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Evaluation of the Bioremediation Potential and Antimicrobial Activity of *Pseudomonas* and *Bacillus* Bacteria Isolated from Landfills in Brazzaville, Congo

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Abstract

The pollution of ecosystems as a result of urbanization, industrialization and poor agricultural practices is becoming increasingly alarming. This has a major impact on health and the economy. This pollution causes illness in humans and animals, even at low levels of exposure, leading to endocrine disorders, congenital malformations, cardiovascular disease, nervous system damage and cancer. They are a brake on redevelopment because of the threats they pose, generally causing an anaerobic environment by blocking the diffusion of air into the soil pores, thus affecting the microbial communities living there and preventing the infiltration of water necessary for plant growth. In an ecosystem subjected to various disturbances, changes can be observed in ecosystem structure and function, including loss of aesthetic values, changes in biomass or productivity, and changes in species composition. These include loss of aesthetic values, changes in biomass or productivity, and altered species composition, as a result of habitat loss, disruption of food webs and variations in macro- and micro-climatic environmental conditions. Respect for the environment is becoming a major concern in today's society. To remedy this, the concept of biological control was used as an alternative, with the selection of microorganisms of bioremediator interest. Twenty (20) isolates, including 10 (50%) from the Pseudomonas genus and 10 (50%) from the Bacillus genus, were isolated from landfills, identified and tested to assess their biofertilization (phosphate solubilization) and depollution (hydrocarbon degradation) potential, and to inhibit the growth of certain microorganisms. The results showed that all Pseudomonas and Bacillus isolates solubilized inorganic

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phosphate, although this activity was higher in Bacillus. All Bacillus inhibited the growth of all the pathogens included in this study, while Pseudomonas only inhibited the growth of E. coli. With regard to their ability to degrade hydrocarbons, these bacteria all showed exponential growth kinetics in the presence of gasoline. These kinetics evolved as a function of the number of days. The results obtained from this work leave no doubt as to the capacity of microorganisms to be used on a large scale as soil biofertilizers to restore soil integrity and promote sustainable agriculture, but also as biodepollutants to purify ecosystems.

Keywords

Bioremediation, Environment, Pseudomonas and Bacillus

1. Introduction

Humanity is undergoing intensive industrialization and agriculture. Metal waste, fertilizers, pesticides used in agriculture and hydrocarbons, which are major pollutants of nature, accumulate in the soil [1]. Pollutants are mainly organic compounds (hydrocarbons, phenol and chlorine compounds, etc.) and heavy metals [2]. Organic pollutants induce ecological alterations, mainly the loss of key community organisms and the proliferation of opportunistic species in affected habitats [3]. Heavy metals, stable and highly persistent compounds, are environmental contaminants that can be accumulated and transferred to higher organisms in food webs, leading to serious ecological and public health problems [4] [5]. These compounds cause a variety of harmful effects, such as the alteration of DNA structure [6] [7]. Soil pollution by hydrocarbons and heavy metals is caused by the deliberate or unintentional discharge of petroleum products. It is caused by both chemical and organic pollution on the one hand, and by the use of chemical fertilizers and pesticides on the other [8].

Today, the various industrial and agricultural pollutants are becoming a crucial problem for cities, as man is unable to get rid of them. These wastes have a negative impact on activities directly dependent on the land, and on human health [9]. According to the US Environmental Protection Agency's (EPA) National Priority List, 40% of hazardous waste sites are co-contaminated with organic pollutants and heavy metals. Remediation of these sites poses a complex problem due to the mixed nature of the contaminants [10]. The ARIA database (Analyse, Recherche et Information sur les Accidents - Analysis, Research and Information on Accidents), operated by the French Ministry of Ecology, Sustainable Development and Town and Country Planning, has recorded accidental events in France and abroad that have or could have affected public health and safety, agriculture and the environment. Since 1992, these accidents, mainly due to industrial and agricultural activities and the transport of hazardous materials, have affected more than 32,000 sites [11]. Of all these accidents, 40% are thought to have resulted in contamination of land or water sites. The Republic of Congo, like most oil-producing countries, is faced with the problem of environmental pollution by hydrocarbons. Slicks of oil and/or bitumen periodically wash up on the Atlantic coast at Pointe-Noire, the country's oil-producing city. In this city, several sites polluted by hydrocarbons are observed in areas where the oil industry is developing. To counter this pollution, new processes involve the use of microorganisms that can reduce the action of pollutants and fertilize the soil. Particular interest is being paid to the bioremediation mechanisms of microorganisms. They are more cost-effective, less toxic, compatible with the environment and can be applied over vast areas [12] [13]. Remediation of these sites by bioremediation requires the selection of indigenous or allochthonous hydrocarbonoclastic microorganisms suited to tropical conditions. In addition, there are microorganisms in the environment capable of using hydrocarbons as their sole source of carbon and energy [13] [14]. The ability of microorganisms to eliminate pollutants and promote plant growth through various mechanisms (nutrient solubilization, hydrocarbon degradation) is based on their diverse metabolic capacities. Landfills are reservoirs of microorganisms, in terms of density and diversity. Some of these microorganisms play a key role in the acquisition of plant nutrients, and in the depollution of hydrocarbons and metal wastes, making them ideal substrates for the search for ideal bioremediation bacteria. The aim of this work is to contribute to the selection of microorganisms capable of bioremediation and producing antimicrobial substances.

2. Materials and Methods

2.1. Biological Material

Biological material consisted of Pseudomonas and Bacillus strains isolated from landfills.

2.2. Methods

2.2.1. Isolation

Pseudomonas and Bacillus strains were isolated on selective cetrimide and Mossel media respectively, using conventional microbiology methods.

100 µL of the stock solution and of the different dilutions 10^{-2} to 10^{-5} was taken and pipetted into the center of Petri dishes containing the different culture media previously poured and solidified, then spread with a rake and incubated for 24 h or 48 h depending on the requirements of the microorganisms. Colonies were purified on Cetrimide and Mossel medium [15].

2.2.2. Identification

Identification was based on cultural, morphological and biochemical characteristics.

- Cultivation characteristics: Determination of cultivation characteristics was based on macroscopic observation of colonies by analysis of shape, appearance, relief, consistency and color on Mossel and Cetrimide culture media [16].

- Caractères morphological: was based on observation of the microscopic characteristics of fresh cells under a 40X objective light microscope. The following characteristics were sought: cell shape, mobility, and arrangement [17].

- Biochemical characteristics: Identification was carried out using a conventional gallery containing citrate and kligler media. The gallery was completed by catalase test and Gram staining [18]. Gram staining was also performed, followed by observation under an immersion light microscope.

2.2.3. Investigation of the Bioremediation Potential of the Isolated Bacteria

The study of the bioremediation potential of bacterial strains was based on the identification of two parameters: biofertilization potential (inorganic phosphate solubilization test and antibacterial activity) and biodepollution potential.

1) Biofertilisation potential

a) Phosphate solubilisation test

This test was used to solubilize phosphate into phosphorus. 100 µL of each bacterial suspension from an overnight culture was introduced into wells made on NBRIP agar (National Botanical Research Institute's phosphate growth medium) supplemented with 5% phosphate previously poured onto Petri dishes, then incubated for 24 hours in the oven; the appearance of a translucent zone around the wells justifies the solubility of phosphate in phosphorus. [19].

b) Antibacterial activity

Antibacterial activity was performed to assess the ability of bacteria to inhibit the growth of plant pathogens. The inoculum of pathogenic bacteria to be tested (E. coli and Staphylococcus aureus) was prepared with 5 mL of 0.9% physiological water and the optical density was adjusted to 0.1 using a spectrophotometer at a wavelength of 625 nm, a value equivalent to 0.5 Mc Farland [20] [21]. Each inoculum was inoculated by swabbing onto a Petri dish containing Muller Hinton agar poured beforehand. Wells were made, into which a 0.1 mL volume of the bacterial suspension was introduced. The plates were incubated at 37˚C in the oven for 24 hours. The growth inhibition diameters of the pathogenic bacteria to be tested around the wells showing a clear halo were measured.

2) Clean-up potential

Research into the clean-up potential of bacterial strains was carried out using the hydrocarbon biodegradation test. This test demonstrated the growth of bacteria in a very carbon-rich medium. 1 mL of the various overnight culture bacterial suspensions were introduced into shaking Erlenmeyer flasks containing 14 mL of liquid medium with 1% petrol, then incubated for 5 days in an oven while measuring the optical density (OD) of each day with a spectrophotometer (ZUZI SPECTROPHOTOMETER Model 4211/50) at 600 nm [22].

3. Resultats

3.1. Hydrocarbon Degradation in *Pseudomonas*

A total of 20 bacteria were isolated and identified from the landfills, including 10 (50%) Pseudomonas and 10 (50%) Bacillus (**Figure 1**).

3.2. Biofertilisation Potential

3.2.1. Solubilisation of Inorganic Phosphate

The ability of Pseudomonas and Bacillus strains to solubilize phosphate was demonstrated by the appearance of clear halos around the wells after 24 hours of incubation. Figure 2 shows phosphate solubilization by two strains of Pseudomonas and two strains of Bacillus.

The different diameters in millimeters (mm) of solubilization of different strains of Pseudomonas and Bacillus are shown in Figure 3 and Figure 4. Figure 3 shows that in Pseudomonas, solubilization was greatest in strain Ad88 and lowest in strain Ad75, with diameters of 20 and 11 mm respectively.

Figure 4 shows that in Bacillus, phosphate solubilization diameters range from 14 mm (Ad69) to 22 mm for strain Ad37. Diameters of 20 mm are also found in strains Ad34 and Ad68.

3.2.2. Antibacterial Activity

Figure 5 shows that all Pseudomonas strains have antibacterial activity on Escherichia coli, with inhibition diameters ranging from 12 to 11.6 mm respectively, whereas Pseudomonas strains have no activity on Staphylococcus aureus.

Figure 6 shows that some Bacillus strains exhibit antibacterial activity on Escherichia coli and Staphylococcus aureus with inhibition diameters greater than 8.5 mm.

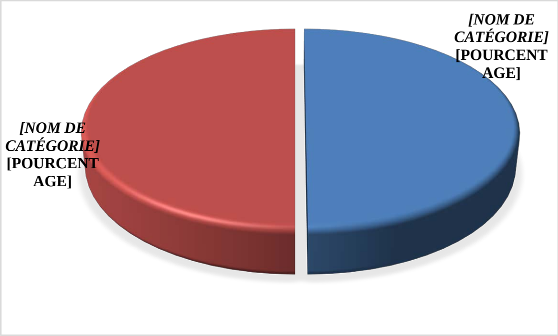

Figure 1. Distribution of *Pseudomonas* and *Bacillus* strains isolated from landfills.

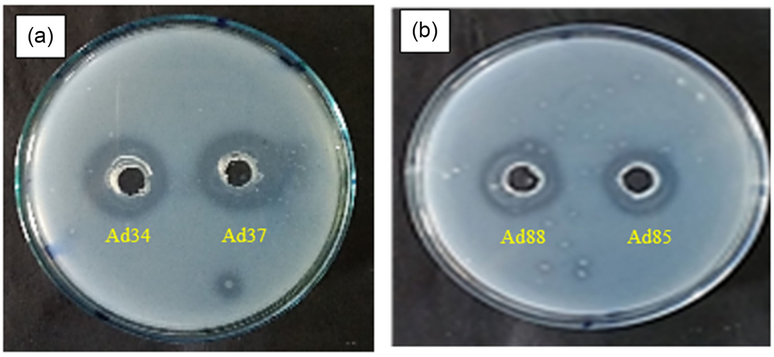

Figure 2. Phosphate solubilisation: (a) *Bacillus* sp Ad34 and *Bacillus* sp Ad37; (b) *Pseudomonas* sp Ad88 and *Pseudomonas* sp Ad85.

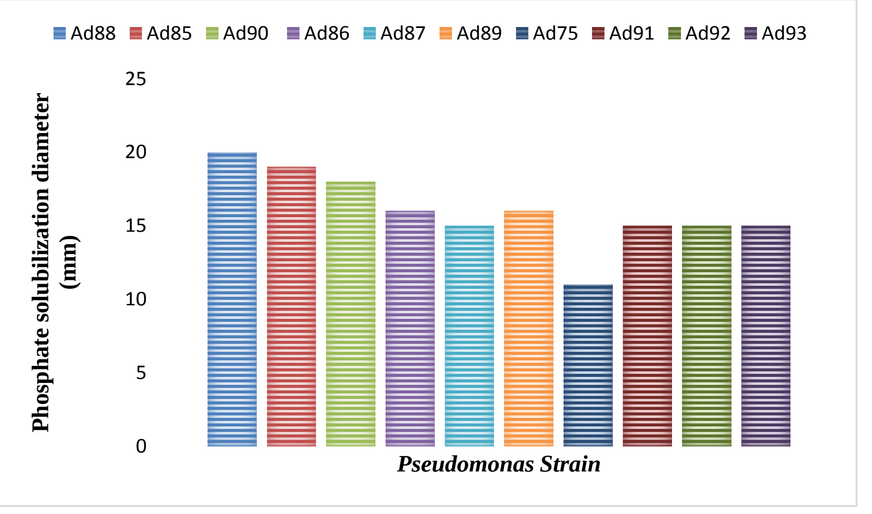

Figure 3. Phosphate solubilisation diameters by *Pseudomonas*.

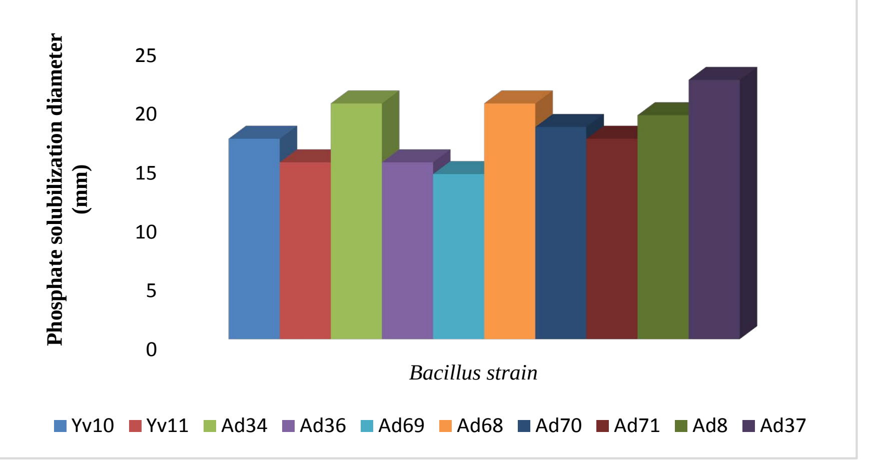

Figure 4. Phosphate solubilisation diameters by *Bacillus*.

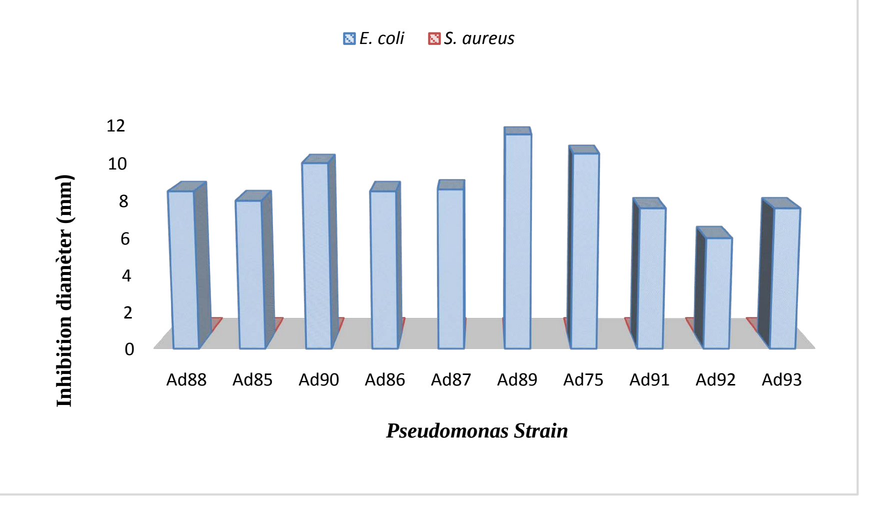

Figure 5. Antibacterial activity in *Pseudomonas*.

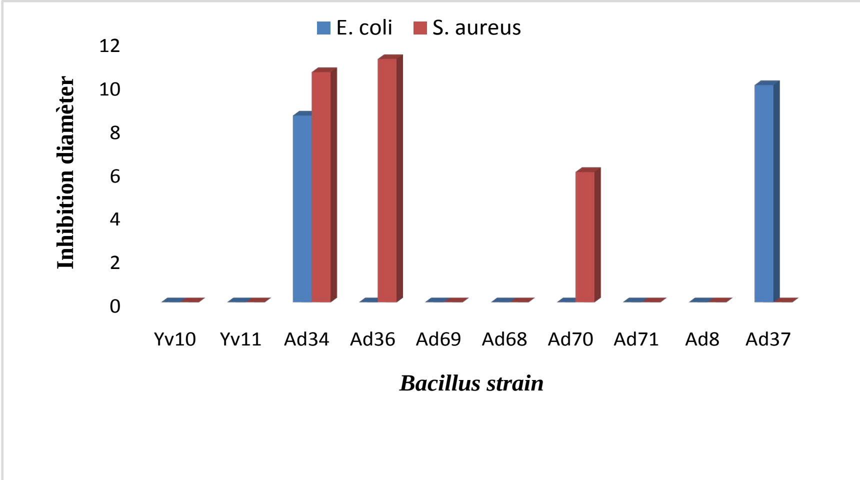

Figure 6. Antibacterial activity in *Bacillus*.

3.3. Hydrocarbon Degradation in *Pseudomonas*

Figure 7 shows the growth kinetics of Pseudomonas strains as a function of time in a medium containing gasoline. The figure shows that the strain curves are very different. In strains Ad93, Ad85, Ad86, Ad89, Ad90, Ad87, Ad75 from T0 h to T 24 h, growth increases progressively, and is proportional to time, from T24 to T48 h a slowdown in growth is observed.

In strains, A88, A91, A92 from TO to T48 growth increases progressively, and is proportional to time. However, from T48 h to 72 h, despite the increase in time, all Pseudomonas strains show a stabilization of optical density, expressing the stabilization of growth.

3.4. Hydrocarbon Degradation in *Bacillus*

Figure 8 shows Bacillus growth kinetics as a function of time in a medium containing petrol. The curves vary from strain to strain. In strains Ad34, Yv11, Yv10, Ad36, Ad69, Ad68, Ad71, Ad8; from T0 to T48 h, growth increases progressively, and is proportional to time. In strains Ad70, Ad37 T0 to T24 h growth increases progressively and is proportional to time, and from T24 to T48 h growth slows. However, from T48 h to T72 h, despite the increase in time, in strains Ad34, Yv11, Yv10, Ad36, Ad69, Ad68, Ad71, Ad8, Ad37 a stabilization of optical density is observed, expressing the stabilization of growth.

4. Discussions

The results obtained showed that all isolates were able to solubilize inorganic phosphate. However, in Bacillus, strains Ad37, Ad68, Ad34, Ad8 Ad70, Yv10, Ad8 showed significant phosphate solubilization, followed by Ad36, Yv11 and Ad69. In Pseudomonas, strains Ad88, Ad85, Ad90, Ad89, Ad86, Ad87, Ad91, Ad92, Ad93 showed high phosphate solubilization, followed by Ad75, which showed low solubilization. This microbial phosphate solubilization may be due

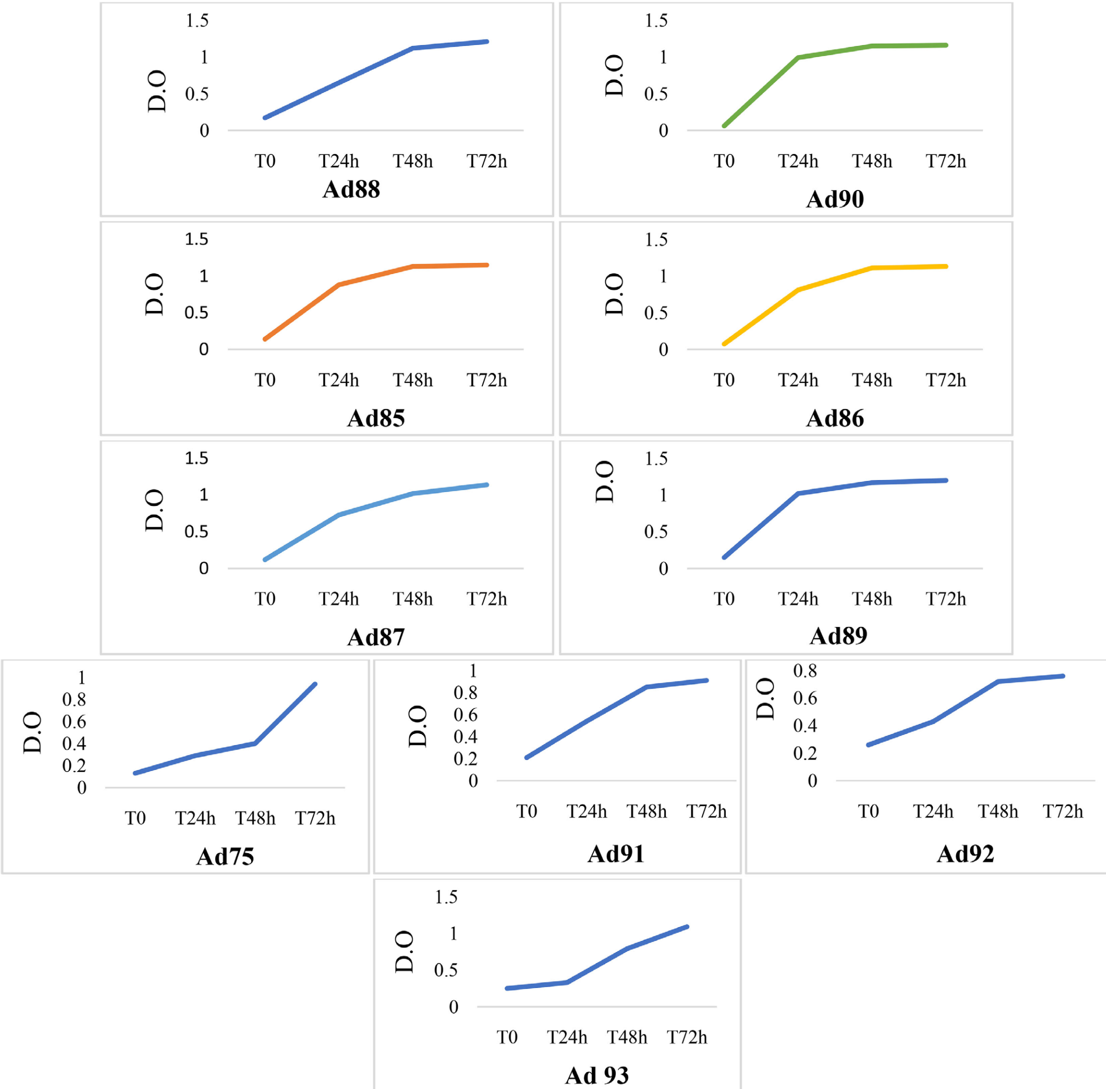

Figure 7. Growth kinetics of *Pseudomonas* as a function of time in a medium containing petrol.

to excretion of organic acids leading to acidity of the external environment [23], or to phosphatases that convert insoluble phosphate forms into soluble phosphate ions. These results are consistent with other authors, who have shown that Bacillus species [24] [25] and Pseudomonas species [26] are among the most efficient bacterial communities for phosphate solubilization. Concerning the ability of bacteria to degrade gasoline, all bacteria showed biodegradation of this pollutant by following their exponential growth kinetics as a function of time with gasoline as substrate.

In Bacillus, the curves obtained from strains Ad36, Yv11, Ad34, Yv10, Ad69, Ad71, Ad8 and Ad68 show the same phases: exponential growth phase and stationary phase. Strain Ad70 shows an exponential growth phase, a slowdown phase and a stationary phase. Strain Ad37 has an exponential growth phase, followed by a slowdown phase and an exponential growth phase (Figure 6). In Pseudomonas, the curves obtained for strains A85, A86, A89, A87 and A90 showed

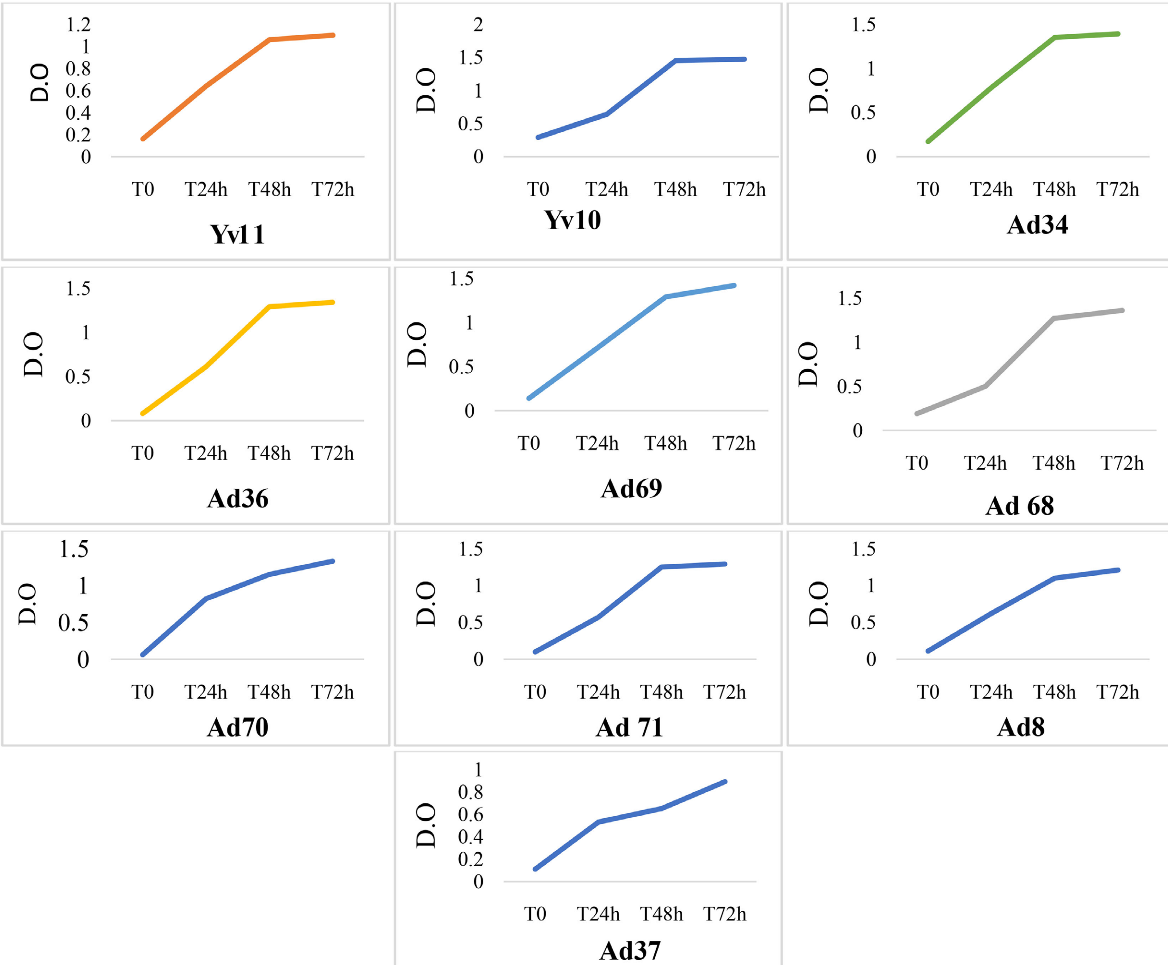

Figure 8. Growth kinetics of *Bacillus* as a function of time in a medium containing petrol.

the same phases: an exponential growth phase, a slowdown phase and a stationary phase. Strains Ad88, Ad91 and Ad92 showed an exponential and stationary growth phase (**Figure 7**). strain Ad93 showed a latent phase and an exponential growth phase. Strain Ad75 has a slowdown phase and an exponential growth phase. An analysis of these curves reveals that there is no lag phase, certainly, because we had not followed the growth kinetics as a function of hours. In fact, in the exponential growth phase, bacterial cells divide non-stop, as long as nutrients are available and toxic substances are absent at the optimal neutral pH (7). As the physiological state is maximal, so is the growth rate [27] [28]. In the slowdown phase, there is depletion of nutritional resources in the culture medium and/or an accumulation of waste products produced by the bacteria [29].

In the stationary phase, nutrients are depleted and toxic products accumulate. The number of cells no longer varies. There is as much cell division as cell death. The rate of growth is constant. This is known as cryptic growth, where cells feed on the contents released by dead cells. These results differ from those obtained by [30], with the absence of a lag phase in the growth curve and a different method in terms of the time interval for optical density (OD) sampling.

Regarding the ability of bacteria to inhibit the growth of pathogens, bacteria of the Pseudomonas genus showed no antibacterial activity on Staphylococcus aureus, whereas these strains showed antibacterial activity on Escherichia coli with inhibition diameters ranging from 6 to 11.6 mm. However, among Bacillus, strains Ad37, Ad36 and Ad70 showed antibacterial activity on Escherichia coli and Staphylococcus aureus respectively. Strain Ad34 showed antibacterial activity on Escherichia coli and Staphylococcus. This shows that Bacillus and Pseudomonas produce lytic enzymes such as glucanases, chitinases and lysozymes to degrade pathogen walls [31]. These results differ from those obtained by [32] who showed that Bacillus and Pseudomonas exhibit inhibitory activities on Staphylococcus aureus and Escherichia coli.

5. Conclusion

This work enabled us to identify strains of biotechnological interest for bioremediation. The results obtained revealed that Pseudomonas and Bacillus are capable of solubilizing phosphate, degrading hydrocarbons and inhibiting the growth of pathogens (Staphylococcus aureus and Escherichia coli). These bacteria, isolated from landfills, have a bioremediation potential that can be put to good use in the treatment of various types of waste, and in agriculture as a biological fertilizer. The antibacterial activity of Bacillus can be exploited to alleviate the problems of antibiotic resistance in certain pathogenic bacteria a real public health problem these days and even against plant pathogens.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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