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DETERMINATION OF BIOTECHNOLOGICAL POTENTIAL OF POULTRY LITTER ISOLATED BACTERIA

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Abstract

Poultry litter is composed of residues from industrial wood processing, serving as a bed for the animals. It has also been widely used as an agricultural biofertilizer, increasing its economic importance. Few studies report the microbiological composition of this material or its biotechnological potential, which was the objective of this study. The microbiological composition was obtained by 16sRNA genetic characterization, as well as, phosphate solubilization capacity, indole-3-acetic acid (IAA) production, fungal antagonism, and enzymatic capacity. As a result, 67 isolates were obtained, of which 30 were taxonomically positioned and 27 were biochemically characterized. The microbiota genera consisted of *Staphylococcus* (17%), *Brevibacillus* (27%), *Bacillus* (37%), *Mammaliicoccus* (7%), *Isoptericola, Microbacterium,* and *Oxalicibacterium* (3.5% each). None of the isolates presented phosphate solubilization capacity. Regarding IAA production, UFPRCA19 showed the highest IAA content (16.60 µg.mg^{-1} protein). Eleven isolates were positive for antagonism with *Aspergillus* spp. and 25 for *Trichoderma* spp. Activity of proteases, caseinases, lipases, amylases, and cellulases were observed with the relative frequencies of 59%, 29%, 11%, 32%, 18%, and 21%. IAA and enzyme-producing bacteria as well as fungal antagonism were found among the isolates. Among all the isolated genera, Bacillus for synthesizing IAA and commercial enzymes becomes an interesting source for biotechnological purposes.

Keywords: Broiler. Enzymes. Microbiota. Plant Growth Promoting Bacteria.

1. Introduction

Various groups of bacterial biomolecules are attractive and useful for industrial, pharmaceutic, and agronomic purposes, mainly due to their stability, catalytic activity, and ease of production and optimization compared to plant and animal enzymes (Singh et al. 2016; Rani et al. 2021). Many bacteria genera can produce a great range of enzymes (amylases, proteases, pectinases, lipases, laccases, cellulases, xylanases, and among others) which present advantages such as high yield, and ease of product modification and optimization allowing for biochemical diversity. Those enzymes are also susceptible to gene manipulation; particularly selected strains can produce purified, well-characterized enzymes on a large scale (Anbu et al. 2015; Liu and Kokare 2017).

Another feature of bacteria is the ability to interact with plants by fixing nitrogen, producing hormones (auxins, gibberellins, abscisic acid, cytokinins, ethylene), and enzymes that can help plant growth, as they increase root surface area, solubilize macronutrients, reduce pathogens effect by antagonistic capacity, among others (Goswami et al. 2016; Santos et al. 2019). The isolation of bacteria strains from raw material in order to obtain inoculants, probiotics, and enzyme sources for industrial and agronomic interest has been gaining ground in biotechnological studies (Lobo et al. 2018; Indira et al. 2019).

Brazil is the third largest poultry producer in the world (Embrapa 2021) and poultry farming is an activity that generates a large amount of waste, namely poultry litter. Poultry litter constitutes all the material in an aviary that serves as a bed for the animals and receives excreta, feed, and feathers residues (Avila et al. 1992). It is composed of residues from the industrial processing of wood such as chips, shavings, sawdust, and sanding dust. Poultry litter has also been widely used as an agricultural biofertilizer which increases its economic importance. Yet, few studies report the material's microbiological composition or its biotechnological potential in bacterial groups (Valadão et al. 2011).

The present work aimed to isolate, identify, and characterize bacterial isolates from poultry litter regarding the potential of promoting plant growth as well as the search for bacterial enzymatic capacity for biotechnological purposes.

2. Material and Methods

Isolation of bacteria

Research activities were carried out at Labiogen (Laboratory of Biochemistry and Genetics) at UFPR - Setor Palotina. The windrowed poultry litter samples (0.25g), were collected from Ross (Aviagen™) broiler houses managed following technical recommendations. The samples were weighed in microtubes, then 1ml of peptone water was added. The samples were vortexed for 2 min and centrifuged for 10 min. In the laminar flow chamber, 100µL of the supernatant was removed from the tube and dispensed into Petri dishes containing culture medium (LB, YGA, NFB, DYGS, and JMV) (Dobereiner et al. 1999) in tetraplicate. The plates were incubated at 30 ºC for 24h. Every 2 days, colonies were subcultured for 3 weeks until pure colonies (visible mass of microorganisms all originating from a single mother cell with similar features) were obtained. The isolates were characterized according to the Gram coloration.

Sequencing and molecular characterization

Bacterial genomic DNA was extracted in accordance with the DNA extraction protocol of the Quick DNA Fecal/Soil Microbe Microprep Kit. PCR amplification of the 16S rRNA gene was carried in 20 µL final volume containing 3 µL 10x Taq buffer; 2.25 µL of 25 mM MgCl2; 1 µL of 10 mM of each deoxyribonucleotide; 1 µL of each oligonucleotide primer (primer 27F and 1492R at 4mM concentration) (Senthilraj et al. 2016); 2 µl Taq DNA polymerase; 2 µL of DNA (50 to 100 ng); and 8.75 µL sterile milli-Q water. The amplification program was: 93 ºC, for 2 min, then 35 cycles of 93 ºC for 45 s, 62 ºC for 30 s, 72 ºC for 2 min, followed by a final extension for 5 min at 72 ºC. To confirm the amplification, 5 µL of the reaction were subjected to electrophoresis on a 0.8% agarose gel in TBE buffer, which resulted in an amplification product comprising almost the entire gene, with approximately 1.5 Kb.

Sample sequencing was performed by ACTGene Análises Moleculares Ltd. (Center for Biotechnology, UFRGS, Porto Alegre, RS, Brazil) using the automatic sequencer AB 3500 Genetic Analyzer (Applied Biosystems). DNA templates were purified with the ExoSAP-IT™ PCR Product Cleanup reagent (Applied Biosystems) and quantified on Nanodrop 2000 c (Thermo Scientific). Subsequently, they were labeled with 2.5 pmol of the specific primer and 0.5 µL of BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), which resulted in a final volume of 10 µL. Labeling reactions were performed in an LGC XP Cycler with an initial denaturing step of 96 ºC for 3 min, followed by 25 cycles of 96 ºC for 10 sec; 55 ºC for 5 sec; and 60 ºC for 4 min. Sequencing data were collected using the software Data Collection 3 (Applied Biosystems). The resulting Data Collection files (.ab1; electropherograms) were converted into FASTA files

(.seq; text) by the Sequence Analysis Software v. 6 (Applied Biosystems) using MEGA software v. 7 (Tamura et al. 2007).

Biochemical characterization of isolates

Indole-3-acetic acid (IAA) production

The bacterial indole-3-acetic acid (IAA) production was carried out following the colorimetric methodology proposed by Sarwar and Kremer (1995) using the Salkowski reagent. The isolates were inoculated in 15 ml falcon tubes containing 10 ml of LB medium with and without L-Tryptophan (TRP) supplementation (0.2M). Incubated tubes in quintuplicate were under constant agitation at 120 rpm, for 24 h and 48 h. After 2 mL of solution were centrifuged (10.000g) for 10 min and the supernatant and pellet were used for different purposes: IAA production was performed by reading the supernatant absorbance at 535 nm (Asghar et al. 2002) using a standard curve of indole-3-acetic acid (0.3 mg mL^{-1}). From the pellet, total protein was evaluated according to the protocol proposed by Lowry et al. (1951) in order to normalize the IAA production readings.

Phosphate solubilization capacity

After being isolated in pure colonies, the bacteria were submitted to the test of phosphate solubilization in a solid NBRIP culture medium (Nautiyal et al. 2000). The inoculated plates were incubated at 30 ºC for 6 days. Then, solubilization was evaluated by measuring both the diameter of the solubilization and the colony halo with the aid of a graduated ruler. The Solubilization Indexes (SI) were obtained using the formula: SI = fHalo (mm) / fColony (mm), where f is the diameter of the halo or colony (Berraquero et al. 1976).

Antagonistic or anti-fungal capacity

Fungal antagonism was tested by the paired culture method: the direct confrontation between the antagonist (isolates) and the fungi *Trichoderma* spp. and *Aspergillus* spp. (Freitas et al. 2016). The fungi were cultivated in Petri dishes with Sabouraud Agar medium at 28 ºC, for 7 to 14 days. With the aid of a platinum loop, agar cubes of a 5 mm diameter were removed from the edges of the colonies of the growing fungi and placed in Petri dishes with a solid LB medium. Bacterial isolates were inoculated on two sides of the fungi colonies with the aid of wooden sticks. Afterward, Petri dishes in triplicate were incubated for 10 days at 30 ºC. The results were evaluated visually.

Detection of enzymatic activity in solid media

Caseinolytic, lipolytic, amylolytic, cellulolytic, and protease activities of the selected strains were measured using the agar diffusion method on agar-modified Basal Medium (MM) (1 g glucose, 2.5 g yeast extract, and 14 g agar) (Pérez-Borla et al. 2009) following the protocol proposed by Mazzucotelli et al. (2013). Bacteria were inoculated in the center of Petri dishes quarters, in 3 plates, which computed 12 repetitions. Petri dishes were incubated for 48 h at 30 ºC.

The qualitative assay of protease activity was evaluated in the MM medium supplemented with 6.2 g. L^{-1} of skim milk protein and by the formation of a clear zone in the medium around the bacterial colony. This indicated positive protease activity. Caseinolytic activity assays were performed by inoculating the isolates in MM supplemented with 1% casein. Positive caseinolytic activity was detected by the formation of a turbidity zone (white halo) around the well. The amylolytic activity assays were performed with inoculation of the isolates in MM and supplemented with 1% soluble starch as substrate. Cellulolytic activity assays were performed by inoculating the isolates in MM supplemented with 7.5% cellulose biomass (2.5 g of cellulose in the form of shredded paper with 75 ml of water). Plates were flooded with

1% iodine (2% KI), which resulted in a clear zone around the bacterial colony, indicating positive amylase and cellulolytic activities.

To do a qualitative assay of lipase activity, the Tween Agar Medium was used (10 g of peptone, 0.1 g of $CaCl_2.2\ H_2O$, 5 g of NaCl, 10 mL of Tween 80, and 15 g of agar-water at pH 7-7.4) (Harrigan 1998). After 15 days of bacteria incubation, a white precipitate formed around the well, resulting from the deposit of calcium salt crystals formed by the fatty acid released by the enzyme. This indicated positive lipolytic activity. The Enzyme Index (EI) was expressed by the ratio between the average diameter of the degradation halo and the average diameter of the colony growth (Carrim et al. 2006).

Statistical Analysis

Data were submitted to Analysis of Variance (ANOVA) and the means were grouped using Scott-Knott at 5% probability, using the GENES statistical program (Cruz 2013).

3. Results

Isolation of bacteria

Sixty-seven isolates were obtained as pure colonies. Among them, 55% were Gram-negative and 45% were Gram-positive. Visually, the appearance of colonies showed that 49% were white and opaque, 24% were colorless, and 27% were yellow. Considering isolation media, 39% grew in the NFB medium, 27% in the DIGS medium, 21% in the LB medium, 9% in the YGA medium, and 3% in the JMV medium. Thirty pure colonies (isolates) were randomly chosen to be used with the molecular characterization. From these, 27 were biochemically tested.

Molecular characterization of isolates

The results of 16sRNA genetic characterization are shown in Table 1. The 30 isolates were classified as *Staphylococcus* (27%), *Brevibacillus* (27%), and *Bacillus* (37%). The rest consisted of genera *Mammaliicoccus* (7%) *Isoptericola*, *Microbacterium*, and *Oxalicibacterium* (3.5% of each).

Biochemical characterization of isolates

The biochemical characterization results are demonstrated in Table 2. From all isolates evaluated, none showed the ability to solubilize phosphate. The IAA content varied between 24h and 48h, with and without the addition of TRP as an inducer (Table 2). Among all the isolates, UFPRCA19 showed better results compared to the others, presenting the highest IAA values in the treatments applied (3.65 to 16.60 $\mu g.mg^{-1}$ protein). It is also noteworthy that the isolates UFPRCA06 and UFPRCA27 without TRP and 48h of growth presented high IAA levels (3.68 and 3.41 $\mu g.mg^{-1}$ protein, respectively). On the other hand, UFPRCA128 presented results close to zero at 24h and very low IAA content at 48h.

Eleven isolates were positive for antagonism with the fungus *Aspergillus* spp. and 25 were positive for *Trichoderma* spp. (Table 2). The halo visual aspect from bacterial inoculation in a specific medium (which proves the enzymatic capacity) was demonstrated in Figure 2. The enzymatic assays showed that 23 of 27 isolates (85%) presented the hydrolytic potential for at least one of the tests (Table 3). Among the isolates, 41% were able to express enzymatic activity in two tests or more, indicating multi-enzymatic activity, thus being of great importance in substrate recycling or reprocessing (Mazzucotelli et al. 2013). The proteolytic activity in 16 isolates (59%) was observed. UFPRCA83 (*Brevibacillus laterosporus)* reached the highest protease EI (3.04cm) followed by UFPRCA18 with 2.45cm, which is different from the other isolates. Caseinase was found in 29% (8 isolates), and the highest level of caseinase EI (2.37cm) was found in isolate UFPRCA83 (*Brevibacillus laterosporus)*. For lipolytic activity, only 3 isolates (11%) showed positive activity (isolates UFPRCA01, 47, and 70); isolate UFPRCA01 (*Staphylococcus cohni*) reached the highest amount of lipase (2.87cm).

Table 1. Probable taxonomic classification of poultry litter bacterial isolates.

Isolate	Classification	Fragment size	E value	Identity %	GenBank Access (OQ)
1	*Staphylococcus cohnii*	1494	1,00E-138	92.70	195171
2	*Mammaliicoccus lentus*	1480	2,00E-179	96.62	195172
5	*Bacillus sonorensis*	1475	8,00E-140	96.97	195173
6	*Bacillus subtilis*	150	0.0	97.85	195174
8	*Bacillus subtilis*	1550	0.0	88.99	195175
12	*Bacillus subtilis*	1550	0.0	96.28	195176
18	*Bacillus haynesii*	1508	4,00E-46	86.71	195175
19	*Bacillus haynesii*	1508	2,00E-165	97.92	195178
21	*Bacillus haynesii*	1508	0.0	95.77	195179
24	*Microbacterium maritypicum*	1437	6,00E-15	94.00	195180
27	*Bacillus haynesii*	1508	8,00E-140	96.97	195181
46	*Staphylococcus cohnii*	1494	2,00E-100	91.29	195182
47	*Staphylococcus cohnii*	1494	0.0	90.39	195183
50	*Brevibacillus halotolerans*	1440	2,00E-118	91.69	195184
56	*Brevibacillus halotolerans*	1486	0.0	89.85	195185
57	*Mammaliicoccus lentus*	1480	0.0	96.56	195186
60	*Staphylococcus equorum*	1494	1,00E-88	88.66	195187
66	*Brevibacillus laterosporus*	1461	8,00E-180	88.57	195188
70	*Staphylococcus epidermidis*	1476	0.0	90.77	195189
81	*Brevibacillus halotolerans*	1440	0.0	99.85	195190
83	*Brevibacillus laterosporus*	1461	0.0	99.37	195191
94	*Bacillus subtilis*	1474	3,00E-70	90.50	195192
100	*Isoptericola nanjingensis*	1404	6,00E-91	80.43	195193
107	*Brevibacillus panacihumi*	1473	0.0	96.62	195194
108	*Oxalicibacterium faecigallinarum*	1489	3,00E-138	85.56	195195
119	*Bacillus velezensis*	1550	0.0	90.45	195196
125	*Brevibacillus laterosporus*	1461	0.0	93.55	195197
126	*Bacillus subtilis*	1550	0.0	94.44	195198
128	*Brevibacillus laterosporus*	1461	3,00E-163	89.20	195199
134	*Staphylococcus cohnii*	1494	6,00E-102	93.46	195200

Fragment Size: Sequence size. E Value: probability of randomly finding the same alignment between two sequences. Identity %: percentage of identity between the isolate's sequence and the related organism. GenBank Access: Sequence accession number of the related organism.

Regarding amylolytic activity, 18% of the isolates were able to hydrolyze starch molecules. Isolate UFPRCA100 (*Isoptericola nanjingensis*) was the highest amylase producer (2.63cm), and 22% of the isolates showed positive activity for cellulolytic capacity, which means that enzymes were able to break down the cellulose molecule. Isolate UFPRCA60 stood out statistically from the other positive isolates, as it presented an EI of 3.11cm (Table 3).

4. Discussion

The isolates were distributed in the phyla Firmicutes (94%) and Actinobacteria (6%). These data correspond with those obtained by Vaz Moreira et al. (2008), who reported Firmicutes as the main phylum in the litter. Among the isolates, genera common to massive sequencing studies of poultry litter samples, such as *Listeria* spp*., Clostridium, Campylobacter, and Salmonella* were not observed (Lu et al. 2003; Guttala et al. 2017). The absence of these groups could possibly be explained, for example, by the composition of the culture medium used for the isolation process, the absence of heat treatment of the samples (Trawińska et al. 2016), and the need for anaerobiosis for the full development of some species such as *Campylobacter* and *Clostridium* (Voss-Rech and Vaz 2012).

Table 2. Biochemical characterization of poultry litter bacterial isolates

Isolate UFPRCA	P solubilization	Antagonism		IAA/ Protein (µg.mg^{-1}) (24h)		IAA/Protein (µg.mg^{-1}) (48h)	
		Aspergillus spp	*Trichoderma spp*		TRP (0.2M)		TRP (0.2M)
01	-	-	+	4.00^{c}	2.21^{d}	2.68^{b}	3.96^{b}
02	-	-	+	3.95^{c}	2.47^{d}	2.84^{b}	2.43^{c}
06	-	+	+	3.88^{c}	4.91^{b}	3.68^{a}	4.15^{b}
08	-	-	+	3.98^{c}	1.96^{d}	1.75^{b}	2.40^{c}
12	-	+	+	3.28^{d}	3.04^{c}	2.76^{b}	3.44^{b}
18	-	-	+	6.18^{b}	3.50^{c}	2.82^{b}	2.84^{c}
19	-	-	+	7.85^{a}	16.60^{a}	3.65^{a}	16.02^{a}
21	-	-	+	0.00^{f}	0.61^{e}	2.02^{b}	1.46^{d}
24	-	-	+	0.15^{f}	1.01^{e}	0.88^{c}	1.13^{d}
27	-	+	+	0. 14^{f}	1.40^{e}	3.41^{a}	2.55^{c}
46	-	+	+	1.43^{e}	0.05^{f}	0.99^{c}	1.91^{c}
47	-	-	+	0.25^{f}	0.62^{e}	0.54^{c}	0.93^{d}
50	-	+	+	1.01^{e}	0.33^{f}	0.70^{c}	1.29^{d}
56	-	+	+	1.21^{e}	0.09^{f}	0.77^{c}	0.44^{e}
57	-	-	+	0.10^{f}	0.02^{f}	1.41^{c}	0.71^{e}
60	-	-	+	0.62^{f}	0.81^{e}	0.16^{c}	0.54^{e}
70	-	-	-	0.01^{f}	1.35^{e}	0.16^{c}	0.33^{e}
81	-	+	+	1.89^{e}	1.06^{e}	0.00^{c}	0.48^{e}
83	-	+	+	0.01^{f}	0.03^{f}	0.35^{c}	0.70^{e}
94	-	-	+	0.05^{f}	0.24^{f}	2.47^{b}	1.16^{d}
100	-	-	-	0.03^{f}	0.04^{f}	0.46^{c}	0.09^{e}
107	-	-	-	0.00^{f}	0.03^{f}	0.18^{c}	0.22^{e}
108	-	-	-	0.26^{f}	0.12^{f}	0.40^{c}	0.37^{e}
125	-	+	+	0.00^{f}	1.13^{e}	0.00^{c}	0.10^{e}
126	-	+	+	0.00^{f}	0.08^{f}	0.56^{c}	0.29^{e}
128	-	+	+	0.00^{f}	0.00^{f}	0.20^{c}	0.49^{e}
134	-	-	+	0.14^{f}	0.11^{f}	1.09^{c}	0.75^{e}
A.brasilense	Nd*	Nd	Nd	1.25^{e}	0.68^{e}	0.16^{c}	0.16^{e}

* Nd — not determined. Averages followed by the same letter did not differ among themselves (Scott-Knott, $p < 0.05$).

The bacterial genera surveyed in this work are similar to those obtained by Sule et al. (2019), who observed several *Staphylococcus* and *Bacillus* species in the composition of poultry litter collected in the Kwara region (Nigeria). Dumas et al. (2011) related the genus *Staphylococcus* as the second most abundant in poultry litter, as this group is related to pathogenicity in aviaries (Szafraniec et al. 2020).

Brevibacillus (*laterosporus, panacihumi, halotolerans*), were also found in great abundance among the isolates. This genus possibly originated from the animals' excreta since the application of probiotics produced by *Brevibacillus laterosporus* is recommended for animals handling, as it provides protection against *Salmonella* infections (Purba et al. 2020) and reduces the incidence of flies on the farm (Ruiu et al. 2013). The control of pathogens happens through the production of antibiotics of laterosporamine, laterosporin, tauramamide, and loloatin types (Djukic et al. 2011, Hassi et al. 2012). Similar to *Brevibacillus*, the *Bacillus* group probably originated from excreta, since the administration of probiotics with *B. subtillis* is widely recommended by veterinarians, as it could replace antibiotics and promote the greater performance of body and visceral growth (Ciurescu et al. 2019; Yu et al. 2020).

Regarding plant growth promotion characteristics, the main ability was to solubilize inorganic phosphate from the soil and make it available to the plants. None of the 28 isolates showed this capacity, which could be explained by the type of organic substrate from bacteria that were isolated (poultry litter). This substrate is mostly composed of wood shavings rich in lignin and hemicellulose (Avila et al. 1992) and poor in macronutrients, including inorganic P. There is also a close relationship between the niche occupied by a microorganism and the chemical compounds produced in order to adapt (Berrada et al. 2012).

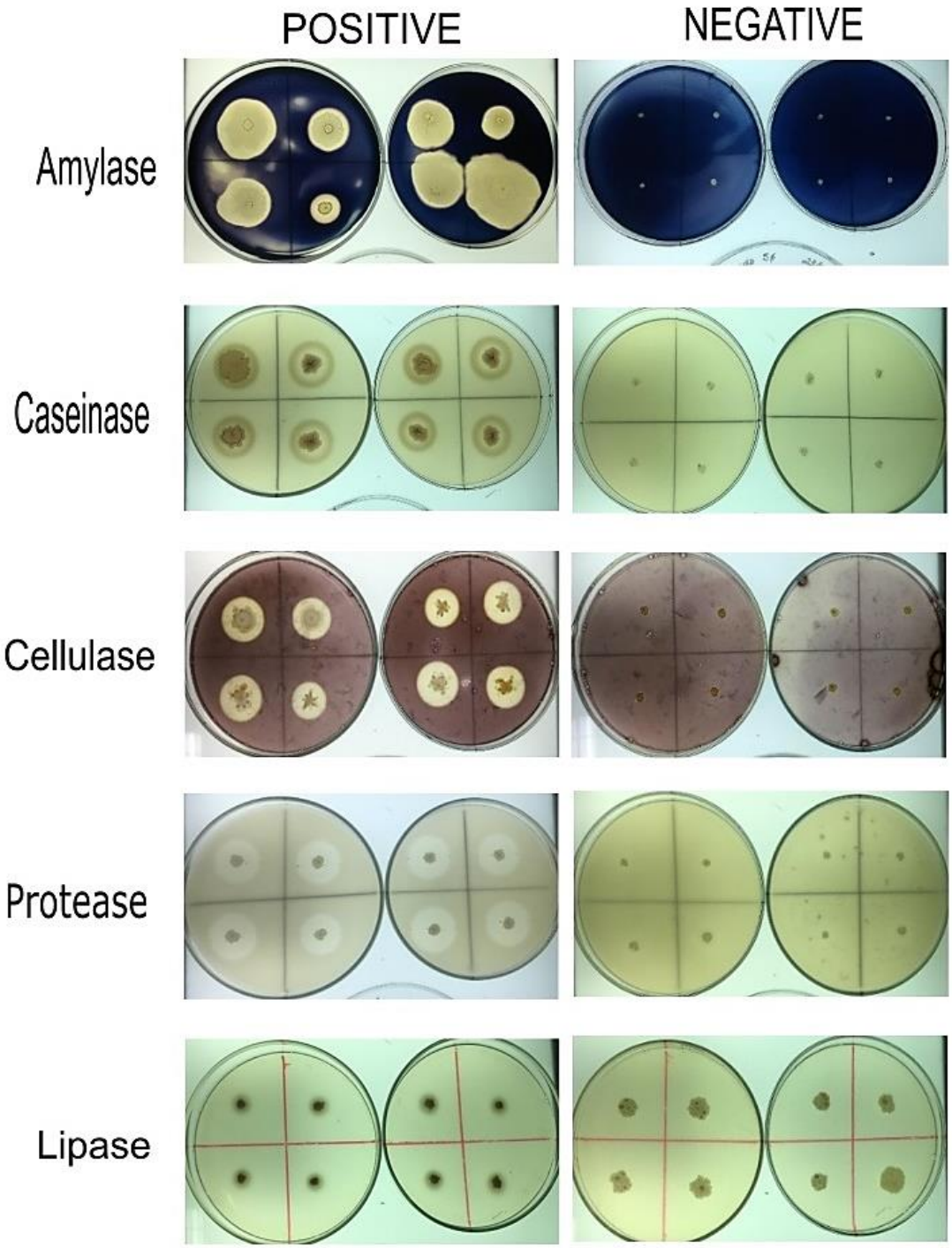

Figure 2. Visual aspects of positive and negative responses from the enzyme essays

Another attribute required of a PGPB is the ability to synthesize IAA, which stimulates and eases plant growth, increasing root surface area (Grover et al. 2021). The IAA secreted by the rhizosphere bacteria, along with the plant's endogenous supply, can impact root growth positively or negatively (Duca et al. 2014). IAA biosynthesis occurs through the transformation of chorismate into tryptophan (Spaepen et al. 2007), and the addition of TRP to the growth medium may favor IAA synthesis. Five different pathways have been described for the production of IAA in bacteria: indole-3-acetamide (IAM), indole-3-pyruvic acid (IPyA), indole-3-acetonitrile (IAN), tryptamine (TAM), and side-chain oxidase of tryptophan (TSO) (Kochar et al. 2013; Li et al. 2018). Among the isolates obtained, the *Bacillus* genus presented the highest IAA contents. In this genus, IAA can be synthesized from IPyA, IAM, IAN, and a TRP-independent pathway, however, IPyA was the most significant pathway for this species (Antonia and Jos 1995; Kunkel and Harper 2018). Isolates UFPRCA18 and 19 (*Bacillus haynesii*), and UFPRCA06, 08, and 12 (*Bacillus subtilis)* showed the highest levels of IAA. The results showed that among isolates, *Bacillus sp* showed the higher IAA capacity to biosynthesize auxin (Chagas et al. 2015; Ozdal et al. 2016; Wagi and Ahmed 2019; Abo Elsoud et al. 2023).

Table 3. Enzymatic activity of poultry litter bacterial isolates.

		Enzymatic Index (cm)*				
Isolates UFPRCA	Probable taxonomy classification	Lipase	Caseinase	Protease	Amilase	Celulase
1	*Staphylococcus cohnii*	2.87^{a}	0^{c}	0^{d}	0^{e}	0^{e}
2	*Mammaliicoccus lentus*	0^{c}	0^{c}	0^{d}	0^{e}	0^{e}
6	*Bacillus subtilis*	0^{c}	1.96^{b}	1.33^{c}	0^{e}	2.27^{c}
8	*Bacillus subtilis*	0^{c}	0^{c}	0.93^{c}	0.88^{c}	1.31^{d}
12	*Bacillus subtilis*	0^{c}	0^{c}	1.41^{c}	0^{e}	1.49^{d}
18	*Bacillus haynesii*	0^{c}	1.64^{b}	2.45^{a}	0^{e}	0^{e}
19	*Bacillus haynesii*	0^{c}	0^{c}	0^{d}	1.71^{b}	0^{e}
21	*Bacillus haynesii*	0^{c}	1.69^{b}	1.26^{c}	0^{e}	0^{e}
24	*Microbacterium maritypicum*	0^{c}	0^{c}	1.02^{c}	0.74^{d}	0^{e}
27	*Bacillus haynesii*	0^{c}	0^{c}	0^{d}	0^{e}	0^{e}
46	*Staphylococcus cohnii*	0^{c}	0^{c}	0^{d}	0^{e}	2.50^{c}
47	*Staphylococcus cohnii*	1.44^{b}	0^{c}	0^{d}	0^{e}	0^{e}
50	*Brevibacillus halotolerans*	0^{c}	1.86^{b}	1.58^{c}	0^{e}	0^{e}
56	*Brevibacillus halotolerans*	0^{c}	0^{c}	1.49^{c}	0^{e}	0^{e}
57	*Mammaliicoccus lentus*	0^{c}	0^{c}	0^{d}	0^{e}	0^{e}
60	*Staphylococcus equorum*	0^{c}	0^{c}	0^{d}	0^{e}	3.11^{a}
70	*Staphylococcus epidermidis*	1.65^{b}	0c	0^{d}	0^{e}	0^{e}
81	*Brevibacillus halotolerans*	0^{c}	1.80^{b}	2.13^{b}	0^{e}	0^{e}
83	*Brevibacillus laterosporus*	0^{c}	2.37^{a}	3.04^{a}	0^{e}	0^{e}
94	*Bacillus subtilis*	0^{c}	0^{c}	1.12^{c}	0^{e}	0^{e}
100	*Isoptericola nanjingensis*	0^{c}	0^{c}	0^{d}	2.63^{a}	2.81^{b}
107	*Brevibacillus panacihumi*	0^{c}	0^{c}	0^{d}	0^{e}	0^{e}
108	*Oxalicibacterium faecigallinarum*	0^{c}	0^{c}	1.86^{b}	0^{e}	0^{e}
125	*Brevibacillus laterosporus*	0^{c}	0^{c}	1.89^{b}	0^{e}	0^{e}
126	*Bacillus subtilis*	0^{c}	2.00^{b}	1.91^{b}	0^{e}	0^{e}
128	*Brevibacillus laterosporus*	0^{c}	1.90^{b}	2.01^{b}	0^{e}	0^{e}
134	*Staphylococcus cohnii*	0^{c}	0^{c}	1.21^{c}	1.00^{c}	0^{e}

* The enzymatic index represents the halo diameter of degradation/diameter of colony in cm. Averages followed by the same letter did not differ among themselves (Scott-Knott, $p < 0.05$).

IAA biosynthesis has also been reported in *Staphylococcus* species (isolates UFPRCA01, 46, 47, 60, and 134) (Shahzad et al. 2017; Mike-Anosike et al. 2018). Furthermore, this species is considered endophytic in maize, which points to its association nature (Mendonça et al. 2020) and potential to promote growth in plants (Tiwari et al. 2019). Some isolates classified as *Brevibacillus* sp. (UFPRCA83, 107, 125 and 128) did not seem to have the ability to synthesize IAA, as the values were close to zero (Table 1).

The concentration of IAA varied through the growth periods: 10 isolates (UFPRCA06, 08, 12, 18, 19, 27 (*Bacillus sp*); UFPRCA01, 46 (*Staphylococcus sp*); UFPRCA50,56, 128 (*Brevibacillus sp*); showed higher IAA contents after 48h bacterial growth. Of these, UFPRCA19 (*Bacillus haynessi*) presented higher values of IAA with/without the addition of TRP to the culture medium (Table 1). Chagas et al. (2015) also observed that 48h of bacterial growth after adding TRP to the medium provided higher volumes of bacterial IAA in *Bacillus* spp. The other isolates UFPRCA18, 19 (*Bacillus sp*), UFPRCA01, 46 (*Staphylococcus sp*) and UFPRCA56, 81 (*Brevibacillus sp*) had the highest IAA rates with 24h of growth. The addition of TRP only allowed IAA increases in 3 isolates with 24h of growth (UFPRCA06, 19, and 60). The possible explanation is that IAA synthesis is regulated by the enzyme tryptophan 2-monooxygenase and is limited by the levels of TRP, working in a feedback inhibition by the concentration of indole-acetamide and IAA (Hutchesons and Kosuge 1985; Patten and Glick 1996).

Comparing the isolate UFPRCA19 (higher IAA content) and *A. brasilense* (control sample), the former showed IAA increments of 6x without TRP and 24x with TRP after 24h of growth. After 48h of growth, the increases were 23x and 100x, without and with TRP, respectively. It is also noteworthy that the isolates UFPRCA06 and 27, had a significantly higher IAA production after 48h of growth without the inducer (21x and 23x, respectively) compared to the *A. brasilense* (Table 2).

One of the indirect mechanisms of plant growth promotion is the ability to biocontrol phytopathogens such as bacteria, fungi, and nematodes (Tiwari et al. 2019). The antagonism test occurs because *Aspergillus* and *Trichoderma* generally release antibiotics and other chemicals that are harmful to

bacteria and that inhibit their growth (antibiosis) (Leelavathi et al. 2014). Regarding the antagonist capacity, it is possible to observe a positive antagonistic response from isolates classified as *B. subtilis* and *B. haynessi* (UFPRCA06, 08, 12, 27, and 126) and the partial response of isolates UFPRCA08, 18, 19, and 21 against *Trichoderma*. The ability of *Bacillus* to biocontrol fungal growth is explained by the production of molecules such as iturins, engicins, surfactins, mycosubtilins, lipopeptides, and bacillomycins that are effective in controlling fungi (Hashem et al. 2019; Sahu et al. 2020). Isolates from the *Staphylococcus* genus (UFPRCA 01, 02, 46, 47, 57, and 134) also present mostly positive biocontrol for the fungus *Trichoderma* and negative for *Aspergillus*. Leelavathi et al. (2014) cited that *Staphylococcus aureus* was susceptible to *Trichoderma* mainly at high concentrations of the extract (100µl.ml^{-1}).

Isolates of the genus *Brevibacillus* (UFPRCA50, 81, 83, 125, and 128) were also effective in antagonizing *Trichoderma* and *Aspergillus* fungi (Table 3). These results agree with the ones obtained by Joo et al. (2015) and Saikia et al. (2011) who observed growth inhibition of several fungal species including *Fusarium* sp*., Alternaria* spp*., Aspergillus* spp*., Rhizoctonia* sp*.,* and *Penicillium* spp. by the presence of this genus. The isolates UFPRCA100 (*Isoptericola nanjingensis*) and 108 (*Oxalicibacterium faecigallinarum*) showed a negative response to antagonism for both fungi evaluated. The isolate UFPRCA24 (*Microbacterium maritypicum*) showed only *Aspergillus* antagonism. The antagonism test was still not performed on these last 3 isolates, which hinders comparison with other studies.

Regarding bacterial hydrolytic capacity, isolated bacteria from poultry litter successfully demonstrated a variety of enzymatic activities. It was revealed that proteases, caseinases, lipases, amylases, and cellulases were produced with relative frequencies of 59%, 29%, 11%, 32%, 18%, and 21%, respectively (Table 3). Bacterial proteases are among the most important hydrolytic enzymes with great potential for use in various biotechnological processes such as food processing, pharmaceutical products, and waste degradation. Several other industries (Uddin et al. 2017) are increasing rapidly due to reduced processing time, low energy input, cost-effectiveness, and nontoxic and eco-friendly characteristics (Singh et al. 2016).

Caseinase can be used for many purposes in the food industry, mainly as a coagulant in cheese manufacturing or for other purposes in the healthcare industry (Dalmaso et al. 2015; Bhaturiwala et al. 2017). Jaouadi et al. (2013) and Hussein et al. (2020) showed the caseinolytic attributes in *Brevibacillus* and *Bacillus* genera (Table 3).

Proteases have been broadly used in a wide range of industries such as textile, pharmaceutical, leather, food, and detergents (Razzaq et al. 2019). Proteases are complex enzymes that have different properties such as substrate specificity, active site, and mechanism of action (Rao et al. 1998). In addition, proteases control the activation, synthesis, and turnover of proteins to regulate physiological processes (Rawlings et al. 2004), which explains the high number of isolates found (*Bacillus, Brevibacillus, Microbacterium, Oxalicibacterium*-59%) with proteolytic ability, the majority being *Bacillus* sp. Many authors cited this genus as a producer of alkaline proteases (active at alkaline pH ranging from 9 to 11), which are vital for industrial biocatalysis (Singhal et al. 2012; Wang et al. 2016).

Amylolytic capacity was observed in *Bacillus* (*subtilis* and *haynessi), Isoptericola nanjingensis,* and *Microbacterium maritypicum* species. Far et al. (2020) related the high capacity of the genus *Bacillus* (*B. amyloliquefaciens*, *B. licheniformis*, and *B. stearothermophilus*) in expressing unique alpha-amylases with thermostable and halotolerant characteristics. Alpha-amylase is currently used in a broad array of industrial applications, namely the production of ethanol, high fructose corn syrup, food, textile, paper, and detergent (Rana et al. 2013).

The synergistic action of cellulolytic/xylanolytic enzymes produced by lignocellulolytic bacteria and fungi are capable of robust cellulosic biomass deconstruction. Hydrolytic enzymes such as cellulases and xylanases break down cellulose and hemicellulose, which serve as building blocks for numerous industrial products (Petridis and Smith 2018). Amongst the microbes, *Bacillus subtilis*, *Staphylococcus* sp., and *Isoptericola nanjingensis* are able to demonstrate cellulolytic capacity. Thapa et al. (2020) described *Bacillus sp* (*B. licheniformis, B. cereus, B. subtilis)* as showing cellulase production. Lipases catalyze the hydrolysis of triacylglycerol into glycerol and fatty acids (Liu and Kokare 2017). Among the isolates, *Staphylococcus* sp. showed lipolytic capacity, agreeing with other authors that added *Bacillus* sp as a lipolytic genus (Odeyemi et al. 2013).

Isolates from *Bacillus* seem to be the most affordable source of industrial enzymes due to their unique properties, such as stability in a wide range of pH and temperatures, high specificity, and biodegradability of a wide range of substrates (Danilova and Sharipova 2020). Commercial enzymes from *Bacillus* spp. comprise about 50% of the enzyme market (Ghazala et al. 2016).

Not all isolates from the same genus showed the same biochemical or enzymatic ability. It was evidenced that isolates belonging to the same taxonomic class showed distinct EI to a determined enzyme (Table 3). This can be partially explained by horizontal gene transfer making bacterial genomes more dynamic through gains and losses of genes, as well as imposing transcriptional and translational changes on them (Bedhomme et al. 2019; Lee et al. 2021).

Poultry litter is still mostly unexplored regarding its microbial potential. We identified isolates that meet important biotechnological characteristics, such as being sources of bioactive compounds of agricultural and industrial importance.

5. Conclusions

Bacterial isolates from poultry litter are mainly composed of *Brevibacillus, Staphylococcus,* and *Bacillus* genera. The plant growth-promoting attributes evaluated highlighted the *Bacillus* genus as the highest IAA producer. In addition, the majority of isolates presented the antagonistic capacity to at least one of the fungi genera, but no isolate showed P solubilization ability. Regarding enzymatic activities, the data revealed that proteases, caseinases, lipases, amylases, and cellulases were produced with relative frequencies of 59%, 29%, 11%, 32%, 18%, and 21%, among isolates. Isolates from the *Bacillus* genus presented the capacity to synthesize more than one enzyme, such as the isolate UFPRCA08 (proteolytic, amylolytic, and cellulolytic). The results demonstrated that poultry litter could be an important source of bacterial genera for agricultural and industrial purposes.

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