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Biotechnological enhancement of coffee pulp residues by solid-state fermentation with *Streptomyces*. Py–GC/MS analysis

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Abstract

The ability of three *Streptomyces* strains to upgrade the nutritional value of coffee pulp residues from Nicaragua in solid-state fermentation (SSF) conditions was analyzed by Py–GC/MS. The presence in these residues of compounds such as polyphenols, tannins, chlorogenic acids and caffeine prevents their utilization as domestic fodder. The characteristic pyrolysis products derived from polyphenols and polysaccharides were identified both in control and treated coffee pulp being remarkable the decrease achieved in the total polyphenols derived compounds after the growth of the strains. The analysis of these compounds demonstrated that both monomethoxy- and dimethoxy-phenols were degraded. In addition, an increase in the microbial treated coffee pulp of protein content was detected by Kjeldahl method. In summary, changes evidenced in coffee pulp treated by *Streptomyces* through the application of analytical pyrolysis reveal the biotechnological interest of these bacteria to upgrade a usefulness and pollutant residue to be used for feeding purposes.
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Keywords: Coffee pulp; *Streptomyces*; Solid-state fermentation; Pyrolysis

1. Introduction

Coffee pulp is an abundant agricultural by-product derived from wet processing of coffee berries and constitutes a potential pollution risk in producer countries. The recent interest to look for an economic use of this residue is impelling to develop biological processes for its exploitation. Thus, efforts to recycle the coffee pulp include activities such as composting, feeding to animals, and production of organic fertilizers, single-cell proteins and biogas [1,2]. Taking into account the average contents of about 50, 10, 2.5 and 18% for carbohydrate, protein, fat and fibres, coffee pulp appear to be a useful feed supplement for animals [1].

In addition, one of the more promising potential uses of coffee pulp that seems to be especially attractive for developing countries is to grow edible mushrooms on this residue in order to convert an agricultural waste to human food [3]. However, the use of coffee pulp for food and feed purposes is restricted by the presence of undesirable factors such as polyphenols, tannins and caffeine, among others [4,5]. Nowadays, several biological treatments including the use of microorganisms such as yeast, filamentous fungi and bacteria are being applied to improve the nutritional value of coffee pulp [2,3,6,7]. Although solid-state fermentation (SSF) has been used for specific biological detoxification of coffee pulp using filamentous fungi at laboratory scale [8], no data on the suitability of streptomycetes for this purpose has been reported.

Streptomycetes are a group of filamentous bacteria which present a series of advantages to be used for biotechnological purposes such as abundant colonization of solid residues, production of a wide range of degradative enzymes, high resistance to extreme conditions, etc. In fact, previous studies demonstrated their suitability to upgrade agricultural residues in SSF [9,10]. The ability of these microorganisms to colonize agro-industrial residues and to produce a wide range of enzyme activities related with lignocellulose degradation make them good candidates for biotechnological recycling of coffee pulp.

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Since several decades pyrolysis technique have been widely used to analyse the composition of different natural and synthetic polymers. Analytical pyrolysis combined with gas chromatography–mass spectrometry is being used to characterize a wide series of biopolymers, additives and impurities present in different types of woods and grass residues [11–13]. The interest of this analytical technique is increasing during the last years due to its suitability to characterize the chemical modifications produced in these materials by different microorganisms.

In this work, the analysis of the chemical modifications produced in the polyphenolic compounds from coffee pulp residues by different *Streptomyces* strains in SSF conditions was investigated through Py–GC/MS. In addition, the ability of these strains to increase the protein content of these residues was also screened through Kjeldahl analysis.

2. Experimental

2.1. Bacterial strains

For this study the actinobacteria strains *Streptomyces chattanoogensis* CECT 3336, *Streptomyces* sp. UAH 47 and *Streptomyces* sp. UAH Nic-C were used. The first two strains were previously selected upon their ability to transform wheat straw in SSF and the last one was isolated from a volcanic soil sample near Casita volcano at Nicaragua. The strains were routinely grown on GAE medium [14] plates at 28 °C (for the strains *S. chattanoogensis* CECT 3336 and *S.* UAH 47) or 45 °C (for the strain *S.* UAH Nic-C) until sporulation occurred (4–6 days). The strains were maintained as spore suspensions in 20% (v/v) glycerol at −20 °C.

2.2. Substrate for fermentation

Coffee pulp used in this study was obtained from wet processing of coffee (*Coffea arabica* variety) cherries originating from Casita volcano plantations at León (Nicaragua). The residue was placed in plastic bags and frozen until used.

2.3. Substrate pre-treatment

Coffee pulp was washed with distilled water and autoclaved at 121 °C for 1 h. Then, the residue was oven-dried at 70 °C until constant weight, milled in a Janke and Kunkel mill and sieved to obtain fractions of 0.8 mm particle size.

2.4. Inoculum preparation

Spores from Petri dishes from 10 days sporulated cultures of the strains were collected in 0.01% (v/v) Tween 80 with a platinum loop. Five millilitres of spore suspensions (10^7 ufc mL^{-1}) were inoculated in 500 mL Erlenmeyer flasks containing 100 mL saline basal medium (SBM) [15] supplemented with 0.6% yeast extract (w/v). Flasks were incubated at 28 or 45 °C (depending on the strain) and 150 rpm for 48 h. Mycelia obtained by centrifugation (10,000 × *g* for 5 min) was washed with distilled water (2×) and resuspended in SBM supplemented with 0.1 yeast extract (w/v) (inoculum).

2.5. SSF

SSF was carried out in 500 mL Erlenmeyer flasks containing 3 g coffee pulp and 0.5 g cassava and inoculated with 10 mL of the previously obtained inoculum to achieve 60–70% moisture degree. Cultures were incubated for 10 days at the required temperature. Uninoculated flasks containing the same components were used as controls.

2.6. Biomass estimation

The biomass achieved for each strain along the incubation time was estimated as CO_2 released by using a CO_2 analyser (1440 Gas Analyser, Servomex).

2.7. Analytical methods

Dry weight loss of the substrate was estimated gravimetrically as previously described [15]. Control and fermented coffee pulps were oven-dried overnight at 80 °C prior to analysis. Total nitrogen was estimated by the Kjeldahl method [16]. Total protein was calculated as $N_{total} \times 6.25$.

2.7.1. Flash pyrolysis—gas chromatography–mass spectrometry

The pyrolysis of untreated and fermented coffee pulp (after 10 days of incubation) samples (1 mg) was performed with a double-shot pyrolyzer (model 2020, Frontier Laboratories) at 500 °C for 1 min. The pyrolyzer was directly connected to a GC–MS system Agilent 6890 equipped with a fused silica capillary column HP 5MS (30 m × 250 μm × 0.25 μm inner diameter). The detector consisted of an Agilent 5973 mass selective detector (EI at 70 eV). Oven temperature was held at 50 °C for 1 min and then increased up to 100 °C at 30 °C min^{-1}, from 100 to 300 °C at 10 °C min^{-1} and isothermal at 300 °C for 10 min using a heating rate of

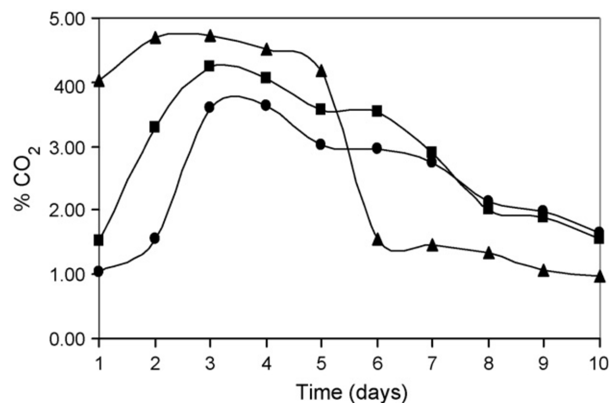

Fig. 1. Carbon dioxide evolution (%) during solid-state fermentation of different *Streptomyces* strains on coffee pulp residues. *Streptomyces chattanoogensis* (●), *Streptomyces* UAH 47 (■), *Streptomyces* UAH Nic-C (▲).

20 °C min^{-1} in the scan modus. The carrier gas used was helium with a controlled flow of 1 mL min^{-1}.

Polyphenols and polysaccharides pyrolysis products were identified on the basis of the Wiley and Nist computer libraries and on the retention times and spectra reported in the literature for lignocellulosic residues.

3. Results and discussion

The time course of growth of the assayed strains on coffee pulp residues supplemented with cassava as co-substrate, as estimated as CO_2 evolution for 10 days of incubation, is shown in Fig. 1. All strains achieved maximum growth at third

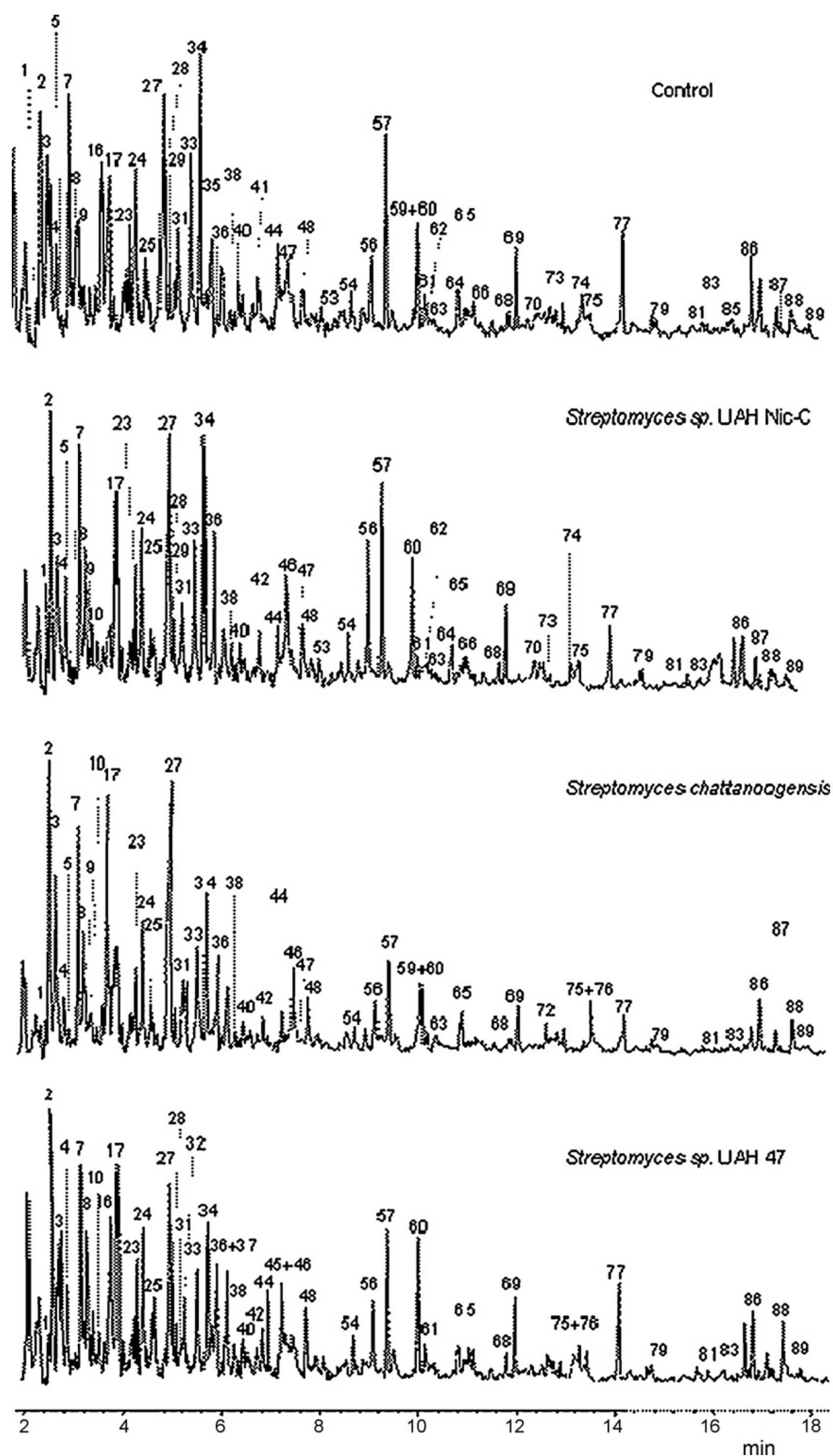

Fig. 2. Py–GC/MS of control and treated coffee pulp by different *Streptomyces* strains. See Table 1 for identification of main Py–GC/MS peaks.

day of incubation, reaching the higher values the strain *S.* UAH Nic-C.

The pyrograms corresponding to untreated and treated coffee pulp by different *Streptomyces* strains after 10 days of incubation are shown in Fig. 2. The identities and the relative abundances of all released pyrolysis products are presented in Table 1. The summed areas of the polysaccharides- and polyphenols-derived peaks were normalized to 100% and the data for two repetitive pyrolysis experiments were averaged. Standard deviation values were 5–8% for all compounds. In this table the percentages corresponding to carbohydrate (range 54.4–67.4), total polyphenols (range 45.6–32.6), monomethoxy-derivatives (range 31.0–22.8), and dimethoxy-derivatives (range 14.6–9.8) pyrolysis products were also shown.

In the pyrograms a series of typical pyrolysis products derived from polysaccharides and polyphenols (monomethoxy- and dimethoxy-derivatives) were detected. These compounds can be considered characteristic markers of grass lignocellulosic residues. It has been described that coffee pulp contains a high amount of polyphenolic polymers (e.g. tannins, lignin and chlorogenic acids) as well as carbohydrates, caffeine and ashes [4,17]. In general, the relative abundance of polyphenols-derived compounds, both monomethoxy and dimethoxy-derivatives was lower in treated coffee pulps than in control pulps. The decrease in these compounds was more remarkable in coffee pulps transformed by the strains *S. chattanoogensis* (30.5%) and *S.* UAH Nic-C (23.9%) as can be calculated from data presented in Table 1. These results could be attributed to the production of oxidative enzyme activities such as laccases by the assayed strains (data not shown). Previous studies have also demonstrated the ability of several *Streptomyces* strains to produce laccases in SSF conditions using wheat straw as substrate and their involvement in the solubilization and mineralization of lignin [9,18].

The decrease produced in the polyphenolic compounds of the coffee pulp by the action of the microorganisms can be considered an important result in order to consider this residue as a domestic fodder to be used in developing countries. In fact, the presence of tannins, polyphenols and caffeine in coffee pulp restricts its economic use and cause severe pollution problems in the environment [1].

It is important to take into account that a sort of compounds detected in the pyrograms cannot be ascribed as certainly to a specific origin. Thus, compounds such as catechol (47) and

Table 1
Semiquantitative analysis of the compounds released after Py–GC/MS of control and treated coffee pulp by different *Streptomyces* strains (area: % of GC–MS total ion peaks)

No.	Compound	Origin[a]	Control	*Streptomyces* UAH Nic-C	*Streptomyces chattanoogensis*	*Streptomyces* UAH 47
1	Pyrrole		0.8	1.4	1.1	1.1
2	Pyridine		5.6	6.4	8.1	8.1
3	Toluene		3.1	4.2	4.2	2.6
4	(3*H*)Furan-2-one	Ps	1.0	1.6	1.7	1.3
5	*N*-Methylpyrrole		0.3	0.5	0.1	0.8
6	*N*-Methylpyridine		0.2	0.3	0.2	0.4
7	Furfural	Ps	3.8	5.9	6.5	5.1
8	Furfurylalcohol	Ps	2.7	3.4	3.3	3.1
9	1-Acetoxypropan-2-one	Ps	0.4	0.3	0.1	0.5
10	2(5*H*) Furanone-5-methyl	Ps	0.5	0.9	0.9	1.3
11	Xylene		0.1	0.1	0.0	0.3
12	Cyclopent-1-ene-3,4-dione	Ps	0.6	0.6	0.6	0.9
13	Styrene		0.6	0.6	1.5	1.0
14	2-Methyl-2-cyclopenten-1-one	Ps	1.3	1.2	1.0	0.8
15	2-Acetylfuran	Ps	2.0	1.1	2.0	2.1
16	(5*H*)Furanone	Ps	1.4	0.5	0.8	1.7
17	2,3-Dihydro-5-methylfuran-2-one	Ps	5.6	6.0	7.6	5.9
18	2(3*H*)Furanone-5-methyl	Ps	0.6	0.0	0.0	0.7
19	Dimethylpyridine		0.0	0.8	0.8	0.0
20	4-Hydroxy-5,6-dihydro-(2*H*)-pyran-2-one	Ps	0.3	0.8	1.0	0.4
21	5-Methyl-2-furfuraldehyde	Ps	0.5	0.6	0.5	0.7
22	2,3-Dimethylcyclopenten-1-one	Ps	0.0	0.0	0.0	0.3
23	Furan-2,5-dimethyl	Ps	1.3	1.7	1.9	1.7
24	Phenol		5.2	3.3	3.8	3.3
25	5,6-Dihydropyran-2,5-dione	Ps	0.6	0.5	0.5	0.9
26	4-Hydroxy-5,6-dihydro-(2*H*)-pyran-2-one	Ps	0.3	0.4	0.0	1.4
27	2-Hydroxy-3-methyl-2-cyclopenten-1-one	Ps	5.1	6.9	9.0	4.6
28	Limonene		1.4	2.0	0.0	1.7
29	2,3-Dimethylcyclopenten-1-one	Ps	0.9	0.9	0.9	0.7
30	Benzeneacetaldehyde		0.4	0.0	0.0	0.0
31	*o*-Cresol		1.0	1.4	1.5	0.7
32	Dimethyldihydropyranone	Ps	0.6	0.0	0.8	0.9
33	*p*/*m*-Cresol		3.1	2.6	3.7	2.4
34	Guaiacol	Ph mono-Me	6.3	5.3	4.7	4.6

Table 1 (*Continued*)

No.	Compound	Origin[a]	Control	*Streptomyces* UAH Nic-C	*Streptomyces chattanoogensis*	*Streptomyces* UAH 47
35	2-Furoic acid methyl ester	Ps	0.3	0.0	0.0	0.9
36	3-Hydroxy-2-methyl-(4*H*)-pyran-4-one	Ps	2.3	2.9	3.4	0.7
37	Isomer-Dimethyldihydropyranone	Ps	0.0	0.0	0.0	1.9
38	Levoglucosenone	Ps	0.7	1.0	0.0	1.0
39	2,6-Dimethylphenol		0.4	0.4	0.3	0.5
40	Phenylacetonitrile		0.9	0.9	1.0	0.0
41	2,4-Dimethyl phenol		0.5	0.5	0.6	0.4
42	4-Ethylphenol		0.7	1.1	0.9	1.1
43	Benzoic acid		0.5	0.0	0.0	0.0
44	4-Methylguaiacol	Ph mono-Me	1.7	1.1	0.8	1.7
45	1,4-Anhydroxylopyranose	Ps	0.0	0.0	0.0	0.7
46	4-Hydroxymethyl-2-furaldehyde	Ps	0.0	4.6	2.2	0.9
47	Catechol	Ps	2.4	0.3	0.3	0.3
48	4-Vinylphenol	Pr	0.6	1.5	1.4	2.2
49	5-Hydroxymethyl-2-furaldehyde	Ps	0.5	0.0	0.0	0.0
50	4-Ethyl-2-methylphenol		0.2	0.2	0.0	0.1
51	*isomer*-Catechol	Ps	0.5	0.2	0.6	0.0
52	*isomer*-4-Ethyl-2-methylphenol		0.2	0.0	0.2	0.0
53	3-Methoxycatechol	Ph mono-Me	0.5	0.5	0.0	0.0
54	4-Ethylguaiacol	Ph mono-Me	1.5	0.9	0.8	1.3
55	2,3-Dihydro-(1*H*)-inden-1-one		0.2	0.5	0.0	0.4
56	Indole		2.1	2.7	1.1	2.6
57	4-Vinylguaiacol	Ph mono-Me	5.9	4.0	3.4	4.3
58	4-Hydroxybenzyl alcohol	Ps	0.2	0.0	0.0	0.0
59	*trans*-4-Propenylphenol		0.3	0.0	0.7	0.0
60	Syringol	Ph di-Me	2.5	2.5	2.5	2.3
61	Eugenol	Ph mono-Me	0.6	0.4	0.2	0.7
62	4-Ethylcatechol	Ps	0.2	0.6	0.0	0.0
63	4-Propylguaiacol	Ph mono-Me	0.2	0.3	0.4	0.2
64	Methylindole		1.3	0.9	0.4	0.7
65	Vanillin	Ph mono-Me	0.3	0.5	1.0	0.3
66	*cis*-Isoeugenol	Ph mono-Me	0.6	0.3	0.2	0.5
67	1,6-Anhydromannopyranose	Ps	0.4	0.0	0.0	0.0
68	4-Methylsyringol	Ph mono-Me	0.6	0.5	0.6	0.4
69	*trans*-Isoeugenol	Ph mono-Me	2.0	1.9	1.9	1.9
70	Levoglucosane	Ps	1.0	0.0	0.0	0.4
71	1-(4-Hydroxy-3-methoxyphenyl)propyne	Ph mono-Me	0.3	0.0	0.0	0.3
72	1-(4-Hydroxy-3-methoxyphenyl)allene	Ph mono-Me	0.2	0.4	0.7	0.0
73	Acetovanillone	Ph mono-Me	0.2	0.1	0.5	0.5
74	4-Ethylsyringol	Ph di-Me	1.0	0.5	0.3	0.4
75	Guaiacylacetone	Ph mono-Me	0.3	0.3	0.2	0.5
76	1,6-Anhydro-β-D-glucopyranose	Ps	0.0	0.0	0.5	1.3
77	4-Vinylsyringol	Ph di-Me	2.8	1.6	1.4	2.3
78	Propiovanillone	Ph mono-Me	0.1	0.1	0.0	0.0
79	4-Allylsyringol	Ph di-Me	0.3	0.3	0.2	0.4
80	4-Propylsyringol	Ph di-Me	0.2	0.2	0.0	0.1
81	*cis*-4-Propenylsyringol	Ph di-Me	0.3	0.3	0.2	0.3
82	Dihydroconiferyl alcohol	Ph mono-Me	0.0	0.0	0.1	0.3
83	Syringaldehyde	Ph di-Me	0.2	0.2	0.2	0.3
84	*cis*-Coniferyl alcohol	Ph mono-Me	0.2	0.2	0.0	0.2
85	1-(3,5-Dimethoxy-4-hydroxyphenyl)propyne	Ph di-Me	0.3	0.0	0.0	0.1
86	*trans*-4-Propenylsyringol	Ph di-Me	1.7	1.0	0.8	1.1
87	Acetosyringone	Ph di-Me	0.2	0.3	0.3	0.3
88	*trans*-Coniferyl alcohol	Ph mono-Me	0.8	0.5	0.7	1.0
89	Syringylacetone	Ph di-Me	0.2	0.2	0.2	0.3
	Carbohydrates peaks (%)		54.4	63.8	67.4	62.0
	Total polyphenols peaks (%)		45.6	36.2	32.6	38.0
	Ph mono-Me peaks (%)		31.0	25.1	22.8	25.9
	Ph di-Me peaks (%)		14.6	11.2	9.8	12.1
	Total polyphenols/carbohydrates ratio		0.8	0.6	0.5	0.6

[a] Origin of compounds: Ph mono-Me, polyphenols monomethoxy-derivatives; Ph di-Me, polyphenols dimethoxy-derivatives; Ps, polysaccharides.

phenol (24) could be assigned to proteins and also to pyrolysis products derived from chlorogenic acids (3-*O*-caffeoylquinic acid), an important constituent of coffee pulp [19]. In order to prevent the ambiguous interpretation of the pyrolysis results concerning to protein estimation in the fermented substrate, Kjeldahl method [16] was applied to analyse the protein enrichment of the substrate. The protein content of untreated coffee pulp was estimated as 10.5% and for transformed coffee pulp by *S. chattanoogensis*, *S.* UAH 47 and *S.* UAH Nic-C were 15.1, 14.0 and 14.9%, respectively. It is noticeable the increase in protein content of transformed residue by the three assayed strains compared with the control (30, 21 and 28% for *S. chattanoogensis*, *S.* UAH 47 and *S.* UAH Nic-C, respectively). These values are higher than those obtained when other lignocellulosic residues such as cassava or banana were fermented by *Aspergillus* or *Rhizopus* strains [20]. These results may confirm the ability of *Streptomyces* strains to increase the protein content of coffee pulp which is considered very important to be used for feeding purposes [21].

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