



Antifungal and antimicrobial proteins and peptides of potato (*Solanum tuberosum* L.) tubers and their applications

Veronika Bártová¹ · Jan Bárta¹ · Markéta Jarošová¹

Received: 27 February 2019 / Revised: 30 April 2019 / Accepted: 1 May 2019 / Published online: 29 May 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Potato proteins are well known for their nutritional, emulsifying, foaming, gel forming or antioxidant properties that all make from them valuable protein source for food industry. Antifungal, antimicrobial and also antiviral properties, described for potato proteins in the review, enrich the possibilities of potato protein usage. Potato proteins were divided into patatin, protease inhibitors and fraction of other proteins that also included, besides others, proteins involved in potato defence physiology. All these proteins groups provide proteins and peptides with antifungal and/or antimicrobial actions. Patatins, obtained from cultivars with resistance to *Phytophthora infestans*, were able to inhibit spore germination of this pathogen. Protease inhibitors represent the structurally heterogeneous group with broad range of antifungal and antimicrobial activities. Potato protease inhibitors I and II reduced the growth of *Phytophthora infestans*, *Rhizoctonia solani* and *Botrytis cinerea* or of the fungi of *Fusarium* genus. Members of Kunitz family (proteins Potide-G, AFP-J, Potamin-1 or PG-2) were able to inhibit serious pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* or *Candida albicans*. Potato snakins, defensins and pseudothionins are discussed for their ability to inhibit serious potato fungi as well as bacterial pathogens. Potato proteins with the ability to inhibit growth of pathogens were used for developing of pathogen-resistant transgenic plants for crop improvement. Incorporation of potato antifungal and antimicrobial proteins in feed and food products or food packages for elimination of hygienically risk pathogens brings new possibility of potato protein usage.

Keywords Potato tubers · *Solanum tuberosum* · L., Bioactive proteins · Antifungal proteins · Patatin · Protease inhibitors · Potato defensin · Potato snakins

Introduction

Plants represent a rich source of nutrients for many organisms and the co-evolution of plants with their pathogens resulted in the development of structural, chemical and protein-based defences designed to detect invading organisms and to stop them (Yan et al. 2015). A large number of proteins, peptides and enzymes active on the fungal cell walls and on intracellular targets have been characterised (Bártová et al. 2012; Heřmanová et al. 2006). Here, we can distinguish antifungal proteins (AFPs) and antimicrobial proteins (AMPs) (Parachin

and Franco 2014). Some AFPs, however, show antimicrobial activities such as some AMPs can dispose of antifungal activities (Hegedüs and Marx 2013).

The plant AFPs are organised into five major groups based on serological and sequence analyses. Such proteins were first detected in tobacco leaves reacting to tobacco mosaic virus and were therefore named pathogenesis-related (PR) proteins (van Loon and van Kammen 1970). The five groups are as follows: PR-1 (cysteine-rich, small proteins of 15–17 kDa), PR-2 (β -glucanases), PR-3 (chitinases), PR-4 (chitin-binding proteins) and PR-5 (thaumatin-like proteins) (Heřmanová et al. 2006; Theis and Stahl 2004). Following research has revealed other types of AFPs comprising protease inhibitors, ribosome inactivating proteins, ribonucleases, lectins, defensins and defensin-like proteins, thionins, peroxidases, cyclophilin-like proteins, lipid transfer proteins, deoxyribonucleases, embryo-abundant proteins and storage 2S albumin (Ng et al. 2013; Yan et al. 2015). The AMPs and AFPs can be used as powerful tools for crop production as markers for

✉ Veronika Bártová
vbartova@zf.jcu.cz

¹ Plant Production Group, Faculty of Agriculture, University of South Bohemia, Na Sádkách 1780, 370 05, České Budějovice, Czech Republic

the selection of plants with resistance against pathogens or for the construction of genetically modified plants expressing AMPs and AFPs in order to reduce crop losses by pathogen infections (Parachin and Franco 2014; Yan et al. 2015).

In addition, the physiological role of plant AMPs and AFPs in human and animal bodies includes endotoxin neutralisation (Rosenfeld et al. 2010), immunomodulating activities (Wiesner and Vilcinskas 2010), induction of wound repair (Baroni et al. 2009), protection against cancer (Mulder et al. 2013; Parachin and Franco 2014) and protection against human and animal pathogens (Paik et al. 2014). The AMPs and AFPs can be therefore used for the production of antimicrobial drugs and conserving agents for their usage in medicine, cosmetics, food and feed industries (Salas et al. 2015).

This review focuses on AMPs and AFPs isolated from potato tubers, on their classification, structure and function description and on search for their various ways of use.

Potato proteins and peptides with antifungal potential

Potato has achieved a worldwide prominence as a foodstuff in European and American diets and potato consumption goes strongly increasing in Asia, Africa and Latin America. Nowadays, it is the fourth largest crop in terms of area cultivation and is the third most consumed food crop in the world (Mäkinen 2014). However, worldwide distribution of potatoes contributed to the spread of numerous pathogens affecting thus potato productivity and quality. In the USA, 50 fungal, 10 bacterial and 30 viral species have been identified to cause economic losses in potato yield (Oerke et al., 2012). Chemical based protection has maintained the amounts of potato production, although continuous use has also lead to cost and energy consumption, increased health risks or emerging pathogen resistance (Burra 2016). Proteins and peptides active in potato defence with antifungal and antimicrobial activities represent good alternative for disease protection and plant durable resistance establishment.

Proteomic aspects of the plant defence and induce protection to *Phytophthora infestans*, a causal agent of late blight in potatoes, have been often studied, because of economic importance of the pathogen (Burra 2016; Feldman et al. 2000; Fernández et al. 2012; Valueva et al. 1998). The further potato fungi and bacterial pathogens with a studied AFP and AMP background are also *Pectobacterium atrosepticum* (Almasia et al. 2008; Barzic and Com 2012), *Rhizoctonia solani* (Almasia et al. 2008; Feng et al. 2003; Gvozdeva et al. 2006), *Spongopora subterranea* subsp. *subterranea* (Perla et al. 2014), *Alternaria solani* (Feng et al. 2003) and *Ralstonia solanacearum* (Feng et al. 2003; Park et al. 2016) and a new bacterial disease of potato caused by *Candidatus*

Liberibacter solanacearum (CLso, zebra chip) (Kumar et al. 2015).

Potato tuber proteins are commonly classified according to their molecular mass and electrophoretic separation characteristics into three groups, namely patatin, protease inhibitors and other proteins (Bárta et al. 2015; Pots 1999). Tuber proteins and peptide that play a role in the defence against potato pathogens belong to (i) protease inhibitors with predominant role of serine, cysteine and Kunitz-type potato protease inhibitors, (ii) patatin proteins or specific isoforms of patatin proteins and (iii) proteins and peptides of the low molecular mass including potato defensins, thionins and snakins (Bártová et al. 2012; Kim et al. 2009). Structural diversity of potato AMPs and AFPs is presented in Fig. 1. Most of the tuber AFPs could be marked as ‘multifunctional’, displaying various other inhibition activities (antimicrobial, antiviral, insecticidal or antiparasitic).

Besides antifungal and antimicrobial activities, many other usable features were described for potato tuber proteins. They are known for their high nutritional value (Bártová and Bárta 2009; Bártová et al. 2015), gel forming (Delahaije et al. 2015); foaming (Løkra et al. 2008; Lomolino et al. 2015; Schmidt et al. 2018) and emulsifying properties (Romero et al. 2011; Schmidt et al. 2018); antioxidant activities (Liu et al. 2003); and anti-obesity and antidiabetic effects (Ku et al. 2016). Potato tuber proteins can be used for the releasing of bioactive peptides with multiple functions such as the antioxidant (Udenigwe et al. 2016) or antihypertensive effects (Mäkinen et al. 2008). The combination of nutritional, functional and defence properties of potato tuber proteins creates unique combination that confer them a new and broad level of interest and usability.

Protease inhibitors of potato tubers

Potato tubers are very good sources of protease inhibitors (PIs) which protect them against disease, pests and herbivores. In the past, the potato PIs were considered primarily as antinutritional factors. However, potato PIs have retained deep interest in recent years for their already established biological activities. Potato protease inhibitors (PPIs) have been examined as therapeutic agents, primarily to deal with various human cancers (Blanco-Aparicio et al. 1998; Huang et al. 1997; Sitjà-Arnau et al. 2005). In addition, potato PIs can be used to reduce food intake in humans for their ability to increase the level of cholecystokinin via trypsin inhibition (Komarnytsky et al. 2011; Ku et al. 2016); they are known for their foaming properties (Schmidt et al. 2018) and for the solubility within a wide pH range (Wagley et al. 2014). Amongst these, PIs are believed to play an important role in the potato plant physiology. They are part of the wound-induced defence response of potato plant against both microorganisms and herbivores (Kim et al. 2006) and they are involved in the blocking UV

irradiation (Huang et al. 1997; Liu et al. 2001). Potato PIs serve as storage proteins and, perhaps, participate in the regulation of endogenous proteases and in the stabilisation of other storage proteins and starch hydrolysis during seed dormancy (Munger et al. 2015).

The PPIs represent the heterogeneous group of potato tuber proteins (Fischer et al. 2015). These type of proteins are primarily classified based on the enzyme type they inhibit. The classification is also performed based on sequence homology, molecular mass, architecture, number of disulphide bridges, isoelectric points and mechanism of action (Meenu Krishnan and Murugan 2016). Potato PIs can be classified into seven (Pouvreau et al. 2001) or into ten different families (Heibges et al. 2003a). Diversity of potato PIs are presented in Table 1.

Antifungal potential of potato protease inhibitors

Phytopathogenic fungi produce extracellular proteinase playing an active role in the development of plant diseases (Jashni et al. 2015). As response to the attack, plants synthesize inhibitory polypeptides that can suppress the proteinase activities (Kim et al. 2009). This phenomenon was firstly recorded in tomatoes infected by *P. infestans* (Woloshuk et al. 1991), in which increased levels of trypsin and chymotrypsin inhibitors were found to correlate with the plants resistance to the pathogen. Inhibition of fungi proteases create a plant-pathogen interaction based on the anti-feedant effect (Vernekar et al. 2001).

The most common potato inhibitors belong to serine proteases of Kunitz-type inhibitors (PKPIs) and potato protease inhibitors I and II (PPI-I and PPI-II). These groups seem to be the most studied classes of PPIs associated with defence and Table 2 shows antifungal or antimicrobial activities, which have been described for these groups of protease inhibitors.

Potato inhibitor I was mentioned to be regulated by wounding and development (Koiwa et al. 1997). Cleveland et al. (1987) reported the presence of six PPI-I genes in potato cv. Russet Burbank tubers. A minimum of 21 PKPI genes have been found to be presented in the potato genome (Heibges et al. 2003a). In addition, Bauw et al. (2006) reported that PKPIs were an extraordinarily dominant protein component in potato tubers of cv. Kuras. Electrophoretic separation revealed five distinct clades termed KPI A, KPI B, KPI C (Heibges et al. 2003b), KPI K (specific for cv. Kuras) and KPI M (miraculin-like sequence) (Bauw et al. 2006). Inhibitors of groups A and B inhibited fungus *Fusarium moniliforme* (Heibges et al. 2003b). In continuity, Valueva et al. (2012) isolated from tubers of cv. Zukov's Jubilee PKPI protein with single polypeptide chain of 23 kDa belonging on the basis of N-terminal sequence structure to the group B. The isolated PKPI inhibited chymotrypsin and trypsin activity.

Two serine protease inhibitors with molecular mass 21 and 22 kDa, termed PSPI-21 and PSPI-22, were found to be

predominantly accumulated in potato tubers that were infected with *P. infestans* zoospores. PSPI-21 molecule consists of two disulphide-linked polypeptide chains (16.5 and 4.5 kDa) and PSPI-22 has one polypeptide chain. Their terminal amino acid residues have significant homology to members of the soybean Kunitz inhibitor family (Valueva et al. 1998). In a subsequent study, Revina et al. (2008) showed antifungal potential of PSPI-21 protein and they termed the isolated inhibitor as potato Kunitz-type subtilisin inhibitor (PKSI). The PKSI belonged to the group C of PKPI and was characterised as protein of one polypeptide chain with Mr 21-kDa inhibiting subtilisin Carlsberg (Revina et al. 2004; Revina et al. 2008). Proteins PSPI-21 and PKSI differed in the ability to inhibit the growth of *P. infestans* and *F. culmorum*. Both proteins inhibited the growth of the *P. infestans* hyphae and induced a complete destruction of *P. infestans* zoospores and a partial destruction of *F. culmorum* macroconidia. The PSPI-21 was the most potent in modification the growth of *P. infestans* mycelium, whereas PKSI primarily affected the growth of the *F. culmorum* mycelium (Revina et al. 2008).

The active mixture of potato (cv. Désirée) sprout proteins showed strong inhibitory activity against *Botrytis cinerea* fungal proteases. The mixture inhibited spore germination, hyphal elongation and the development of necrotic lesions. Both PKPI and PPI-I have been identified in this protein mixture as active components (Hermosa et al. 2006).

Kunitz protease inhibitors isolated from various plant sources are most often active against serine protease (trypsin, chymotrypsin, subtilisin). They also inhibit aspartic and cysteine proteases (cathepsin D, papain). Mode of antifungal action is based on the competitive inhibition that is performed through an arginine and lysine amino acid in position P1, where a complex between inhibitor and enzyme is created (de Souza Cândido et al. 2011). Potato antifungal proteins belonging to the family of PKPI were described by Kim et al. (2005) and (2006) and Park et al. (2005). Protein termed AFP-J was purified from potato tubers (cv. L. Jopung). The protein of Mr 13.5 kDa with 83% homology with a serine protease inhibitor belonging to Kunitz family inhibited chymotrypsin, pepsin and trypsin. The AFP-J inhibited microorganisms, including *Candida albicans*, *Trichosporon beigelii* and *Saccharomyces cerevisiae*, whereas it exhibited no activity against crop fungal pathogens (Park et al. 2005). The protein named Potide-G isolated from potato tubers of cv. Golden Valley was characterised as an antimicrobial protein of Mr 5578.9 Da. The protein suppressed proteolytic activity of trypsin, chymotrypsin and papain. Potide-G inhibited the growth of bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Clavibacter michiganensis* subsp. *michiganense*) and fungi (*C. albicans* and *R. solani*) (Kim et al. 2006), and it also showed potent antiviral activity against potato virus Y infection (Tripathi et al. 2006). Potato tuber protein termed Potamin-1 (PT-1),

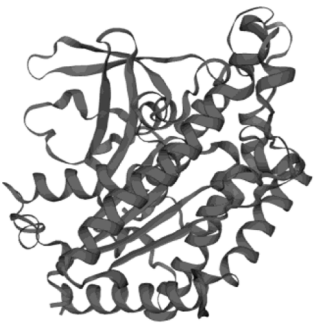
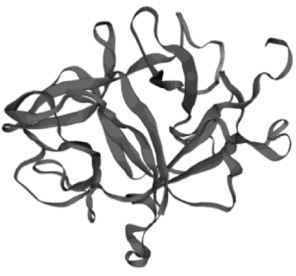
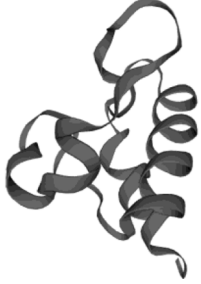
	
Patatin-05 (<i>Solanum tuberosum</i> L.) UniProtKB - Q3YJT5; PAT05; Length: 387, Mass (Da): 42,528 MATTKSVLVLI F M I L A T S S T F A T L G E M V T V L S I D G G G I K G I I P G I I L E F L E G Q L Q K M D N N A D A R L A D Y F D V I G G T S T G G L L T A M I T P N E N N R P F A A A K D I V P F Y F Q H G P H I F N S S T G Q F F G P K Y D G K Y L M Q V L Q E K L G E T R V H Q A L T E V A I S S F D I K T N K P V I F T K S N L A K S P E L D A K M S D I C Y S T A A P T Y F P P H Y F A T N T S N G D K Y E F N L V D G A V A T V A D P A L L S V S V A T R R A E E D P A F A S I R S L N Y K Q L L L L S L G T G T N S E F D K T H T A Q E T A K W G A L Q W M L V I Q Q M T E A A S S Y M T D Y Y L S T V F Q D L H S Q N N Y L R V Q E N A L T G T T K A D D A S E A N M E L L V Q V G E N L L K K P V S K D N P E T Y E E A L K R F A K L L S D R K K F R A N K A S Y Source: https://www.uniprot.org/uniprot/Q3YJT5	Serine protease inhibitor 2 (<i>Solanum tuberosum</i> L.) UniProtKB - P58515; SPI2; Length: 186, Mass (Da): 20,116 L P S D A T P V L D V T G K E L D S R L S Y R I I S T F W G A L G G D V Y L G K S P N S D A P C A N G I F R Y N S D V G P S G T P V R F I G S S S H F G Q G I F E N E L L N I Q F A I S T S K L C V S Y T I W K V G D Y D A S L G T M L L E T G G T I G Q A D S S W F K I V K S S Q L G Y N L L Y C P V T S S S D D Q F C S K V G V V H Q N G K R R L A L V N E N P L D V L F Q E V Source: https://www.uniprot.org/uniprot/P58515
	
Potamin-1 (<i>Solanum tuberosum</i> L.) UniProtKB - P84813; POTM1; Length: 47, Mass (Da): 4,833 D I C T N C C A G T K G C N T T S A N G A F I C E G Q S D P K K P K A C P L N C D P H I A Y A Source: https://www.uniprot.org/uniprot/P84813	Defensin-like protein P322 (<i>Solanum tuberosum</i> L.) UniProtKB - P20346; DF322; Length: 74, Mass (Da): 8,414 M R F F A T F F L L A M L V V A T K M G P M R I A E A R H C E S L S H R F K G P C T R D S N C A S V C E T E R F S G G N C H G F R R R C F C T K P C Source: https://www.uniprot.org/uniprot/P20346
	
Snakin-1 (<i>Solanum tuberosum</i> L.) UniProtKB - Q948Z4; SNAK2; Length: 88, Mass (Da): 9,664 M K L F L L T L L L V L T V I P S L I Q T T M A G S S F C D S K C K L R C S K A G L A D R C L K Y C G I C C E E C K C V P S G T Y G N K H E C P C Y R D K K N S K G K S K C P Source: https://www.uniprot.org/uniprot/Q948Z4	Snakin-2 (<i>Solanum tuberosum</i> L.) UniProtKB - Q93X17; SNAK2; Length: 104, Mass (Da): 11,038 M A I S K A L F A S L L L S L L L L E Q V Q S I Q T D Q V T S N A I S E A A Y S Y K K I D C G G A C A A R C L S S R P R L C N R A C G T C C A R C N C V P P G T S G N T E T C P C Y A S L T T H G N K R K C P Source: https://www.uniprot.org/uniprot/Q93X17

Fig. 1 Tertiary structure of selected potato tuber proteins was created by a fully automated protein structure modelling server SWISS-MODEL (the Biozentrum of the University of Basel) using protein sequences available in database Uniprot (<https://www.uniprot.org/>).

isolated from tubers of cv. Gogu Valley, has an N-terminal sequence with 62% homology with serine protease inhibitor belonging to Kunitz family. The protein of Mr 4833 Da possessed antimicrobial activity, but lacked haemolytic activity. The protein forms a dimeric molecule joined by disulphide bridges and it exhibited activities against chymotrypsin, trypsin and papain. The PT-1 strongly inhibited pathogenic strains including *C. albicans*, *R. solani* and *C. michiganensis* subsp. *michiganense* (Kim et al. 2005). In a subsequent study, the same authors isolated the low molecular protein (Mr 3228.5 Da) from potato tubers of cv. Gogu Valley named PG-2. The best score (43%) of the N-terminal sequence homology of PG-2 was obtained for PPI-I. PG-2 exhibited antimicrobial activity against *C. albicans* (human pathogen yeast strain), *C. michiganensis* subsp. *michiganense* and *S. aureus*, but it did not lyse human red blood cells (Kim et al. 2013).

Feldman et al. (2014) isolated and characterised the serine protease inhibitor termed proteinase K inhibitor (PLPKI) from potato leaves infected with *P. infestans*. Sequencing of the residues at the NH₂ terminus demonstrated that PLPKI is a novel member of the PPI-I family. The PLPKI inhibited the activity of extracellular proteases produced by *P. infestans* and *R. solani*. A positive correlation between the levels of PLPKI and the degree of horizontal resistance was showed and established the fact that it could be used as a suitable biochemical marker to help in selection and breeding approaches. The monomer of PLPKI has Mr of 8.5–9.0 kDa and is highly active against proteinase K but it poorly inhibits trypsin and chymotrypsin.

Less information is available about antifungal activities of other protease inhibitors presented in potato tubers. Amongst them, potato protease inhibitors II (PPI-II) have a potential for defence and antifungal activities. Peña-Cortés et al. (1989) showed increasing concentration of PPI-II in tubers after application of abscisic acid indicating the role of these PPI-II in the wounding and infection defence mechanism. The fungi of *Fusarium* group (various strains of *F. solani* and *F. oxysporum*) were strongly inhibited by protein samples isolated from potato fruit juice at 80 °C. Isolated antifungal proteins of Mr 7–17 kDa were identified, using MALDI-TOFF analysis, as a mixture of PPI-I and PPI-II, with predominating of PPI-II (Bártová et al. 2018).

The potato carboxypeptidase inhibitor (PCI) provided resistance to fungal pathogens when expressed in rice. It was shown that rice plants constitutively expressing *pci* gene exhibited resistance against pathogens *Magnaporthe oryzae* and

Fusarium verticillioides probably due to inhibition of fungal carboxypeptidase B (Quilis et al. 2007).

Antifungal activity of potato defensins and other potato tuber proteins

This variable group includes potato tuber proteins that could not be classified within PIs or patatin family. A simplified characteristic of the group is difficult. Generally, these types of antimicrobial and antifungal agents are mostly characterised as cysteine-rich proteins and peptides, including thionins, defensins and lectins.

Thionins were the first plant peptides reported to have activity against plant pathogens (Fernandez de Caleyra et al. 1972). Thionins are antimicrobial peptides approximately of Mr 5 kDa, with an abundance of arginine, lysine and cysteine residues (Yan et al. 2015). The antimicrobial activity is based on a charge-based interaction between the peptide and phospholipids in pathogen membranes. Next, the positively charged proteins and negatively charged membranes create electrostatic interactions that can lead to the disruption of the plasma membrane integrity, which is mentioned in Fig. 2 (Chang et al. 2008; Wimley 2010). Defensins that are phylogenetically separated but structurally similar to thionins appear not to cause substantial membrane permeabilisation but act on certain membrane-bound receptors (Kim et al. 2009; Yan et al. 2015).

Potato pseudothionin (Pth-St1) with Mr of 5 kDa was found to be active against bacterial and fungal pathogens of potato such as *Clavibacter michiganensis* subsp. *sepedonicus*, *Pseudomonas solanacearum* and *F. solani*. The Pth-St1 does not inhibit trypsin or insect α -amylase activities, and, in contrast with thionins, it does not affect cell-free protein synthesis or β -glucuronidase activity (Moreno et al. 1994).

There is limited information about potato tuber defensins available. Potato defensin named PTH1 has 47 amino acid residues and in combination with potato snak-in-1, it was synergistic against bacteria *C. michiganensis* subsp. *sepedonicus* and *Pseudomonas syringae* pv. *syringae*, and it was additive against the fungi *B. cinerea*, *F. solani* and *Bipolaris maydis* and bacterium *Pseudomonas syringae* pv. *tabaci* (Kovalskaya and Hammond 2009; Segura et al. 1999). Moreover, PTH-1 peptide alone inhibited fungi *Colletotrichum coccodes* and *B. cinerea* (Kovalskaya and Hammond 2009).

Potato defensins DL1 and DL2 families are antipathogenic peptides, which may be phylogenetically related to each other and share common features with snake-venom disintegrins (Calvete et al. 2000). The DL1 and DL2 defensins have been tested against phospholipid vesicles (liposomes) suggesting their ability to interact with membranes. The DL2 induced vesicle aggregation, but not leakage. Potato defensin DL1 failed to show any effect on liposomes (Caaveiro et al. 1997).

Table 1 Classification and characterisation of potato protease inhibitors (Pouvreau 2004; Pouvreau et al. 2001; Bárta et al. 2015)

Protease inhibitor	Molecular mass (kDa)	Isoelectric points (pI)	Number of protein subunits	Enzyme inhibition
Potato inhibitor I (PPI I)	7.68–7.87	5.1–6.3–7.2–7.8	5	T, Ch, HLE
Potato inhibitor II (PP II)	20.02–20.68	5.5–5.8–5.9–6.0–6.1–6.5–6.9	2	T, Ch, HLE
Potato aspartate protease inhibitor (PAPI)	19.87–22.03	6.2–7.5–8.2–8.4–8.6–8.7	1	CaD, T, Ch, HLE
Potato cysteine protease inhibitor (PCPI)	20.1–22.7	5.8–6.6–6.7–7.1–8.0–8.3–8.6→9.0	1	Pap, T, Ch, HLE
Potato Kunitz-type protease inhibitor (PKPI)	20.19–20.24	8.0→9.0	1	T, Ch
Other serine potato protease inhibitors (OSPI)	21.03–21.80	7.5–8.8	1–2	T, Ch, HLE,
Potato carboxypeptidase inhibitor (PCI)	4.27	nd	1	CpA

nd not detected, T trypsin, Ch chymotrypsin, HLE human leucocyte elastase, CaD cathepsin D, Pap papain, CpA carboxypeptidase A

Snakins represent a specific group of basic cell wall-associated antimicrobial proteins. Yan et al. (2015) classified these proteins into ‘another antifungal plant proteins’ because of their minimal similarity to any other antipathogenic proteins isolated from plants. The mechanism of action has not yet been explained. Snakin-1 (SN1) and snakin-2 (SN2) amino acid sequence alignments show a similarity with members of GAST family from tomato (gibberellic acid-stimulated transcript) and GASA family from *Arabidopsis* (gibberellic acid-stimulated transcript in *Arabidopsis*), and they have been classified as members of a novel snakin/GASA family (Berrocal-Lobo et al. 2002) and snakin proteins have been reported to be involved in diverse biological processes, including cell division, cell elongation, cell growth, transition to flowering, signalling pathways and defence (Berrocal-Lobo et al. 2002; Nahirňak et al. 2012; Segura et al. 1999).

The potato SN1 gene is constitutively expressed in different tissues during their development and is not affected by abiotic and biotic stress. Similarly, SN2 represents a widely divergent snakin subfamily, but, in contrast with SN1, expression of the SN2 gene is affected by various external treatments, including pathogen infection. Although SN2 is only 38% homologous with SN1, they have similar properties. They are basic and rich in cysteine residues with similar antimicrobial activity against bacterial and fungal pathogens while different from that of defensin peptides from the same tissues as it can be seen in Table 3 adapted according results of Berrocal-Lobo et al. (2002). Proteins SN1 and SN2 caused an aggregation of both Gram-positive and Gram-negative bacteria (Segura et al. 1999; Berrocal-Lobo et al. 2002; Kovalskaya and Hammond 2009) and inhibition of fungal pathogens of *B. cinerea*, *F. solani* and *F. culmorum*, at a concentration of < 10 µM. The mature peptide of SN2 is basic (pI = 9.16) with 66 amino acid residues, calculated at an Mr of 7025 Da with six cysteines (Berrocal-Lobo et al. 2002). Similarly, SN1 has 63 amino acid residues (Mr 6922 Da) with six cysteines (Segura et al. 1999).

Lectins, carbohydrate-binding proteins that agglutinate cells or precipitate polysaccharides and glycoconjugates, are widely distributed in nature. Lectins probably interact with the fungal membrane by binding to carbohydrates on the surface of the cell wall with indirect impact on fungal growth (Yan et al. 2015). Potatoes contain a number of chitin-binding proteins which have possible functions in their defence against pathogens. Millar et al. (1992) reported an increased synthesis of the hydroxyproline-containing glycoprotein of Mr 57 kDa, which binds to chitin and shares common epitopes with lectin 24 h after wounding. The 20-kDa chitin-binding lectin isolated from potato tubers of cv. Deshi manifested antifungal activity against *Aspergillus niger*, *Penicillium* spp. and *Rhizopus* spp. and antibacterial activity against Gram-positive (*L. monocytogenes*) and Gram-negative (*E. coli*, *Salmonella enteritidis* and *Shigella boydii*) bacteria. The isolated potato lectin was also able to prevent the biofilm produced by *Pseudomonas aeruginosa*. It can thus have possible applications in clinical microbiology and in biomedical science (Hasan et al. 2014). Shah et al. (2016) isolated potato tuber lectin of 20 kDa and identified the fact that it belonged to PKPI. Other biological activities of this protein have, however, not yet been described.

Feng et al. (2003) isolated antimicrobial proteins, termed AP₁, from potato leaves of cv. MS-42.3. The protein had good inhibition activity against five different strains of *R. solanacearum* from potato or other crops and against two fungal pathogens, *R. solani* and *A. solani* from potato. The N terminus exhibits 58% identity with the acid phosphatase from *Mesorhizobium loti*. The authors suggested that AP₁ belongs to a new group of proteins with antimicrobial characteristics and functions in relation to energy metabolism of plants.

Aspartic protease constitutes one of the four super-families of proteolytic enzymes. Three potato aspartic proteases, one from tubers (StAP₁) (Guevara et al. 1999) and two from leaves (StAP₂ and StAP₃) (Guevara et al. 2004), were

Table 2 Potato protease inhibitors with described antifungal or antimicrobial activities

Protein/peptide	Inhibited microorganism	Reference
Serine protease inhibitors		
Mixture of PPI-I and PKPI	<i>B. cinerea</i>	Hermosa et al. 2006
PLPKI	<i>P. infestans</i> , <i>R. solani</i>	Feldman et al. 2014
Thermally stable PPI-II	<i>F. solani</i> , <i>F. oxysporum</i>	Bártová et al. 2018
Kunitz-type protease inhibitors		
PKPI groups A and B	<i>F. moniliforme</i>	Heibges et al. 2003b
PKSI (PKPI group C)	<i>P. infestans</i>	Revina et al. 2004
	<i>F. culmorum</i>	Revina et al. 2008
PSPI-21	<i>P. infestans</i>	Valueva et al. 1998
	<i>F. culmorum</i>	Revina et al. 2008
PSPI-22	<i>P. infestans</i>	Valueva et al. 1998
AFP-J	<i>C. albicans</i> ,	Park et al. 2005
	<i>T. beigelii</i>	
	<i>S. cerevisiae</i>	
Potide-G	<i>S. aureus</i>	Kim et al. 2006
	<i>L. monocytogenes</i>	Tripathi et al. 2006
	<i>E. coli</i>	
	<i>C. michiganensis</i> subsp. <i>michiganense</i>	
	<i>C. albicans</i>	
	<i>R. solani</i>	
PT-1	antiviral activity against potato virus Y	Kim et al. 2005
	<i>C. albicans</i>	
	<i>R. solani</i>	
PG-2	<i>C. michiganensis</i> subsp. <i>michiganense</i>	Kim et al. 2013
	<i>C. albicans</i> (human pathogen strain)	
	<i>C. michiganensis</i> subsp. <i>michiganense</i>	
Carboxypeptidase inhibitor	<i>S. aureus</i>	
PCI	<i>M. oryzae</i>	Quilis et al. 2007
	<i>F. verticillioides</i>	

PPI-I potato protease inhibitor I, *PPI-II* potato protease inhibitor II, *PKPI* potato Kunitz-type protease inhibitor, *PLPKI* potato serine protease inhibitor specific for proteinase K, *PKPI* potato Kunitz-type protease inhibitor, *PKSI* potato subtilisin protease inhibitor from Kunitz-type family, *PSPI-21* potato serine protease inhibitor of 21 kDa, *PSPI-22* potato serine protease inhibitor of 22 kDa, *AFP-J* antifungal protein isolated from cv. L. Jopung, *PT-1* Potamin 1, *PG-2* Potide G-2, *PCI* potato carboxypeptidase inhibitor

presented as bifunctional proteins with both proteolytic and antimicrobial activities (Pagano et al. 2007). Similarly as other plant aspartic proteases, StAPs are glycosylated (Guevara et al. 1999; Guevara et al. 2001). The StAPs are induced by both abiotic and biotic stress and have extracellular localisation (Guevara et al. 2002). The StAP glycosylation was demonstrated as essential to StAP antifungal activity; deglycosylated StAP fungicidal activity is lower than that of the native StAPs at all concentrations. The StAP *in vitro* antifungal activity was recorded with regard to *P. infestans* and *F. solani* (Guevara et al. 2002; Guevara et al. 2004) via direct interaction with microbial surfaces followed by membrane permeabilisation (Mendieta et al. 2006).

Potato patatins and their antifungal potential

Unlike PPIs, patatin constitutes a more uniform group of homologous protein isoforms and represents a major tuber protein component with significant contribution to nutritional (Bártová and Bárta 2009; Bártová et al. 2015) and functional (Bárta et al. 2012; Schmidt et al. 2018) properties of potato tuber proteins. Bárta et al. (2012) found the patatin relative abundance between 5.40 and 35.40% within a group of 20 potato cultivars. Patatins are glycoproteins with a molecular weight of 39–43 kDa (existing as native 80-kDa dimers) with different zero points of charge (pI) between 4.45 and 5.17 and glycosylation patterns (1–3 glycosylation) (Bárta et al. 2012;

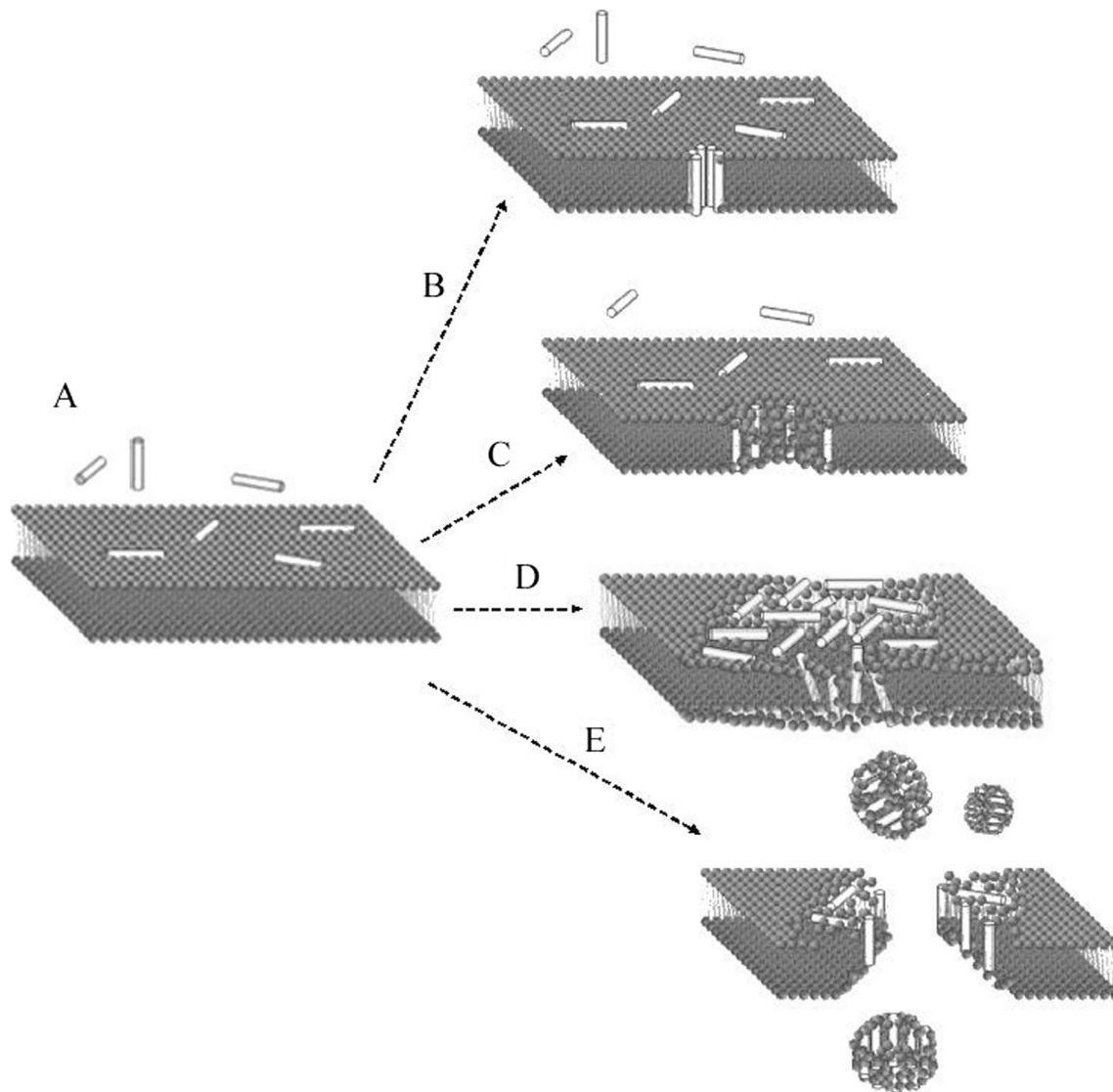


Fig. 2 Models of AMP mode of action with corresponding changes in membranes (figure modification according to Chang et al. 2008; Wimley 2010). The contact of AMPs with the membrane and its adsorption to the membrane (a); reorientation of the AMPs in the lipid bilayer and formation of transbilayer pores (b, c). A barrel-stave pore (b)—AMPs interact laterally with one another when hydrophobic regions of AMPs align with the tails of the lipids and the hydrophilic regions form the inner surface of the pore. The membrane thickness does not change. A toroidal pore (c)—

AMP specific interactions are not present, but hydrophilic parts of the lipids are electrostatically interacted by charged residues of AMPs. The membrane thickness decreases. In the carpet model (d), AMPs are bound to the membrane surface. AMP hydrophobic residues facing the membrane and their hydrophilic surfaces facing the solvent. When a large concentration of peptides is reached, peptide molecules disrupt the membrane in the detergent-like model (e). The lipid bilayer is disrupted into a set of separate micelles

Lattová et al. 2015). Patatin exists in various mass and charge forms which differ amongst potato cultivars and *Solanum* species. Differences of individual isoforms in molar mass are predominantly caused by different numbers of glycosylation in combination with mutations in the primary sequence of the patatin protein chain (Bárta et al. 2012; Pots 1999). Purified samples of patatin obtained from 20 potato cultivars were according to MALDI-TOFF MS analysis, of three mass levels (~40.6, 41.8 and 42.9 kDa); 2D PAGE showed 17–23 detected patatin spots representing various patatin isoforms (Bárta et al. 2012). Such a high patatin variability in combination

with observed patatin enzymatic activities and partly induced synthesis of patatin proteins supported the concept that patatin is not only storage protein but could also be a part of defence mechanisms. The real physiological role of patatin in potato tubers has, however, not yet been completely established (Bártová et al. 2012). Patatin has surprising enzymatic activities of nonspecific lipid acyl hydrolase (LAH) (Bárta et al. 2012; Pots 1999; Racusen 1986), phospholipase A₂ (Jiménez-Atiénzar et al. 2003; Senda et al. 1996), acyl transferase (Jimenez et al. 2001), β -1,3 glucanase (Tonón et al. 2001) and β -1,2 xylosidase (Peyer et al. 2004). LAH activity may

play a role in the defence by means of releasing fatty acids after the damaging of vacuoles. Fatty acids are then oxidised with subsequent production of cytotoxic products and water-insoluble waxes (La Camera et al. 2005; Racusen 1983). Similarly, the activity of patatin phospholipase A₂ (PLA₂) participates in the production of bioactive lipid components involved in defence mechanisms (Matos and Pham-Thi 2009). The β -1,3 glucanases belong to pathogenesis-related proteins with an evident relationship with β -1,3-glucanases of hyphal cell wall (Heřmanová et al. 2006). Clear information about patatin antifungal, antimicrobial or other inhibition activities has been limited.

The participation of patatin proteins in potato defence against fungal pathogens is evident from the patatin-increased secretion in resistant genotypes as response to pathogen attack. The infection with *P. infestans* induced accumulation of patatins in the resistant cultivar while no changes in patatin accumulation were observed in the susceptible cultivar (Fernández et al. 2012). Direct antifungal effects of patatin were documented only in few cases. Tonón et al. (2002) isolated acidic β -1,3-glucanase of 39 kDa from potato cultivar Huinkul with a high level of field resistance after 72 h of infection with *P. infestans* when the β -1,3-glucanase activity increased. The enzyme produced a direct inhibitory effect on

the germination of sporangia of *P. infestans*. Between two potato cultivars of different degrees of field resistance to *P. infestans*, there were different expressions of β -1,3-glucanase observed. The susceptible cultivar Bintje induced a β -1,3-glucanase expression in lower levels than that observed in the resistant cultivar. Additionally, Sharma et al. (2004) prepared somatic hybrids (Wisconsin J series) with durable resistance against *P. infestans*. The purified patatin from the resistant genotype was able to inhibit *P. infestans* spore germination by 70% at a 2.5- μ g ml⁻¹ concentration. The infection of resistant plants with spores of *P. infestans* showed that patatin is expressed in stem tissues 72 h after the plant was inoculated suggesting thus participation of patatin proteins in potato defensive mechanisms.

Antifungal potential of potato protein hydrolysates

Protein hydrolysates have been defined as a mixture of polypeptides, oligopeptides and amino acids that are manufactured from protein source using partial hydrolysis (McCarthy et al. 2013). In the human and animal body, the bioactive peptides display hormone or drug-like activities and can be classified, on the basis of their mode of action, as antimicrobial, anti-thrombotic, antihypertensive, opioid, immunomodulatory, mineral binding and antioxidative, based on the amino acid composition and specific sequence (McCarthy et al. 2013; Sánchez and Vázquez 2017). There is available information about antihypertensive (Mäkinen 2014) and antioxidant (Cheng et al. 2010; Udenigwe et al. 2016) activities of hydrolysed potato proteins, but a very limited information about their antimicrobial or antifungal actions. In the study of Park et al. (2005), an antifungal protein, AFP-J, with activity against yeast strains, was described. The subsequent study brings a description of functional peptide produced by a partial acid digestion of AFP-J. The active peptide was named Potide-J and was active (MIC 6.25 μ g/ml) against *C. albicans*. The amino acid sequence of Potide-J was established as Ala-Val-Cys-Glu-Asn-Asp-Leu-Asn-Cys-Cys. Mass spectrometry showed its Mr 1083.1 Da (Lee et al. 2012).

Application of potato tuber proteins with antifungal activities

In the past few years, great efforts have been made to study the activities of antifungal and antimicrobial plant proteins and peptides which, for their part, can offer anti-infection applications. These compounds bring hopeful solutions in the fight with pathogens in a number of branches where the pathogen elimination is the key step for successful results. The AFPs and AMPs can be used not only in food industry, agricultural biotechnologies and breeding, feedstuffs and aquaculture, but also in medicine and pharmacology (Meng et al. 2010).

Table 3 Inhibition of bacterial and fungal pathogens by snakins StSN2 and StSN1 and defensin StPTH1 from potato. Adapted from Berrocal-Lobo et al. (2002)

Pathogen	Protein (EC ₅₀) ^a		
	StSN1	StSN2	StPTH1
	μM		
Bacteria			
<i>C. michiganensis</i>	1	4	7
<i>R. solanacearum</i>	NA	NA	25
<i>R. solanacearum</i> (<i>rfa</i>)	30	15	25
<i>E. chrysanthemi</i>	NA	NA	NA
<i>R. meliloti</i>	8	nt	NA
Fungi			
<i>B. cinerea</i>	2	0.8	1
<i>F. solani</i>	3	2	0.2
<i>F. culmorum</i>	2	2	NA
<i>F. oxysporum</i> f. sp. <i>conglutinans</i>	10	13	nt
<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	20	13	nt
<i>P. cucumerina</i>	10	10	10
<i>C. graminicola</i>	10	20	2
<i>C. lagenarium</i>	10	10	nt
<i>B. maydis</i>	20	20	10
<i>A. flavus</i>	20	20	nt

^a EC₅₀ effective concentration for 50% inhibition

NA not active at concentration ≤ 20 μ M, nt not tested

Application in phytopathogen control

Phytopathogens are responsible for most crop losses worldwide and have caused devastating famines in the human history. Proteins active against plant pathogens or those involved in signalling transduction have a natural potential to be used in plant breeding or in the production of genetically modified plants of enhanced pathogen resistance. Those can be also used as biochemical markers for the selection of resistant plants and in launching of new cultivars.

Rice plants expressing constitutively a *pci* gene for the carboxypeptidase inhibitor (PCI), showed resistance against pathogens *M. oryzae* and *F. verticillioides* (Quilis et al. 2007). Potato plants (cv. Kennebec) were transformed with a construct encoding *S. chacoense* SN1 genes. Overexpression of SN-1 genes in transgenic lines exhibited a significant symptom reduction of *R. solani* infection and higher survival rates. These lines showed also significant protection against *Erwinia carotovora* (Almasia et al. 2008). Hybrid proteins SN1 and PTH1 expressed in potato and tobacco plants increased the antimicrobial and antifungal potential of both the plants (Kovalskaya et al. 2011). The SN1 genes introduced into wheat plants enhanced resistance to *Rhizoctonia cerealis* and *Bipolaris sorokiniana* (Wang et al. 2012).

Potato plant with the ability to accumulate the key defence proteins with antifungal activities usually exhibited durable field resistance against pathogens and should therefore be used as hopeful biochemical markers in breeding. Protease inhibitor named PLPKI (Feldman et al. 2014), inhibitors PSPI-21 and PSPI-22 (Valueva et al. 2003) and inhibitors PI-I and PI-II (Peña-Cortés et al. 1989) and patatin isoforms (Senda et al. 1998; Sharma et al. 2004; Tonón et al. 2002) were described as potential biochemical markers in horizontal resistance to *P. infestans*.

Application in food industry

People started to use chemicals as food preservatives in food industry long time ago. Since these chemically synthesized preservatives can negatively affect human health and food taste, the demand for food preservatives from natural sources has been increasing (Meng et al. 2010). More strategies can be used for food preservation using AFPs and AMPs. The key request is, however, not only a protein antifungal or antimicrobial efficiency but also a functional stability under different thermal, acidity or salinity conditions.

Rodríguez et al. (2015) described direct application of aqueous extracts enriched in *S. tuberosum* tuber proteins on the lemon surface to control fungi responsible for post-harvest diseases. The PPI fraction IF25 (Mr 15–20 kDa) was able to inhibit *Penicillium digitatum* and *Geotrichum candidum*. The extract built-in to the wax is therefore suitable to create the control coat and to efficiently protect the fruit.

Biodegradable film with incorporated AFPs and AMPs together with antioxidant or with nutritionally improving substances seems to be the resulting tendency of functional food packaging in future. This technique offers the advantage of concentrating the antimicrobial agents on the surface of food products, which is where potential pathogens grow (Shen et al. 2010). An active packaging offers better preservation of sensory attributes, of safety and of product shelf-life. To the best of our knowledge, there is no evidence about such application potato tuber proteins although these seem to be the ideal candidate. The individual components of potato proteins and peptides, how it was mentioned in the previous chapters, have antimicrobial (Bártová et al. 2012) and antioxidant (Liu et al. 2003) activities. The potato tuber proteins are nutritional valuable (Bártová et al. 2015), some of them are thermostable up to 100 °C (e.g. CPI, PI-I and PI-II, hydrolyse protein fragments) (Bártová et al. 2018) and the potato proteins are even able to create bio-based polymer films (Schäfer et al. 2018). Potato AMPs with ability to inhibit pathogenic *Listeria* and *Salmonella* species are alternatively usable as food preservatives. The group of potato proteins with perspective usage for the food industry includes Potide-G, potato lectin or total protein extract obtained from tubers of cv. Gogu Valley.

Application in feedstuffs

The use of antibiotic compounds as animal feed additives has been used to improve ruminal fermentation and the efficiency of nutrient utilisation (Candanosa et al. 2012) and to avoid of risks intestinal pathogens such as *Salmonella* spp. or *Listeria* spp. However, the overuse of antibiotics can disrupt balance of intestinal microbiota of the animals and also can raise animal products safety risk. Therefore, the use of antibiotics as animal feed additives has been restricted in the EU. The use of endogenous AFPs and AMPs as food additives has ability to inhibit corresponding pathogens, while they cannot kill normal intestinal flora and animal cells (Jin et al. 2009; Ohh et al. 2010).

Jin et al. (2009) reported that pigs fed by increasing levels (0 to 600 ppm) of refined potato tuber protein from cv. Gogu Valley demonstrated linear improvements in their performance and a linear decrease in their faecal and intestinal bacteria, suggesting that the potato protein was effective at higher levels. Since the potato tubers of Gogu Valley are known to contain AMP potamin-1 (PT-1), the authors suggested the AMP may have caused the decrease in microbe numbers, contributing towards an increased performance. Protein isolates included in pig diets inhibited the growth of *S. aureus*, *Salmonella choleraesuis*, *Salmonella gallinarum* and *E. coli* and could therefore serve as an alternative to antibiotics. Similarly, potato proteins (again cv. Gogu Valley) were used as alternative antibiotics in the diet of broilers. The birds fed by potato protein diet had less coliforms in the caecal content than those on the control diet. The potato proteins showed the

potential to be used as alternative to antibiotics in the diet of broilers (Ohh et al. 2010).

Application in medicine

The excessive use of antibiotics is not only the problem of feedstuffs. The damage of human intestinal microflora, the allergenicity of antibiotics, the resistance of bacteria to antibiotics and the environmental risk of these use of antibiotics are the key inconveniences also in human medicine and pharmacology. In a study on healthy humans, the administration of potato fruit juice from cv. Gogu Valley increased the growth responses of *Bifidobacterium* and *Lactobacillus* and decreased the growth of *Clostridium perfringens* and *E. coli* (Lee 2005). Potide-G, previously characterised, was able to inhibit pathogenic strains of *S. aureus*, *L. monocytogenes*, *E. coli* and *C. albicans* (Kim et al. 2006). *C. albicans* was inhibited also by PT-1 (Kim et al. 2005) and PG-2 (Kim et al. 2013). A 20-kDa chitin-binding lectin isolated from potato tubers of cv. Deshi inhibited pathogenic strains of *L. monocytogenes*, *E. coli*, *S. enteritidis* and *Shigella boydii*. The isolated potato lectin was also able to prevent the formation of biofilm produced by *P. aeruginosa* and could thus have possible applications in clinical microbiology and biomedical science (Hasan et al. 2014). Data about on the real potential of potato protein usage in human medicine have been limited. Paik et al. (2014) declared the anti-*Leishmania* effect of the fraction rich in serine protease inhibitors obtained from potato tubers. The in vitro and ex vivo assays with *Leishmania donovani*-infected macrophages showed a reduction in the parasite viability and in the intracellular parasite load and the serine protease inhibitors from potatoes were evaluated as a perspective drug against leishmaniasis.

Conclusions

Potato (*Solanum tuberosum* L.) has achieved a worldwide prominence both as food and as industrial crop for starch and protein production. Isolation of potato proteins from starch production waste has opened a new directions of research. Surprising possibilities of them, highly usable in various branches, have gradually been described, such as foaming, emulsifying and gelling abilities, high nutritional value, regulation digestive enzymes, reduction of food intake in humans or blocking UV irradiation. Last but not least, the proteins are part of potato plant defence system and they exhibit antifungal, antibacterial and antiviral activities. The AFPs and AMPs isolated from potato tubers belong to various groups from the point of view of their structure, molecular mass, enzyme activities, thermostability, physiological roles or the intensity of antifungal activity. Some of them could be termed as multifunctional,

having both antifungal and antimicrobial, or even antiviral, abilities. The AFPs occurring in potato tubers include both proteins with storage function presented constitutively in the tubers and proteins synthesized as pathogenesis-related components.

Potato protease inhibitors with their multifunctional activities have the possibility for their use in various applications. Here, we can discuss their possible incorporating as antimicrobial additive into feed or food products or use in breeding programmes for the production of crops with a higher pathogen resistance. Serine and cysteine protease inhibitors and potato protease I and potato Kunitz-type protease inhibitors amongst them seem to be the most studied and perspective inhibitors. Potato Kunitz-type protease inhibitors inhibits the growth of *Phytophthora infestans*, *Rhizoctonia solani* and *Botrytis cinerea* or of the fungi of *Fusarium* genus. Strong inhibitory activities with predominant antibacterial activity were described for the low molecular part of potato protease inhibitors including heat-stable protein Potide-G with the ability to inhibit bacterial pathogenic strains of *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Clavibacter michiganensis* subsp. *michiganense* and *Candida albicans* of fungus *Rhizoctonia solani*. Connected antifungal and antibacterial activities were also described for small (5 kDa maximum), cysteine-rich proteins including potato pseudothionin, defensins and lectins and a very specific group of proteins named potato snakins. These proteins are active against a wide range of pathogenic fungi and Gram-negative as well as Gram-positive bacteria and they often work in synergic effect.

The combination of antifungal and antibacterial activities, the heat stability and other properties of potato tuber proteins have given them the momentous potential for use in various applications in situations where the fight with pathogenic fungi and bacteria is of crucial importance. The possible application of potato tuber proteins is promising in the launching of new crop cultivars of enhanced resistance to pathogens, in food industry to replace chemical preservatives or to create active food packaging and finally in human and animal pharmacology to at least partly replace antibiotics.

Funding information The present work was supported by the Grant Agency of the University of South Bohemia in České Budějovice, project GAJU 027/2019/Z.

Compliance with ethical standards

This paper is not based on any study carried out with human participants or animals by any of the authors.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Almasia NI, Bazzini AA, Hopp E, Vazquez-Rovere C (2008) Overexpression of *snakin-1* gene enhances resistance to *Rhizoctonia solani* and *Erwinia carotovora* in transgenic potato plants. *Mol Plant Pathol* 9:329–338
- Baroni A, Donnarumma G, Paoletti I, Longanesi-Cattani I, Bifulco K, Tufano MA, Carriero MV (2009) Antimicrobial human beta-defensin-2 stimulates migration, proliferation and tube formation of human umbilical vein endothelial cells. *Peptides* 30:267–272
- Bárta J, Bárta V, Zdráhal Z, Sedo O (2012) Cultivar variability of patatin biochemical characteristics: table versus processing potatoes (*Solanum tuberosum* L.). *J Agric Food Chem* 60:4369–4378
- Bárta J, Bárta V, Brabcová A, Diviš J, Horáčková V, Kamenová A, Zdráhal Z (2015) Potential of potato proteins within the frameworks of *Solanum* genus (In Czech). Kurent, České Budějovice, Czech Republic
- Bárta V, Bárta J (2009) Chemical composition and nutritional value of protein concentrates isolated from potato (*Solanum tuberosum* L.) fruit juice by precipitation with ethanol or ferric chloride. *J Agric Food Chem* 57:9028–9034
- Bárta V, Bárta J, Kamenová A, Staňková A, Čurn V (2012) Characteristics of potato antimicrobial proteins and peptides and their application potential (In Czech). *Chem List* 106:365–372
- Bárta V, Bárta J, Brabcová A, Zdráhal Z, Horáčková V (2015) Amino acid composition and nutritional value of four cultivated South American potato species. *J Food Compos Anal* 40:78–85
- Bárta V, Bárta J, Vlačihová A, Šedo O, Zdráhal Z, Konečná H, Stupková A, Švajner J (2018) Proteomic characterization and antifungal activity of potato tuber proteins isolated from starch production waste under different temperature regimes. *Appl Microbiol Biotechnol* 102:10551–10560
- Barzic MR, Com E (2012) Proteins involved in the interaction of potato tubers with *Pectobacterium atrosepticum*: a proteomic approach to understanding partial resistance. *J Phytopathol* 160:561–575
- Bauw G, Nielsen HV, Emmersen J, Nielsen KL, Jørgensen M, Welinder KG (2006) Patatins, Kunitz protease inhibitors and other major proteins in tuber of potato cv. Kuras. *FEBS J* 273:3569–3584
- Berrocal-Lobo M, Segura A, Moreno M, Lopez G, García-Olmedo F, Molina A (2002) Snakin-2, an antimicrobial peptide from potato whose gene is locally induced by wounding and responds to pathogen infection. *Plant Physiol* 128:951–961
- Blanco-Aparicio C, Molina M, Fernández-Sales E, Frazier M, Mas J, Querol E, Avilés FX, de Llorens R (1998) Potato carboxypeptidase inhibitor, a t-knot protein, is an epidermal growth factor antagonist that inhibits tumor cell growth. *J Biol Chem* 273:12370–12377
- Burra DD (2016) Defence related molecular signalling in potato - new perspectives from “-Omics”. Ph.D. thesis, Swedish University of Agricultural Sciences, Alnarp, Sweden
- Caaveiro JM, Molina A, González-Mañas JM, Rodríguez-Palenzuela P, García-Olmedo F, Goñi FM (1997) Differential effects of five types of antipathogenic plant peptides on model membranes. *FEBS Lett* 410:338–342
- Calvete JJ, Moreno-Murciano MP, Sanz L, Jurgens M, Schraker M, Raida M, Benjamin DC, Fox JW (2000) The disulfide bond pattern of catrocollastatin C, a disintegrin-like/cysteine-rich protein isolated from *Crotalus atrox* venom. *Protein Sci* 9:1365–1373
- Candanosa E, Villagodo A, Castillo DA, Mendoza GD (2012) Effect of monensin, virginiamycin and sodium bicarbonate on ruminal fermentation and acid-base status in sheep. *J Anim Vet Adv* 7:184–189
- Chang WK, Wimley WC, Searson PC, Hristova K, Merzlyakov M (2008) Characterization of antimicrobial peptide activity by electrochemical impedance spectroscopy. *Biochim Biophys Acta* 1778:2430–2436
- Cheng Y, Xiong YL, Chen J (2010) Antioxidant and emulsifying properties of potato protein hydrolysate in soybean oil-in-water emulsions. *Food Chem* 120:101–108
- Cleveland TE, Thornburg RW, Ryan CA (1987) Molecular characterization of a wound inducible inhibitor I gene from potato and the processing of its messenger RNA and protein. *Plant Mol Biol* 8:199–207
- de Souza Cândido E, Pinto MFS, Pelegrini PB, Lima TB, Silva ON, Pogue R, Grossi-de-Sá MF, Franco OL (2011) Plant storage proteins with antimicrobial activity: novel insights into plant defense mechanisms. *FASEB J* 25:3290–3305
- Delahajje RJB, Wierenga PA, Giuseppin MLF, Gruppen H (2015) Comparison of heat-induced aggregation of globular proteins. *J Agric Food Chem* 63:5257–5265
- Feldman ML, Oliva CR, Casalagué CA, Daleo GR (2000) Induction of a proteinase K inhibitor in a potato cultivar with a high degree of field resistance against *Phytophthora infestans*. *Physiol Plant* 109:14–20
- Feldman ML, Andreu AB, Korgan S, Lobato MC, Huarte M, Walling LL, Daleo GR (2014) PLPKI: A novel serine protease inhibitor as a potential biochemical marker involved in horizontal resistance to *Phytophthora infestans*. *Plant Breed* 133:275–280
- Feng J, Yuan F, Gao Y, Liang Ch XJ, Zhang Ch HL (2003) A novel antimicrobial protein isolated from potato (*Solanum tuberosum*) shares homology with an acid phosphatase. *Biochem J* 376:481–487
- Fernandez de Caley R, Gonzalez-Pascual B, Garcia-Olmedo F, Carbonero P (1972) Susceptibility of phytopathogenic bacteria to wheat purothionins *in vitro*. *Appl Microbiol* 23:998–1000
- Fernández MB, Pagano MR, Daleo GR, Guevara MG (2012) Hydrophobic proteins secreted into the apoplast may contribute to resistance against *Phytophthora infestans* in potato. *Plant Physiol Biochem* 60:59–66
- Fischer M, Kuckenberger M, Kastilan R, Muth J, Gebhardt C (2015) Novel *in vitro* inhibitory functions of potato tuber proteinaceous inhibitors. *Mol Gen Genomics* 290:387–398
- Guevara MG, Oliva CR, Machinandiarena M, Daleo GR (1999) Purification and properties of an APs from potato tuber that is inhibited by a basic chitinase. *Physiol Plant* 106:164–169
- Guevara MG, Daleo GR, Oliva CR (2001) Purification and properties of an aspartic protease from potato leaves. *Physiol Plant* 112:321–326
- Guevara MG, Oliva CR, Huarte M, Daleo GR (2002) An aspartic protease with antimicrobial activity is induced after infection and wounding in intercellular fluids of potato tubers. *Eur J Plant Pathol* 108:131–137
- Guevara MG, Verissimo P, Pires E, Faro C, Daleo GR (2004) Potato aspartic proteases: induction, antimicrobial activity and substrate specificity. *J Plant Pathol* 86:233–238
- Gvozdeva EL, Volotskaya AV, Sof'in AV, Kudryavtseva NN, Revina TA, Valueva TA (2006) Interaction of proteinases secreted by the fungal plant pathogen *Rhizoctonia solani* with natural proteinase inhibitors produced by plants. *Appl Biochem Microbiol* 42:502–507
- Hasan I, Ozeki Y, Kabir SR (2014) Purification of a novel chitin-binding lectin with antimicrobial and antibiofilm activities from a bangladeshi cultivar of potato (*Solanum tuberosum*). *Indian J Biochem Biophys* 42:462–468
- Hegedüs N, Marx F (2013) Antifungal proteins: more than antimicrobial? *Fungal Biol Rev* 26:132–145
- Heibges A, Glaczinski H, Ballvora A, Salamini F, Gebhardt C (2003a) Structural diversity and organization of three gene families for Kunitz-type enzyme inhibitors from potato tubers (*Solanum tuberosum* L.). *Mol Gen Genomics* 269:526–534
- Heibges A, Salamini F, Gebhardt C (2003b) Functional comparison of homologous member of three groups of Kunitz-type enzyme inhibitors from potato tubers (*Solanum tuberosum* L.). *Mol Gen Genomics* 269:535–541

- Heřmanová V, Bárta J, Čurn V (2006) Antifungal plant proteins - classification, characterization and potential applications. *Chem List* 100: 495–500
- Hermosa MR, Turrà D, Fogliano V, Monte E, Lorito M (2006) Identification and characterization of potato protease inhibitors able to inhibit pathogenicity and growth of *Botrytis cinerea*. *Physiol Mol Plant Pathol* 68:138–148
- Huang C, Ma W-Y, Ryan CA, Dong Z (1997) Proteinase inhibitors I and II from potatoes specifically block UV-induced activator protein-1 activation through a pathway that is independent of extracellular signal-regulated kinases, c-jun N-terminal kinases, and P38 kinase. *Proc Natl Acad Sci U S A* 94:11957–11962
- Jashni MK, Mehrabi R, Collemare J, Mesarich CH, Wit PJGM (2015) The battle in the apoplast: further insights into the roles of proteases and their inhibitors in plant-pathogen interaction. *Front Plant Sci* 6:584
- Jimenez M, Escribano J, Perez-Gilbert M, Chazarra S, Cabanes J, Garcia Carmona F (2001) An octaethylene glycol monododecyl ether-based mixed micellar assay for determining the lipid acyl hydrolase activity of patatin. *Lipids* 36:1169–1174
- Jiménez-Atiénzar M, Cabanes J, Gandía-Herrero F, Escribano J, García-Carmona F, Pérez-Gilbert M (2003) Determination of the phospholipase activity of patatin by a continuous spectrophotometric assay. *Lipids* 38:677–682
- Jin Z, Shide PL, Yang YX, Choi JY, Yoon SY, Hahn T-W, Lim HT, Park YK, Hahm KS, Joo JW, Chae BJ (2009) Use of refined potato (*Solanum tuberosum* L. cv. Gogu Valley) protein as an alternative to antibiotics in weanling pigs. *Livest Sci* 124:26–32
- Kim J-Y, Park S-C, Kim MH, Lim HT, Park Y, Hahm K-S (2005) Antimicrobial activity studies on a trypsin-chymotrypsin protease inhibitor obtained from potato. *Biochem Biophys Res Commun* 330:921–927
- Kim MH, Park S-C, Kim J-Y, Lee SY, Lim HT, Cheong H, Hahm K-S, Park Y (2006) Purification and characterization of a heat-stable serine protease inhibitor from the tubers of new potato variety Golden Valley. *Biochem Biophys Res Commun* 346:681–686
- Kim J-Y, Park S-C, Hwang I, Cheong H, Nah J-W, Hahm K-S, Park Y (2009) Protease inhibitors from plants with antimicrobial activity. *Int J Mol Sci* 10:2860–2872
- Kim J-Y, Park S-C, Kim M-H, Lim H-T, Park Y, Hahm K-S (2013) PG-2, a potent AMP against pathogenic microbial strains from potato (*Solanum tuberosum* L. cv. Gogu Valley) tubers not cytotoxic against human cells. *Int J Mol Sci* 14:4349–4360
- Koiwa H, Bressan RA, Hasegawa PM (1997) Regulation of protease inhibitors and plant defense. *Trends Plant Sci* 2:379–384
- Komamytsky S, Cook A, Raskin I (2011) Potato protease inhibitors inhibit food intake and increase circulating cholecystokinin levels by a trypsin-dependent mechanisms. *Int J Obes* 35:236–243
- Kovalskaya N, Hammond RW (2009) Expression and functional characterization of the plant antimicrobial snak-in-1 and defensin recombinant proteins. *Protein Expr Purif* 63:12–17
- Kovalskaya N, Zhao Y, Hammond RW (2011) Antibacterial and antifungal activity of a Snakin-defensin hybrid protein expressed in tobacco and potato plants. *Open Plant Sci J* 5:29–42
- Ku SK, Sung SH, Choung JJ, Choi J-S, Shin YK, Kim JW (2016) Anti-obesity and anti-diabetic effect of a standardized potato extract in *ob/ob* mice. *Exp Ther Med* 12:354–364
- Kumar GNM, Knowles LO, Knowles NR (2015) Zebra chip disease decrease tuber (*Solanum tuberosum* L.) protein content by attenuating protease inhibitor levels and increasing protease activities. *Planta* 242:1153–1166
- La Camera S, Geoffroy P, Samaha H, Ndiaye A, Rahim G, Legrand M, Heitz T (2005) A pathogen-inducible patatin-like lipid acyl hydrolase facilitates fungal and bacterial host colonization in *Arabidopsis*. *Plant J* 44:810–825
- Lattová E, Brabcová A, Bártová V, Potěšil D, Bárta J, Zdráhal Z (2015) N-Glycome profiling of patatins from different potato species of *Solanum* genus. *J Agric Food Chem* 63:3243–3250
- Lee HS (2005) Clinical effects of intake of juice valley and Gogu valley towards fecal microflora of healthy human volunteers. *Food Sci Biotechnol* 14:540–542
- Lee J-K, Gopal R, Seo CH, Cheong H, Park Y (2012) Isolation and purification of a novel deca-antifungal peptide from potato (*Solanum tuberosum* L. cv. Jopung) against *Candida albicans*. *Int J Mol Sci* 13:4021–4032
- Liu G, Chen N, Kaji A, Bode AM, Ryan CAR, Dong Z (2001) Proteinase inhibitors I and II from potatoes block UVB-induced AP-1 activity by regulating the AP-1 protein compositional patterns in JB6 cells. *Proc Natl Acad Sci U S A* 98:5786–5791
- Liu YW, Han C, Lee MH, Hsu FL, Hou WC (2003) Patatin, the tuber storage protein of potato (*Solanum tuberosum* L.), exhibits antioxidant activity *in vitro*. *J Agric Food Chem* 51:4389–4393
- Løkra S, Holland MH, Clausen IC, Straekvern KO, Egellandsdal B (2008) Chemical characterization and functional properties of potato protein concentrate prepared by large-scale expanded bed absorption chromatography. *LWT-Food Sci Technol* 41:1089–1099
- Lomolino G, Vincenzi S, Gazzola D, Crapisi A, Curioni A (2015) Foaming properties of potato (*Solanum tuberosum*) proteins: a study by the gas sparing method. *Colloids Surf A Physicochem Eng Asp* 475:75–83
- Mäkinen S (2014) Production, isolation and characterization of bioactive peptides with antihypertensive properties from rapeseed and potato protein. Ph.D. thesis. University of Turku, Turun Yliopisto, Finland
- Mäkinen S, Kelloniemi J, Pihlanto A, Mäkinen K, Korhonen H, Hopia A, Valkonen JPT (2008) Inhibition of angiotensin converting enzyme I caused by autolysis of potato proteins by enzymatic activities confined to different parts of the potato tuber. *J Agric Food Chem* 56: 9875–9883
- Matos AR, Pham-Thi A-T (2009) Lipid deacylating enzymes in plants: old activities, new genes. *Plant Physiol Biochem* 47:491–503
- McCarthy AL, O'Callaghan YC, O'Brien NM (2013) Protein hydrolysates from agricultural crops – bioactivity and potential for functional food development. *Agriculture* 3:112–130
- Meenu Krishnan VG, Murugan K (2016) *Solanum* protease inhibitors and their therapeutic potentialities: a review. *Int J Pharm Sci* 8:14–21
- Mendieta JR, Pagano MR, Muñoz FF, Daleo GR, Guevara MG (2006) Antimicrobial activity of potato aspartic proteases (StAPs) involves membrane permeabilization. *Microbiology* 152:2039–2047
- Meng S, Xu H, Wang F (2010) Research advances of antimicrobial peptides and applications in food industry and agriculture. *Curr Protein Pept Sci* 11:264–273
- Millar DJ, Allen AK, Smith CG, Sidebottom C, Slabas AR, Bolwell GP (1992) Chitin-binding proteins in potato (*Solanum tuberosum* L.) tuber. Characterization, immunolocalization and effects of wounding. *Biochem J* 283:813–821
- Moreno M, Segura A, Garcia-Olmedo F (1994) Pseudothionin-Sth1, a potato peptide active against potato pathogens. *Eur J Biochem* 223: 135–139
- Mulder KC, Lima LA, Miranda VJ, Dias SC, Franco OL (2013) Current scenario of peptide-based drugs: the key roles of cationic antitumor and antiviral peptides. *Front Microbiol* 4:321
- Munger A, Simon M-A, Khalif M, Goulet M-C, Michaud D (2015) Cereal cystatins delay sprouting and nutrient loss in tubers of potato, *Solanum tuberosum*. *BMC Plant Biol* 15:296
- Nahiriak V, Almasia NI, Fernandez PV, Hopp HE, Estevez JM, Carrari F, Vazquez-Rovere C (2012) Potato snakin-1 gene silencing affects cell division, primary metabolism, and cell wall composition. *Plant Physiol* 158:252–263
- Ng TB, Cheung RCF, Wong JH (2013) Recent progress in research on plant antifungal proteins: a review. In: *Antifungal metabolites from plants*. Springer, Berlin, Heidelberg, pp 221–241

- Oerke EC, Dehne HW, Schönbeck F, Weber A (2012) Crop production and crop protection: estimated losses in major food and cash crop. Elsevier, Amsterdam, The Netherlands
- Ohh SH, Shide PL, Choi JY, Jin Z, Hahn TW, Lim HT, Kim GY, Park YK, Hahm KS, Chae BJ (2010) Effect of potato (*Solanum tuberosum* L. cv. Golden Valley) protein on performance, nutrient metabolizability, and cecal microflora in broilers. Arch Geflügelk 74:30–35
- Pagano MR, Mendieta JR, Munoz FF, Daleo GR, Guevara MG (2007) Roles of glycosylation on the antifungal activity and apoplast accumulation of StAPs *Solanum tuberosum* aspartic proteases. Int J Biol Macromol 41:512–520
- Paik D, Das P, De T, Chakraborti T (2014) *In vitro* anti-leishmanial efficacy of potato tuber extract (PTE): Leishmanial serine proteases as putative target. Exp Parasitol 146:11–19
- Parachin NS, Franco OL (2014) New edge of antibiotic development: antimicrobial peptides and corresponding resistance. Front Microbiol 5:147
- Park Y, Choi BH, Kwak JS, Kang CW, Lim HT, Cheong HS, Hahm KS (2005) Kunitz-type serine protease inhibitor from potato (*Solanum tuberosum* L. cv. Jopung). J Agric Food Chem 53:6491–6496
- Park S, Gupta R, Krishna R, Kim ST, Lee DY, Hwang D-J, B S-Ch S-C, Ahn I-P (2016) Proteome analysis of disease resistance against *Ralstonia solanacearum* in potato cultivar CT206-10. Plant Pathol J 32:25–32
- Peña-Cortés H, Sánchez-Serrano JJ, Mertens R, Willmitzer L, Prat S (1989) Abscicic acid is involved in the wound-induced expression of the proteinase inhibitor II gene in potato and tomato. Proc Natl Acad Sci U S A 86:9851–9855
- Perla V, Jayanty SS, Holm DG, Davidson RD (2014) Relationship between tuber storage proteins and tuber powdery scab resistance in potato. Am J Potato Res 91:233–245
- Peyer C, Bonay P, Staudacher E (2004) Purification and characterization of a beta-xylosidase from potatoes (*Solanum tuberosum*). Biochim Biophys Acta 1672:27–35
- Pots AM (1999) Physico-chemical properties and thermal aggregation of patatin, the major potato tuber protein. Ph.D. thesis. Wageningen Agricultural University, Wageningen, The Netherlands
- Pouvreau L (2004) Occurrence and physico-chemical properties of protease inhibitors from potato tubers (*Solanum tuberosum*). Ph.D. thesis. Wageningen Agricultural University, Wageningen, The Netherlands
- Pouvreau L, Gruppen H, Piersma SR, van den Broek LAM, van Koningsveld GA, Voragen AGJ (2001) Relative abundance and inhibitory distribution of protease inhibitors in potato juice from cv. Elkana. J Agric Food Chem 49:2864–2874
- Quilis J, Meynard D, Vila L, Avilés FX, Guiderdoni E, Segundo San B (2007) A potato carboxypeptidase inhibitor gene provides pathogen resistance in transgenic rice. Plant Biotechnol J 5:537–553
- Racusen D (1983) Lipid acyl hydrolase of patatin. Can J Bot 62:1640–1644
- Racusen D (1986) Esterase specificity of patatin from two potato cultivars. Can J Bot 64:2104–2106
- Revina TA, Speranskaya AS, Kladnitskaya GV, Shevelev AB, Valueva TA (2004) Subtilisin protein inhibitor from potato tubers. Biochem Mosc 69:1092–1098
- Revina TA, Gerasimova NG, Kladnitskaya GV, Chalenko GI, Valueva TA (2008) Effect of proteinaceous proteinase inhibitors from potato tubers on the growth and development of phytopathogenic microorganisms. Appl Biochem Microbiol 44:89–92
- Rodríguez IF, Sayago JE, Torres S, Zampini IC, Isla MI, Ordóñez RM (2015) Control of pathogens by protein extracts from *Solanum tuberosum* tubers. Eur J Plant Pathol 141:585–595
- Romero A, Beaumal V, David-Briand E, Cordobés F, Guerrero A, Anton M (2011) Interfacial and oil/water emulsions characterization of potato protein isolates. J Agric Food Chem 59:9466–9474
- Rosenfeld Y, Lev N, Shai Y (2010) Effect of the hydrophobicity to net positive charge ratio on antibacterial and anti-endotoxin activities of structurally similar antimicrobial peptides. Biochemistry 49:853–861
- Salas CE, Badillo-Corona JA, Ramírez-Sotelo G, Oliver-Salvador C (2015) Biologically active and antimicrobial peptides from plants. Biomed Res Int 2015:1–11
- Sánchez A, Vázquez A (2017) Bioactive peptides: a review. Food Qual Saf 1:29–46
- Schäfer D, Reinelt M, Stäbler A, Schmid M (2018) Mechanical and barrier properties of potato proteins isolate-based films. Coatings 8:58
- Schmidt JM, Damgaard H, Greve-Paulsen M, Larsen LB, Hammershøj M (2018) Foam and emulsion properties of potato protein isolate and purified fractions. Food Hydrocoll 74:367–378
- Segura A, Moreno M, Madueno F, Molina A, Garcia-Olmedo F (1999) Snakin-1, a peptide from potato that is active against plant pathogens. Mol Plant-Microbe Interact 12:16–23
- Senda K, Yoshioka H, Doke N, Kawakita K (1996) A cytosolic phospholipase A2 from potato tissues appears to be patatin. Plant Cell Physiol 37:347–353
- Senda K, Doke N, Kawakita K (1998) Effect of mastoparan on phospholipase A2 activity in potato tubers treated with fungal elicitor. Plant Cell Physiol 39:1080–1086
- Shah KR, Patel DK, Pappachan A, Prabha CR (2016) Characterization of a Kunitz-type serine protease inhibitor from *Solanum tuberosum* having lectin activity. Int J Biol Macromol 83:259–269
- Sharma N, Gruszeński HA, Park SW, Holm DG, Vivanco JM (2004) Purification of an isoform of patatin with antimicrobial activity against *Phytophthora infestans*. Plant Physiol Biochem 42:647–655
- Shen XL, Wu JM, Chen Y, Zhao G (2010) Antimicrobial and physical properties of sweet potato starch films incorporated with potassium sorbate or chitosan. Food Hydrocolloid 24:285–290
- Sitjà-Arnau M, Molina MA, Blanco-Apericio C, Ferrer-Soler L, Lorenzo J, Avilés FX, Querol E, de Llorens R (2005) Mechanism of action of potato carboxypeptidase inhibitor (PCI) as an EGF blocker. Cancer Lett 226:169–184
- Theis T, Stahl U (2004) Antifungal proteins: targets, mechanisms and perspective applications. Cell Mol Life Sci 61:437–455
- Tonón C, Daleo G, Oliva C (2001) An acidic β -1,3 glucanase from potato tubers appears to be patatin. Plant Physiol Biochem 39:849–854
- Tonón C, Guevara G, Oliva C, Daleo G (2002) Isolation of a potato acidic 39 kDa β -1,3-glucanase with antifungal activity against *Phytophthora infestans* and analysis of its expression in potato cultivars differing in their degrees of field resistance. J Phytopathol 150:189–195
- Tripathi GR, Park J, Park Y, Hwang I, Park Y, Hahm KS, Cheong H (2006) Potide-G derived from potato (*Solanum tuberosum* L.) is active against potato virus YO (PVYO) infection. J Agric Food Chem 54:8437–8443
- Udenigwe CC, Udechukwu MC, Yiridoe C, Gibson A, Gong M (2016) Antioxidant mechanism of potato protein hydrolysates against in vitro oxidation of reduced glutathione. J Funct Foods 20:195–203
- Valueva TA, Revina TA, Kladnitskaya GV, Mosolov VV (1998) Kunitz-type proteinase inhibitors from intact and *Phytophthora*-infected potato tubers. FEBS Lett 426:131–134
- Valueva TA, Revina TA, Gvozdeva EL, Gerasimova NG, Ozeretskovskaya OL (2003) Role of proteinase inhibitors in potato protection. Bioorg Khim 29:499–504
- Valueva TA, Parfenov IA, Revina TA, Morozkina EV, Benevolensky SV (2012) Structure and properties of the potato chymotrypsin inhibitor. Plant Physiol Biochem 52:83–90
- van Loon LC, van Kammen A (1970) Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var “Samsun” and “Samsun NN”. Virology 67:566–75
- Vernekar JV, Tanksale AM, Ghatge MS, Deshpande VV (2001) Novel bifunctional alkaline protease inhibitor: protease inhibitory activity

- as the biochemical basis of antifungal activity. *Biochem Biophys Res Commun* 285:1018–1024
- Wagley A, Karboune S, Alli I (2014) Potato protein isolates: Recovery and characterization of their properties. *Food Chem* 142:373–386
- Wang JF, Du LP, Li Z, Huang SP, Ye XG, Feng D, Zhang ZY (2012) Development and characterization of SN1 transgenic wheat plants with enhanced resistance to *Rhizoctonia cerealis* and *Bipolaris sorokiniana*. *Acta Agron Sin* 38:773–779
- Wiesner J, Vilcinskas A (2010) Antimicrobial peptides: the ancient arm of the human immune system. *Virulence* 1:440–464
- Wimley WC (2010) Describing the mechanism of antimicrobial peptide action with the interfacial activity model. *ACS Chem Biol* 15:905–917
- Woloshuk CP, Meulenhoff JS, Sela-Buurlage M, van den Elzen PJ, Cornelissen BJ (1991) Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. *Plant Cell* 3:619–628
- Yan J, Yuan S-S, Jiang L-L, Ye X-J, Ng TB (2015) Plant antifungal proteins and their applications in agriculture. *Appl Microbiol Biotechnol* 99:4961–4981

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.