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Purifying Potential of *Streptomyces albidoflavus* Strain DSM 40455T and *Streptomyces antibioticus* Strain NBRC 12838T in Wastewater Treatment

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Abstract The ability of Actinomycetes strains to degrade pollutant matters and to reduce or eliminate pathogens microorganisms from domestic wastewater of an industrial site (oilfield of Tsimiroro-Madagascar) at the laboratory scale is demonstrated in the present work. Two most active Actinomycetes isolates (*Streptomyces albidoflavus* strain DSM 40455T and *Streptomyces antibioticus* strain NBRC 12838T) against test-pathogens were selected for the purification treatment. The analysis of physico-chemical (COD, BOD, pH, conductivity, color, TDS, nitrite, nitrate, phosphate and chloride rates) and microbiological parameters (sulphite reducing anaerobe, fecal coliforms, fecal *Streptococcus* and *Escherichia coli* rates) allowed to evaluate the quality of the wastewater. Physico-chemical results revealed that purified water is qualitatively improved view that 60.86% of TDS, 71.61% of its color, 25.55% of its chloride rate, 45.32% of its nitrate rate, 99.9% of its nitrite rate, 26.25% of its phosphate rate, 46.53% of its initial COD and 58.11% of its BOD were eliminated at the end of the treatment. Only, the conductivity increased compared with the guideline values for all treatment. The process improved also microbiological quality of the wastewater with total elimination of fecal *Streptococcus* and diminution of fecal coliforms, sulphite reducing anaerobe and *Escherichia coli* concentrations. The experiment proved that biological treatment using Actinomycetes strains is a promising, less expensive and simple technology for wastewater recycling ensuring thus their reuse for other activities.

Keywords: *wastewater, biological treatment, Actinomycetes, microbiological quality, physico-chemical quality*

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1. Introduction

Water covers 71% of the earth's surface, only 1% of this volume is available to humans and the rest of the ecosystem [1]. That's the reason it can't be considered as a simple commercial product, it must be classified as an universal heritage which must be protected, defended and treated as such [2].

Water is used in several sectors such as agriculture, livestock, industry, and other domestic uses. All these activities result in water pollution, or precisely they produce wastewaters which are often rejected in the receiving environment (sea, rivers, and soils) without prior treatment causing then an enormous degradation of physico-chemical and biological qualities of this environment and generating many human, animal and plant diseases [3].

During the last 50 years, global water consumption has continued to increase [4]. This is due to the rapid growth of the population and the intensification of agriculture and breeding. As a result, environmental degradation is constantly evolving because of the important rejection of toxic liquid, solid and gaseous waste.

Unlike water-scarce countries that are paying close attention to their resources by incorporating wastewater reuse into their watershed management, Madagascar's problem lies mainly to the irrational management of water resources of which consequences are harmful. Many industrial wastes become a considerable source of polluted water. Generally, these industries dump their wastes in the receiving environment without the lesser treatment precaution. Moreover, domestic wastewater forming enormous effluents which are sources of many environmental problems is added to these pollution sources. In recent years, the interest of solid or liquid waste recycling is beginning to be felt at the industries level and city managers in Madagascar. Committed to the environmental protection policies and conscious of untreated waste dangers, some industrialists installed their wastewater ponds. The technologies used concern only

mechanical and physico-chemical treatments of which results don't usually respond to environmental requirements. They are efficient for some pollutant elements but the approaches don't eliminate totally wastewater dangers. On this point, biological treatments have shown their performance in several countries [5]. Many bacteria are actually used according to their respective role in the biological treatment of the wastewater. In most of the cases, they constitute the activated sludge composition. *Acinetobacter* and *Moraxella* are known for their ability to eliminate phosphate from wastewater, *Methanococcus*, *Methanobacterium* and *Methanobacillus* as carbonate reducing bacteria; *Thiobacillus* as nitrate reducing bacteria and many other roles [6].

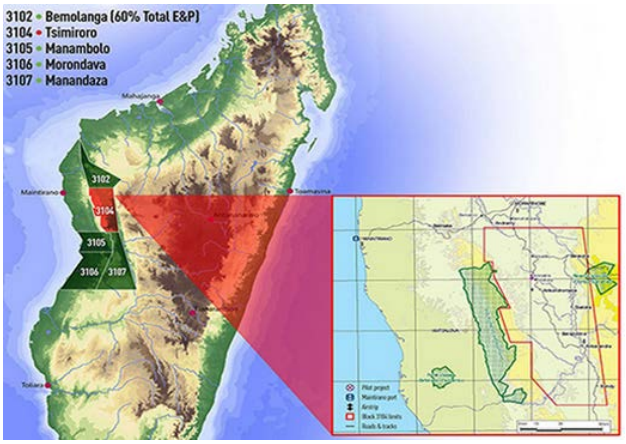
The present work was undertaken to reduce environmental and health risks caused by waste rejection in the receiving environment and to manage sustainably water resources. This study aims to test out the efficiency of microorganisms particularly Actinomycetes group to degrade pollutant matters and to eliminate pathogens in the wastewater. Our approach consists in selecting the most active strains against pathogens and a describing the efficiency of the biological treatment.

2. Materials and Methods

2.1. Biological Materials

2.1.1. Water Sampling

The water subjected to the purification treatment was constituted by the domestic wastewater of an industrial site (oilfield of Tsimiroro located in the North West of Madagascar, approximately at 100km of the West Coast in the sedimentary basin of Morondava in the South of Bemolanga and Morafenobe with 18°21'47.16''S of latitude, 45°3'56.49'' of longitude and 324m of altitude) (Figure 1). This wastewater included toilet, laundry, cooking and sewage waters. Water sampling was carried out with 7 sterile bottles of 1l in the sewage system previously undergone a primary treatment; water samples were then kept at +4°C in an icebox and transported to the laboratory.



Source: Madagascar Oil, Impact Environmental Study of Tsimiroro, BD 500 FTM

Figure 1. Localisation of Tsimiroro

2.1.2. Actinomycetes Isolates

The Actinomycetes strains used for wastewater treatment came from microbial strain collection of the Laboratory of Environmental Microbiology/National Center of Environmental Research (Antananarivo-Madagascar). They were isolated from different ecological niches as ginger rhizomes (S1 and S2), ginger rhizospheric soil (S18, S24, S31, S43, S44 and S51) [7] and marine sponges (M2 and M20) [8].

2.2. Biological Screening of Actinomycetes Isolates

The screening was based on the ability of Actinomycetes strains to inhibit test-pathogens growth according to Acar and Goldstein's method [9] with some modifications [10]: the agar cylinder method. Test-pathogens included germs belonging to *Streptococcus* and coliform group. This choice responded to the standard recommendation for wastewater microbiological criteria outlined in the Malagasy decree n°2003/464. Furthermore, other bacteria were tested to evaluate activity spectrum of the Actinomycetes isolates. The pathogens were also provided by the Laboratory of Environmental Microbiology and included 4 Gram negative bacteria (*Klebsiella oxytoca* ATCC 8724, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 700323 and *Salmonella enteridis*) and 3 Gram positive bacteria (*Streptococcus pneumoniae* ATCC 6301, *Staphylococcus aureus* ATCC 11632 and *Bacillus cereus* ATCC 13061). For the test, agar cylinders (6mm in diameter) of Mueller-Hinton medium previously inoculated by test-pathogens were taken with sterile cork borers and substituted by agar cylinders of pure Actinomycete strains. Petri dishes were, then, placed at +4°C for 4h to allow the prediffusion of bioactive substances produced by Actinomycetes strains and incubated at 37°C for 24h [11]. All tests were carried out in triplicate and antagonistic activity was evaluated by the measure of the inhibition zone around Actinomycetes colonies after 24h of incubation. Only, isolates showing higher inhibition zone than 8mm were considered as active isolates [12].

2.3. Biological Treatment of the Wastewater

The most active strains (with broad activity spectrum or high inhibition zone) were selected for the treatment of the wastewater. Two types of experiments were carried out: treatment with inoculation of each selected Actinomycete strain and treatment with mix inoculation of potent Actinomycetes strains.

Five hundred milliliters (10^3cfu/ml) of Actinomycete suspension were prepared as preculture and poured into a recipient containing 5000ml of wastewater. For mix inoculation, the same volumes of preculture and wastewater were maintained. The pH was adjusted at 7 to promote bacterial growth and the culture was incubated at 30°C with shaking at 150rpm. Experimentation was carried out in a closed system.

2.4. Evaluation of Water Quality

Every 3 days for 3 weeks, microbiological and physico-chemical quality analyses of the water were

carried out to assess the effectiveness of the treatment and the performance of the technique used. Non treated wastewater was used as control.

The parameters cited in the Malagasy decree n°2003/464 for water quality and classification were considered for the measures. These parameters include physic-chemical parameters (color, temperature, pH, conductivity, total dissolved solids, nitrate, nitrite, chloride, phosphate, and BOD and COD rates) and microbiological parameters (research and count of fecal *Streptococcus*, fecal coliforms, *Escherichia coli* and spores of sulphite reducing anaerobe bacteria). Analyses were performed in accordance with protocols described in the standard methods for water analysis. Analytical method and standard reference used were summarized in the Table 1.

2.5. Molecular characterization of active isolates

Two strains (M20 and S43) showing ability to degrade pollutant matters and inhibit pathogens of the wastewater were subjected to molecular identification by 16S rDNA gene sequencing. Briefly, pure colonies of isolates M20 and S43 were picked, suspended into colony lysis buffer (10 mM TrisCl pH 8, 1mM EDTA, 50 mM KCl, 0.1% Tween 20) and boiled for 10min. The solution was then directly used for PCR using the primers pA (AGAGTTTGATCCTGGCTCAG) and pH (AAGGAGGTGATCCAGCCGCA) for 16S rRNA gene partial amplification. PCR products sequencing was performed by Sanger method using the primer pH.

2.6. Phylogenetic Analysis

Sequencing products were checked and assembled with CLC Workbench program for a final size of 1427pb for the isolate M20 and 1421pb for the isolate S43. Assembled sequences were then analyzed by EZ Taxon to identify the closest relatives in the Genbank database and the NCBI Genbank. The sequences were aligned with the program MUSCLE [13] and the alignment was examined manually with the program MEGA 6 [14]. For each phylogenetic analysis by Maximum likehood, the substitution model by the lowest Akaike Information Criterion was performed.

2.7. Statistical analysis

Data were analyzed using ANOVA Microsoft. The difference was considered as significant at $p<0,05$.

3. Results

3.1. Antimicrobial activity of Actinomycetes

Among the 10 Actinomycetes strains tested, 6 (60%) exhibited antimicrobial activity against at least one test-germ (Table 2). The isolates were more active against Gram positive bacteria than Gram negative bacteria and two strains (M2 and M20) inhibited the growth of alltestedpathogens. Compared to the reference antibiotics used, the isolates M2 and M20 were more active than Tetracycline (30µg) and Nalidixic acid (30µg) against *Streptococcus pneumoniae* and *Escherichia coli* respectively; S18 and S43 exhibited strong activity than Fusidic acid (10µg) against *Staphylococcus aureus*. The isolate S43 displayed high inhibition growth of *Bacillus cereus* than Netilmicine (30µg).

According to these results, two isolates were selected for wastewater biological treatment. The isolate M20, for its large activity spectrum exhibiting strong activity against test germs used compared with the isolate M2 and the reference antibiotics and the isolate S43, for its strong activity against Gram positive bacteria used (*Streptococcus*, *Staphylococcus* and *Bacillus*).

3.2. Water Physico-chemical Quality

Physico-chemical quality of raw and treated wastewaters (with the isolate M20, the isolate S43 and mix inoculation M20/S43) was assessed by considering the parameters cited above. The Table 3 summarizes the results of water physico-chemical quality for each treatment.

From these results, it could be deduced that most of the parameters responded to the treatment, a qualitative improvement was observed.

Table 1. Analytical method, standard reference for wastewater analysis

Analyzed parameters	Analytical method	Standard reference
Color	Spectrophotometric	Palintest
pH	Electrometric	NFT 90-008
Conductivity	Electrometric	NF EN 27888
Total dissolved solids	Filtration and Gravimetric	NF EN 872
Nitrate	Spectrophotometric	T90-045 / ISO 7890-3
Nitrite	Spectrophotometric	NF T90-012
Chloride	Titration	NF T 90-014
Phosphate	Spectrophotometric	NF T 90-023
BOD	Dilution	NF T 90 - 103
COD	Digestion and titration	NF T 90 - 101
Fecal *Streptococcus*	Membrane filtration	ISO 7899-2: 2000
Fecal coliforms	Membrane filtration	ISO 9308-1: 2000
Spores of sulphite reducing anaerobe bacteria	Membrane filtration	ISO 6461-2: 1968
Escherichia coli	Membrane filtration	ISO 9308-1: 2000

Table 2. Antimicrobial activity of Actinomycetes strains on test-germs

Isolates	Diameter of inhibition zone (mm):						
	Gram negative bacteria				Gram positive bacteria		
	Escherichia coli ATTC 25922	*Klebsiellaoxytoca* ATTC 8724	*Salmonella enteridis*	*Enterobacter cloacae* ATCC 700323	*Staphylococcus aureus* ATTC 11632	*Streptococcus Pneumonia* ATTC 6301	*Bacillus cereus* ATTC 13061
S18	12.5 ± 0.0^{c}	12 ± 0.0^{c}	-	-	28.5 ± 2.1^{b}	-	12.5 ± 0.7^{c}
S43	-	-	-	-	38 ± 2.8^{a}	29 ± 1.0^{a}	52.5 ± 3.5^{a}
S44	-	-	-	-	-	-	13 ± 0.7^{c}
S51	-	-	-	-	-	-	14 ± 0.7^{c}
M2	16 ± 0.5^{b}	$13,6 \pm 0.5^{b}$	19.3 ± 0.5^{b}	18.6 ± 0.1^{a}	11 ± 1.0^{d}	18.6 ± 0.5^{b}	21 ± 1.1^{b}
M20	18 ± 1.1^{a}	22.3 ± 0.5^{a}	21 ± 1.0^{a}	13.6 ± 1.1^{b}	19.3 ± 0.5^{c}	29 ± 1.0^{a}	20 ± 1.0^{b}
NA	18(S)	23(S)	-	-	-	-	-
NET 30	-	-	-	-	-	-	27(S)
FA 10	-	-	-	-	25(S)	-	-
TETRA 30	-	-	-	-	-	38(S)	-

Table 3. Recapitulative results of wastewater physico-chemical analysis for each treatment

Parameters/ Treatment	pH	Conductivity (µs/cm)	TDS (mg/l)	Color (mg of Pt/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphate (mg/l)	Chloride (mg/l)	BOD_5 (mg/l)	COD (mg/l)
E1	8.56	10935	115	200	1.39	291.33	7.39	275.38	77	177
E2	8.01	10250	185	145.56	1.01	0.11	6.96	269.09	70.5	145.67
E3	8.37	9250	96	79.33	1.28	11.44	9.14	262.76	58.6	132.67
E4	7.95	8770	45	56.77	0.76	0.07	5.45	205.02	32.25	94.64
Criteria values for wastewater	6<pH <9	<1500	<60	<70	<20	<0,2	<10	<250	<50	<150
Percentage of elimination (%) for E4	7.12	6.27	60.86	71.61	45.32	99.9	26.25	25.55	58.11	46.53

3.3. Physico-chemical Parameters

For the tested parameters, significant differences were noted between treated waters.

Concerning the pH, compared with the raw water (E1), an improvement was noted for the wastewater treated with the isolate M20 (E2) where the pH value was 8.01. For the wastewater treated with the isolate S43 (E3), a slight augmentation of the pH value (8.37) was observed compared with that of E2. For mix treatment (M20/S43), the pH decreased significantly with a value of 7.95.

The amount of total dissolved solids in E2 was 185mg/l which was greatly higher than E1. On the contrary, TDS amount decreased with E3 (96mg/l) and reduced significantly until 45mg/ml for the mix treatment.

Regarding to the color, the effectiveness of the treatment was determined through its intensity for each treated sample. Compared with E1 color, that of E4 was clearer; for E2 and E3, a large difference of intensity color was noted, E2 color was more intense than E3 color.

About the conductivity, that of treated water decreased slightly than the conductivity value of the raw water (E1). Any significant difference wasn't observed for E2 and E3 conductivity values. A weak decrease of this value was recorded for E4.

For the nitrite, a significant improvement was noted for the water samples treated with the three treatments compared with the raw water (E1). This improvement touched particularly E4 where the nitrite amount was 0.07mg/l. Those of E2 and E3 were 0.11mg/l and 11.44mg/l respectively.

An improvement was also emphasized for the nitrate amount in the three treated wastewater samples. However, it is very significant for E4 with the nitrate amount of 0.76mg/l.

About the phosphate, E2 showed a weak decrease rate of phosphate (6.96mg/l); it was 9.14mg/l for E3 and this rate was significantly reduced for E4 (5.45mg/l).

The chloride rate of E2 and E3 was weak compared with E1, E4 exhibited yet a satisfied value (205.02mg/l) than E2 (269.09mg/l), E3 (262.76mg/l) and the standard (<250mg/ml).

The COD rate of E4 showed a significant improvement of wastewater quality view its value (94.64mg/l) decreasing progressively compared with E2 (145.67mg/l) and E3 (132.67mg/l).

For the BOD rate, the recorded values showed a decrease for E2 (70.5mg/l) and E3 (58.6mg/l) samples compared with the raw water. Compared to the BOD value of E1 (77mg/l), that of E4 was half-reduced (32.25mg/l).

3.4. Microbiological Parameters of the Wastewater for Each Treatment

An analysis before treatment and an analysis after treatment were realized in a view to assess wastewater microbiological parameters quality and treatment efficiency by Actinomycetes strains. Microorganisms' rate in E1 was strongly higher than Malagasy decree n°2003/464 guideline values for wastewater which means that the water was much polluted. Treatment by each isolate or mix treatment improved wastewater quality. The results obtained are presented in the Table 4.

Table 4. Wastewater microbiological quality before and after 3 weeks of treatment

Germs \ Treatments	E1	E2	E3	E4	Guideline values
Sulphite reducing anaerobe bacteria	$8.0.10^4$	$6.3.10^2$	52	8	100
Fecal coliforms	$2.1.10^5$	$3.4.10^3$	46	39	100
Fecal *Streptococcus*	$7.4.10^5$	<1	<1	<1	100
Escherichia coli	$3.0.10^5$	$2,7.10^3$	68	11	100

According to these results, the number of sulphite reducing anaerobe bacteria colonies in treated water decreased. Whatever the treatment, wastewater quality showed a remarkable improvement, particularly, for E4 (8FCU/ml at the end of the treatment) compared with the raw water.The same tendency of results was noted for fecal coliforms and *Escherichia coli*; the best result was recorded for E4. For fecal *Streptococcus*, no colony was observed after only 3 days for the three treatments.

3.5. Molecular Identification of Potent Actinomycetes Isolates

Molecular characterization using 16S rRNA gene sequences of the two efficient Actinomycetes isolates showed that they belong to the genus *Streptomyces*. The marine isolate M20 matched with high similarity with *Streptomyces albidoflavus* strain DSM 40455T whereas the telluric isolate S43 was identified as *Streptomyces antibioticus* strain NBRC 12838T (Figure 2 and Figure 3).

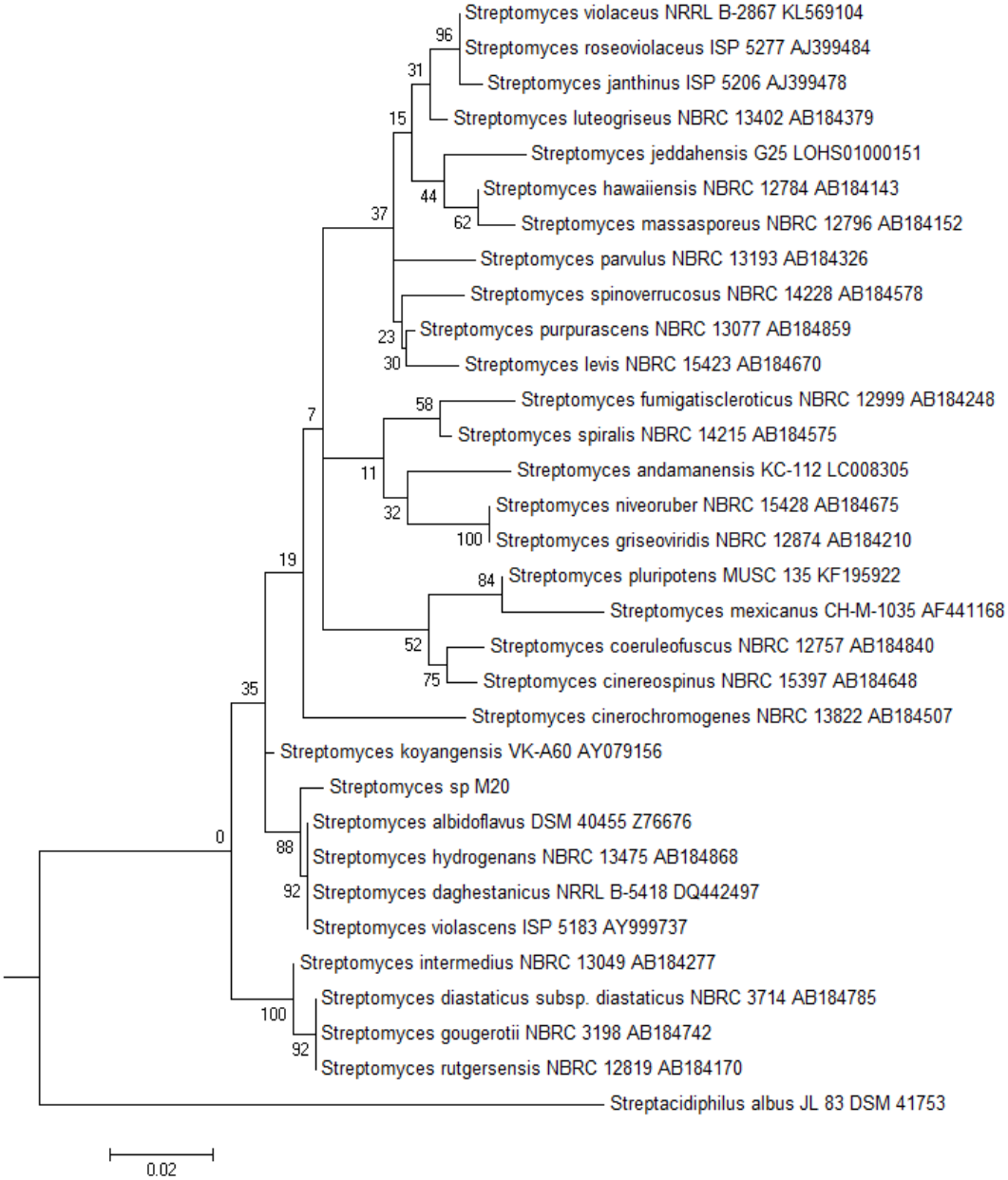


Figure 2. Maximum likehood tree based on partial 16SrDNA gene sequences of potent isolate M20

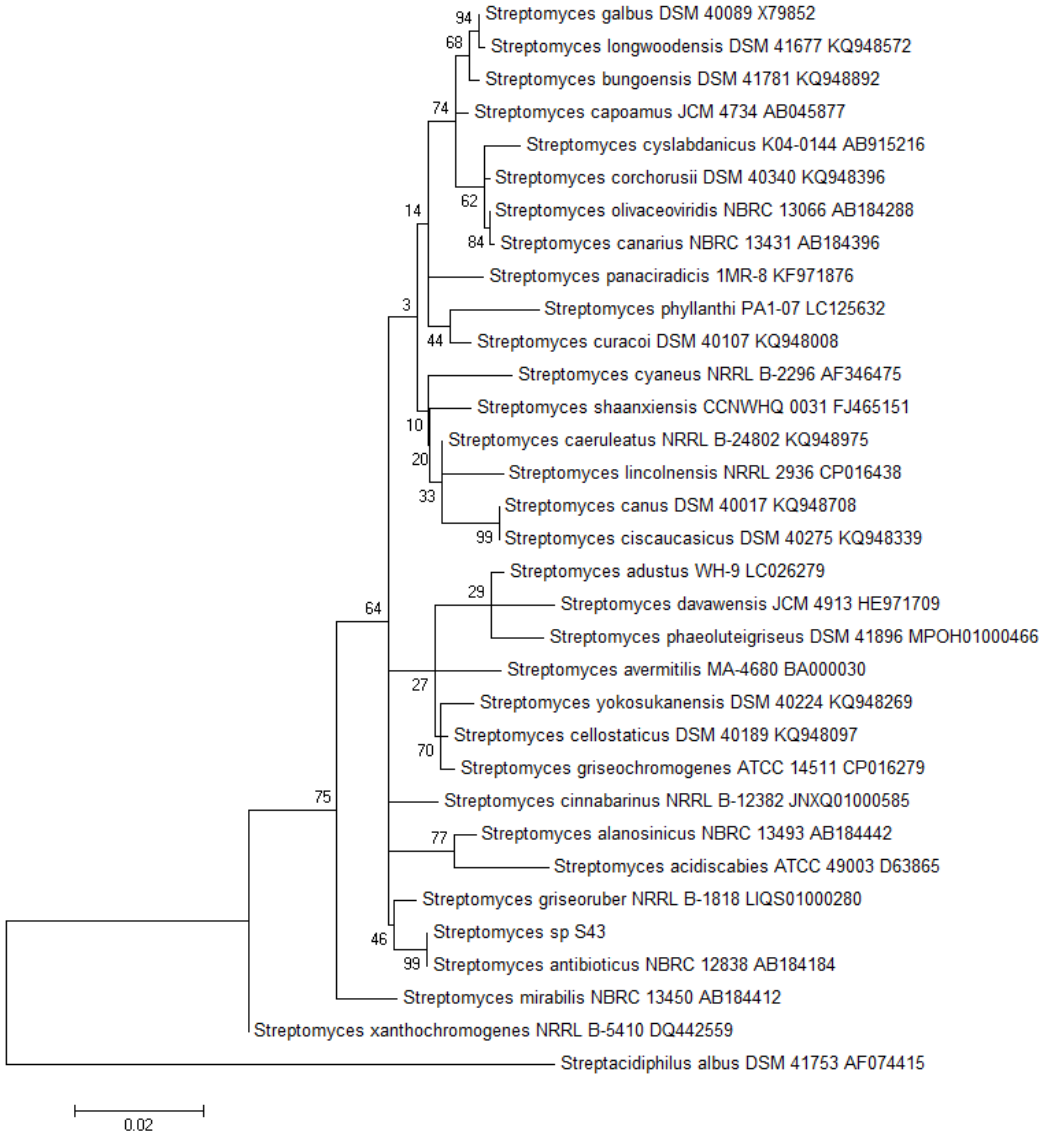


Figure 3. Maximum likehood tree based on partial 16S rDNA gene sequences of potent isolate S43

4. Discussion

The main objective of the present work was to develop a reliable approach of wastewater treatment based on Actinomycetes potentiality to degrade pollutant matters and to eliminate pathogens of the wastewater. The choice of the biological agent used for the treatment lies in the fact that Actinomycetes strains are known by their capacity to product secondary metabolites such as antibacterials, antifungals [15,16,17,18,19] and antipollutants [20]. It's the first study demonstrating the utilization of Actinomycetes strains as biological agent for wastewater treatment.

In the first part of the work, the study was mainly focused on the selection of active Actinomycetes strains, antimicrobial assay was carried out. The results showed that among the 10 tested Actinomycetes strains, 6 inhibited one or several test-germs growth. Actinomycetes isolates were particularly active against Gram positive bacteria than Gram negative bacteria. The difference of pathogens sensitivity against Actinomycetes isolates could be explained by the difference of cell membrane composition [21,22]. Gram positive bacteria cell membrane is constituted only by a peptidoglycane layer [23], whereas that of Gram negative bacteria is formed by an external layer of lipopolysaccharide complexe, phospholipids and proteins. This layer has a selective barrier role making then Gram negative bacteria resistant to Actinomycetes strains [24]. This explains the high number of antagonistic Actinomycetes against Gram positive bacteria compared with Gram negative bacteria. However, it could be emphasized that 3 strains inhibited at least one test-Gram negative bacteria growth. The isolates M2 and M20 showed antagonistic activity against all tested Gram negative bacteria. These results are in agreement with those of Thenmozhi and Kannabiran [25] who demonstrated that ethyl acetate extract of marine Actinomycetes, *Streptomyces* VITSTK7 was active on all test-germs used including Gram negative bacteria.

The second part of the work developed the possible capacity of Actinomycetes strains to purify wastewater. Two parameters were analyzed: the effect of Actinomycetes utilization on physico-chemical properties of the water and the effect of Actinomycetes activity on pathogens microorganisms' development (microbiological parameters).

For the physico-chemicals parameters, analyses were based on the variation of pH, phosphate, nitrate, nitrite,

chloride, total dissolved solids and oxygen needs. During the 3 treatments, wastewater pH showed a slight change but it is conform to the norms. This modification could be due to the increase in $C0_2$ and 0_2 productions [26].

A decrease of TDS amount was recorded especially for mix treatment with M20 and S43 strains. This would be explained by thedecomposition of dissolved solids by the two tested strains which used them as source of nutriments. In parallel, color intensity of treated wastewater decreased during the treatment because dissolved substances offering the real color of wastewater were eliminated. The conductivity decreased slightly after the treatments but its values were not conform compared with the guideline value for wastewaster conductivity. This high value allows qualifying the tested wastewater as mineralized water [27].

The nitrate rate was low and the nitrite rates decreased significantly for each treatment. Laverman [28] in his work demonstrated that some antibiotics as Vancomycin, Erythromycin and Chloramphenicol inhibited the denitrification mechanism to the diminution of nitrate reduction and the augmentation of nitrite production. This observation allows deducing that antibiotics produced by Actinomycetes isolates hadn't any effects on the denitrification. Concerning the wastewater quality improvement, mix treatment with the isolates M20 and S43 were proved efficient conducting to a considerable decrease of the nitrite and the nitrate rates largely inferior to the norms.

The treatment with each isolate seems inefficient for chloride, the values decreased slightly whereas mix treatment with the both isolates reduced largely this rate to the normal. For the phosphate rate, its value varied for one treatment to another.

Among the most remarkable improvements, the cases of BOD_5 and COD rates can be noted especially for E4. These results illustrate thatthe different forms of nitrogen (nitrate and nitrite) in the wastewater during the treatment were only the intermediary forms of this element involved directly or indirectly in the wastewater biological and/or chemical oxygen demand and the rates of these elements depend on the evolution of living organisms composition in the wastewater [29].

Considering the ten biological and physico-chemical parameters tested in this study and referring to the guide values defined by Malagasy decree for reject waters, the treatment of wastewater performed allows tracing a promising way for the improvement of water quality. However, the absence of relative specific norms for the re-use of treated wastewater for other purposes is an handicap for the exploitation of the results. For industrial re-use, Lee et *al.* [30] suggested to consider only nine physico-chemical and biological parameters such as pH, turbidity, total dissolved solids, color, alkalinity, iron, magnesium, chloride and COD for treated water quality assessment.

During the biological treatment, four microorganisms were considered: sulphite reducing anaerobe bacteria, *Escherichia coli*, fecal *Streptococcus* and coliforms. They are frequently used for water qualification but they are not exhaustive to represent microorganisms causing environmental danger (pollution of receiver site, propagation of pathogens, …). A remarkable improvement of wastewater quality was noted during the treatment: total elimination of pathogens (fecal *Streptococcus* case) or reduction of pathogens rate (the case of the three other microorganisms). These results are comparable to those of Hossain et *al.* [31] which showed that the inoculation of some purifying microbial strains in a system using fermentation tank facilitates the elimination of some pathogens as fecal *Streptococcus* and coliforms.

Another remarkable aspect of this work is the synergy between the two Actinomycetes strains used. It was demonstrated that instead of the competition frequently observed by inoculating simultaneously two bacterial strains, a synergic effect was noted. This positive effect could be attributed to the functional complementarity between the Actinomycetes strains.

It would be emphasized that usually, biological treatment is followed by other treatments to remove others remaining chemical elements and microorganisms of the previous treatment. According to the results obtained, this treatment proved that the use of Actinomycetes strains as biological treatment precisely in the secondary treatment level reduces the cost in terms of water treatment facility and environmental requirements respect.

5. Conclusion

It would be concluded from this study that Actinomycetes strains are able to purify wastewater, on the one hand, by degrading pollutant matters resulting by their reduction after the treatment and on the other hand, by eliminating (fecal *Streptococcus*) or reducing fecal contamination indicating germs. For all of the considered parameters except conductivity, our results showed that wastewater treatment through Actinomycetes is a promising and convenient technology for developing country. However, further investigations will have to be conducted on the optimization of treatment conditions, the purifying effect of Actinomycetes strains on other types of water and the pilot test.

Abbreviations

S: sensible
NA: nalidixic acid
NET: netilmicine
FA: fusidic acid
TET: tetracycline
E1: raw water
E2: wastewater treated with the isolate M20
E3: wastewater treated with the isolate S43
E4: wastewater treated both with the isolate M20 and the isolate S43.

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