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Recycling potato waste for the production of blue pigments by *Streptomyces lydicus* PM7 through submerged fermentation

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Abstract

Background Discarded potato is the most abundant potato waste and represents a worldwide disposal problem to the potato industry. This agricultural waste contains valuable nutrients that could be used as substrate to obtain diverse high value-added microbial products, such as biopigments. The aim of this work was to evaluate the use of discarded potato as a sole substrate source for producing blue pigments by *Streptomyces lydicus* PM7 through submerged fermentation.

Results Initially, the traditional culture medium ISP2 was established as suitable for inoculum preparation, as it allowed high growth rates and consumption of ~ 75% reducing sugar, leading to 1.3 g L^{-1} dry biomass at 72 h of incubation. The formulated discarded potato broth (DPB) medium was evaluated together with five other traditional liquid culture media (potato dextrose broth, ISP2, ISP3, ISP4, and ISP5) for producing blue pigments by *S. lydicus* PM7. The highest blue pigment production was obtained by using DPB medium, reaching ~ 0.97 g L^{-1}, followed by ISP5 (~ 0.36 g L^{-1}). In terms of evaluating the concentration of discarded potato powder, the highest concentration of blue pigments was obtained with 16 g L^{-1}, compared to concentrations of 4, 8, and 32 g L^{-1}. In general, a notable increase in total proteins (~ 14 g L^{-1} in biomass; ~ 8 g L^{-1} in medium) and reducing sugars (~ 5 g L^{-1}) on the fifth day of DPB fermentation was observed, at which time the production of blue pigments began. These data proved that *S. lydicus* PM7 is able to degrade potato wastes during submerged fermentation and to direct metabolism towards the formation of biopigments. Chromatographic analysis revealed that the main blue pigment produced by new strain in this complex medium is actinorhodin.

Conclusions Discarded potato favored the production of blue pigments by *S. lydicus* PM7 under submerged fermentation, leading to final product concentration almost three times higher than others traditional *Streptomyces* culture media. To the best of our knowledge, this is the first report on the production of actinorhodin by the specie *S. lydicus*, as well as on this pigment synthesis based on an agricultural waste as a sole nutrient source for fermentation process. The findings showed that potato waste could be a potential byproduct for replacement of commercial culture media using for this same purpose.

Keywords Actinobacteria, Agricultural waste, Bioconversion, Actinorhodin

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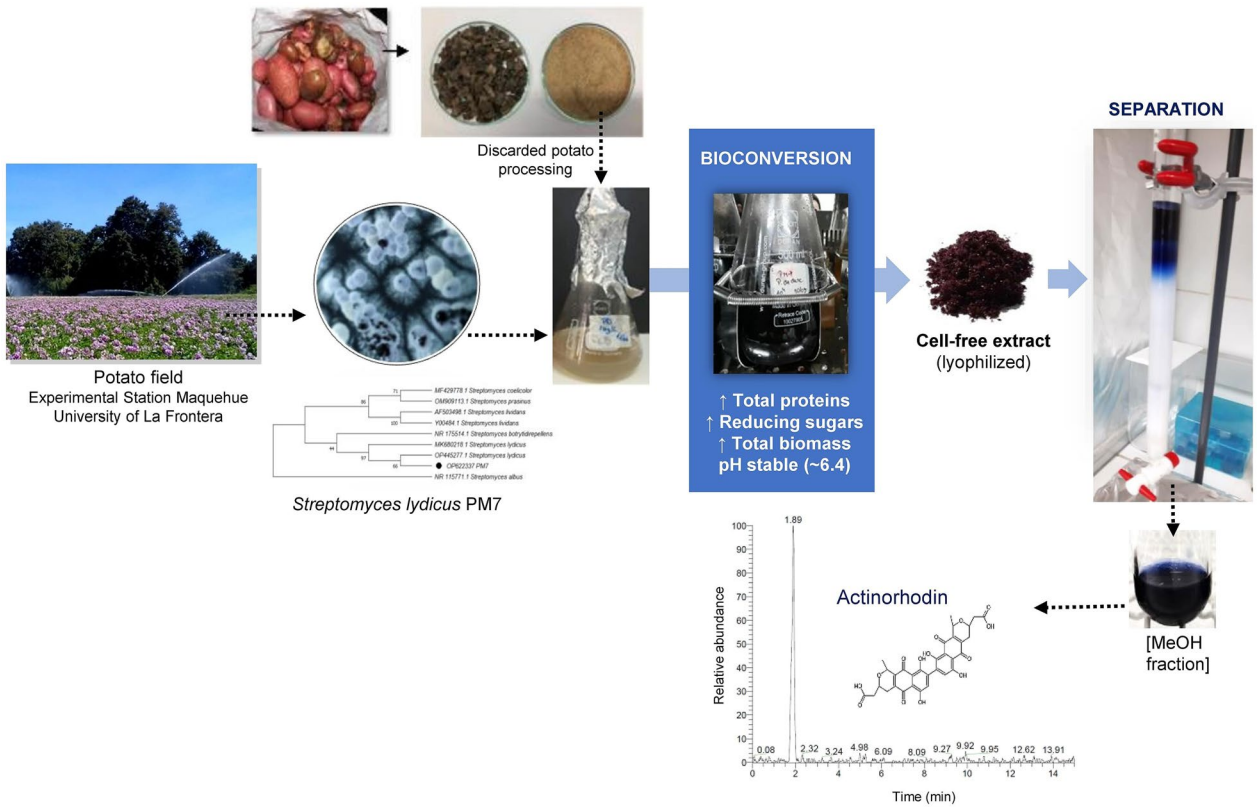
Graphical Abstract



Background

Potatoes are the fourth most important world crop for human consumption behind wheat, rice, and corn. However, ~ 30% of the total potato production is not suitable for human consumption or seed, and therefore eliminated or sold at very low cost for animal feed [1]. Most of the potato wastes are generated during potato harvesting, when potatoes are damaged or left in the field [2]. Thus, discarded potato represents a disposal problem to the potato industry in diverse regions, since the wet tuber is prone to rapid microbial spoilage and constitutes a source of plant pathogen microorganisms [3].

An alternative to valorize discarded potato and convert them into a valuable byproduct of the potato industry is to recycle their nutrients for being use as a substrate in the synthesis of microbial products with high added value [4]. In general, discarded potato has the same nutritional components those potato tubers suitable for human consumption, with differences in size, form, and damage in peel. The approximate composition of raw potato tubers is (in 100 g): water 83.3 g, carbohydrate 12.4 g, protein 2.6 g, ash 1.6 g, total dietary fiber 2.5 g, and total lipid 0.1 g [3]. On dry basis, the most predominant nutrient component of potato tubers is starch (60–71%), followed by protein (4.9–6.5%) [5]. Besides, potato tubers damages (abrasions, cuts, bruises) during harvest allows the loss of a large amount of nutrients, and the profitability of the production system decreases.

Potato starch is an important component in various traditional culture media, including Potato Dextrose Agar (PDA), widely used for the cultivation of bacteria, fungi, yeast, and molds. The potato extract in culture media serves as a source of carbon, nitrogen, minerals, and vitamins. However, traditional starch-based culture media, such as PDA, can cost more than USD$400 per kg. In fact, Panesar et al. [6] reported that the cost of the culture media used to produce microbial products represent between 38% and 72% of the total production costs. In this way, in recent decades, research has been intensified in the recycling of waste from both food production and processing for obtaining microbial products, such as enzymes, biosurfactants, antibiotics and biopigments [7–10].

Nowadays, biopigments are highly required by different industries to replace artificial pigments, because many of them have been banned due to their high risks to human

health and behavior. These unwanted side effects range from the inhibition of key enzymatic processes at the cellular level, allergic reactions, and possible contributions to development of attention deficit and hyperactivity to teratogenicity and carcinogenicity [11–13]. Microbial pigments have proven to be an eco-friendly and non-toxic alternative, being low-cost and safe industrial production needed to enable a commercial production [14].

The reduction of costs associated with the substrate in microbial pigment production processes could mean a great step towards the color additive market, which has been dominated by low-cost artificial dyes from the nineteenth century. Thus, numerous reports indicated the need for natural color additives in diverse industrial areas, and appropriate fermentation strategies for low-cost processes that replace commercial growing media with nutrient-rich waste-based substrates [15–18].

An interesting source of natural pigments is the *Streptomyces* genus, which comprises aerobic, Gram-positive and filamentous bacteria with special features and versatility. The *Streptomyces* genus has been recognized for its production of antibiotics but is also being explored as a source of blue pigments suitable for use in food processing industry, such as actinorhodin (ACT) [19] and 4,8,13-trihydroxy-6,11-dione-trihydrogranaticins A (TDTA) [20]. Although microbial pigments with a wide range of colors can be produced, blue colors are rare in nature because their electronic configurations required to absorb photons at 560–700 nm, which is complex and occur infrequently [21]. ACT is a dimeric benzoisochromanequinone antibiotic mainly produced by *Streptomyces coelicolor*, one of the most well-characterized Actinobacteria [22], with yields of ~3 g L^{-1} [19] and that can be produced intra-or extracellularly [23]. Another ACT-producer is *Streptomyces lividans*, but unlike *S. coelicolor*, this specie produces very low levels and has been less studied as blue pigment producer [24].

Streptomyces prasinus was recently reported as a novel *Streptomyces* specie able to produce ACT under extreme or stress conditions [25]. Zhang et al. [19] indicated that the color of ACT isomers is pH dependent, and the compound is stable to light, heat, and common food additives, being also non-toxic. Moreover, ACT has been reported as a potent bacteriostatic and pH-responsive antibiotic [26]. As far as we know, there are no reports on both the production of ACT by *Streptomyces lydicus* and the use of potato waste as basal support for the microbial production of this pigment. Therefore, the aim of this study was to evaluate the use of potato wastes as basal substrate for producing blue pigments by *S. lydicus* PM7 through submerged fermentation.

Methods

Materials

All reagents and chemicals used for extractions and preparation of traditional culture media were purchased from Sigma-Aldrich/Merck (Darmstadt, Germany). Standards and solvents for preparative column chromatographic extractions were gradient grade for liquid chromatography.

Potato waste preparation

Discarded potatoes (*Solanum tuberosum*) were obtained from a local potato-producing company near Temuco City (Chile). The fresh discarded potatoes were cut in small pieces (1 cm^3), dried at 70 °C and ground to a fine powder as described previously [4]. The proximate composition of the discarded potato powder is moisture 7.8%, fat 6.2%, protein 7.9%, crude fiber 0.4%, ash 3.9% and N-free extract 73.7% [4]. The obtained powder was stored in glass bottles at room temperature and used to prepare the discarded potato medium (named DPB) at the required concentrations in the fermentation assays. In all assays, the culture media were sterilized by autoclaving at 121 °C for 20 min before their use.

Bacterial strain

The bacterial strain PM7 was isolated from a soil sample of a potato field at the Experimental Station Maquehue–Universidad de La Frontera, Temuco city (Chile) and identified as a member of the genus *Streptomyces* in our previous work [4]. The strain was kept on slants of International *Streptomyces* Medium No. 2 (ISP2; glucose 4 g, yeast extract 4 g, malt extract 10 g, distilled water 1 L, pH 7.0) jellified with agar 15 g L^{-1} at 4 °C and maintained in the strain collection at the Laboratory of Environmental Biotechnology, Universidad de La Frontera.

Bacterial phylogenetic analysis

A phylogenetic tree was constructed by the neighbor-joining method to identify the bacterial isolate at the species level and to determine the distance among blue pigment-producing *Streptomyces* species.

DNA extraction

A sterile glass tube with 5 mL of Luria–Bertani (LB) broth was inoculated with spores of *S. lydicus* PM7 and incubated in an orbital shaker-incubator at 28 °C and 120 rpm for 72 h. After the incubation time, 1.8 mL was taken and added to the collection tube for the genomic DNA extraction using the DNeasy PowerLyzer Microbial Kit (Hilden, Germany), according to the manufacturer's instructions.

Molecular identification and phylogenetic tree construction

The 16S rRNA was selectively amplified from genomic DNA by polymerase chain reaction (PCR) using universal primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5′-GGTTACCTTGTTACGACTT-3′). PCR amplification was performed in a Multigene Optimal Thermal Cycler (Labnet, USA) in 50 µL of Master Mix PCR mix comprising 25 µL mix reaction buffer 2x (AmpliTaq™), 22 µL ultra-pure water, 1 µL of each primer (10 µM) and 1 µL of DNA. The temperature and cycling conditions were as follows: preheating at 94 °C for 2 min; 30 cycles at 94 °C for 1 min; 55 °C for 1 min; 72 °C for 1.5 min; and incubation at 72 °C for 10 min. The presence of PCR products was assessed by electrophoresis on a 1% agarose gel stained with GelRed™. Sequencing was done with a dye Terminator Cycle Sequencing Kit and an ABI 3730XL DNA Sequencer (Applied Biosystems) by Macrogen (Korea). The nearest taxonomic group was identified by 16S rRNA nucleotide sequence BLASTN (http://www.ncbi.nlm.nih.gov/blast) using DDBJ/EMBL/GenBank nucleotide sequence databases. A phylogenetic tree was constructed in MEGAX [27] by using the neighbor-joining method [28]. The bootstrap consensus tree inferred from 1,000 replicates [29] was taken to represent the evolutionary history of the taxa analyzed [29]. The evolutionary distances were computed using the maximum composite likelihood method [30].

Inoculum preparation

To standardize the incubation time to the inoculum preparation, a growth curve was constructed from the average dry biomass of the bacterial strain cultured in ISP2 medium. A pre-inoculum consisting of a suspension of *S. lydicus* PM7 spores was prepared in 5 mL of 0.9% NaCl solution by transferring a loopful culture from 7-day-old culture in Petri dishes with ISP2 medium from cultures grown on slants. The obtained spores were transferred into ISP2 broth medium at concentration of 10^4 mL^{-1} in 250 mL Erlenmeyer flasks with a working volume of 100 mL. The flasks were closed with a gauze-covered cotton-wool plug and incubated in darkness at 28 °C and 120 rpm for four days. Dry biomass, pH and reducing sugars were measured each 8 h.

After each incubation time, bacterial cultures were centrifuged at 8000 rpm for 15 min, and the pellet was used for measuring dry weight using an analytic balance after drying at 105 °C until constant weight. Reducing sugar consumption in cell free supernatants (CFSs) was determined using the dinitrosalicylic acid (DNS) method [31], and changes in pH were determined using a pH meter. Growth curve assay was carried out in triplicate under destructive sampling mode.

The maximum specific growth rate (μ_{max}) was calculated from the plot of ln[x] versus t using the following equation:

$$\ln x_t = \ln x_0 + \mu t$$

where x_t = biomass concentration (g L^{-1}), x_0 = biomass concentration (g L^{-1}) after t days, and t = time (h). In this plot, in a period of time with linear trend, a linear equation ($y = mx + c$) can be obtained. The slope (m) of this straight line corresponds to μ_{max} value, which characterizes the exponential phase of growth.

Batch fermentation assays to assess growth and blue pigment production by *S. lydicus* PM7

Pigment production in potato waste medium and in traditional culture media

Different liquid culture media were evaluated for producing blue pigments by *S. lydicus* PM7: potato dextrose broth (PDB), International *Streptomyces* project (ISP2 to ISP5) and discarded potato broth (DBP), to study the replacement of traditional culture media with discarded potato. DPB medium was prepared at 16 g L^{-1} DP powder according to Schalchli et al. [4]. PDB (Difco) medium contained (per L) potato starch (from infusion) 4 g and dextrose 20 g; ISP2 medium contained (per L) glucose 4 g, yeast extract 4 g, malt extract 10 g; ISP3 medium contained (per L) oatmeal 20 g and trace salt solution 1 mL; ISP4 medium contained (per L) soluble starch 10 g, $CaCO_3$ 2 g, K_2HPO_4 1 g, $MgSO_4 \times 7H_2O$ 1 g, NaCl 1 g, $(NH_4)_2SO_4$ 2 g, trace salt solution 1 mL; ISP5 medium contained (per L) L-asparagine 1 g, glycerol 10 g, K_2HPO_4 1 g, trace salt solution 1 mL. The trace salt solution contained $FeSO_4 \times 7$ H_2O 0.1 g, $MnCl_2 \times 4H_2O$ 0.1 g, $ZnSO_4 \times 7$ H_2O 0.1 g. The pH of all the culture media was adjusted to 7.0 with either NaOH or HCl 0.1 M.

The culture media (100 mL) were placed in 250 mL Erlenmeyer flasks closed with a gauze-covered cotton-wool plug, sterilized by autoclaving, inoculated with *S. lydicus* PM7 inoculum at 4% v v^{-1} and incubated at 28 °C and 120 rpm for 10 days. After the fermentation time, the supernatants of culture media were separated from the biomass to obtain cell-free supernatants (CFSs) by centrifugation at 8000 rpm for 10 min at 4 °C, filtered through syringe filter (0.45 and 0.22 µm) and stored at 4 °C for spectrophotometric measurement.

The CFS obtained from the *S. lydicus* PM7 culture using DPB medium, with visible blue pigment production, was

used to determine the maximum absorption wavelength (λ max) by scanning the blue CFS in the range 400 to 800 nm against control medium (without inoculum) using UV–visible spectrophotometer (Optizen POP, Mecasys Co., Ltd., Korea). Then, the CFS was lyophilized and different solutions in range 10–500 ppm of blue extract with pH adjustment to 12 using KCl/NaOH buffer (200 mM) were prepared to obtain a calibration curve.

To determine the blue pigment content in CFS of all the previous culture media, an aliquot of the respective CFS (500 µL) was adjusted to pH 12 using KCl/NaOH buffer 200 mM according to Zhang et al. [19], with modifications. Then, the absorbance spectrum of blue pigments was adjusted to 0.1–1.0 and recorded with a spectrophotometer at 640 nm. The results of the extracellular pigments were expressed in quantity of blue pigments produced (in mg L^{-1}) based on the calibration curve ($R^2 = 0.9996$).

Effect of potato waste powder concentration on blue pigment production

The DPB medium formulated with the processed discarded potato powders was placed into 250 mL Erlenmeyer flasks with a working volume of 100 mL at different concentrations (2, 4, 8, 12, and 16 g L^{-1}) without pH adjustment (initial pH 6.2 ± 0.2). After sterilization, the flasks were inoculated with *S. lydicus* PM7 at 4% v v^{-1}, closed with a gauze-covered cotton-wool plug and incubated at 28 °C and 120 rpm for 10 days. Each assay was carried out in triplicate under destructive sampling mode.

Kinetics of fermentation using defined potato waste powder concentration

A fermentation assay was carried out to study the bioconversion process of discarded potato into blue pigments by *S. lydicus* PM7 in 250-mL Erlenmeyer flasks containing 100 mL of DPB at 16 g L^{-1} without pH adjustment (initial pH 6.3). After sterilization, the flasks were inoculated with *S. lydicus* PM7 at 4% v v^{-1}, closed with a gauze-covered cotton-wool plug and incubated at 28 °C and 120 rpm for 10 days in darkness. The control treatment was DPB medium without actinobacterial inoculum. The traditional culture medium ISP2 with and without *S. lydicus* PM7 inoculum was used as negative control for comparative purposes considering its traditional use for the growth of diverse *Streptomyces* species and our previous assays, where no blue pigment production was obtained. Each assay was carried out in triplicate under destructive sampling mode. Three flasks were removed every 24 h for analytical measurements. The extraction and measurement of blue pigment content in each fermentation time was determined as described above.

Determination of total biomass. Total biomass (bacteria + DP) was separated by centrifugation at 8000 rpm and dried at 105 °C until a constant weight for measuring dry weight.

Determination of total soluble proteins. A part of total biomass (1 g) was washed three times with pure water and centrifuged at 6000 *g* for 5 min to analyse total proteins in biomass (bacterial and residual potato biomass). Total soluble proteins were extracted by using a buffer solution (NaCl 300 mM, NaH_2PO_4 50 mM, Tween 20 0.1%, phenylmethylsulfonyl fluoride (PMSF) 5 mM, pH 8) and determined by Bradford standard assay [32]. The concentration of total extracellular proteins was determined directly in the CFSs by the Bradford standard assay.

Determination of reducing sugars. Reducing sugars were measured in CFSs using the DNS method [31].

Determination of pH. The pH was directly measured in CFSs using a pH-meter.

Blue pigment extraction and identification

Five 500 mL Erlenmeyer flasks with 200 mL of DPB medium at 16 g L^{-1} of discarded potato powders without pH modification (initial pH 6.2 ± 0.2) were sterilized and inoculated with *S. lydicus* PM7 at 4% v v^{-1}. The flasks were closed with a gauze-covered cotton-wool plug and incubated in orbital shaker-incubator at 28 °C and 120 rpm for 10 days. After the fermentation period, the supernatant was separated from the biomass by centrifugation at 8000 rpm for 10 min at 4 °C and filtered through syringe filter (0.45 and 0.22 µm) to obtain the CFS. The CFS was lyophilized and stored at 4 °C for further analyses. Then, blue pigments were fractionated using a chromatographic column (10 × 2.5 cm) with 10 g of LiChroprep RP-18 (15–25 µm). As mobile phase, 50 mL of solvents of increasing polarity were used [MeOH 100; MeOH:H_2O 90:10; MeOH:H_2O 50:50; H_2O 100]. The fractions were collected with volumes of 10 mL. The fractions obtained were concentrated to dryness in a SPD121P SpeedVac® Concentrator (Thermo Scientific Savant®) and stored at 4 °C in darkness.

The MeOH fraction containing blue pigments was analysed by HPLC–ESI–MS to identify the main compounds contained. The conditions were: ACE Excel 2 C18 column (3 × 100 mm, 3 µm); solvents (A) H_2O with 0.1% formic acid, and (B) acetonitrile with 0.1% formic acid; 40 °C column temperature; flow rate of 0.2 mL min^{-1}; 50:50 (v/v) (A:B) isocratic run for 15 min. The mass spectrometer registered between 300 and 1300 *m/z* in negative mode.

Statistical analysis

Data from all the experiments were averaged ($N=3$) and the standard errors (SD) of the means were calculated. Differences among treatments were assessed with one-way analysis of variance (ANOVA) and post hoc analysis of differences in means was conducted with the Tukey test using JMP 11.0 software (SAS Institute Inc., NC, USA) with statistical significance $p<0.05$.

Results

Bacterial phylogenetic analysis

Molecular identification of *Streptomyces* sp. PM7 through the sequencing of the 16s rRNA gene resulted in a clear phylogenetic relation with the Actinobacteria phylum and a closest identity relation with the specie *Streptomyces lydicus* (Fig. 1). The GenBank accession number for the sequence *S. lydicus* PM7 is OP622337.

Inoculum preparation

The growth curve of *S. lydicus* PM7 and changes in pH and reducing sugar content in CFS during 4 days of incubation in ISP2 medium are shown in Fig. 2. The ISP2 medium contain glucose, an easily metabolizable carbon source; therefore, it has been commonly used for development of *Streptomyces* inoculum. In our study, ISP2 medium provided an adequate *S. lydicus* PM7 biomass production with a consequent reducing sugar consumption for the inoculum preparation.

The lag phase (12 h) was followed by an exponential phase with $\mu=0.08$ h^{-1}. A progressive decrease in reducing sugar concentration was observed from 4 h, followed by an increase in biomass concentration, which reached 1.33 g L^{-1} at 72 h of incubation. The pH of the culture medium also decreased from 7.05 to 6.34. Based on the growth curve and the content of residual reducing sugars, the incubation time for inoculum preparation was defined as 72 h.

Effect of culture media on blue pigment production by *S. lydicus* PM7

S. lydicus PM7 was able to produce extracellular blue pigments using DPB medium under the fermentation conditions employed. The pigments exhibited maximum absorption (λ max) at 580 nm by scanning the samples in the range 400–800 nm (Fig. 3). The λ max was not clearly evidenced in samples of fermentation broth employing the traditional culture media in the visible wavelength range, presumably due to the low blue pigment content, excepting ISP5 medium that also showed λ max of 580 nm.

The highest blue pigment production was obtained by using DPB medium, reaching 973.6 ± 32.3 mg L^{-1} (Table 1). Among the traditional culture media, the pigment production did not occur or was poorly produced, in exception of the ISP5 medium that allowed to a pigment concentration of 363.0 ± 17.7 mg L^{-1}. The DPB medium also led to the highest biomass concentration (4.9 g L^{-1}), followed by the commercial PDB and the IPS4

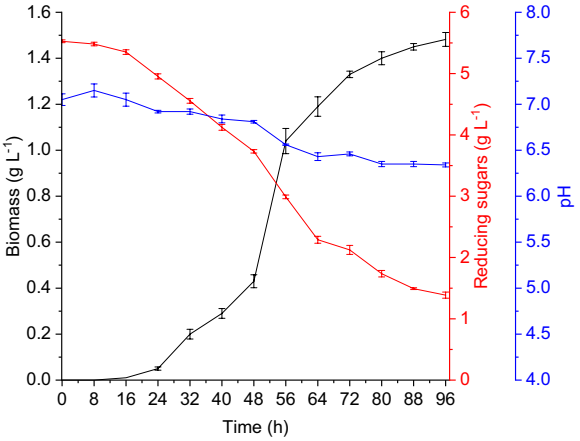


Fig. 2 Changes in pH, reducing sugars and bacterial biomass during the fermentation of *Streptomyces lydicus* PM7 cultured in International *Streptomyces* Project No. 2 (ISP2) medium at 28 °C and 120 rpm for 96 h. Inoculum size: 10^4 spores m L^{-1}

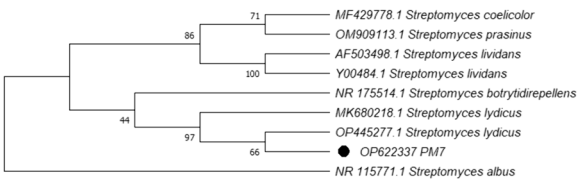


Fig. 1 Maximum likelihood phylogeny based on the nearly full length of the 16S rRNA gene of *Streptomyces lydicus* PM7

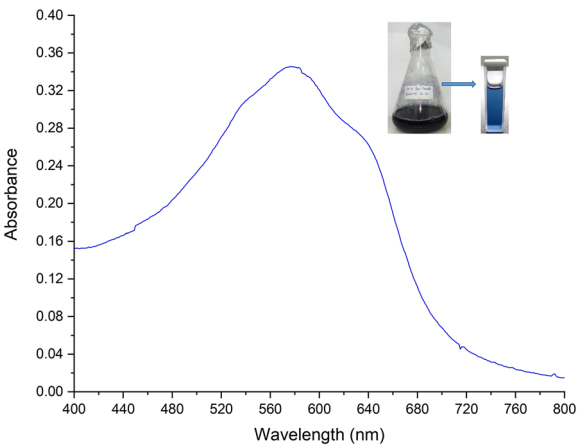


Fig. 3 Absorbance spectrum for cell-free supernatant (final pH 6.4) of *Streptomyces lydicus* PM7 cultured in discarded potato broth (DPB) at 28 °C and 120 rpm for 10 days. Inoculum size: 4% v v^{-1} (~ 5 mg L^{-1} dry weight)

Table 1 Blue pigment production by *Streptomyces lydicus* PM7 using potato wastes and traditional culture media after 10 days of incubation at 28 °C and 120 rpm

Analyses	Waste medium	Traditional *Streptomyces* culture media				
	DPB	PDB	ISP2	ISP3	ISP4	ISP5
Blue pigments (mg L^{-1})	973.7* ± 32.3	21.2 ± 2.7	0.0 ± 0.0	6.1 ± 4.0	6.1 ± 6.2	363.0 ± 17.8
Biomass (g L^{-1})	4.9* ± 0.9	2.4 ± 0.3	1.5 ± 0.2	0.2 ± 0.1	2.5 ± 0.1	0.3 ± 0.0

The culture media correspond to discarded potato broth (DPB), potato dextrose broth [PDB) and the International *Streptomyces* Project (ISP) media 2–5 (initial pH 7.0)

* Total Biomass corresponds to residual potato waste and bacterial biomass. Inoculum size: 4% v v^{-1} (~ 5 mg L^{-1} dry weight). The asterisks show significant difference among the culture media ($p > 0.05$, one-way ANOVA)

media (~ 2.5 g L^{-1}), while the growth was negligible in the IPS3 and IPS5 media.

Effect of discarded potato concentration on blue pigment production

Blue pigment production by *S. lydicus* PM7 varied with the concentration of potato powder (Fig. 4). A significantly higher concentration (0.87 g L^{-1}) of blue pigments was obtained at the end of incubation time by using 16 g L^{-1} of the potato powder (Fig. 4**).** Although pigment production began earlier with the use of 4 g L^{-1} of the waste, the final concentration did not show significant differences from those obtained with 4, 8 and 32 g L^{-1} (0.60, 0.65 and 0.75 g L^{-1}, respectively).

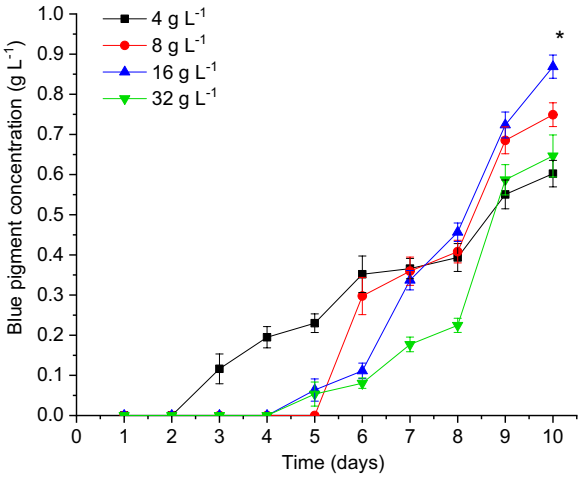


Fig. 4 Production of blue pigments by *Streptomyces lydicus* PM7 using different concentrations of discarded potato. Culture conditions: 28 °C, 120 rpm, 10 days of incubation and inoculum size of 4% v v^{-1} (~ 5 mg L^{-1} dry weight). The asterisk indicates that 16 g L^{-1} of discarded potato at 10 d of incubation was significantly higher than the other discarded potato concentrations ($p > 0.05$, one-way ANOVA)

Batch fermentation assay

To study the kinetic profiles of the fermentation process with *S. lydicus* PM7 in DPB medium (16 g L^{-1}), the changes in total biomass, pH, total proteins, reducing sugars and blue pigment concentration were assessed along the process time (Fig. 5). An increase in total biomass (waste and bacterial biomass) was observed from the 2nd day of fermentation in DPB medium, varying from 4.2 to 5.5 g L^{-1} at day 0 and 7 of incubation, respectively (Fig. 5a). In the negative control (ISP2 medium), total biomass reached a maximum of 1.8 g L^{-1} dry weight at day 5 of incubation. In control DPB medium, without *S. lydicus* PM7 inoculum, no significant differences were obtained in total biomass during all incubation times. The pH decreased during fermentation of *S. lydicus* PM7 into ISP2 medium from 7.0 to 5.0 at day 4 of incubation (Fig. 5b). In contrast, the pH of DPB medium with and without inoculum remained constant (pH above 6.4) until the end of the fermentation assay.

The changes in total proteins in both ISP2 and DPB media are shown in Fig. 5c, d. Total proteins in biomass and CFS increased slightly in the DPB medium inoculated with *S. lydicus* PM7 until day 4 of incubation reaching values of 5.7 and 2.7 g L^{-1}, respectively. From this point, total proteins in both the biomass and the CFS increased considerably, reaching 14.1 and 8.2 g L^{-1}, respectively. The maximum value of total protein in biomass obtained in ISP2 medium was 2.0 g L^{-1} at day 7 of fermentation. In this control medium, the content of reducing sugars decreased steadily from 5.7 (at day 0) to 2.4 g L^{-1} at day 10 of incubation (Fig. 5e). On the other hand, a low initial reducing sugar content was detected in DPB medium inoculated with *S. lydicus* PM7, which increased from 2 h to achieve a maximum of 5.0 g L^{-1} on the fifth day of incubation. The reducing sugar content in control DPB remained constant (above 0.4 g L^{-1}) until the end of the fermentation assay.

The synthesis of blue pigments by *S. lydicus* PM7 began between day 4 to 5 of incubation (when total proteins and reducing sugars in the CFS increase) and increased exponentially until the end of the fermentation process, reaching 0.87 ± 0.05 g L^{-1} at the end of the fermentation assay (Fig. 5f). Figure 5g shows a dark blue coloration in the DPB medium after 6 days of incubation while no blue coloration was obtained in

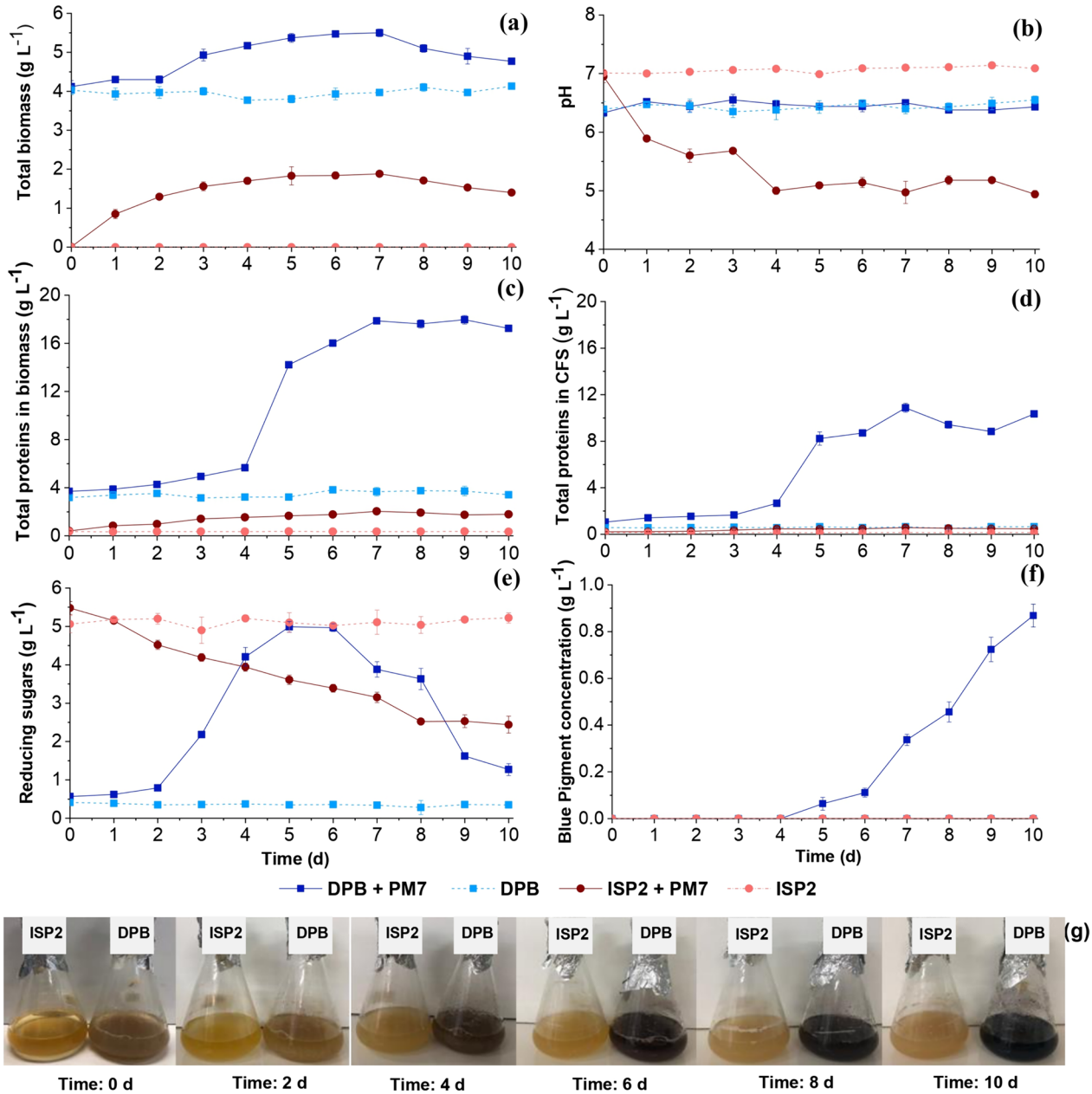


Fig. 5 Changes in total biomass (dry weight) (**a**), pH (**b**), total proteins in biomass (**c**), total proteins in cell free supernatant (CFS) (**d**), reducing sugars (**e**) blue pigment concentration (**f**) and pigment formation (**g**) by *Streptomyces lydicus* PM7 cultured at 28 °C and 120 rpm for 10 days through submerged fermentation using discarded potato broth (DPB) at 16 g L^{-1} of potato powder. Inoculum size: 4% v v^{-1} corresponding to ~ 5 mg L^{-1} dry weight. Control treatments were commercial medium ISP2 and DPB without inoculum

the traditionally used ISP2 culture medium (negative control).

Identification of the main blue pigment produced by *S. lydicus* PM7

The HPLC–ESI–MS analysis revealed that the main blue pigment produced by *S. lydicus* PM7 using discarded potato as the sole carbon source was ACT (Fig. 6). This compound was identified in the main blue fraction (MeOH fraction), with a retention time of 1.9 min and m/z value at 634.1 as the parent ion and 635.1 and 636.1 as $[M+H^+]$ and $[M+2H^+]$, respectively.

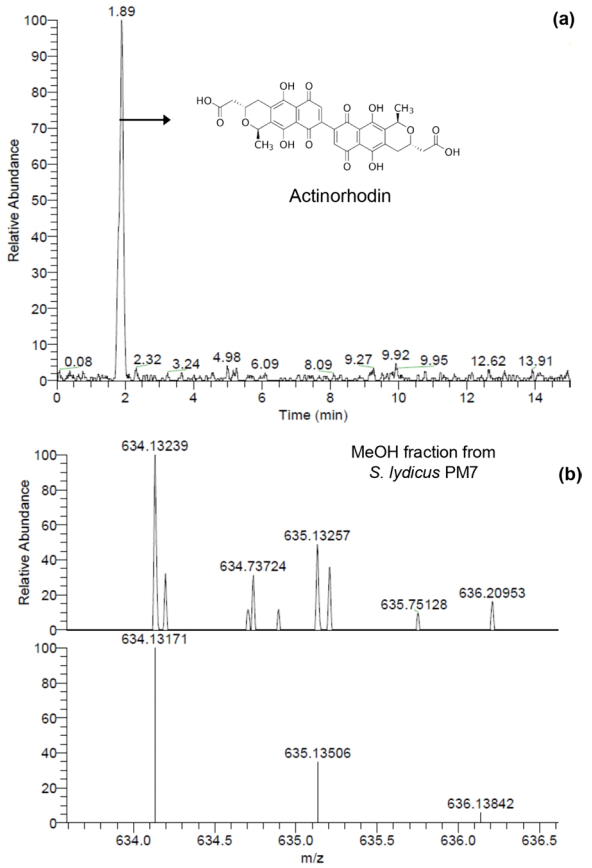


Fig. 6 HPLC chromatogram (**a**) and mass spectrum (**b**) of the MeOH fraction of blue pigments produced by *Streptomyces lydicus* PM7 using discarded potato broth as a sole nutritional source

Discussion

The production of valuable natural products from *Streptomyces* spp. for multiple applications has been widely reported using traditional culture media as substrate and in a lesser extent agroindustrial wastes [33–36]. In our previous work, we reported that *Streptomyces* spp. were able to biotransform potato solid wastes into intra- and extracellular pigments [4]. Besides, morphological and biochemical characteristics for the blue pigment producer *Streptomyces* sp. PM7 were determined, being positive for fermentation of all the tested carbohydrates (glucose, ribose, xylose, mannitol, maltose, lactose, sucrose, glycogen) and enzymes esculin (β-glucosidase), α-glucosidase, β-galactosidase, alkaline phosphatase, pyrrolidonyl arylamidasen and pyrazinamidase. The present work further investigated the bioconversion process of discarded potato into blue pigments by *Streptomyces* sp. PM7 through submerged fermentation and the chemical composition of the main blue fraction.

The phylogenetic analysis reveals a considerable distance to the well-known blue pigment producer *S. coelicolor*, *S. prasinus* and *S. lividans*. In this way, the Actinobacteria *S. lydicus* has been studied over decades as an antibiotic producer (e.g., chandramycin, natamycin, and streptolydigin) [37–39] and an effective biocontrol agent [40]. In our study, *S. lydicus* PM7 was not able to produce blue pigments when it was cultured into ISP2 medium at pH 7.0. However, the kinetic profile of the fermentation evidenced high growth rates during exponential phase, as well almost constant uptake rates of the reducing sugars, resulting in low residual level of this substrate; therefore, ISP2 medium was used for inoculum preparation and as negative control in the fermentation assay. The specific growth rate of *S. lydicus* PM7 (0.08 h^{-1}) in ISP2 medium was similar to those reported in other *Streptomyces* spp., reaching the stationary phase after three days of incubation. For example, specific growth rates of Antarctic *Streptomyces* grown in M1 broth medium (peptone 2 g L^{-1}, yeast extract 4 g L^{-1}, starch 10 g L^{-1}; pH 7.0) were reported in range of 0.08–0.18 h^{-1} [41]. The specific growth rates of the blue pigment-producing *S. coelicolor* have also been reported in similar range (from 0.03 to 0.11 h^{-1}) with different values depending on culture conditions [42, 43].

Variations in cultivation parameters such as initial pH or nutrient content can significantly modulate not only growth kinetics but also enzymatic profiles of *Streptomyces* spp., consequently influencing the synthesis and yield of pigments during the fermentation process [23, 44]. For example, the effect of different growing parameters on blue pigment production from *S. prasinus* was investigated by Azizan et al. [25]. The highest production of the antibiotic blue pigment ACT was recorded at 33 °C (1.95×10^{-6} mol L^{-1}), pH 5 (7.1×10^{-6} mol L^{-1}) and 50% w v^{-1} glucose (9.56×10^{-6} mol L^{-1}) for 60 h of incubation. In general, the yields of microbial pigments are highly dependent on culture conditions and fermentation technologies, being most of them secreted under stress conditions [45]. In this way, most of the traditional culture media contain rapidly metabolizable carbon sources (e.g., glucose, maltose, and sucrose) that could interfere with the synthesis of secondary metabolites [46]. The highest pigment production was observed when DPB and ISP5 media were used. The DPB medium is a complex source of nutrients, which mainly contains starch (soluble and insoluble in water) and proteins as carbon and nitrogen source, respectively. On the other hand, ISP5 contains glycerol and asparagine as carbon and nitrogen source. The level of blue pigments obtained in traditional culture media agree with reports indicating that in media containing a mixture of a rapidly and slowly used carbon sources, the rapidly metabolizable nutrient source is used first to produce cells but little or no secondary metabolites are synthesized [45, 46]. Interestingly, Riascos et al. [47] pointed out that fourteen

XRE/DUF397 proteins pairs participate in the regulation of antibiotic production and this regulation could be culture medium dependent. In our work, when PDB (Difco) medium was used, a low content of blue pigments was produced after 10 days of fermentation by *S. lydicus* PM7, which was associated with the depletion of glucose after this fermentation time [46]. Likewise, this strain was not able to produce blue pigments using ISP2 as culture medium presumably due to a repression by the presence of glucose as carbon source. On the other hand, starch has been reported as one of the best carbon sources for pigment production by *Streptomyces flavofuscus* [48]. In our study, the traditional culture media containing potato starch from infusion (PDB medium) or soluble starch from potato (ISP4 medium) as carbon source did not promote a noticeable production of blue pigments by *S. lydicus* PM7. Thus, among the traditional culture media, ISP5 was the best substrate for producing blue pigments by *S. lydicus* PM7, which contained glycerol, L-asparagine, K_2HPO_4 and trace salt solution. Interestingly, Kim et al. [49] reported that the use of glycerol instead of glucose as carbon source dramatically relieved the repression by carbon source in *S. lividans*, leading extensive synthesis of ACT blue pigment by this specie. The authors explained that that glucose prevents ACT production by repressing the synthesis ofafsR2 mRNA. In addition, Babitha et al. [45] suggested a positive effect of glycerol on both pigment yield and microbial biomass that can act as an osmolyte and serves as a carbon source with a metabolic function.

Numerous authors highlight the metabolic versatility of *Streptomyces*, which is mainly due to a large number of secreted proteins. Specifically, Spasic et al. [50] indicated that *Streptomyces* are producing all major classes of biocatalysts, having potent biodegradation abilities coupled with powerful biosynthetic capability. In nature, *Streptomyces* spp. secrete and release specific proteins for substrate-binding and hydrolysis to obtain nutrients and to survive the hostile environment of different niches [51]. The strain *S. lydicus* PM7 was isolated from soil samples of potato roots, which suggest that it could has developed a metabolic diversity to exploit this complex organic material in the soil for nutrient acquisition. Our results showed that proteins produced by *S. lydicus* PM7 after two days of fermentation were able to break down carbohydrates into reducing sugars, favoring the production of blue pigments. The increment of total proteins in CFS could be due to the absence of the outer membrane in *S. lydicus* PM7, allowing a direct release of secreted carbohydrate-processing proteins responsible for the breakdown of potato starch into the culture medium [51].

Along with the production of proteins and the breakdown of potato components, the production of extracellular blue pigments by *S. lydicus* PM7 was observed after the third day of fermentation. The increase in biomass and absence of blue pigments when *S. lydicus* PM7 was cultured in ISP2 medium established that this pigment should be considered a secondary metabolite. Of the two reported blue pigments produced by *Streptomyces* spp. (ACT and TDTA) [19, 20] we identified ACT as the main blue pigment produced by *S. lydicus* PM7 since the main fraction displayed the m/z value at 634, agreeing to data for γ-ACT [52]. This compound is known to exhibit a pH-dependent absorption spectrum, with its λ max varying depending on the pH of the solution. Zhang et al. [19] investigated the pH dependence of ACT's absorption spectrum and reported variations in λ max ranging from 484 nm at pH 3 to 623 nm at pH 12. In our study, we observed a λ max of 580 nm in the CFS with a pH of 6.4 (final pH at the end of the incubation period). This result is consistent with the reported range, considering the pH-dependent nature of ACT.

As far as we know, the production of ACT by *S. lydicus* has not yet been reported, nor has the use of agricultural wastes as basal substrate for the production of ACT by *Streptomyces* spp. The ACT derived from *S. coelicolor* A3(2) has been recognized as an important biocatalyst. Nishiyama et al. [53] demonstrated that oxidation reactions producing H_2O_2 proceed upon addition of ACT to the reaction mixture, ACT is not consumed during the reaction, and a small amount of ACT consume an excess amount of the substrates. Thus, the released H_2O_2 in the presence of a reducing agent could be associated to oxidative degradation of reducing sugars [54] and/or participation in the hydrolysis of starch [55]. In this way, *Streptomyces* bacteria can respond to biotic and abiotic factors via two-component systems (TCS), consisting of a sensor histidine kinase and a cytoplasmic response regulator that typically modulates target gene expression [56]. Many of these TCS are involve to either directly or indirectly in antibiotic production [57]. Honma et al. [58] showed that the blue-pigmented antibiotic ACT production was regulated by endogenously produced nitric oxide via the heme-based DevS/R TCS.

The characteristics of ACT reported by numerous works include pH-redox activity, stability to light, heat and commonly used food additives, non-toxic (LD50 > 15 mg g^{-1} in acute toxicity test), bacteriostatic and pH-responsive antibiotic effect [19, 26]. In consequence, ACT has been considered a valuable natural compound with multiple applications and safe to replace blue synthetic dyes. In addition to the production of ACT by a new specie of the genus *Streptomyces*, this study reports for the first time the production of ACT using an agricultural waste as the only substrate

in a liquid state fermentation process. Therefore, although other *Streptomyces* species like *S. coelicolor* can produce blue pigments, *S. lydicus* PM7 exhibits a clear advantage for sustainable pigment production. This study further highlights the potential of *S. lydicus* PM7 for environmentally friendly ACT production. The utilization of potato waste as a sole nutrient source could simplifies the fermentation process and reduces reliance on expensive traditional culture medium.

Conclusion

Discarded potato favored the production of blue pigments by *S. lydicus* PM7 under submerged fermentation, being even better than several traditional *Streptomyces* culture media. The results showed that *S. lydicus* PM7 produce and release functional proteins required to break down the nutrients provided by discarded potato into reducing sugars. In general, the production of blue pigments by *S. lydicus* PM7 begin the fifth day of fermentation, when total proteins and reducing sugars are at the highest concentration. The blue pigment actinorhodin was identified in the main fraction analyzed by HPLC–ESI–MS. To the best of our knowledge, this is the first report on the production of actinorhodin through a bioprocess based on the use of an agricultural waste as a sole nutrient source for microbial growth, as well as on the actinorhodin production by the specie *S. lydicus*.

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Author contributions
H.S., M.C.D., E.H. and O.R. planned experiments and wrote the main manuscript text. A.A. and H.S. performed experiments. A.Q. and H.S. contributed to data analysis and visualization. C.L. performed evolutionary analyses. R.A. performed HPLC–ESI–MS analysis and G.B. reviewed the final manuscript version and prepared Figs. 2–5.

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Availability of data and materials
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Declarations

Ethics approval and consent to participate
Not applicable.

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Competing interests
The authors declare no competing interests.

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