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Selection and agricultural use of potential phosphate-solubilizing bacteria isolated from poultry slaughterhouse sludge in maize

Seleção e uso agrícola de potenciais bactérias solubilizadoras de fosfato isoladas de lodo de abatedouro de aves

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Highlights

Poultry abattoir sludge is a potential material for bioprospecting microorganisms.
The isolates demonstrated the ability to solubilize phosphate in selective media.
UFVLSOF-7 (*Sphingomonas* sp.) was what promoted greater initial growth of corn.

Abstract

TIn the farming, increasing demands for higher yields has put pressure on land resources which eventually increased demand for phosphate fertilizers (non-renewable resource), given that low availability of phosphorus in many soils is often compensated by the application high doses phosphate fertilizers. Complementary, studies have been made with a view to select phosphate-solubilizing bacteria. The purpose of his study was to: (i) isolate and characterization potential phosphate-solubilizing bacteria from sludge from the wastewater treatment plant of a poultry slaughterhouse; (ii) evaluate the ability of bacteria to solubilize phosphate rock and promoting plant growth. The bacteria were isolated in culture medium containing calcium phosphate. Morphological characterization consisted in cell shape, Gram staining and the characteristics of the colonies. To assess natural phosphate (NPh) solubilization, maize seeds were inoculated together with Araxá phosphate. The experiment consisted of 10 treatments: eight bacterial isolates + NPh, NPh and a control. The experiment was carried out in a greenhouse for 45 days, in a randomized block design, with six replications. The variables measured were the growth characteristics of the maize. Eight strains were isolated, all stained gram-positive, and 90% were rod-shaped and 10% coccoid-shaped. Inoculation with isolate LSOF-7 (*Sphinghomonas* sp.) combined with NPh induced increased maize dry matter by 20% compared to the treatment containing only NPh and by 67% compared to the control. It was concluded that sludge from wastewater treatment plants of poultry slaughterhouses contains bacterial

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strains with potential to phosphate-solubilizing and them also probably present promoting plant growth and promote maize plant growth.
Key words: *Zea mays* L. *Sphinghomonas.* Plant growth-promoting bacteria. Rock phosphate. Solid waste..

Resumo

Na agricultura, a busca por altas produtividades exerce pressão nas reservas de fosfato (recurso não renovável), visto que, muitas das vezes a baixa disponibilidade de fósforo de muitos solos são compensadas pela aplicação de elevadas doses de fertilizantes. De modo complementar, estudos vêm sendo feitos com intuito de selecionar bactérias solubilizadoras de fosfato. O trabalho objetivou: (i) isolar e caracterizar bactérias solubilizadoras de fosfato oriundas de lodo de estação de tratamento de efluentes de abatedouro de aves; (ii) avaliar a capacidade dos isolados na promoção do crescimento de plantas. As bactérias foram isoladas em meio de cultura contendo fosfato de cálcio. A caracterização morfológica consistiu no formato celular, coloração de Gram e nas características das colônias. Para avaliar a solubilização de fosfato natural (FN), realizou-se inoculação em sementes de milho na presença de fosfato de Araxá. O experimento consistiu em dez tratamentos: oito isolados bacterianos + FN, FN e controle. Foi conduzido em casa-de-vegetação por 45 dias, em delineamento inteiramente casualizado, com seis. As variáveis mensuradas foram as características de crescimento da planta. Foram isoladas oito estirpes, todas apresentaram coloração gram-positiva e 90% forma de bastonete e 10% cocos. A inoculação com isolado LSOF-7 (*Sphinghomonas* sp.) junto com FN promoveu incrementos na matéria seca de plantas de milho de 20% frente ao tratamento contendo apenas FN e 67% comparado ao controle. Conclui-se que os lodos de estações de tratamento de efluentes de abatedouro de aves contêm estirpes bacterianas com potencial de solubilização de fosfato e as mesmas também apresentam provável efeito promoção do crescimento de plantas de milho.
Palavras-chave: *Zea mays* L. *Sphinghomonas.* Bactéria promotoras de crescimento de planta. Fosfasto de rocha. Resíduos sólidos.

Low soil phosphorus (P) availability is a major constraint for plant production worldwide (Drohan et al., 2019). In tropical soils, the lack of this element is regulated by the application of soluble phosphate fertilizers at high rates. However, only about 10 to 30% of the applied phosphate mineral fertilizer can be recovered by the crop grown after the fertilization, while the rest is fixed in the soil by physicochemical reactions (Holford, 1997). Phosphate solubilizing microorganisms (PSMs) can solubilize less labile P forms, increasing P availability to plants (Rawat et al., 2021). These organisms can produce organic acids (e.g.: citric, lactic, formic, and malic acid) and enzymes (e.g., acid and alkaline phosphatases), that contribute to the release P from more recalcitrant inorganic and organic soil P pools, some PSMs can also produce plant growth regulators that stimulate root growth which in turn contributes to increase the acquisition of P and other nutrients by plants (Rawat et al., 2021).

Poultry slaughterhouse can be a potential material for the biopropensity of phosphorus-solubulizing bacteria, for two reasons: (i) The by-product is rich in carbon with an abundance of proteins, vitamins and nutrients (Lozada et al., 2017), but few studies have investigated the microbial community with pathways of interest in use in plant production (Bunraksa et al., 2020); (ii) In Brazil there is a large generation of the by-product, given that the country is the 1st largest exporter of Poultry Meat in the world [Associação Brasileira de Proteína Animal [ABPA], 2023].

The purposes of this study was to: (i) isolate and characterization potential phosphate-solubilizing bacteria from sludge from the wastewater treatment plant of a poultry slaughterhouse; (ii) evaluate the ability of bacteria to solubilize phosphate rock and promoting plant growth

Samples of poultry slaughterhouse sludge, a waste generated in the treatment plant effluents from the type activated sludge - extended aeration, were provided by the companies Francap company located in the municipality of Para de Minas (Minas Gerais, Brazil), 19°51'34" S and 44°34'29" W. Samples were transported to the Universidade Federal de Viçosa of Florestal (Minas Gerais, Brazil) 19°82'92" S and 44°42'54" W, where microbiological analyses were. The chemical properties of the sludge were determined as follows: (N 9.5, P 1.1, K 0.45, Mg 1.35, S 0.18, C 18.72) dag/Kg; (Zn 517, Fe 1908, Mn 114, Cu 342, B 8.3) mg/Kg; pH 6.7; C/N 1.96 (Lozada et al., 2017).

Samples of 10 g of sludge were diluted in 90 ml saline solution (0.85 g L^{-1} NaCl) and from this dilution (10^{-1}) serially diluted to 10^{-12}. Samples of 100 uL of each dilution were transferred to Petri dishes containing solid medium consisting of 10 glucose, 5 g ammonium chloride, 1 g sodium chloride, 1 g magnesium sulfate heptahydrate, 1 g calcium phosphate, 15 g bacteriological agar, 2 mL fungicide Cerconil (0.1 g L^{-1}), and 1000 ml of distilled water; the pH was adjusted to 6.5 with sulfuric acid and/or potassium hydroxide and the samples were incubated at 30°C for 3 days. The formation of a transparent halo around the colony indicated phosphate-solubilizing activity. Individual colonies were transferred to liquid DYGS medium and maintained at 30°C, at 120 rpm, for 24 h (Döbereiner et al., 1995). Then the bacteria were transferred to Petri dishes containing the above medium to determine the halo of phosphate solubilization and for purification of the isolates. The solubilization halo was calculated by the formula: mean diameter of solubilization halo - mean diameter of colony halo. The values were averaged, and the standard error of the mean calculated for each treatment. The tests were carried out in three replications.

After isolation, the bacteria were cultured in liquid DYGS medium for 24 hours at 30ºC and 120 rpm and were placed on Petri dishes containing solid DYGS medium. The plates were set in a bacteriological incubator at 30°C for 7 days and the colonies characterized based on the characteristics of the cells (shape and Gram staining) and the colonies (shape, color, size, colony surface, edge, surface, and mucus).

Each bacterial isolate was labeled with the acronyms: UFV LSOF-X (UFV=Federal University of Viçosa; L = sludge, SOF = phosphate-solubilizing, and X = number of the isolate in the collection).

The experiment consisted of 10 treatments: eight bacterial isolates + natural phosphate (NPh), one treatment containing only NPh, and a control (no bacteria and no NPh). The experiment was conducted for 45 days in a greenhouse in a completely randomized design with six replications, in experimental units consisting of one pot containing two maize plants.

To obtain the pre-inoculum, bacteria were grown in 5 mL liquid DYGS medium for 24 h at 30°C and 120 rpm. Then, the pre-inoculum was poured into Erlenmeyer flasks containing 200 ml liquid DYGS medium. The flasks were shaken for 24 hours at 120 rpm and 30°C to obtain the inoculum. Inoculation consisted of immersion of the maize seeds (*Zea mays* L. variety AG1051) for 2 hours in the flask, followed by application of the bacterial medium on the substrate. Controls were immersed in autoclaved liquid DYGS. Later, the seeds were transferred to 0.7dm^3 plastic pots containing substrate with the following chemical properties: pH 4.1; (P 6.8, K 78) mg/dm^3; (Ca 0.6, Mg 0.3, 1.4 Al, H + Al 7.59, SB 1.10, $CEC_{(t)}$ 2:50, $CEC_{(t)}$ 8.69) $cmol_c$ / dm^3; V 13, m 56%. Natural phosphate Araxá (fluorapatite, with 22.7% total P_2O_5, 4.3% P_2O_5 soluble in 2% citric acid (1: 100), in powder) was applied at a ratio of 10 g/kg soil (Ribeiro et al., 1999). Forty-five days after planting, the plants were harvested to measure the variables: plant height (PH); stem diameter (DIAM); number of leaves (NL). Root dry matter (RDM); shoot dry matter (SDM), determined by oven-drying by forced air ventilation at 65 °C for 72 hours, followed by weighing on a precision scale. The total dry matter (TDM) by the sum of RDM and SDM. The data were subjected to ANOVA to test the effect of treatment on ability of bacteria to solubilize phosphate rock using maize plants by the Tukey test at 5% probability. Characterization of phosphate-solubilizing bacteria data were subjected to descriptive statistical analysis.

Research has demonstrated a rich variety of shapes, sizes, colors and biochemical reactions of bacteria that can solubilize inorganic and organic phosphate. The study shows a tendency in the cell and colony morphology of bacterial strains to grow in round, white or cream-colored colonies, with entire edges, with coccoid-shaped cells with gram-positive staining. All had stained gram positive and over 85% were coccoid-shaped. With regard to the colony morphology, all had a round shape, smooth surface and no mucus. The white color was predominant (87.5%), followed by yellow (12.5%). Half of the isolates had a colony diameter of 1.5 mm, 25% a diameter of more than 1 mm and the remaining 25% a diameter of less than 1mm. The colony edges were predominantly entire (87.5%), followed by jagged edges (12.5%).

As well as our results, Bunraksa et al. (2020) demonstrated a great diversity of microorganisms isolated in the poultry slaughterhouse sludge, and some microorganisms have the effect of synthesizing substances that promote growth in plants. Also reported a great diversity of bacteria isolated in poultry slaughterhouse sludge, some of which have the ability to fix atmospheric N (Lozada et al., 2018).

All bacteria isolated *in vitro* from poultry slaughterhouse sewage sludge solubilize phosphate in selective media (using medium with calcium phosphate), this capacity is detected by the formation

of a transparent halo around the bacterial colonies (Figure 1). In the present study, the three largest halos formed followed in the following ascending order: UFV LSOF-1; UFV LSOF-8; UFV LSOF-7 (Figure 1). However, the UFV LSOF-7 isolate (with two isolates producing larger halos compared to the same one) was the one that most increased the growth of corn plants (Table 1).

The isolate LSOF-7 led to significant increases in root dry matter (RDM) and total dry matter (TDM) compared to the control. The isolate LSOF-1 led to significant increases in stem diameter (DIAM) compared to the control. No significant differences were found between treatments with bacterial inoculation and the control for the variables: plant height (PH), number of leaves (NL), shoot fresh matter (SFM), shoot dry matter (SDW), total dry matter (TDM). The isolate LSOF-7 led to significant increases in maize biomass (Table 1) and was the only treatment that reached a weight of 1.00 g, superior to 0.60g of the control and 0.83g of the treatment containing only NPh, i.e., a yield of 40 and 17%, respectively.

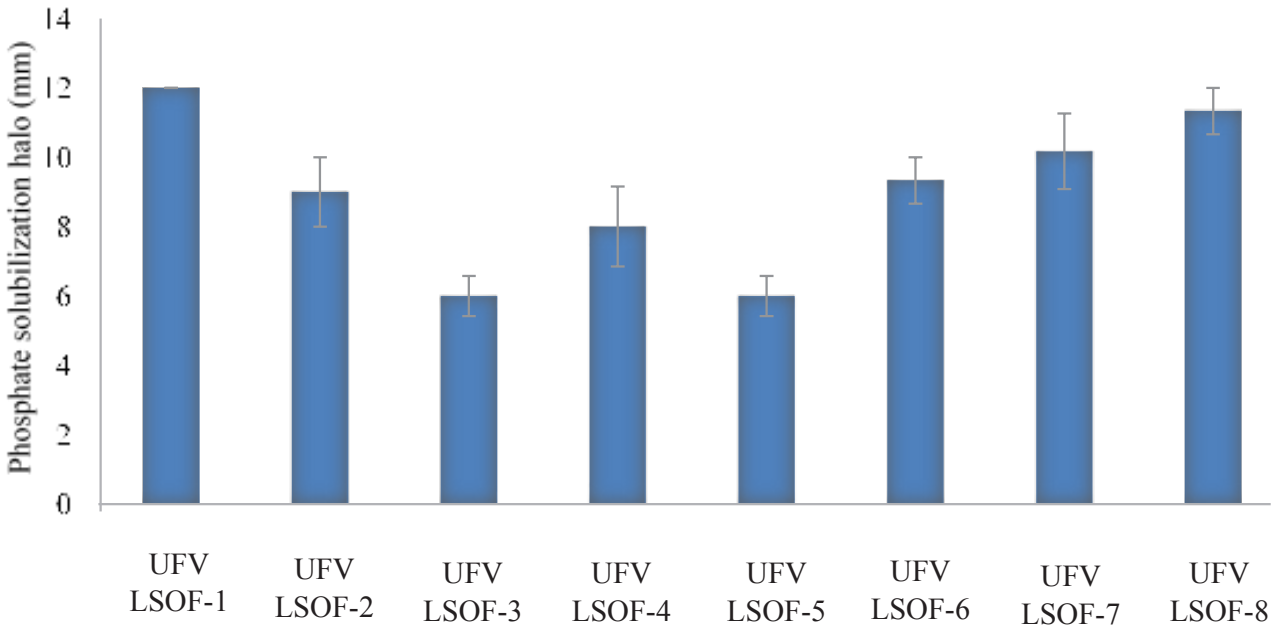

Figure 1. Halo diameters phosphate solubilization by bacteria isolated from a wastewater treatment plant of a sludge poultry slaughterhouse. Bars denote the standard deviation around the mean (n = 3).

Table 1
Growth characteristics: plant height (PH), stem diameter (DIAM), number of leaves (NL), root dry matter (RDM), shoot dry matter (SDW), total dry matter (TDM) of maize (*Zea mays*) in response to inoculation with phosphate-solubilizing bacteria together with rock phosphate (NPh)

Treatments	PH	DIAM	NL	RDM	SDM	TDM
	---------- cm ----------		unint	---------- g plant ----------		
Control	36.4 a	2.91c	4 a	0.38 b	0.22 a	0.60 b
NPh	35.3 a	3.15 bc	4 a	0.51 ab	0.33 a	0.83 ab
UFVLSOF-1 + NPh	40.4 a	4.00 a	5 a	0.49 ab	0.35 a	0.84 ab
UFVLSOF-2+NPh	41.3 a	3.75 ab	4 a	0.52 ab	0.33 a	0.85 ab
UFVLSOF-3+ NPh	40.5 a	3.68 abc	4 a	0.40 b	0.32 a	0.73 ab
UFVLSOF-4 + NPh	41.5 a	3.6 abc	4 a	0.51 ab	0.33 a	0.84 ab
UFVLSOF-5 + NPh	39.9 a	3.6 abc	4 a	0.41 b	0.32 a	0.38 ab
UFVLSOF-6 + NPh	39.0 a	3.5 abc	4 a	0.42 bc	0.36 a	0.74 ab
UFVLSOF-7 + NPh	29.0 a	3.5 abc	4 a	0.69 a	0.30 a	1.00 a
UFVLSOF-8 + NPh	38.0 a	3.6 abc	4 a	0.49 ab	0.35 a	0.84 ab
CV (%)*	18.0	11.7	6.5	27.5	27.1	17.1

*CV = coefficient of variation. Means followed by equal letters do not differ by Tukey test at 5% probability level;

There is no guarantee that the bacterial strains with the highest *in vitro* phosphate solubilization rates obtain the best results in nursery or field tests, since the agri-environment and symbiosis conditions affect the performance of inoculation (Vasseur-Coronado et al., 2021). Isolation sites and the environment of the bacteria, which influence the solubilizing ability, since there are inducing mechanisms or genetic suppressors controlled by the phosphorus availability in the environment (Nahas, 1996). In other words, the ability to solubilize phosphorus depends on the genetic background of the bacterium and the environment in which it is established. Reports in the literature have shown that associations between plants and bacteria may involve specific interactions (Giassi et al., 2016), and that differences in the composition of root exudates may influence bacterial colonization of the rhizosphere (Lugtenberg et al., 2001).

Another hypothesis for the greater increase in corn growth of the UFV LSOF-7 isolate compared to the UFV LSOF-1 and UFV LSOF-8 isolates (that produced larger halos in the presence of phosphate) (Figure 1), may be related to the effect of promoting plant growth provided by the isolate UFV LSOF-7. The UFV LSOF-7 isolate was identified through genomic characterization using commercial service of MACROGEN Ltd., Korea, at the genus level as: *Sphingomonas* sp (similarity of 99%). There are reports that the genus *Sphingomonas,* in addition to solubilizing phosphates (Lukesch et al., 2020) has the capacity to promote plant growth (Lombardino et al., 2022) fixating nitrogen (Yang et al., 2014) inducing stress response

pathways, or acting as biocontrol agents (Kim et al., 2020).

Other studies reported that the genus *Sphingomonas* when inoculated in maize seeds increased plant growth. In greenhouse test, Wang et al. (2022) found increments of the root fresh weight and shoot dry weight 39.1% and 34.8% respectively compared with non-inoculated plant. Also, in greenhouse test, Pedrinho et al. (2010) reported that the genus *Sphingomonas* has high potential for use in formulations of inoculants and biofertilizer due to the fact that microorganisms are able to solubilize phosphates and have a promoting effect on plant growth. The authors verified that the inoculation of *Sphingomonas* in corn seeds has the same growth response when compared to the inoculation of *Azospirillum brasilense,* which are bacteria that are more widely used, including in commercial formulations.

Our findings also suggest that environmentally friendly fertilizers with improved agronomic efficiency can be produced with UFVLSOF-7 (*Sphingomonas* sp.), bacteria of the genus have the ability to solubilize phosphorus (Damo et al., 2022). In summary, our main findings add evidence that poultry slaughterhouse sludge is a potential material for bioprospecting microorganisms for use in plant production. However, survival tests and field studies are still necessary to assess the activity of the fungus in environmental conditions. Furthermore, it is encouraged that new studies be carried out to investigate whether there are other mechanisms of P mobilization (*e.g.,* production of phosphatases, phytases, phosphohydrolase, and exopolysaccharides.

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