNPs) into rapeseed cells which could be later used for genome editing without integrating foreign genetic material[1] into the rapeseed genome. For this purpose, two different cell types (microspores and protoplasts) and two different delivery methods were tested.

First, isolation protocols for highly viable protoplasts and highly embryogenic microspores were optimized. Up to 84% viable protoplasts were produced with overnight incubations of etiolated hypocotyls and the most embryogenic microspores (at late uninucleate to early binucleate developmental stage) were obtained from 3-4 mm flower buds. Several sgRNAs were constructed and *in vitro* synthesised targeting exons of phytoene desaturase gene (*pds*) encoding an important enzyme in the carotenoid biosynthetic pathway which mutation results in albino and dwarf phenotypes[4]. Before transformation, sgRNA and Cas9 were preassembled in molar ratios from 1:10 to 1:20[5]. They were introduced in microspores with electroporation and in protoplasts with PEG. After 24 hours, on target mutations were detected using T7E1 endonuclease assay, restriction enzymes site loss assay and Sanger sequencing. Preliminary results showed targeted mutagenesis of protoplasts in the region of exon 1 of the *pds* gene using the T7E1 assay. New experiments are currently underway. With further optimization, these techniques could be used for basic research and for plant breeding.

REFERENCES

1. Kanchiswamy CD. DNA-free genome editing methods for targeted crop improvement, Plant Cell Rep.**2016**, 35, 1469–1474
2. Custers J. “Microspore culture in rapeseed (*Brassica napus* L.),” in Doubled Haploid Production in Crop Plants, eds Maluszynski M, Kasha KJ, Forster BP, Szarejko I. Dordrecht,Kluwer Academic Publishers **2003**, 185–193.
3. Wang YP, Sonntag K, Rudlogg E. Development of rapeseed with high erucic acid content by asymmetric somatic hybridization between *Brassica napus* and *Crambe abyssinica*. Theoretical and Applied Genetics **2003**, 106, 1147-1155.
4. Qin G, Gu H, Ma L, Peng Y, Deng XW, Chen Z, Qu LJ. Disruption of phytoene desaturase gene results in albino and dwarf phenotypes in Arabidopsis by impairing chlorophyll, carotenoid, and gibberellin biosynthesis. Cell Res. **2007**, 17, 471-82.
5. Woo JW, Kim J, Kwon SI, Corvalan C, Cho SW, Kim H, Kim SG, Kim ST, Choe S, Kim JS. DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nat Biotechnol.**2015** 33(11):1162–1164.

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Abstract

DISTRIBUTION ANALYSIS AND PRODUCTION OF FUNGAL ACTINOPORIN- AND PERFORIN-LIKE PROTEINS

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ABSTRACT

Pore-forming proteins are currently of great interest due to their role in fungal pathogenicity, which is recognized as a serious threat to food safety[1] and public health[2]. The aim of our study was to analyze the prevalence of aegerolysins, MACPF (Membrane Attack Complex component/PerForin) and NLP (NEP-1-like protein) proteins within the fungi kingdom. We attempted to express proteins B473 (a PlyB homolog) and NLP from fungus *Aspergillus niger* in bacterium *Escherichia coli*, in order to confirm the results of bioinformatic analysis, and characterize the properties of the proteins.

We found that the prevalence of the proteins does not correlate with the fungal lifestyle. Individual organisms possess several different copies of the genes encoding the proteins under study, which are spread throughout the genome and the phylogenetic tree. This suggests the possibility of different gene donors. Synteny analysis showed that in 38 cases, homolog PlyA is found adjacent to homolog PlyB, like in the fungus *Pleurotus ostreatus.*

There is an increasing body of evidence to suggest that HGT is an important mechanism in eukaryotic genome evolution[3]. Researchers connected HGT to emergence of new diseases[2] and expansion of host range[4]. We used *in silico* methods to discover a potential horizontal gene transfer event in fungus *A. niger*, and noted repeated sequences in its vicinity that could be due to transposon activity.

This study revealed some interesting starting points for further research. It would be advantageous to optimize the production and isolation of target pore-forming proteins, as they offer a great potential for biotechnological innovations[5,6].

REFERENCES

1. Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ. Emerging fungal threats to animal, plant and ecosystem health. *Nature*. **2012**, 484, 7393: 186–194.
2. Pfaller MA, Diekema DJ. Rare and emerging opportunistic fungal pathogens: concern for resistance beyond *Candida albicans* and *Aspergillus fumigatus*. *Journal of clinical microbiology*. **2004**, 42, 10: 4419–4431.
3. Fitzpatrick DA. Horizontal gene transfer in fungi. *FEMS Microbiology Letters*. **2012**, 329, 1: 1–8.
4. Mehrabi R, Bahkali AH, Abd-Elsalam KA, Moslem M, Ben M'barek S, Gohari AM, Jashni MK, Stergiopoulos I, Kema GHJ, de Wit PJGM. Horizontal gene and chromosome transfer in plant pathogenic fungi affecting host range. *FEMS Microbiology Reviews*. **2011**, 35, 3: 542–554.
5. Majd S, Yusko EC, Billeh YN, Macrae MX, Yang J, Mayer M. Applications of biological pores in nanomedicine, sensing, and nanoelectronics. *Current Opinion in Biotechnology.* **2010**, 21, 4: 439–476
6. Provoda CJ, Lee KD. Bacterial pore-forming hemolysins and their use in the cytosolic delivery of macromolecules. *Advanced Drug Delivery Reviews*. **2010**, 41, 2: 209–221.

ABSTRACTS of LECTURES

MOLECULAR BASIS OF DISEASES & GENOMICS

Miha Modic

TDP-43 safeguards pluripotency by regulating alternative polyadenylation and repressing paraspeckles

Urša Lampreht Tratar

Gene electrotransfer of antibiotic-free IL-12 plasmid induces antitumor effect in B16F10 melanomas

Katja Uršič

Peritumoral gene electrotransfer of IL-12 as adjuvant immunotherapy to intratumoral electrochemotherapy with cisplatin for treatment of murine B16F10 melanoma

Katarina Žnidar

The effect of gene electrotransfer of blank pDNA on tumor cells' survival and expression of cytosolic DNA sensors

Vasja Progar

Functional enrichment of differentially expressed genes in hop infected by *V. nonalfalfae*

Kristina Marton

Verticillium nonalfalfae and its candidate secreted effector proteins

Marija Rogar

Polymorphisms in segregation genes involved in the development of gastric cancer in Slovenian population

Alja Zottel

Glioblastoma multiforme and nanobodies

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Abstract

TDP-43 SAFEGUARDS PLURIPOTENCY BY REGULATING ALTERNATIVE POLYADENYLATION AND REPRESSING PARASPECKLES

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ABSTRACT

A fundamental question of stem cell biology is what dissolves the self-renewal program to facilitate exit from pluripotency. Here we show that TDP-43 regulates alternative polyadenylation (APA) during early differentiation, including of the pluripotency factor *SOX2*. A decrease of TDP-43 during early differentiation leads to loss of SOX2 due to the coupled action of *miR-21*. Hence, overexpression or reduction of TDP-43 promotes somatic cell reprogramming/pluripotency or differentiation, respectively. Moreover, TDP-43 stimulates production of an unstable short isoform of the lncRNA *NEAT1*. Reduced TDP-43 allows formation of the stable full-length isoform of *NEAT1*, which is required for efficient differentiation by scaffolding the nuclear domains named paraspeckles. Taken together, our work reveals a post-transcriptional axis that stabilizes the states of pluripotency and differentiation independently of any specific lineage.

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Abstract

GENE ELECTROTRANSFER OF ANTIBIOTIC-FREE IL-12 PLASMID INDUCES ANTITUMOR EFFECT IN B16F10 MELANOMAS

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ABSTRACT

The use of plasmids encoding interleukin-12 (IL-12) in gene electrotransfer studies has shown great promise in treating tumors induced in experimental animals, and in human and veterinary clinical studies[1]. These studies were using plasmids carrying the gene for antibiotic resistance, which is not recommended for human therapy. Therefore, the aim of this study was to construct an antibiotic-free plasmid and to evaluate its antitumor effectiveness in murine B16F10 melanoma. The antibiotic-free plasmid encoding IL-12 (pORFmIL-12ORT) was successfully constructed using ORT technology (Cobra bio, Keele, UK). Gene electrotransfer of pORFmIL-12ORT was performed in B16F10 tumors growing subcutaneously in C57Bl/6 mice. The antitumor effectiveness was evaluated by tumor growth delay assay and local immune response was detected by hemotoxylin eosin (HE) staining and immunohistochemical (IHC) staining of F4/80 and MHCII. Systemic effect was evaluated by the IL-12 and IFNγ determination in blood. All the IL-12 treated tumors responded completely to the therapy by the day 14. HE staining showed infiltration of immune cells, which lasted from day 4 to day 14. IHC staining for F4/80 and MHCII showed higher intensity of positive cells at day 4, which lasted until day 14, compared to control. Serum IL-12 and IFNγ showed increased levels of IL-12 at days 1 and 4 and of IFNγ at days 4 and 8. Our results showed complete tumor response after gene electrotransfer of pORFmIL-12ORT and increased immune cells infiltration into the tumors. Also, high infiltration of MHCII positive cells in the IL-12 treated tumors proposes a M1 polarization of macrophages, that is important for the tumor elimination[2].

REFERENCES

1. Cemazar M, Jarm T, Sersa G. Cancer electrogene therapy with interleukin-12. *Curr Gene Ther*. **2010**, 10(4), 300 - 11
2. Chanmee T, Ontong P, Konno K, Itano N. Tumor-Associated Macrophages as Major Players in the Tumor Microenvironment. *Cancers*. **2014**, 6(3), 1670 - 90

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Abstract

PERITUMORAL GENE ELECTROTRANSFER OF IL-12 AS ADJUVANT IMMUNOTHERAPY TO INTRATUMORAL ELECTROCHEMOTHERAPY WITH CISPLATIN FOR TREATMENT OF MURINE B16F10 MELANOMA

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ABSTRACT

Treatment of metastatic melanoma has undergone rapid renovation, especially in the light of combining established therapies with recently discovered immunotherapies. Our research group has been studying intratumoral electrochemotherapy (i.t. ECT; a combination of chemotherapeutic drug and electric pulses) as a local ablative therapy which is a well-established therapy in preclinical models as well as in clinics.[1] ECT with cisplatin (CDDP) results not only in direct cytotoxic effect on cancer cells, but also indirectly affects the immune system.[2,3] However, after applying low-dose i.t. ECT with CDDP (2 mg/kg) to murine B16F10 melanoma, we observed only a delayed growth of primary tumors with no complete responses. To achieve both better local tumor growth control and an effect on distant non-treated metastasis (abscopal effect), we applied peritumoral gene electrotransfer (p.t. GET) of plasmid encoding interleukin-12 (IL-12) as an adjuvant immunotherapy.[4] IL-12 is a proinflammatory cytokine which monitors inflammation via innate and adaptive immune system responses.[5] With p.t. GET of therapeutic plasmid encoding mouse IL-12 under constitutive EF-1α/HTLV hybrid promoter, we achieved low dose expression of the transgene in surrounded (p.t.) skin tissue, without any systemic cytotoxicity. Treatment by p.t. GET (50 µg/tumor) alone resulted in only tumor growth delay but no complete responses, whereas with combined therapy (i.t. ECT with CDDP and p.t. GET with IL-12), we cured 37.5% of animals. Further, we also observed abscopal effect in combined therapy. Thus we conclude that p.t. GET of IL-12 potentiates the effect of i.t. ECT with CDDP on local and systemic level.

REFERENCES

1. Yarmush ML, Golberg A, Serša G, Kotnik T, and Miklavčič D. Electroporation-Based Technologies for Medicine: Principles, Applications, and Challenges. *Annu. Rev. Biomed. Eng.* **2014**, 16, 295–320.
2. Kelland L. The resurgence of platinum-based cancer chemotherapy. *Nat. Rev. Cancer*. **2007**, 7, 573–584.
3. Hato SV, Khong A, De Vries IJM, and Lesterhuis WJ. Molecular pathways: The immunogenic effects of platinum-based chemotherapeutics. *Clin. Cancer Res*. **2014**, 20, 2831–2837.
4. Sersa G, Teissie J, Cemazar M, Signori E, Kamensek U, Marshall G, and Miklavcic D. Electrochemotherapy of tumors as in situ vaccination boosted by immunogene electrotransfer. *Cancer Immunol. Immunother*. **2015**, 64, 1315–1327.
5. Tugues S, Burkhard SH, Ohs I, Vrohlings M, Nussbaum K, Vom Berg J, Kulig P, and Becher B. New insights into IL-12-mediated tumor suppression. *Cell Death Differ*. **2015**, 22, 237–246.

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Abstract

THE EFFECT OF GENE ELECTROTRANSFER OF BLANK pDNA ON TUMOR CELLS' SURVIVAL AND EXPRESSION OF CYTOSOLIC DNA SENSORS

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ABSTRACT

Gene electrotransfer (GET) is an effective non-viral delivery method for introducing plasmid DNA (pDNA) into cells and tissues. After exposure of cells to the electric pulses, pDNA enters the cells and reaches the cytosol via endocytosis or through electroporation induced pores [1]. Preclinical studies showed tumor growth delay and regression after GET of the blank pDNA, however the possible mechanisms responsible for tumor regression are not known [2,3]. One of the possible mechanism could be the activation of DNA-specific receptors in the cytosol referred to as cytosolic DNA sensors. Their response to DNA leads to upregulation of interferons and cytokines as well as cell death [4].

In our study, the presence of cytosolic DNA sensors in different mouse tumor cells (B16F10, TS/A, WEHI 164) were demonstrated using RT-PCR and western blot. After GET of control pDNA several DNA sensors (DDX60, DAI, p204) and Interferon β (IFN β) were upregulated on mRNA and protein level. pDNA concentration-dependent decrease in cell survival after pDNA GET was obtained in all cell lines, with TS/A cell line the least susceptible. After GET of control pDNA, a higher number of necrotic cells were observed in two cell lines (B16F10 and WEHI 164) compared to TS/A cells as determined by flow cytometry using annexin V and 7AAD assay to characterize apoptotic or necrotic cell death.

To conclude, the increased expression of several DNA sensors and IFNβ after GET of blank pDNA indicate that this might be the possible mechanism for observed cell death of tumor cells.

REFERENCES

1. Rosazza C, Phez E, Escoffre JM, Cezanne L, Zumbusch A, and Rols MP. Cholesterol implications in plasmid DNA electrotransfer: Evidence for the involvement of endocytotic pathways. *Int J Pharm* **2012**, 423: 134-143.

2. Heller L, Todorovic V, Cemazar M. Electrotransfer of single-stranded or double-stranded DNA induces complete regression of palpable B16.F10 mouse melanomas. *Cancer Gene Ther* **2013**, 20:695-700.

3. Ugen KE, Kutzler MA, Marrero B, Westover J, Coppola D, Weiner DB*, et al*. Regression of subcutaneous B16 melanoma tumors after intratumoral delivery of an IL-15-expressing plasmid followed by in vivo electroporation. *Cancer Gene Therapy* **2006**, 13: 969-974.

4. Desmet CJ, Ishii KJ . Nucleic acid sensing at the interface between innate and adaptive immunity in vaccination. *Nat Rev Immunol* **2012**, 12:479-491.

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Abstract

FUNCTIONAL ENRICHMENT OF DIFFERENTIALLY EXPRESSED GENES IN HOP INFECTED BY *V. nonalfalfae*

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ABSTRACT

Hop (*Humulus lupulus* L.) is an agriculturally important plant with uses in brewing and pharmacy. Its production is hampered by Verticillium wilt, predominantly caused by a soil-borne pathogenic fungus *Verticillium nonalfalfae*. Some hop cultivars exhibit resistance against this disease but underlying molecular mechanisms of resistance remain to be determined.

In this study, resistant and susceptible hop plants were infected with a lethal strain of *V. nonalfalfae*. RNA-Seq was performed on roots and shoots of infected and control plants harvested at 6, 12, 18 and 30 days post inoculation.

Sequenced reads were mapped to the gene models of hop draft genome[1], which were then assigned Arabidopsis gene IDs based on sequence similarity. Differentially expressed genes (DEGs) were determined using FunPat[2] and subjected to functional network analysis using ClueGO[3].

The susceptible hop cultivar mounted a strong basal defence response against V. nonalfalfae, with enhanced chitinase and oxidoreductase activity and downregulation of photosynthetic processes in shoots. In the resistant cultivar, cutin metabolism and cell wall biogenesis were enriched in DEGs in shoots and terpenoid biosynthesis and polysaccharide catabolism in roots. Additionally, the network analysis exposed several DEGs at intersections of the enriched functional groups. These potentially code for hub proteins coordinating the hop response to V. nonalfalfae and identification of their exact roles in this pathosystem should be pursued in further studies.

REFERENCES

1. HopBase v1.0. http://hopbase.org/

2. Sanavia T., Finotello F., Di Camillo B. FunPat: function-based pattern analysis on RNA-seq time series data. *BMC Genomics* **2015**, 16(Suppl 6), S2.

3. Bindea G., Mlecnik B., Hackl H., Charoentong P., Tosolini M., Kirilovsky A., Fridman W.H., Pages F., Trajanoski Z., Galon J. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. 2009. *Bioinformatics* **2009**, 25(8), 1091-1093.

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Abstract

Verticillium nonalfalfae AND ITS CANDIDATE SECRETED EFFECTOR PROTEINS

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ABSTRACT

Verticillium nonalfalfae (*Vna*)[1] is a soil born phytopathogenic ascomycete that causes verticillium wilting on a wide range of crops, including hop. Apart from phytosanitary measures, the only effective approach is planting resistant varieties. In modern resistance breeding programs, plant pathogen effectors are exploited as a tool to detect resistance (R) genes and functionally characterize R proteins that recognize different effectors[2]. Effectors are small secreted proteins that modulate host immune responses and can also serve as avirulence factors. We report here an in-house pipeline for effector detection of phytopathogenic fungi.

The putative *Vna* proteome containing 9,269 gene models was functionally annotated with a wide range of analyses using blast and hmmer searches against different databases. Based on signal peptide, transmembrane domain predictions and putative cellular localization, we determined the *Vna* secretome, comprising 962 gene models. Three hundred and thirty CSEPs (candidate secreted effector proteins) were identified after omitting gene models with carbohydrate-active enzyme annotation and retaining gene models with none or only effector-specific PFAM domains. For the 49 best ranked CSEPs (based on *in planta* expression, transcriptomic data of differentially expressed gene models, no sequence homology to other known genes, lineage-specificity for highly virulent *Vna* etc.), we examined their temporal and spatial expression during hop infection using RT-qPCR and identified the six best effector candidates. Two of them were knocked out from *Vna* conidia, which were used for inoculation of susceptible hop plants. Disease symptoms evaluation revealed reduced virulence of knock outs.

REFERENCES

1. Inderbitzin P, Bostock RM, Davis RM, Usami T, Platt HW, Subbarao K V. Phylogenetics and taxonomy of the fungal vascular wilt pathogen Verticillium, with the descriptions of five new species. *PLoS One*. **2011**, 6(12), e28341.
2. Klosterman SJ, Subbarao K V, Kang S, et al. Comparative genomics yields insights into niche adaptation of plant vascular wilt pathogens. Dangl JL, ed. *PLoS Pathog*. **2011**, 7(7), e1002137.

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Abstract

POLYMORPHISMS IN SEGREGATION GENES INVOLVED IN THE DEVELOPMENT OF GASTRIC CANCER IN SLOVENIAN POPULATION

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ABSTRACT

Gastric cancer is very complex and heterogeneous disease, and while numerous studies focusing on its development were carried out, the detailed mechanisms still remain unclear[1]. Genomic instability is one of the main topics of gastric cancer genetic studies[2]. Single nucleotide polymorphisms (SNPs) in genes encoding mitotic kinases as well as of other segregation genes, or their combinations, may result in the development of cancer[3].

Polymorphisms were determined using quantitative real-time PCR (qPCR), restriction fragment length polymorphism (RFLP) and DNA sequencing.

The association between polymorphisms rs2277559 (*BUB1B*), rs2241666 (*ZWINT*), rs11858113 (*CASC5*) and rs11855334 (*CASC5*) and increased risk of developing gastric cancer in male population was determined. Genotypes of six polymorphisms (in *BUB1B* gene) rs2277559, rs2290551, rs1801376, rs1047130, rs1565866, rs2277560 were associated with Lauren classification. On the other hand, the link between genotypes of polymorphisms rs1801376 (*BUB1B*), rs11855334 (*CASC5*), rs2241666 (*ZWINT*), rs2910101 (*PTTG1*) and rs1047130 (*BUB1B*) and tumour differentiation was observed. Survival analysis revealed association between the lymph node involvement and perineural invasion. Statistically higher frequencies of haplotypes G-A-G-T-G-G-A, G-G-A-G-A-A-G and A-G-G-T-A-G-A in gene *BUB1B* and of haplotypes A-A-A-C and C-C-G-T in gene *ESPL1* were observed in gastric cancer patients, while haplotypes A-C-A-T and C-A-G-T in gene *ESPL1* were found frequently present in the group of controls.

Specific genotypes of selected polymorphisms in chromosome segregation genes were found associated with development of gastric cancer in Slovenian population of gastric cancer patients. Polymorphisms linked to gastric cancer could serve as potential diagnostic and prognostic biomarkers.

REFERENCES

1. Hudler P.: Genetic aspects of gastric cancer instability. The Scientific World Journal. 2012; 2012: 761909.
2. Lengauer C., Kinzler K. W., Vogelstein B.: Genetic instability in colorectal cancers. Nature. 1997; 386(6625): 623-7.
3. Frank S. A.: Genetic predisposition to cancer - insights from population genetics. Nature reviews Genetics. 2004; 5(10): 764-72.

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Abstract

GLIOBLASTOMA MULTIFORME AND NANOBODIES

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ABSTRACT

Glioblastoma multiforme (GBM) is an astrocytoma type of brain cancer and is one of the deadliest cancers.[1] In spite of the standard therapy that includes surgery, radiotherapy and chemotherapy, most of the patients do not survive more than two years.[1] There are many reasons why the therapies fail. One of the biggest reasons is the existence of glioblastoma stem cells that are resistant to chemotherapy and radiotherapy and are able to form a new tumor.[2] The other reason is that it is difficult to surgically remove tumor completely.[3] One of most promising devices for targeting GBM are nanobodies, small antigen-recognizing part of heavy chain antibody, that is mostly found in llamas, camels and sharks.[4] The are many advantages of nanobodies regarding classical antibodies such as high yield in production, low immunogenicity, solubility and stability at high temperature as extreme pH.[4,5] The aim of our work was to evaluate the effect of four nanobodies on U87MG, U251MG and NCH glioblastoma cells lines. Furthermore, we have determined the location of specific antigens using FITC bound nanobodies and have verified our results with commercial antibodies. We confirmed the results for three nanobodies and one has to be confirmed in future. Nanobodies for different antigens effect cells in different way. We conducted two different metabolic assays: WST test and AlamarBlue test. The results show that the most cytotoxic for NCH cells is Nb206 and for U87MG and U251MG are the most cytotoxic Nb10, Nb79 and Nb206 nanobodies.

REFERENCES

1. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352(10):987-96.

2. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 2006;444(7120):756-60.

3. Van Meir EG, Hadjipanayis CG, Norden AD, Shu HK, Wen PY, Olson JJ. Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma. CA Cancer J Clin. 2010;60(3):166-93.

4. Deffar K, Shi H, Li L, Wang X, Zhu X. Nanobodies - the new concept in antibody engineering. African Journal of Biotechnology. 2009;8(12):2645-52

5. Oliveira S, Heukers R, Sornkom J, Kok RJ, van Bergen En Henegouwen PM. Targeting tumors with nanobodies for cancer imaging and therapy. J Control Release. 2013;172(3):607-17.

POSTERS

ABSTRACTS and PAPERS of POSTERS

MOLECULAR BASIS OF DISEASES

Marko Vidak, Damjana Rozman, Mirjana Liović, Radovan Komel

Search of new glioblastoma stem cell markers by comparing microarrays data from the NCBI GEO database, and experimental validation of those markers in glioma cell lines

Matej Rebek, Eva Jakljevič, Darja Žgur-Bertok, Marjanca Starčič Erjavec

Phylogenetic groups and *clbAQ*, *usp*, *hlyA*, *cnf1* genes among commensal escherichia coli strains isolated from feces of healthy humans of both genders and both age groups

Tinkara Prijatelj, **Danijela Vočanec**, Tadej Pajič, Martina Fink, Irena Preložnik Zupan, Peter Černelč, Radovan Komel, Tanja Kunej, Nataša Debeljak

Novel molecular-genetic diagnostic test for JAK2 negative erythrocytosis

Rok Končnik, **Barbara Krajnc**, Jovan Krsteski, Uroš Potočnik

Polymorphism rs1056837 in *CYP1B1* encoding an estrogen metabolizing enzyme presents a risk for ULM among Slovenian women

Matjaž Deželak, **Žan Hribar**, Carina E. P. Kozmus, Uroš Potočnik

A polymorphism located in 3'UTR of *PITHD1* near cannabinoid receptor 2 gene *CNR2* is associated with severe forms of Crohn's disease and *CNR1* gene expression

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Abstract

SEARCH OF NEW GLIOBLASTOMA STEM CELL MARKERS BY COMPARING MICROARRAYS DATA FROM THE NCBI GEO DATABASE, AND EXPERIMENTAL VALIDATION OF THOSE MARKERS IN GLIOMA CELL LINES

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ABSTRACT

Glioblastoma multiforme (GBM) is the most lethal brain tumor. Glioblastoma stem cells are believed to be the reason for the tumor's malignancy. Identification of stem cells among other cells in the tumor mass would therefore facilitate GBM therapy. Most likely there are different groups of stem cells within a single tumor, each such group expressing its own set of markers[1]. Our goal is to find new markers that would enable targeting of stem cell populations not expressing any of already known markers.

Gene expression profiles of glioblastoma cell lines will be compared with the profiles of non-malignant brain cells from the same dataset. All the profiles have been obtained from microarrays and uploaded to the NCBI GEO database. The goal is to find genes that are significantly overexpressed in malignant cells in most or all the analyzed datasets. The proteins coded by these genes will be assessed by their function, intracellular location and expression frequency in brain and other tissues. Most interesting candidates, i.e. surface proteins that are not too commonly expressed outside the brain and whose function is related to growth and proliferation, will have their expression investigated in the U87 glioma cell line and the NCH CD133+ purported glioblastoma stem cell line, as well as in a new cell line derived from the U87. Compared to the original U87 line, this new cell line is believed to be enriched in stem-cell like cells, including CD133- stem cells not present in the NCH line.

REFERENCES

1. Wang J, Sakariassen P, Tsinkalovsky O, Immervoll H, Bøe SO, Svendsen A, et al. CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. *Int J Cancer*. **2008**, 122(4), 761-768.

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Article

PHYLOGENETIC GROUPS AND *clbAQ, usp, hlyA, cnf1* GENES AMONG COMMENSAL *Escherichia coli* STRAINS ISOLATED FROM FECES OF HEALTHY HUMANS OF BOTH GENDERS AND BOTH AGE GROUPS

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The bacterium *E. coli* and its host usually coexist in mutual benefit; *E. coli* is known to be an important member of the gut microbiota. But there are several *E. coli* strains that have specific virulence factors and can cause a variety of different intestinal and extraintestinal infections. It is known that the gut microbiota can be the reservoir of such strains. The aim of our study was to reveal if carriage of such potential pathogenic *E. coli* strains in the gut differs among females and males and among children (0-18 years) and adults (>18 years). To fulfill this aim we isolated on MacConkey plates *E. coli* from feces of healthy humans of both genders and both age groups. To confirm the identification of *E. coli* the indole test, URISELECT plates and LAMP assay were used. ERIC-PCR was performed to distinguish different isolates/strains. We obtained a collection of 56 different *E. coli* strains. All strains were screened for phylogenetic groups by the triplex PCR as well as the extended quadruplex PCR phylotyping method and the following virulence-associated genes: *clbA, clbQ, cnf1, hlyA* and *usp*. The results showed that around 50% of all tested strains belonged to the phylogenetic group B2. The genes *clbA, clbQ* were detected in 62%,*usp* in 55%, *hlyA* in 20% and *cnf1* in 18% of all tested *E. coli* strains. There was no statistical significant difference in carriage of potential pathogenic *E. coli* strains among females and males and among children and adults. Our results showed that in the intestine of healthy humans of both genders and age groups there is a high virulence potential for extraintestinal pathogenic*E. coli* strains.

INTRODUCTION

Escherichia coli (*E. coli*) is the commensal species of the facultative anaerobic microbiota in the lower intestine of healthy warm-blooded organisms. It colonizes the gastrointestinal tract of infants within a few hours after birth and mostly coexists with its host in mutual benefit for decades[1]. However, it is assumed that the intestinal microbiota is also a reservoir of the so called extraintestinal pathogenic *E. coli* (ExPEC) strains that can, due to specific virulence factors instigate an impressive variety of extraintestinal infections[2]. Characteristic virulence traits that are present in most ExPEC include various adhesins, factors to avoid or subvert host defence systems (e.g. capsule, Toll/interleukin-1 receptor domain–containing protein), mechanisms for nutrient acquisition (e.g. siderophores), and toxins (e.g. hemolysin, cytotoxic necrotizing factor 1)[3]. It is known that ExPEC mainly belong to phylogenetic group B2 and to a lesser extent to the group D. In addition, many virulence factors are associated with the B2 group[3, 4].

Yet, data on the prevalence of potential ExPEC strains among the bowel microbiota from healthy humans are very limited[2]. To further our understanding of the potential of commensal *E. coli* to cause extraintestinal infections and to compare their virulence potential in relation to host gender/age we screened a collection of 56 *E. coli* strains, isolated from feces of healthy humans of both genders and both groups (0-18 and >18), for the prevalence of several extraintestinal virulence-associated genes. Statistical analysis was performed to reveal a possible difference in ExPEC carriage among females and males and both age groups.

METHODS

E. coli strains were isolated as lactose-positive colonies on MacConkey agar plates from feces of 56 individuals. The indole test was performed to ascertain the *E. coli* species. The investigated strains were cultivated in Luria Bertani medium or agar at 37˚C.

We used ERIC-PCR to differentiate *E. coli* isolates/strains. The ERIC-PCR profiles, following agarose gel electrophoresis, were determined manually. We used the same method as described in Fajs et al., 2013[5]. Prior to profiling, boiled lysates from isolates were prepared[6] to be used in PCR reactions.

To verify the identification of *E. coli* we used the LAMP assay. LAMP assay method was performed as described in Hill et al., 2008[7]. The lysates, as well as PCR reactions, were prepared in duplicates.

E. coli strains were screened for phylogenetic groups with the triplex PCR method differentiating the phylogenetic groups A, B1, B2, D[8] and with the extended quadruplex PCR method differentiating the phylogenetic groups A, B1, B2, C, D, E, F[9]. We also performed a screening for the following virulence-associated genes: *clbAQ, cnf1, hlyA* and *usp.* The primers and PCR programs used were described previously[3, 10, 11, 12, 13]. The lysates, as well as PCR reactions, were prepared in duplicates.

To analyze the data we used Fisher's exact test (two-tailed). The threshold for statistical significance was set at p values of < 0.05.

RESULTS

ERIC-PCR was performed on all 56 isolates. ERIC-PCR produced profiles with bands ranging from less than 250 bp to approximately 3000 bp. Based on analysis of ERIC-PCR profiles, all the isolates had different ERIC-PCR profiles and were thus considered genetically distinct.

Products of the LAMP assay were analyzed with agarose gel electrophoresis (Figure 1) and we were able to verify the identification of *E. coli* in all but two isolates. These isolates were not *E. coli,* therefore we excluded them from this research.

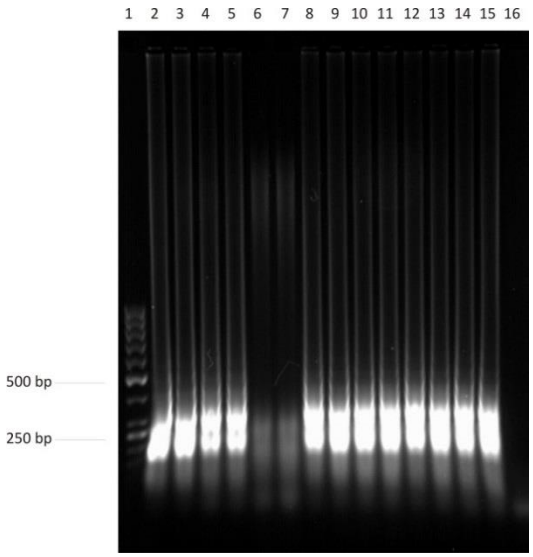

Figure 1: Agarose gel electrophoresis of LAMP products. LAMP products were analyzed on a 1% agarose gel. 1 – GeneRuler™ 50 bp DNA ladder (Fermentas, St. Leon-Rot, Germany); 2,3 – isolate 2001 ž; 4,5 – isolate 1999ž/8; 6,7 – not E. coli*; 8,9 – isolate 1998ž/2; 10,11 – isolate 1999ž/5; 12,13 – isolate 1999ž/4; 14, 15 – isolate 1969m; 16 – negative control.*

Our analysis showed that the majority of the strains, studied with the triplex PCR method[1], belonged to groups B2 and A, 27 (48%) were assigned to the group B2 and 11 (20%) were assigned to group A. Ten (18%) isolates were grouped as D and 8 (14%) belonged to the B1 group (Figure 2). As expected, when tested with the extended quadruplex PCR method[2], the results were quite similar. Twenty-six

(46%) of the studied strains belonged to the group B2, 14 (25%) of them belonged to the group A and 8 (14%) of the strains belonged to the group B1. Only a few of the strains belonged to groups C, D, E and F (Figure 2).

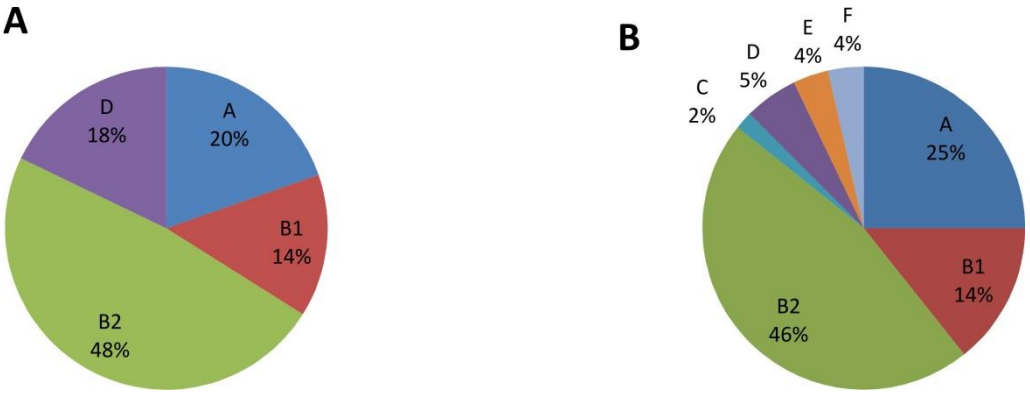

Figure 2: Prevalence of E. coli *strains by phylogenetic groups using the triplex PCR method (A) and the extended quadruplex PCR method (B).*

The most prevalent virulence-associated genes among the studied strains were *clbAQ*, which was found in 35 (62%) of the tested strains, followed by *usp*, detected in 31 (55%) strains, *cnf1* was found in 10 (18%) strains and *hlyA* in 52 (58%) strains (Figure 3).

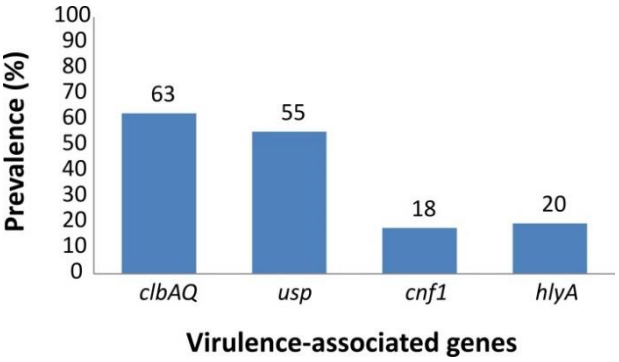

Figure 3: Prevalence of virulence-associated genes, found among studied E. coli *strains*

Analysis of the distribution of virulence-associated genes in relation to age revealed that no statistically significant differences were found between the age group 0-18 years and the age group >18 years (Table 1).

Table 1: Positive E. coli *strains for virulence-associated genes in relation to age*

	No. (%) of positive strains		
	0–18 years (n = 27)	>18 years (n = 29)	*p* value[1]
Virulence genes			
clbAQ	20 (74)	15 (52)	0.104
cnf1	5 (19)	5 (17)	1
hlyA	6 (22)	5 (17)	0.742
usp	17 (63)	14 (48)	0.296

[1] Calculated by Fisher's exact test.

The performed analysis of the distribution of virulence-associated genes in relation to gender showed that the studied traits were rather equally distributed among the *E. coli* isolates from males and females (Table 2).

Table 2: Positive E. coli *strains for virulence-associated genes in relation to gender*

	No. (%) of positive strains		
	Female (n = 41)	Male (n = 15)	*p* value[1]
Virulence genes			
clbAQ	25 (61)	10 (67)	0.764
cnf1	9 (22)	1 (7)	0.259
hlyA	10 (24)	1 (7)	0.255
usp	21 (51)	9 (60)	0.763

[1] Calculated by Fisher's exact test.

DISCUSSION

As our analysis showed that more than 60% of the studied isolates belonged to either the B2 or D phylogenetic groups when studied with triplex PCR method[8], and almost 50% of isolates belonged to the B2 phylogenetic group when studied with extended quadruplex PCR method[9], it can be assumed that the majority of the tested isolates exhibit a high virulence potential[4]. Accordingly, a high prevalence of several virulence-associated genes associated with the B2 group was detected. Thus, many of the studied strains harbored genes characteristic of ExPEC. Due to morphological differences and differences in dynamics (transit time), related to gender as well as age[14, 15], we expected to find several statistically significant associations in relation to host gender and age. However, in our study, among isolates from males and females no statistically significant differences were found (Table 1 and Table 2). To conclude, the review article Starčič Erjavec and Žgur-Bertok, 2015[16] showed that there is high virulence potential for extraintestinal infections among commensal *E. coli* isolated from healthy humans and our study showed that healthy females and males and both age groups have no statistical significant difference in carriage of potential ExPEC.

REFERENCES

1. Kaper J, Nataro J, Mobley H. Pathogenic *Escherichia coli.Nat. Rev. Microbiol*. **2004**, 2, 123–140.

2. Tenaillon O, Skurnik D, Picard B, Denamur E. The population genetics of commensal *Escherichia coli*. *Nat. Rev. Microbiol*. **2010**, 8, 207–217.

3. Russo T, Carlino U, Johnson J. Identification of a new iron-regulated virulence gene, *ireA*, in an extraintestinal pathogenic isolate of *Escherichia coli*. *Infect. Immun.* **2001**, 69, 6209–6216.

4. Picard B, Garcia J, Gouriou S, Duriez P, Brahimi N, Bingen E, Elion J, Denamur E. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. *Infect. Immun.* **1999**, 67, 546–553.

5. Fajs L, Jelen M, Borić M, Đapa T, Žgur-Bertok D, Starčič-Erjavec M. The discriminative power in determining genetic diversity of *Escherchia coli* isolates: Comparing ERIC-PCR with AFLP. *Afr. J. Microbiol. Res.* **2013**, 70, 20, 2416-2419.

6. Le Bouguenec C, Archambaud M, Labigne A. Rapid and specific detection of the *pap*, *afa*, and *sfa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. *J. Clin. Microbiol.* ***2009,*** 30, 1189–1193.

7. Hill J, Beriwal S, Chandra I, Paul V, Kapil A, Singh T, Wadowsky R, Singh V, Goyal A, Jahnukainen T, Johnson J, Tarr P, Vats A. Loop-mediated isothermal amplification assay for rapid detection of common strains of *Escherichia coli*. *J. Clin. Microbiol.* **2008**, 46, 8, 2800-2804.

8. Clermont O, Bonacorsi S, Bingen A. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *App. Environ. Microbiol.* **2000***,* 66, 10, 4555-4558.

9. Clermont O, Christenson J, Denamur E, Gordon D. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ. Microbiol. Rep*. **2013**,5, 1, 58-65.

10. Johnson J, Russo T, Tarr P, Carlino U, Bilge S, Vary Jr. J, Stell A. Molecular epidemiological and phylogenetic associations of two novel putative virulence genes, *iha* and *iroN*$_{(E.\ coli)}$, among *Escherichia coli* isolates from patients with urosepsis, *Infect. Immun.* **2000**, 68, 3040–3047.

11. Johnson J, Stell A, Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise., *J. Infect. Dis*. **2000**,181, 261–272.

12. Schubert S, Rakin A, Karch H, Carniel E, Heesemann J. Prevalence of the "high-pathogenicity island" of *Yersinia* species among *Escherichia coli* strains that are pathogenic to humans. *Infect. Immun*. **1998**, 66, 480–485.

13. Starčič Erjavec M, Arbiter T, Žgur-Bertok D. Pathogenicity islands, plasmids and iron uptake systems in extraintestinal pathogenic *Escherichia coli* strains*. Acta biol. slov*. **2009**, 52, 73–83.

14. Graff J, Brinch K, Madsen J. Gastrointestinal mean transit times in young and middle-aged healthy subjects. *Clin. Physiol*. **2001**, 21, 253–259.

15. Hounnou G, Destrieux C, Desmé J, Bertrand P, Velut S. Anatomical study of the length of the human intestine. *Surg. Radiol. Anat*. **2002**, 24, 290–294.

16. Starčič Erjavec M, Žgur-Bertok D. Virulence potential for extraintestinal infections among commensal *Escherichia* coli isolated from healthy humans – the Trojan horse whitin our gut. *FEMS Microbiol. Lett*. **2015**, 362, 1-9.

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Abstract

NOVEL MOLECULAR-GENETIC DIAGNOSTIC TEST FOR JAK2 NEGATIVE ERYTHROCYTOSIS

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ABSTRACT

Erythrocytosis is heterogeneous group of disorders characterized by the expansion of the erythrocyte compartment including elevated red blood cell (RBC) number, haematocrit, and haemoglobin content in the peripheral blood. Familial erythrocytosis (FE) is a group of rare congenital disorders with various genetic background. Erythropoietin receptor gene (*EPOR*) mutations are the indicator for primary familial erythrocytosis. Secondary erythrocytosis syndromes are typically associated with a defect in various genes included in oxygen sensing pathway that leads to the increased erythropoietin production (*1*). Current diagnostic procedure in Slovenia enables exclusion of *JAK2* gene mutations, the cause of polycythaemia vera (PV). Our study was the basis of new molecular-genetic test for *EPO* and *EPOR* genes in *JAK2* negative erythrocytosis.

Sequence analysis of *EPO* promoter and 3' enhancer and *EPOR* exon 8 was performed on two related patients with erythrocytosis with unknown cause.

Primary familial erythrocytosis due to *EPOR* mutation was excluded by sequence analysis in both patients. So far, 24 mutations in *EPOR*, located in exon 8, have been associated with erythrocytosis. Mutations are leading to cytoplasmic truncation of the receptor and loss of the C-terminal negative regulatory domain (*2*).

However, sequence analysis revealed mutation in 3' enhancer region of the *EPO* gene in both patients, previously described in blood donors with upper limit haematocrit. The role of erythropoietin in erythrocytosis is indirect and previously had not been linked to the disease.

New molecular-genetic test for analysis of the *EPO* (promoter and 3' enhancer) and *EPOR* (exon 8) mutations was successfully implemented in clinical use.

REFERENCES

1. G. Lee, M. O. Arcasoy, The clinical and laboratory evaluation of the patient with erythrocytosis. *Eur J Intern Med* **26**, 297-302 (2015).
2. C. Bento *et al.*, Genetic basis of congenital erythrocytosis: mutation update and online databases. *Hum Mutat* **35**, 15-26 (2014).

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Abstract

POLYMORPHISM rs1056837 IN *CYP1B1* ENCODING AN ESTROGEN METABOLIZING ENZYME PRESENTS A RISK FOR ULM AMONG SLOVENIAN WOMEN

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ABSTRACT

Uterine leiomyomas (ULM) are the most common benign uterine neoplasms affecting a majority of reproductive aged women[1]. The aetiology of ULM is poorly understood, especially the molecular bases for these tumors. Several studies suggest that ULM are influenced by genetic risk factors[2, 3, 4]. One of the molecular mechanisms that may be involved are polymorphisms of genes involved in estrogen synthesis and/or metabolism (*CYP1A1*, *CYP2A13*, *CYP1B1* and *COMT*) [2, 5].The aim of our study was to investigate whether polymorphisms in the selected genes, coding for *CYP1A1* (rs1799814), *CYP2A13* (rs13809886), *CYP1B1* (rs1056837) and *COMT* (rs4680) are associated with ULM susceptibility in Slovenian women with solitary and multiple leiomyomas.

We investigated 180 women with clinically diagnosed ULM and 134 controls from Slovene population. Genotypization of rs1799814, rs13809886, rs1056837 and rs4680 was performed using polymerase chain reaction followed by either restriction fragment length polymorphism or high-resolution melting analyses. When examining genotype frequencies of all patients and general control, AA genotype of rs1056837 was associated with ULM susceptibility compared to AG and GG genotypes in dominant genetic model (p = 0.0001; OR = 3.315; 95% CI 1.788-6.146). Conversely, in recessive genetic model, GG genotype was associated with ULM susceptibility compared to AG and AA genotypes (p = 0.026; OR = 2,033; 95% CI = 1.099-3.761). Analysis of associations between SNPs and clinical data within particular ULM patient group revealed that multiple ULM patients with AA genotype of were more likely to have previous inflammation of genital tract (p = 0.002; OR = 0.0262; 95% CI = 0.112-0.613). There were no statistically significant associations of other polymorphisms.

In our study we noted a significant difference between patients with ULM and general subjects in *CYP1B1* rs10568 regarding expression of AA and GG genotypes. Results suggest co-dominant influence of alleles A and G on ULM phenotype where only a combination of both alleles have protective function from ULM development.

REFERENCES

1. Segars JH, Parrott EC, Nagel JD, Guo XC, Gao X, Birnbaum LS et al. Proceedings from the Third National Institutes of Health International Congress on Advances in Uterine Leiomyoma Research: comprehensive review, conference summary and future recommendations. *Hum Reprod Update.* **2014**, 20(3), 309-33.

2. Bulun SE. Uterine fibroids. *N Engl J Med*. **2013**, 3;369(14), 1344-55.

3. Blake RE. Leiomyomata uteri hormonal and molecular determinants of growth. *J Natl Med Assoc*. **2007,** 99(10),1170-84.

4. Pakiz M, Potocnik U, But I. Solitary and multiple uterine leiomyomas among Caucasian women: two different disorders? *Fertil Steril*. **2010**, 94(6), 2291-5.

5. Walker CL, Stewart EA. Uterine fibroids: the elephant in the room. *Science.* **2005**, 308, 1589–92.

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Abstract

A POLYMORPHISM LOCATED IN 3'UTR OF *PITHD1* NEAR CANNABINOID RECEPTOR 2 GENE *CNR2* IS ASSOCIATED WITH SEVERE FORMS OF CROHN'S DISEASE AND *CNR1* GENE EXPRESSION

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ABSTRACT

Inflammatory bowel disease (IBD) is a chronic inflammatory and autoimmune disease of gastrointestinal tract. Crohn's disease (CD) and ulcerative colitis (UC) are the principal types of inflammatory bowel disease. CD patients that do not respond to standard therapy (corticosteroids, aminosalicylates, immunosuppressives, etc.) need treatment with biological drugs. The so-called 'refractory CD patients' can be regarded as a yet another subtype of IBD. The search for molecular biomarkers that would identify individuals refractory to standard therapy is mandatory to save costs on therapeutics as well as to improve patient's well-being. Several reports have suggested that the endocannabinoid system (ECS) is involved in pathology of intestinal inflammation[1] and that mRNA expression of cannabinoid receptor 2 gene *CNR2* correlates with the seriousness of symptoms[2]. A single nucleotide polymorphism (SNP) rs4237 located in the 3' untranslated region of *PITHD1* and in the vicinity of *CNR2*, was reported to be an expression quantitative trait locus (eQTL) of *CNR2* mRNA expression levels[3].

Therefore, we tested the hypothesis that rs4237 and *CNR2* mRNA expression are specifically associated with refractory CD but not with other types of IBD and that rs4237 is associated with *CNR2* mRNA expression. We studied case-control cohorts of 276 healthy individuals, 61 UC and 113 CD patients on standard therapy and 119 refractory CD patients on adalimumab (Humira®, Abbot Laboratories) therapy. Relative mRNA expressions of *CNR1* and *CNR2* were measured in PBMCs with qPCR using -ΔΔCq method[4]. Genotyping was performed by High Resolution Melting (HRM) analysis.

Between-cohort analyses confirmed that rs4237 was associated only with refractory CD ($p = 3.50 \times 10^{-2}$; odds ratio = 0.435; 95% confidence interval: 0.204-0.924) but not with other types of IBD. According to the recessive genetic model, individuals with CT or TT genotypes were more likely to be refractory to standard therapy (28.9%) compared to individuals with CC genotype (15.0%). *CNR1* and *CNR2* mRNA expressions differed significantly between healthy individuals and refractory CD patients ($p = 1.66 \times 10^{-9}$ and $p = 5.37 \times 10^{-21}$, respectively). In refractory patients, median *CNR1* and *CNR2* mRNA expressions were lower (-1.236 ± 2.006 and -1.586 ± 2.071, respectively) compared to healthy individuals. Within-cohort analyses confirmed eQTL relation between rs4237 and *CNR1* mRNA expression ($p = 2.10 \times 10\text{-}2$) specifically for refractory CD but not for other types of IBD. According to the recessive genetic model, refractory CD patients with CT or TT genotypes had lower median *CNR1* mRNA expression (-1.510 ± 1.795) compared to patients with CC genotype (-0.266 ± 4.064).

Our results suggest that CT or TT genotype of rs4237 in *PITHD1* as well as 2- and 3-fold lower *CNR1* and *CNR2* mRNA expressions, respectively, increase the risk for refractory CD. In addition, eQTL relation between rs4237 and *CNR1* mRNA expression was established for refractory CD patients.

REFERENCES

1. Fichna J, Bawa M, Thakur GA, et al. Cannabinoids alleviate experimentally induced intestinal inflammation by acting at central and peripheral receptors. PLoS ONE 2007, 9(10), e109115.
2. Wright KL, Duncan M, Sharkey KA. Cannabinoid CB2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation. Br. J. Pharmacol. 2008, 153, 263-270.
3. Dixon AL, Liang L, Moffatt MF et al. A genome-wide association study of global gene expression. Nat. Genet. 2007, 39, 1202-7.
4. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001, 25, 402-408.

ABSTRACTS and PAPERS of POSTERS

POPULATION GENETICS & GENOMICS

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Genotypization and morphological characterization of six Slovenian landraces of proso millet (*Panicum miliaceum* L.)

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Abstract

DISCOVERY OF SMALL RNAs IN *Verticillium nonalfalfae*

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ABSTRACT

RNA interference is an evolutionary conserved eukaryotic mechanism regulating gene expression post-transcriptionally[1]. The process of gene regulation is mediated by small RNAs[2], including microRNA-like small RNAs (milRNAs), recently shown to exist in filamentous fungi[3,4,5,6]. However, to date no milRNAs have been reported in the phyto-pathogenic fungus *Verticillium nonalfalfae*, a soil borne plant pathogen causing vascular wilt in many important crops worldwide. Two pathotypes of *V. nonalfalfae*, mild strain and lethal strain, have been isolated from Slovenian hop fields, with the lethal strain causing severe symptoms in hop plants, resulting in complete dieback of the plant[7]. With the accessible genome sequence and detailed transcriptome of the two strains, we aim to identify milRNAs in of *V. nonalfalfae* and to elucidate their possible involvement in the pathogenesis process. Discoveries of novel microRNAs correlate with the advent of new NGS sequencing and bioinformatics technologies, also applied for the purposes of this study. Two Slovenian isolates, Rec (mild) and T2 (lethal), were acquired from the fungal genebank of the Slovenian Institute for Hop Research and Brewing. Small RNA fractions were isolated from four different sources: spores, mycelia, mycelia grown on simulated xylem fluid medium (SXM) and resting mycelia and used for small RNA library construction and sequenced using the Ion Proton sequencing platform. Fungal milRNA precursors were predicted using MIReNA software[8] and the results were further validated and candidates selected manually using criteria for plant miRNA candidates. Several candidate milRNA precursors were selected for both pathotypes and have now been validated with stem-loop RT-PCR.

REFERENCES

1. Hannon GJ. RNA interference. *Nature* **2002**, 418, 6894, 244-51.
2. Hamilton AJ, Blaucombe DC. A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* **1999**, 286, 5441, 950-2.
3. Zhou J, Fu Y, Xie J, Li B, Jiang D, Li G, Cheng J. Identification of microRNA-like RNAs in a plant pathogenic fungus *Sclerotinia sclerotiorum* by high-throughput sequencing. *Mol Genet Genomics* **2012**, 287, 4, 275-82.
4. Chen R, Jiang N, Jiang Q, Sun X, Wang Y, Zhang H, Hu Z. Exploring microRNA-like small RNAs in the filamentous fungus *Fusarium oxysporum. PLoS One* **2014**, 9, 8, e104956.
5. Mueth NA, Ramachandran SR, Hulbert SH. Small RNAs from the wheat stripe rust fungus (*Puccinia striiformis* f.sp. tritici). *BMC Genomics* **2015**, 16, 718.
6. Liu T, Hu J, Zuo Y, Jin Y, Hou J. Identification of microRNA-like RNAs from *Curvularia lunata* associated with maize leaf spot by bioinformation analysis and deep sequencing. *Mol Genet Genomics* 2016, 291, 2, 587-96.
7. Radišek S, Jakše J, Simončič A, Javornik B.Characterization of *Verticillium albo-atrum* Field Isolates Using Pathogenicity Data and AFLP Analysis. *Plant Disease* **2003**, 87, 6, 633-638.
8. Mathelier A ,Carbone A. MIReNA: finding microRNAs with high accuracy and no learning at genome scale and from deep sequencing data. *Bioinformatics* **2010**, 26, 18, 2226-34.

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Abstract

NEW METHOD FOR STATISTICAL PATTERN RECOGNITION USAGE IN CANCER ASSOCIATED microRNAs

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ABSTRACT

MicroRNAs (miRNAs) are small 22-25 nucleotides long noncoding RNA molecules that regulate gene expression and that are present and stable in the bloodstream. They are evolutionally conserved, and control gene expression in metazoan animals, plants, viruses, and bacteria primarily at post-transcriptional and transcriptional levels. In this study, we present a novel 2D graphical representation of miRNAs sequences based on graph theory. Graph theory is one of most tools in mathematics, which are used in many cases, because we can simple present very complex problems. In recent years, a very large variety of statistical methodologies, at various levels of complexity, have been put forward to analyse genotype data and detect genetic variations that may be responsible for increasing the susceptibility to cancer. In this article, we present new method for statistical pattern recognition. Statistical pattern recognition relates to the use of statistical techniques for analysing data measurements in order to extract information and make justified decisions. We considered construction of n × 4 matrix to represent DNA primary sequences. Four column of matrix present 4 nucleotides of DNA base. First column present A, second C, third G and last column present T. We traveled through DNA sequences to determinate each nucleotide from DNA base in matrix. Denoted nucleotides present vertex of new graph. We calculated topological properties of this graph and compared it for different genomic miRNAs sequences. The novelty of graphical representations of miRNAs sequences is very useful for prediction cancer association. We tested the proposed method on hsa-mir-423, hsa-mir-27a. The importance of topological modeling of miRNAs is distinguished, as it presents simplified visual characteristics for the rapid and efficient modeling, comparison of miRNAs and researching effort to establish their roles in cancer.

REFERENCES

1. Gregory, SG; Barlow, KF; McLay, KE; Kaul, R; Swarbreck, D; Dunham, A; Scott, CE; Howe, KL; et al. (2006). "The DNA sequence and biological annotation of human chromosome 1". Nature. 441 (7091): 315–21. Bibcode:2006Natur.441..315G. doi:10.1038/nature04727. PMID 16710414.

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Article

IDENTIFICATION OF NON-SYNONYMOUS POLYMORPHISMS WITHIN REGIONS CORRESPONDING TO PROTEIN INTERACTION SITES

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Background: Protein-protein interactions (PPI) play an important role in function of all organisms and enable understanding underlying metabolic processes. Predictions of protein interactions are an important aspect in proteomics, as experimental methods may result in high degree of false positive results and are more expensive than computational predictions. Prediction of PPIs has been a topic of increased interest in the last few years. Although there are many databases collecting predicted PPIs, exploration of genetics information underlying PPI interactions has not been investigated thoroughly. The aim of the present study was to identify: 1. genomic locations corresponding to regions involved in predicted interaction sites, 2. Non-synonymous polymorphisms within interacting sites (PPI-SNPs), 3. examples in which predicted PPIs sites overlap with previously reported experimentally validated interactions. Methods: Datasets representing predicted PPIs were obtained from PiSITE database (http://pisite.hgc.jp). Non-synonymous polymorphisms mapped on protein structural data (PDBs) were obtained from the UCSC's ftp server. Polymorphism locations on protein structures were mapped to predicted PPI regions in order to identify PPI-SNPs. Locations of experimentally validated PPIs were extracted from published literature (http://www.ncbi.nlm.nih.gov/pubmed). Location of polymorphisms on 3D protein structures was visualized using PyMol software. Results: We developed a scalable method for identification of PPI-SNPs, which was further used to identify 662 polymorphisms located within the predicted PPI sites that map to 196 genes that are associated with different diseases, mainly with different types of cancer. Additionally, 13 polymorphisms in seven genes within predicted PPIs are also located within experimentally validated interaction regions. Conclusions: The present study presents novel SNP prioritization approach for functional studies and potential candidates for disease biomarkers.

INTRODUCTION

The study of protein interactions (PIs) provides insights into biological processes. Proteins can interact with DNA, smaller ligands, RNA or with other proteins (protein-protein interactions; PPIs). Protein interaction malfunctions can result in loss of phenotypic trait, which many times results in a disease[1]. Relevance of protein interaction prediction is situated on the fact, that obtaining empirical evidence in great quantity is currently a challenging task. Additionally, accuracy of high-throughput experiments still yields many false-positive and false-negative results[2]. Using the data of predicted protein interactions many a times provides additional evidence in explaining various biological phenomena[3], or facilitate planning experimental validation of experimental PPIs[4]. Use of modern prediction techniques such as machine learning, graph theory, Bayesian prediction and pattern recognition have been used in tools, such as Struct2Net[5], PiPs[6], HitPredict[7], PRISM[8], DOMINE[9], ProBis[10], and PiSITE[11].

Previous studies on domain altering SNPs in human proteome[12] identified many polymorphisms which alter specific protein domains and can therefore have clinically significant effects. Non-synonymous polymorphisms (nsSNP) have been shown to cause pathological effects, for example in hypercholesterolemia[13], incident coronary heart disease[14], breast cancer[15] and many other types of cancer.

Prediction of the effect of amino acid substitution on protein function is an important task in systems biology. One of the most widely used tools in this field is SIFT algorithm, which uses homology based approach to identify amino acid loci, which could potentially affect protein function. SIFT tool has already been successfully used to analyze effects of possible cancer mutations[16]; yet identified mutations were not directly associated with protein-protein interaction sites. However, significant 3D clustering of missense mutations in several previously known oncoproteins has been detected[17]. In our previous study we reanalyzed genomic regions involved in interactions between transcription factor and its targets and identified polymorphisms located within hypoxia response elements (HREs)[18].

Although there are already many datasets providing predicted PPI interactions, those predictions remain in the protein domain – they are not associated with underlying nucleotide sequence and genetic variability. To our knowledge, there are no reported studies related with polymorphisms located within regions corresponding to predicted protein-protein interaction information performed for nsSNPs, mapped onto protein structure (PDB)[16].
Protein-protein interaction data used in this study was obtained from the PiSITE database. Interactions were predicted using BLAST queries, residue specific interactions were identified based on distance criteria, where distance between residues needed to be smaller than 4 Å[11]. The aim of this study was to identify nsSNPs, occurring within predicted protein-protein interaction sites.

METHODS

Non-synonymous polymorphisms mapped onto PDB structures (n=11551) were obtained from UCSC institute's ftp server[19]. Regions involved in predicted protein-protein interactions were obtained from PiSITE[11]. Mutations' datasets were preprocessed and further merged using algorithms, implemented in R language (https://www.r-project.org/). Using Python scripting (https://www.python.org/), we extracted all interactions from the PiSITE dataset, consisting of 110325 chains. Association of genes with diseases was analyzed using functional annotation tool DAVID (https://david.ncifcrf.gov/home.jsp). The predicted protein-protein interaction interfaces were further analyzed for overlap with experimentally validated interactions in previously published literature (http://www.ncbi.nlm.nih.gov/pubmed). In addition, we selected nsSNPs that are located within predicted as well as experimentally validated PPI regions. Selected results were visualized using open-source PyMol software (https://sourceforge.net/projects/pymol/).

RESULTS

In the present study we identified nsSNPs located within regions corresponding to predicted protein-protein interaction interfaces. For some selected cancer genes we also confirmed that predicted interaction sites overlap with previously reported experimentally validated interaction sites. Main workflow of the process is presented in **Figure 1**.

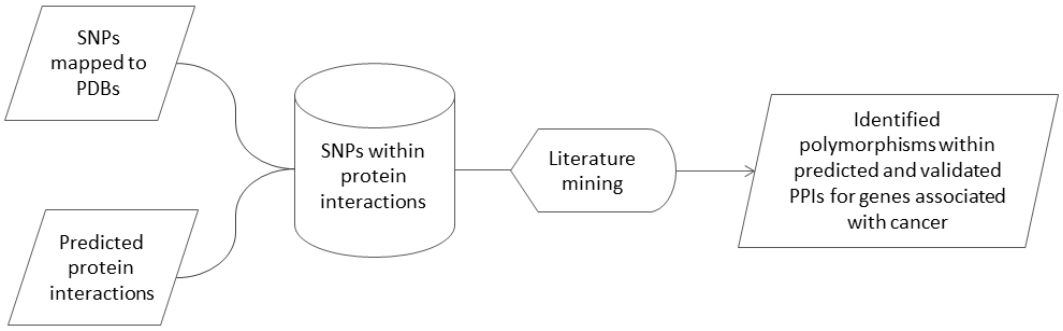

Figure 1: Main workflow of the study.

Mapping of nsSNPs to predicted interaction sites resulted in the catalog of 662 nsSNPs with matching reference SNP ID (rs) accession numbers from dbSNP. Collected 662 PPI-SNPs within predicted interaction sites map to 196 protein-coding genes. Further data processing revealed that from 196 identified genes, 48 are statistically significant associated with at least one type of cancer. Additionally, genes were also associated with Alzheimer's disease, rheumatoid arthritis, diabetes type 1 and type 2, asthma, cardiovascular disease, cholesterol, depression, and Crohn's disease. Ten cancer associated genes with the highest number of SNPs located within predicted PPIs are presented in **Table 1**.

Table 1: Ten genes with the highest number of SNPs, located within predicted PPIs..

Gene name	CFTR	TCAP	XRCC5	FGFR2	MICA	SERPINA1	VHL	TP53	BRCA1
Number of PPI-SNPs	*5*	*5*	*5*	*6*	*6*	*8*	*8*	*10*	*15*
rs SNP ID numbers (examples)	*rs74571530* *rs35032490* *rs1800100* *rs80055610* *rs11531593*	*rs45495192* *rs45458802* *rs17851031* *rs45614536* *rs45513698*	*rs1805380* *rs11558396* *rs2287558* *rs11575909* *rs61758380*	*rs55977237* *rs755793* *rs77543610* *rs4647921* *rs79184941* *rs55689343*	*rs3819268* *rs17200179* *rs17206575* *rs17206589* *rs17200207* *rs17200186*	*rs17580* *rs28929471* *rs28929473* *rs11575873* *rs1131154* *rs55819880* *rs72552401* *rs12077*	*rs17855706* *rs5030812* *rs5030818* *rs28940297* *rs5030811* *rs5030804* *rs5030805* *rs5030822*	*rs28934875* *rs28934572* *rs28934575* *rs11540652* *rs28934573* *rs28934576* *rs28934578* *rs28934571* *etc.*	*rs80357316* *rs80357031* *rs80357054* *rs80357017* *rs80357406* *rs80357438* *rs1800062* *rs80356929* *etc.*

Legend: CFTR: Cystic fibrosis transmembrane conductance regulator; TCAP: Telethonin, XRCC5: X-ray repair cross-complementing protein 5, FGFR2: Fibroblast growth factor receptor 2, MICA: MHC class I polypeptide-related sequence A, SERPINA1: Alpha-1-antitrypsin, VHL: von Hippel-Lindau tumor suppressor; TP53: tumor protein p53; BRCA1: BRCA1, DNA repair associated.

Using data from the literature we found 7 genes in which predicted PPI sites also corresponded to experimentally validated interaction sites: *TCAP*, *APOA1, CRP*, *ESR1*, *TP53, VHL*, and *SHFM1*. Out of 662 PPI-SNPs 13 nsSNPs are present within predicted and validated PPIs (**Table 2**).

Table 2: Identified nsSNPs in genes associated with cancer that are located within predicted as well as experimentally validated protein-protein interaction interfaces.

Gene name	***rs SNP ID numbers***	**Associated cancer type (According to DAVID functional annotation analysis).**	**Interaction sites found in the literature**
TCAP	rs45495192 rs45614536 rs45513698	breast cancer	**1–53** (binding to the very N-terminus of titin[20])
APOA1	rs28931573	colorectal cancer	**161–240** (binding to NDRG1[21])
CRP	rs34200896	lung cancer	**109-132** (constitutive binding region[22])
ESR1	rs79374934	bone cancer, breast cancer, colon cancer, endometrial cancer, prostate cancer, uterine cancer etc.	**185–595** (binding to MUC1-CD[23]) **410–731** (binding to HPIP[24])
TP53	rs28934576	bladder cancer, leukemia, lung cancer, brain cancer, breast cancer, cervical cancer, stomach cancer etc.	**256 – 294** (Binding to p120E4F[25])
VHL	rs17855706 rs5030812 rs5030818 rs28940297	brain cancer, breast cancer, renal cancer, kidney cancer	**100-155** (binding to TRiC[26]) **157–172** (binding to elongin BC[26]),
SHFM1	rs11553943 rs1802882	breast cancer	**7-25**, **37-63** (binding to BRCA2[27])

For example, in *VHL* gene there are four nsSNPs within predicted PPI and also located within previously experimentally validated interaction regions. Graphic presentation for *VHL* gene is presented in **Figure 2**. Polymorphisms are located on locus 110 (rs17855706), 115 (rs5030812), 161 (rs5030818) and 163 (rs28940297). Experimentally validated PPI sites encompass amino acids 100-155 and 157-172[26].

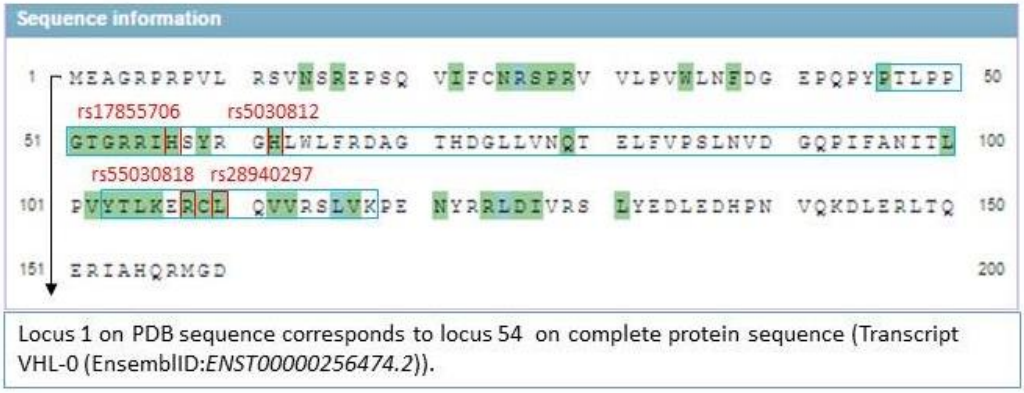

Figure 2: Visualization of polymorphisms within predicted and experimental interaction regions in *VHL* gene (PDB accession 1lm8, chain V) on modified figure from PiSITE server. Predicted PPI region is marked with green color and experimental PPI region with blue line. Polymorphisms are marked with red boxes.

Three dimensional visualization of *VHL* gene and four polymorphisms located within predicted and validated interaction sites are presented in **Figure 3**.

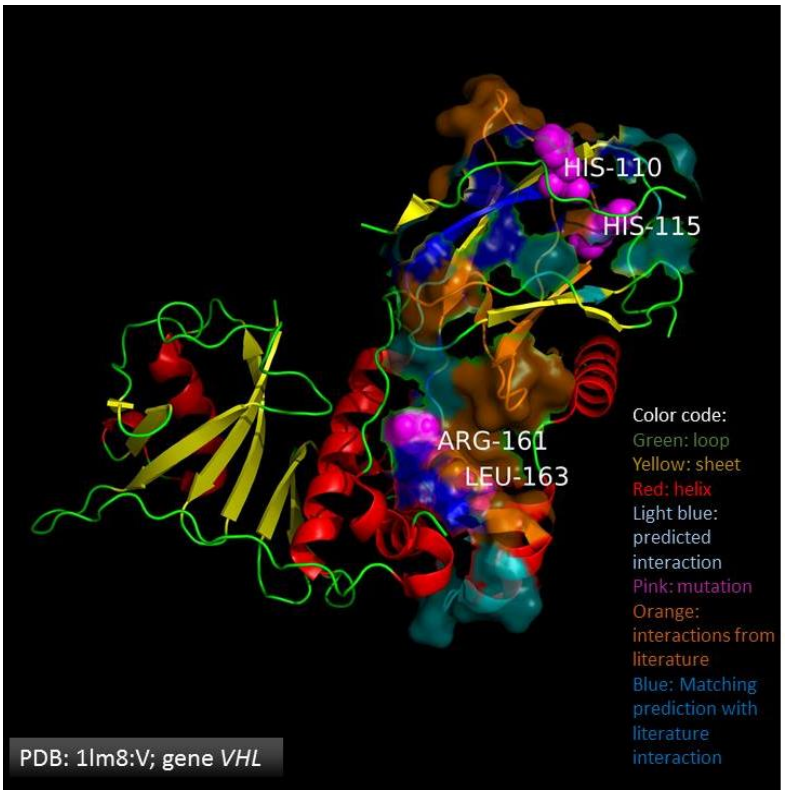

Figure 3: Representation of different layers of interaction information in this study on example protein *VHL*. Four polymorphisms located within both, predicted and experimentally validated interaction sites are marked in pink color: rs17855706 (His110Tyr), rs5030812 (His115Arg), rs5030818 (Arg161Ter) and rs28940297 (Leu163Pro).

DISCUSSION

Identification of nsSNPs located within predicted protein-protein interaction sites provides additional information on biological phenomena and enables more detailed explanation of disease development. Within locations of predicted protein interactions we identified 662 polymorphisms mapping to 196 genes, which showed significant clustering in terms of cancer and other disease associations. The

results of the present study are in concordance with an observation by Kamburov et al. (2015), who proved significant clustering patterns of polymorphisms emerged in cancer related protein interactions. Oncoproteins *VHL*, *TP53* and *BRCA1* were in the present study additionally confirmed as proteins with the highest number of predicted PPI-SNPs. In addition, regions involved in predicted PPI also overlapped with experimentally validated interactions in seven proteins, including VHL and TP53. By literature mining we found two interaction sites for protein VHL. VHL binds to elongin BC via a linear sequence known as "BC box", consist of amino acids 157–172[26]. We have detected two nsSNPs (rs5030818, rs28940297) located within this region, which is known as a hot spot for disease-causing inherited point mutations[26]. Furthermore we have identified two nsSNPs (rs17855706, rs5030812) in the region of VHL comprising amino acids 100-155, that mediates TRiC binding[26]. Within previously reported experimentally validated interaction region of oncoprotein TP53, we have identified one nsSNP (rs28934576). This TP53 region is important for binding to the E1A-regulated transcription factor p120E4F, a transcriptional repressor of the adenovirus E4 promoter. The interaction involves carboxy-terminal half of p120E4F and sequences located at the end of the sequence-specific DNA-binding domain of TP53, which encompassing amino acids 256 – 294[25].

Similarly to our previous SNP prioritization studies in which we collected polymorphisms located within miRNA genes[28] and within transcription factor-target interaction[18] in the present study we collected polymorphisms, located within predicted PPI sites, associated with cancer risk. Additionally we have also confirmed that predicted interaction sites overlap with experimentally validated interactions in previously published literature. In conclusion, we identified 13 nsSNPs located within both, predicted and previously experimentally validated interaction sites that map to seven cancer associated genes. In addition, an investigation whether other identified nsSNPs within PPI sites are associated with cancer should also follow. The results of the present preliminary study could serve as a basis for planning more targeted experimental designs, functional experiments and might present novel drug targets and therapeutic strategies for cancer.

REFERENCES

1. Gonzalez M W, Kann M. G. Chapter 4: Protein interactions and disease. *PLoS Comput Biol*. **2012**, 8, 12.
2. Rao V S, Srinivas K, Sujini G N, Kumar G N. Protein-protein interaction detection: methods and analysis. *Int J Proteomics*. **2014**.
3. Zahiri J, Bozorgmehr J H, Masoudi-Nejad A. Computational prediction of protein-protein interaction networks: algorithms and resources. *Curr Genomics.* **2013**, 14, 6, 397-414.
4. Jessulat M, Pitre S, Gui Y, Hooshyar M, Omidi K, Samanfar B et al. Recent advances in protein-protein interaction prediction: experimental and computational methods. *Expert opinion on drug discovery*. **2011,** 6, 9, 921-935.
5. Singh R, Park D, Xu J, Hosur R, Berger B. Struct2Net: a web service to predict protein-protein interactions using a structure-based approach. *Nucleic Acids Res*. **2010**, 38, 508-515.
6. McDowall M D, Scott M S, Barton G J. PIPs: human protein-protein interaction prediction database. *Nucleic Acids Res*. **2009**, 37, 651-656.
7. López Y, Nakai K, Patil A. HitPredict version 4: comprehensive reliability scoring of physical protein-protein interactions from more than 100 species. *Database (Oxford)*. **2015**.
8. Baspinar A, Cukuroglu E, Nussinov R, Keskin O, Gursoy A. PRISM: a web server and repository for prediction of protein-protein interactions and modeling their 3D complexes. *Nucleic Acids Res.* **2014**, 42, 285-289.
9. Yellaboina S, Tasneem A, Zaykin D V, Raghavachari B, Jothi R. DOMINE: a comprehensive collection of known and predicted domain-domain interactions. *Nucleic Acids Res*. **2011**, 39, 730-735.
10. Konc J, Janežič D. ProBiS-ligands: a web server for prediction of ligands by examination of protein binding sites. *Nucleic Acids Res*. **2014**, 42, 215-220.
11. Higurashi M, Ishida T, Kinoshita K. PiSite: a database of protein interaction sites using multiple binding states in the PDB." *Nucleic acids research*. **2009**, 37, 360-364.
12. Liu Y, Tozeren A. Domain altering SNPs in the human proteome and their impact on signaling pathways. *PLoS One*. **2010**, 5, 9.

13. Angarica V E, Orozco M, Sancho J. Exploring the complete mutational space of the LDL receptor LA5 domain using molecular dynamics: linking SNPs with disease phenotypes in familial hypercholesterolemia. *Hum Mol Genet*. **2016**, 25, 6, 1233-1246.
14. Jensen M K, Pers T H, Dworzynski P, Girman C J, Brunak S, Rimm E B. Protein interaction-based genome-wide analysis of incident coronary heart disease. *Circ Cardiovasc Genet*. **2011**, 4, 5, 549-556.
15. Milne R L, Burwinkel B, Michailidou K, Arias-Perez J I, Zamora M P, Menéndez-Rodríguez P et al. Common non-synonymous SNPs associated with breast cancer susceptibility: findings from the Breast Cancer Association Consortium. *Hum Mol Genet*. **2014**, 23, 22, 6096-6111.
16. Kumar P, Henikoff S, Ng P C. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nature protocols*. **2009**, 4, 7, 1073-1081.
17. Kamburov A, Lawrence M S, Polak P, Leshchiner I, Lage K, Golub T R et al. Comprehensive assessment of cancer missense mutation clustering in protein structures. *Proc Natl Acad Sci U S A*. **2015**, 112, 40, 5486-5495.
18. Slemc L, Kunej T. Transcription factor HIF1A: downstream targets, associated pathways, polymorphic hypoxia response element (HRE) sites and initiative for standardization of reporting in scientific literature. *Tumor Biology*. In press.
19. Ryan M, Diekhans M, Lien S, Liu Y, Karchin R. LS-SNP/PDB: annotated non-synonymous SNPs mapped to Protein Data Bank structures. *Bioinformatics*. **2009**, 25, 11, 1431-1432.
20. Pinotsis N, Petoukhov M, Lange S, Svergun D, Zou P, Gautel M et al. Evidence for a dimeric assembly of two titin/telethonin complexes induced by the telethonin C-terminus. *J Struct Biol*. **2006**, 155, 2, 239-250.
21. Hunter M., Angelicheva D, Tournev I, Ingley E, Chan D C, Watts G F et al. NDRG1 interacts with APO A-I and A-II and is a functional candidate for the HDL-C QTL on 8q24. *Biochem Biophys Res Commun*. **2005**, 332, 4, 982-992.
22. Zhang J, Yang L, Ang Z, Yoong S L, Tran T T, Anand G S et al. Secreted M-ficolin anchors onto monocyte transmembrane G protein-coupled receptor 43 and cross talks with plasma C-reactive protein to mediate immune signaling and regulate host defense. *J Immunol*. **2010**, 185, 11, 6899-6910.
23. Wei X, Xu H, Kufe D. MUC1 oncoprotein stabilizes and activates estrogen receptor alpha. *Mol Cell*. **2006,** 21, 2, 295-305.
24. Manavathi B, Acconcia F, Rayala S K, Kumar R. An inherent role of microtubule network in the action of nuclear receptor. *Proc Natl Acad Sci U S A*. **2006**, 103, 43, 15981-15986.
25. Sandy P, Gostissa M, Fogal V, Cecco L D, Szalay K, Rooney R J et al. p53 is involved in the p120E4F-mediated growth arrest. *Oncogene*. **2000**, 19, 2, 188-199.
26. Feldman D E, Thulasiraman V, Ferreyra R G, Frydman J. Formation of the VHL-elongin BC tumor suppressor complex is mediated by the chaperonin TRiC. *Mol Cell*. **1999**, 4, 6, 1051-1061.
27. Yang, H, Jeffrey P D, Miller J, Kinnucan E, Sun Y, Thoma, N H et al. BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. *Science*. **2002**, 297, 5588, 1837-1848.
28. Zorc M, Obsteter J, Dovc P, Kunej T. Genetic Variability of MicroRNA Genes in 15 Animal Species. *J Genomics*. **2015**, 3, 51-56.

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Abstract

GENOTYPIZATION AND MORPHOLOGICAL CHARACTERIZATION OF SIX SLOVENIAN LANDRACES OF PROSO MILLET (*Panicum miliaceum* L.)

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ABSTRACT

Proso millet (*Panicum miliaceum* L.) is one of the world's oldest cultivated cereals. It appeared as a staple crop in northern China 10,000 years ago[1] and later spread to other parts of the world, including Slovene territory where it was grown as early as a thousand years B.C. by the Celts[2].

Proso millet has two main advantages over other cereals: firstly, it is considered a health-food crop owing to its unique nutritional value[3] and secondly, it is very tolerant of various abiotic stresses, e.g., salinity and drought. In addition, it can grow well in poor soils[4].

In Slovenia, the cultivation of millet reached its peak in the 19th century, covering 18,000 ha. In 2016, proso millet is cultivated on only around 200 ha, mostly by organic farmers, who use landraces of yellow and white proso millet from Prekmurje and Gorenjska regions, the Austrian cultivar 'Kornberger Mittelfrühe' domesticated in Slovenia as 'Kornberg millet' and, in recent years, also the cultivar 'Sonček'[2].

In the current study, we analysed the genetic diversity of six Slovenian landraces of proso millet together with the cultivar 'Sonček' by utilizing both molecular markers and morphological traits. The use of microsatellites (SSRs), which have proved to be a powerful tool for genetic diversity analyses in various crops, also resulted in high discrimination with proso millet. We successfully amplified 11 SSR loci and, of these, 7 loci were polymorphic. Only accessions of the cultivar 'Sonček' formed a uniform cluster, all other landraces were very diverse. Nevertheless, we were able to differentiate six different clusters of genotypes, representing six landraces, which differ also in the morphology of the panicle. Moreover, yield stability and 1000-seed weight, as well as other morphological characteristics, were determined in a 2-year field experiment, also showing very diverse results between years as well as among samples within some landraces.

1. Lu H, Zhang J, Liu K-B, Wu N, Li Y, Zhou K, Ye M, Zhang T, Zhang H, Yang X. Earliest domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10,000 years ago. *Proc Natl Acad Sci USA*. **2009**, 106, 7367–7372.

2. Kocjan Ačko D. Importance and possibilities of proso millet (*Panicum miliaceum* L.) production for human nutrition and animal feed in Slovenia. *Int. j. food, agric. environment*. **2012**, 10(2), 636-640.

3. Liu M, Qiao Z, Zhang S, Wang Y, Lu P. Response of broomcorn millet (*Panicum miliaceum*L.) genotypes from semiarid regions of China to salt stress. *Crop J*. **2015**, 3, 57–66.

4. Sabir P, Ashraf M, Akram NA. Accession variation for salt tolerance in proso millet (*Panicum miliaceum* L.) using leaf proline content and activities of some key antioxidant enzymes. *J. Agron. Crop Sci.* **2011**, 197, 340–347.

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