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AKADÉMIAI KIADÓ

Examples of potato epidermis endophytes and rhizosphere microbes that may be human pathogens contributing to potato peel colic

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SHORT COMMUNICATION

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ABSTRACT

Potato tubers defend themselves against herbivores with endogenous secondary compounds such as solanine and scopolamine. They also recruit endophytes and members of the tuberosphere to repel herbivores. Many of these endophyte defence features are overcome by cooking, with some notable exceptions that have been identified by rDNA analysis of potato peel samples and may account for some previously unrecognised features of potato peel colic. This is relevant regarding the rather modern way of cooking, where the potato peel is left intact in food and consumed.

KEYWORDS

potato, tuber, colic, mycotoxin, endophyte, rDNA

1. INTRODUCTION

Potato tubers are an excellent source of nutrients, though they do have some drawbacks. Especially parts near the epidermis can cause bowl irritation and colic, though this tissue has traditionally been peeled and disposed of. Unfortunately, since the 1940s, potato peels have been popularly, but wrongly, accepted as the most nutritious part of this food (Gale, 1941;

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Camire et al., 2009). However, this part of the potato tuber can also be the most hazardous, where the associated human pathology is usually attributed entirely to alkaloids (Iablokov et al., 2010), especially if the tubers are allowed to green. In addition to alkaloids, but much less well defined, there is additional pathological material at the surface of tubers. These include microbial endophytes, microbial toxins, heat resistant toxins and spores, which may also contribute to colic and irritable bowel syndrome caused by the ingestion of these microbial elements in the potato peel, even if cooked (Altayar and Sutherland, 2006; Iablokov et al., 2010).

Metabarcoding or analysis of environmental samples for rDNA (ribosomal DNA) is an excellent method for determining the entire microbial community in a sample, including recalcitrant species that cannot be cultivated. This paper presents some examples of the normal microbial components of potato peel. This supplements the abundant body of research concerned with conventional microbiological methods used to study plant pathogenic endophytes (Stone et al., 2004). In nature, however, such sharp and anthropocentric distinctions are not seen, and instead there is a natural spectrum of endophyte functions from pathological, to "normal", to symbiotic, and endophytes sometimes even protect plants because they repel herbivores (Durham and Tannenbaum, 1998; Fortier et al., 2001). With this method, in some cases, even human bacterial pathogens can be detected (Brandl et al., 2013; van Overbeek et al., 2014), because of the practice of using animal dung or even human manure for fertilisation (Bryan, 1977). Sometimes even fungal human pathogens are detected (Doan and Davidson, 2000), though fungi are less commonly cited. Here we report examples of bacterial and fungal endophytes from potato peels, emphasising organisms capable of causing colic and bowel irritation even after cooking. We hope that in the future, people will avoid food like this, which could cause harm to their health.

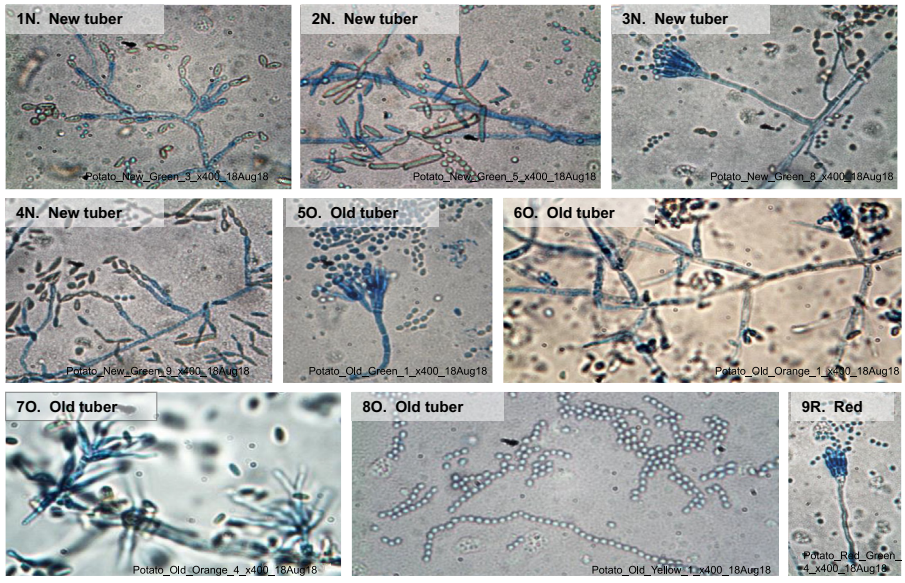

Fig. 1. Endophytes grown out of tubers to reveal reproductive structures, then stained using the Aniline Lactophenol Blue Cellophane Tape method (Carmichael, 1956). Numerals show species number. N: new; O: old, R: red

2. MATERIALS AND METHODS

Red (Petite Red), New (Publix White), and Old (Russet, aged at 4 °C for ≥ one month) potato tubers were purchased from Publix Supermarkets (Lakeland, Fl). For microscopic studies Red, New, and Old potatoes were halved and incubated at 25 °C, in a glass dish, under plastic wrap until fungal endophytes produced visible conidiospores (day 5). These fungal colonies were

Table 1. Most abundant prokaryotes

Species	New	Red	Old	Notes	Reference
Arthrobacter sp.	0.0	0.0	0.2	Normal soil microbiome members	Mongodin et al. (2006)
Bacillus sp.	8.6	41.6	24.4	Normal soil microbe with heat resistant spores. Heat resistant toxins in some species	Vary et al. (2007); Taylor et al. (2005); Rasimus-Sahari et al. (2015); Altayar and Sutherland (2006)
Enterobacter sp.	46.1	32.6	12.7	Indicator of faecal material, some species produce heat stable carbohydrate endotoxins	Alegbeleye et al. (2018); Guo et al. (2018)
Enterococcus casseliflavus	0.1	0.5	0.0	*Enterococcus* indicate faecal material, *E. casseliflavus* has a heat stable small protein toxin	Rajarajan et al. (2018)
Escherichia hermannii	0.1	1.3	0.3	*Escherichia* is an indicator of faecal material, *E. hermannii* is an opportunistic pathogen	Yamanaka et al. (2010)
Kluyvera intermedia	0.4	0.2	0.1	*Kluyvera* indicate faecal material	Balzer et al. (2010)
Microbacterium sp.	0.0	0.1	0.2	*Microbacterium* sp. indicate faecal material and may be opportunistic pathogen	Balzer et al. (2010)
Pantoea sp.	34.1	18.6	58.1	*Pantoea* sp. is an enterobacterial opportunistic pathogen	Liberto et al. (2009)
Pseudomonas sp.	0.3	0.1	0.3	Some rhizosphere *Pseudomonas* spp. are opportunistic pathogens. Some produce heat resistant toxins	Berg et al. (2005); Heckly (1970)
Serratia proteamaculans	0.4	0.1	0.0	Some rhizosphere *Serratia* spp. may be opportunistic pathogens	Berg et al. (2005)
Staphylococcus epidermidis	9.6	4.1	3.3	Part of normal skin microbiom but may be an opportunist	Fey and Olson (2010)

All prokaryotes detected by rDNA analysis were ranked by relative abundance (numbers shown above) and species that ranked ≥0.1 were selected for this table. Species were then reordered alphabetically.

Table 2. Most abundant fungi

Species	New	Red	Old	Notes	Reference
Acremonium sp.	2.3	0.9	0.1	Common endophytes. Some are opportunist pathogens. Some produce heat stable alkaloids	Wicklow et al. (2005); Perdomo et al. (2011)
Alternaria sp.	0.0	3.4	0.0	Some are opportunists. Some produce several toxic heat stable secondary products	Davis and Stack (1994); De Hoog and Horré (2002); Terminiello et al. (2006)
Athelia bombacina	0.0	0.0	2.3	Normal soil fungus and plant pathogen	Heye and Andrews (1983)
Chaetomium sp.	0.3	0.0	3.1	Soil fungus, endosymbiont and plant pathogen. Rare human pathogen	Haruma et al. (2018); Barron et al. (2003), Sodeoka et al. (2012)
Cladosporium sp.	0.3	0.0	0.0	Soil microbiom member and plant pathogen. Rarely human pathogenic. Heat stable mycotoxins	Ogórek et al. (2012); Cheng et al. (2015); Ma et al. (2017)
Colletotrichum coccodes	0.0	0.0	1.1	Soil microbiom member and plant pathogen	Cummings and Johnson (2014)
Cryptococcus sp.	2.4	83.2	0.7	Yeast soil microbiom member and plant pathogen. Some species are human pathogens	Fonseca et al. (1999)
Debaryomyces hansenii	7.4	0.1	0.1	Soil yeast. Some strains are human pathogenic	Breuer and Harms (2006)
Doratomyces sp.	0.0	0.0	0.3	Soil fungus	Jiang and Zhang (2008)
Emericellopsis stolkiae	0.0	0.3	0.0	Soil fungus	Davidson and Christensen (1971)
Erysiphe huayinenesis	4.2	0.1	0.1	Most *Erisiphe* spp. are plant pathogens causing powdery mildews	Saenz and Taylor (1999)
Filobasidium floriforme	0.0	2.1	0.0	Environmental basidiomycetous yeast	Bandoni et al. (1991)
Fusarium sp.	1.1	0.2	9.1	Soil fungus. Some are human opportunists. Some *Fusarium* spp. produce heat stable toxins	Babic et al. (2015); King (1981)
Geomyces sp.	0.8	0.1	2.5	Soil fungus. Some are animal opportunists	Gargas et al. (2009)
Helminthosporium solani	0.1	4.0	0.0	Aetiological agent of Silver Scurf disease of potato	Errampalli et al. (2001)
Lactarius volemus	1.5	0.0	0.0	Widespread ectomycorrhizal fungus	Shimono et al. (2007)
Meyerozyma guilliermondii	0.0	0.3	0.0	Soil fungus. Some *Meyerozyma* spp. are opportunists	Coda et al. (2013); Babic et al. (2015)
Penicillium sp.	0.1	0.1	3.7	Environmental ascomycetous fungus. Some produce heat stable mycotoxins	Pitt (1987); Cruz et al. (2013)

(continued)

Table 2. Continued

Species	New	Red	Old	Notes	Reference
Plectosphaerella sp.	0.5	0.1	17.7	Soil fungi, many are plant pathogens	Raimondo and Carlucci, 2018
Preussia sp.	0.0	0.0	0.1	Dung and soil fungi	Cain (1961); Mapperson et al. (2014)
Rhodotorula sp.	0.0	0.6	0.0	Environmental basidiomycetous yeast, some species are opportunists	Zaas et al. (2003)
Stemphylium solani	0.0	0.5	0.0	Aetiological agent of Grey Leaf Spot and other diseases in several plant species. Heat stable phytotoxin	Zheng et al. (2009, 2010)
Termitomyces sp.	0.0	1.1	0.0	Symbionts with termites	Pegler and van Haecke (1994)
Trichocladium asperum	0.6	0.3	54.9	Soil fungus, some dermatophyte species	Góralska et al. (2015)
Trichosporon sp.	0.9	0.4	0.2	Environmental basidiomycetous yeast, some species are human pathogens	Gemeinhardt (1965); Sugita et al. (2000); Archer-Dubon et al. (2003),
Umbelopsis sp.	1.6	0.0	0.0	Soil fungus	Meyer and Walter (2003)
Volutella ciliata	0.8	0.0	0.1	Plant litter fungus	Collado et al. (2007)

All fungi detected by rDNA analysis were ranked by relative abundance (numbers shown above) and species which ranked ≥0.1 were selected for this table. Species were then reordered alphabetically.

counted. No bacterial colonies were visible. The fungal structures were harvested using cellophane tape (Carmichael 1956), stained with Lactophenol Blue (Linder, 1929), and examined using bright field microscopy.

For molecular analysis, endophytes in the tuberosphere were harvested by peeling the outer 1.5 mm using a kitchen potato peeler. The peel was placed on paper towels and dried at 45 °C, 50 °C, or 55 °C to optimise the drying process and to find conditions for drying at the lowest temperature to avoid microbial decomposition. Appearance, odour, and mass were recorded periodically, and according to the results, 50 °C was selected for drying potato peel for subsequent analyses. For DNA analysis tuber peel was dried at 50 °C for 2 days and then sequence analyses were performed using the Illumina sequencing platform (Reeder and Knight, 2010) optimised for prokaryote or fungal rDNA targets (Scott Dowd, personal communication). DNA primers used for detection of bacteria were bac799F 5′-ACCMGGATTAGATACCCKG-3′ and illbac1193R 5′-CRTCCMCACCTTCCTC-3′. Primers used for detection of fungi were ITS1F-Bt1 5′-CTTGGTCATTTAGAGGAAGTAA-3′ and ITS2R 5′-GCTGCGTTCTTCATC-GATGC-3′.

Sequence relative abundance was screened for values above 0.1, and graphs were drawn using the Excel program. After identification of the microbes, literature databases were searched to determine if relevant microbes were known to produce, especially heat resistant, substances that are toxic, allergenic, or irritating.

3. RESULTS AND DISCUSSION

When endophytes were grown out of potato tubers, only a few types were identifiable using the unaided eye (three from Red, three from New, and five from Old potatoes) or bright field microscopy (one from Red, four from New, and four from Old potatoes, see Fig. 1). All these fungal types were recognised as separate species because of their different types of conidia and

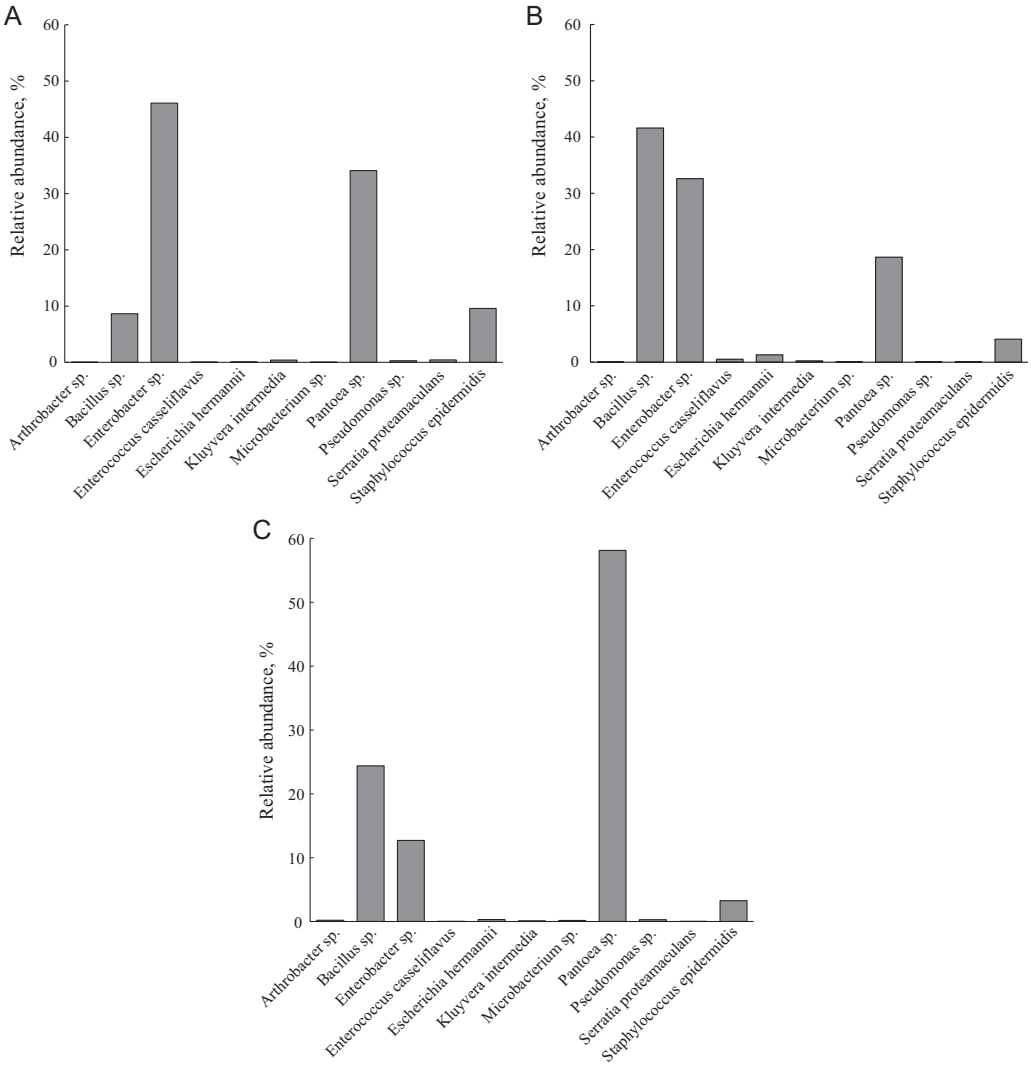

Fig. 2. A: Four important prokaryote species were detected by rDNA analysis of A: new potato peel, B: red potato peel; C: old potato peel

conidiophores. Because of this obvious underestimation of microbial load, detection of microbes using rDNA analysis was done.

Nucleotide sequencing data were ranked by abundance because it was assumed that the more abundant examples were more important. This showed a ranked list of eleven prokaryotes with relative abundance higher than 0.1, four of which were implicated in producing heat resistant pathological substances (Table 1). A similar process of ranking showed twenty seven fungi with relative abundance higher than 0.1, five of which were able to produce heat resistant pathological substances (Table 2). Literature search has shown that many of these species are characteristic of tubers used for food and cooking because they have been linked to research that on heat resistant pathological features. Relevant papers are listed in Tables 1 and 2.

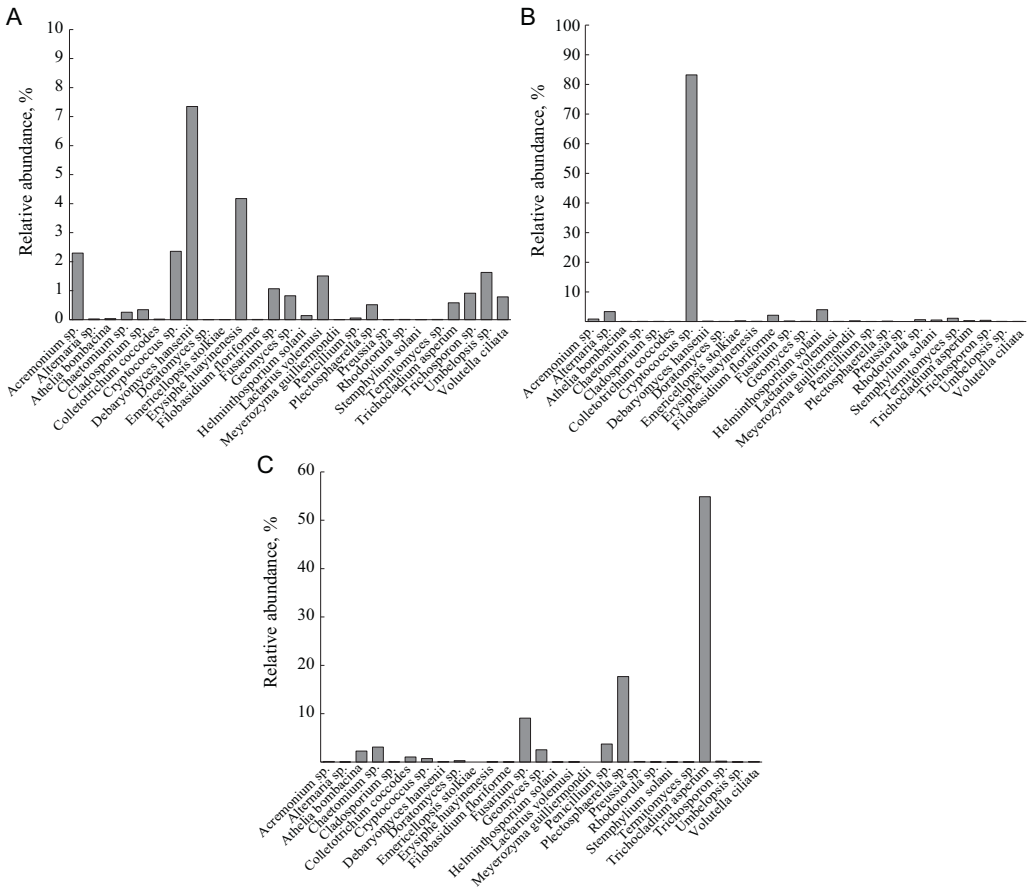

Fig. 3. Fungi in potato peel. A: More than ten important fungal species were detected by rDNA analysis of new potato peel. B: One important fungal species was detected by rDNA analysis of red potato peel; C: Seven important fungal species were detected by rDNA analysis of old potato peel

4. CONCLUSIONS

For endophyte growth, physiological state of tubers is more important than genetic cultivar, and that is why the three groups of tubers were selected for DNA analysis: new, red, and old. The number of fungal colonies that could be used for microscopy was very small and could be prepared for the examinations with difficulty. Many more species were identified by rDNA analysis, as shown by comparison of the small number of species seen in Fig. 1 and the ranked abundance data recorded in Tables 1 and 2. This shows that rDNA sequencing analysis was needed to avoid underestimates of species present and also to avoid the bias that always comes from culturing microbes, i.e. some important fungi may be slow to grow and some irrelevant fungi may grow very well under the particular conditions of cultivation.

The tuber peel samples were prepared to obtain material similar to peels served as potato peels and chips, i.e. residual soil, epidermis, and buds were retained. Air drying at the optimal temperature of 50 °C was enough to obtain material suitable for sequencing, providing water removal quickly enough to prevent microbial decomposition, as detected by strong amine odour development.

Considering the diverse origin of these potato peels, it is quite an oddity that the major bacterial species are the same in all three cases (Fig. 2A-C), i.e. *Bacillus* sp., *Enterobacter* sp., *Pantoa* sp., and *Staphylococcus epidermidis*. Faecal species were found like *Escherichia, Enterobacter* and *Enterococcus* spp., even though potatoes grown in the USA do not use solid human waste as a fertiliser. These may have originated in wild animal faeces. *S. epidermidis* is a part of normal human skin microbiom and may have originated from handling of the tubers at some point between the field and the laboratory. This is in contrast to fungi, where much greater variation of species was seen between the three samples (Fig. 3A-C). With the exception of *Cryptococcus,* no fungal species associated with human pathology have been observed. However, many species implicated in producing heat stable mycotoxins and allergens (Ogórek et al., 2012) have been detected (Table 2). This study is obviously not representative of all potato tuber peels, but does show that disturbing microbial species are fairly easy to find.

Obviously, cooking potato peels kills almost all live vegetative cells associated with conventional food poisoning (Doan and Davidson, 2000). However, the data in Tables 1 and 2 show that heat resistant irritants, allergens, alkaloids, and possibly unknown irritating compounds may be present, which could survive cooking and account for some human pathology observed from eating potato peels, even after cooking (Altayar and Sutherland, 2006; Iablokov et al., 2010). This is all over and above the fact that bitter tuber alkaloids are found mostly in the epidermis (Friedman and Dao, 1992; Deußer et al., 2012; Zhang and Peterson, 2018) and contribute significantly to potato peel colic from green tubers. Endophytes may account for the colic observed when consuming non-green cooked potato peels. This is enough to come to the obvious conclusion that potato tubers defend themselves in several ways including cooperation with symbiotic endophytes. It is not unexpected, as plants are already known to defend themselves using symbiotic fungi (Hardoim et al., 2015). Little research has been done to determine if potato endophytes are similar worldwide, so the particular species described here might be limited to North American potatoes and cultivation methods. However, caution is advisable, because eating unknown microbes is a risky activity. Therefore, to improve food safety, we should do the sensible thing, which is to peel potato tubers and throw the poisonous epidermal defence layer away rather than expose ourselves to the danger of eating it.

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