SHORT COMMUNICATION

Cyttaria hariotii E.Fisch. as a promising source of pullulan and Mn(II)-pullulan complexes for Mn-deficiency remediation in winter cereals

M. Carmen Ramos-Sánchez, Jesús Martín-Gil, Laura Buzón-Durán and Pablo Martín-Ramos

ABSTRACT

Pullulan, a water-soluble polysaccharide consisting of maltotriose units used in the preparation of edible films and drug delivery, is generally produced from starch by Aureobasidium pullulans (de Bary & Löwenthal) G.Arnaud fungus. In this article, the characterisation of an alternative pullulan source – the stromata of Cyttaria hariotii E.Fisch. fungus – by elemental analysis, infrared spectroscopy and thermal analysis techniques is reported. With a view to a possible valorisation of this pullulan and its derivatives as bioactive formulations in agriculture, low-molecular-weight pullulan (<7 kDa) complexes with Mn(II), suitable for the remediation of Mn-deficiencies in winter cereal by foliar application, were synthesised and characterised by mass spectrometry.

ARTICLE HISTORY

Received 11 May 2020
Accepted 13 September 2020

KEYWORDS

E1204; FTIR spectroscopy; llao-llao; mass spectrometry; thermal analysis

CONTACT

Pablo Martín-Ramos pmr@unizar.es

Supplemental data for this article can be accessed at http://sci-hub.st/10.1080/14786419.2020.1831493.

© 2020 Informa UK Limited, trading as Taylor & Francis Group
1. Introduction

_Cyttaria hariotii_ E.Fisch. is one of the most common fungi in Andean-Patagonian forests. General information on this fungus is presented in Supplementary Material. The main carbohydrate component of _C. hariotii_ is pullulan, a homo-polysaccharide composed by maltotriosyl repeating units and a small number of maltotetraose residues (Shingel 2004). Pullulan is usually biosynthesized by strains of _Aureobasidium pullulans_ (de Bary & Löwenthal) G.Arnau d, and has applications in blood plasma substitutes, edible coatings for fruits, additives and cosmetics (Piergiovanni and Mascheroni 2007).

Upon subjection to the action of enzymes or by treatments with chemicals, low-molecular pullulan may be obtained, which can find applications through complexation with divalent transition metal ions (Mitić et al. 2011). Although synthetic procedures for the formation of complexes between M(II) transition metal ions and pullulan are already available in the bibliography (Mitić et al. 2011, 2018), the one corresponding to Mn(II)-pullulan complexes has not been reported so far.

The aim of the study presented herein has been to investigate the vibrational and thermal properties of _C. hariotii_ stromata, assessing its suitability for its potential use as raw material. Given the large number of active centres in pullulan that are capable of bond formation with M(II) ions, the preparation of Mn(II) ions complexes with low-molecular-weight pullulan from _C. hariotii_ has also been investigated. Such complexes may find application in foliar Mn fertilisation.
2. Results and discussion

2.1. Characterisation of pullulan from C. hariotii

2.1.1. Elemental analysis
Experimental values of CHON for C. hariotii stromata (36.8% in C, 7.0% in H, 44.5% in O, and 0.6% in N) were in agreement with calculated values on basis of a ~73 wt% content in pullulan.

2.1.2. Vibrational characterisation
The infrared spectra of the samples from C. hariotii were compared with those of the commercial pullulan and maltotriose (Figure S2). Band assignments are summarised in Table S2. The spectra of the samples from C. hariotii exhibited very similar features to those of commercial pullulan, although the bands at 720 and 755 cm\(^{-1}\) (maltotetraose units) and 1080 cm\(^{-1}\) (\(\alpha\)-(1→4) glycosidic linkages) were missing. The absence of bands assignable to ester functionality (1653 cm\(^{-1}\)) suggests low esterification or etherification degree, which confers high solubility. Thus, the concurrence of low contents in \(\alpha\)-(1→4)-D-glucosidic bonds and a low esterification degree would explain the moderate-high solubility in water exhibited by pullulan from C. hariotii.

2.1.3. Thermal characterisation
The thermal curves of C. hariotii stromata samples in inert and oxidative conditions (Figure S3), together with their interpretation, are presented in Supplementary Material. A good correspondence with pullulan obtained from A. pullulans was found for the TG thermograms and the maxima of the DTG peaks and DSC effects (Table S3) (Ramos Sánchez 1990; Katsikas et al. 1993).

2.1.4. Comparison with pullulan obtained from other sources
According to both the literature data and results presented above, stromata of C. hariotii can be an excellent source of pullulan, although it exhibits differential characteristics as compared to pullulans from other origins, such as its higher water solubility and lower functionalization degree. In addition, while the \(M_W\) of pullulan obtained from A. pullulans ranges from thousands to 20,00,000 Da (Rekha and Sharma 2007), pullulan from C. hariotii can have a \(M_W\) as low as 140 kDa (Oliva et al. 1986). Moreover, some of the drawbacks detected when A. pullulans is chosen for pullulan production (Mishra 2017) would be avoided.

2.2. Potential applications of pullulan from C. hariotii: complexes of low-molecular-weight pullulan with Mn(II)
As discussed above, low-\(M_W\) pullulan can find applications through complexation with divalent transition metal ions (Mitić et al. 2011).

The MALDI-TOF/TOF MS spectra of the Mn(II)-pullulan complexes (Figure S4) showed repeating units of 230 Da and 232 Da, which correspond to Glcp(OH)\(_4\) and MnGlcp(OH), respectively, where Glcp is the anhydro-glucopyranose moiety. Since peaks with \(m/z\) difference of 54.9 Da (which would correspond to Mn) did not appear,
one may conclude that Mn(II)-RLMP complexes were very stable. According to Mitić et al. (2011), a tentative structure with tetragonal distorted $O_6$ coordination with O ligand atoms can be proposed for the $[\text{Mn(Glc}p]_3$ and $[\text{Mn}_2(\text{Glc}p)_3(\text{H}_2\text{O})_2]$ complexes.

These Mn(II)-pullulan complexes may be useful as a bioactive formulation in agriculture, given that manganese deficiency remains a major unsolved nutritional problem in agricultural plant production. Such deficiency causes substantial yield reductions, especially in the case of winter cereals cultivated on sandy and calcareous soils (Hebbern et al. 2005; Mousavi et al. 2011; Schmidt et al. 2013), or as a result of increasing phosphorus status due to the application of high levels of animal manure and P-fertilisers (Schjoerring et al. 2011). This deficiency is traditionally corrected by repeated foliar Mn applications (Schmidt et al. 2016; Ullah et al. 2018). Given the high solubility of the Mn(II)-pullulan complexes reported herein, the pullulan obtained from $C. hariotii$ may thus find application in such agricultural practices, using it as a carrier for Mn(II) delivery and expanding its current applications as a carrier in drug delivery (dos Santos and Grenha 2015; Pandurangan et al. 2016; Nasrollahzadeh et al. 2019).

Alternatively, the pullulan for $C. hariotii$ may also be used for other previously reported applications in agriculture: for instance, as a binding agent for solid fertilizers, providing higher water solubility and allowing time-released N fertilisation (Matsunaga et al. 1977), or in seed coatings (Matsunaga et al. 1978).

3. Conclusions

The present study puts forward the use of $C. hariotii$ as a cheap source of pullulan, using a green chemistry technique (sonication) and avoiding some of the drawbacks detected when $A. pullulans$ is chosen for pullulan production. Moreover, pullulan from $C. hariotii$ exhibits higher water solubility and a lower functionalization degree than the pullulan obtained from $A. pullulans$ or $R. paludigenum$, making it more suitable for processing. Whereas hydrophobised pullulan is used preferably as a coating material in drug delivery applications, pullulan with higher water solubility and low viscosity, as the one obtained from $C. hariotii$, may have countless industrial applications as a food additive, blood plasma substitute, flocculant and adhesive. Relative to its potential applications in agriculture, complexes of Mn(II) ion with low-MW pullulan (6 kDa) from $C. hariotii$ were synthesised and characterised by MALDI-TOF/TOF MS. By repeated foliar spraying, these complexes may be suitable for the timely alleviation of latent Mn-deficiency in winter cereals, with a view to ensuring winter survival, and increasing grain yields.

Disclosure statement

The authors declare no conflict of interest.

References

dos Santos MA, Grenha A. 2015. Polysaccharide nanoparticles for protein and peptide delivery. Chapter 7. In: Donev R, editor. Protein and peptide nanoparticles for drug delivery. Amsterdam: Elsevier; p. 223–261.
Hebbern CA, Pedas P, Schjoerring JK, Knudsen L, Husted S. 2005. Genotypic differences in manganese efficiency: field experiments with winter barley (Hordeum vulgare L.). Plant Soil. 272(1-2):233–244.

Katsikas L, Jeremic K, Jovanovic S, Velickovic JS, Popovic IG. 1993. The thermal degradation kinetics of dextran and pullulan. J Therm Anal. 40(2):511–517.

Matsunaga H, Fujiyama S, Namikawa N, Tsuji K, Watanabe M, inventors. 1977. Sumitomo Chemical Company Ltd, assignee. Fertilizer composition. USA.

Matsunaga H, Tsuji K, Watanabe M, inventors. 1978. Sumitomo Chemical Co Ltd, assignee. Coated seed containing pullulan-based resin used as binder. USA.

Mishra B. 2017. Major problems addressed in pullulan production: a review. Adv Biotechnol Microbiol. 6(5):555696.

Mitić Z, Cakić M, Nikolić GM, Nikolić R, Nikolić GS, Pavlovic R, Santaniello E. 2011. Synthesis, physicochemical and spectroscopic characterization of copper(II)-polysaccharide pullulan complexes by UV-vis, ATR-FTIR, and EPR. Carbohydr Res. 346(3):434–441.

Mitić Z, Nikolić GM, Cakić M, Nikolić GS, Živanović S, Mitić S, Najman S. 2018. Synthesis, spectroscopic and structural characterization of Co(II)-pullulan complexes by UV-Vis, ATR-FTIR, MALDI-TOF/TOF MS and XRD. Carbohydr Polym. 200:25–34.

Mousavi SR, Shahsavari M, Rezaei M. 2011. A general overview on manganese (Mn) importance for crops production. Aust J Basic Appl Sci. 5(9):1799–1803.

Nasrollahzadeh M, Sajjadi M, Sajadi SM, Issaabadi Z, et al. 2019. Green nanotechnology. Chapter 5. In: Nasrollahzadeh M, Sajadi M, Atarod M, editors. An introduction to green nanotechnology. Amsterdam: Elsevier; p. 145–198.

Oliva EM, Fernandez Cirelli A, de Lederkremer RM. 1986. Characterization of a pullulan in Cyttaria darwinii. Carbohydr Res. 158:262–267.

Pandurangan AK, Kanagesan S, Narayanaswamy R, Mohd. Esa N, Parasaruman P. 2016. Nanobiomaterial-based delivery of drugs in various cancer therapies. Chapter 11. In: Grumezescu AM, editor. Nanobiomaterials in cancer therapy. Oxford: William Andrew-Elsevier; p. 331–365.

Piergiovanni L, Mascheroni E. 2007. Impiego delle biotecnologie per la produzione di imballaggi per alimenti. Chapter 15. In: Gigliotti C, Verga R, editors. Biotecnologie alimentari. Padova, Italy: Piccin Nuova Libraria SpA; p. 323–334.

Ramos Sánchez MC. 1990. Utilidad del análisis térmico en microbiología. Aplicación de las técnicas TG-DTG, DTA y DSC en la caracterización de polisacáridos de paredes fúngicas. Valladolid, Spain: Universidad de Valladolid.

Rekha M, Sharma CP. 2007. Pullulan as a promising biomaterial for biomedical applications: a perspective. Trends Biomater Artif Organs. 20(2):116–121.

Schjoerring JK, Skytte K, Husted S, Pedas P. 2011. Elevated phosphorus impedes manganese acquisition by barley plants. Front Plant Sci. 2:37.

Schmidt SB, Pedas P, Laursen KH, Schjoerring JK, Husted S. 2013. Latent manganese deficiency in barley can be diagnosed and remediated on the basis of chlorophyll a fluorescence measurements. Plant Soil. 372(1-2):417–429.

Shingel KI. 2004. Current knowledge on biosynthesis, biological activity, and chemical modification of the exopolysaccharide, pullulan. Carbohydr Res. 339(3):447–460.

Ullah A, Farooq M, Rehman A, Arshad MS, Shoukat H, Nadeem A, Nawaz A, Wakeel A, Nadeem F. 2018. Manganese nutrition improves the productivity and grain biofortification of bread wheat in alkaline calcareous soil. Ex Agric. 54(5):744–754.