Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.6, No.12 2016

Polyhydroxyalkanoate Production by Streptomyces plumbiresistensCCNWHX 13-160^{T}

Maytham Ayuob Al-Hamdani*
Dept. Biology, College of Education for Pure Sciences, University of Basrah-Iraq

Abstract
A novel actinomycete isolated from sandy soil of Basrah province, Iraq, was identified by 16S rRNA sequencing as *Streptomyces plumbiresistens*CCNHWX 13_160^{T}. Polyhydroxyalkanoate (PHA) production by this strain was investigated. The extracted PHA was characterized by FTIR spectroscopy. It was found that this strain is PHA producer.
Keywords: polyhydroxyalkanoate, *Streptomyces plumbiresistens*

1. Introduction
Among bacteria, many genera were known to produce many substances several of them have a significant value in industry. One of these products is polyhydroxyalkanoate (PHA), a polymer known as biodegradable plastic (Chen 2009). Valappil (2007) reviewed data on PHA producers with special consideration on *Bacillus* and *Streptomyces*. *Streptomyces*, a genus belongs to actinomycetes, aerobic Gram positive bacteria predominantly found in soil. Members of the actinobacteriahave been known to be polyhydroxyalkanoate producers (Matias *et al*. 2009).

During the study of soil bacteria in Basrah Province, a novel isolate of *Streptomyces* was isolated. The present study was conducted to investigate the ability of this isolate to produce PHA.

2. Materials and methods:
2.1. Collection of soil sample:
Soil sample was collected from Al-Zubair area west of Basrah city- Iraq. The texture of the soil was sandy soil. The sample was taken 3cm beneath the surface of the soil, placed in a disposable plastic vial and brought to the laboratory.

2.2. Isolation and identification of bacterial isolates:
Soil sample was cultured after serial dilutions by spreading on nutrient agar . Plates was incubated in an incubator at 37 C for 72 hours. Then, the colonies were studied and identified morphologically, by Gram stain and by genomic DNA extraction and amplification of 16S rRNA. The obtained product of PCR has been sent to Bioneer company Labortories, South Korea for purification and sequencing. Data of sequencing have been manipulated.

2.3. Detection of PHA bacterial isolates:
Colonies were cultured on nutrient agar plates enriched with 1% glucose. The plates wereincubated at 37^{0}C for 72hrs. Then, the culture plates were flooded with 0.02% alcoholic sudan black B to stain bacterial colonies followed the method of Juan *et al.* (1998). The plates were kept undisturbed for 30 min. The excess dye was then decanted , rinse with 100% ethanol . PHA producing colonies appeared bluish black. Isolates that show positive reaction with sudan black B were checked forpha production by using specific stain , nile blue A stain. A medium containing the same ingredients with the dye nile blue A in a concentration of 0.5ug/ml was incorporated. The PHA accumulating colonies fluorescence when exposed to uv light.

2.4. PHA production and extraction In liquid culture:
A culture medium of the same previous ingredients except agar was used. The flasks containing 100 ml of liquid medium were inoculated with 0.1 ml of 1 O.D . at 540 nm of the bacterial suspension and incubated in a shaker incubator at 37C,150rpm for 7 days. Cells were harvested by centrifuging. PHA was extracted using sodium hypochlorite method by Ramsy *et al*. (1990).

2.5. Fourier Transform Infrared Spectroscopy Analysis(FTIR):
FTIR analysis of the polymer that extracted from this bacterial strain was carried out by Jasco FTIR 4200 spectroscopy, Japan. in IR range of 4000-400 cm^{-1} . Peak values obtained were analysed to interpret the presence of specific functional groups in the extracted polymer.

3. Results and Discussion:
Colonies of Streptomyces in the present study was found to be sticky to the surface of the agar medium(nutrient

agar), white in colour, cone in shape, Gram-positive , composed of branched white aerial mycelium and brownish substrate mycelium on nutrient agar. Spores are produced, white in colour. With age, a brown diffusible pigment is produced. This isolate of Streptomyces was classified based on 16S rRNA, shows 100% identity to *Streptomyces plumbiresistens* 13-160^T after manipulation of gene sequencing data. These characters are similar to that descrided firstly isolated from lead polluted soil in north west China by Guo *et al*. (2009). In the present study, this strain was isolated from sandy soil of Iraq not contaminated with heavy metals. The strain in the present study was classified based on 16S rRNA. Gene sequencing was shown in Table 1.

Table 1. Gene sequencing of *Streptomyces* isolated in the present study.

Streptomyces plumbiresistens strain CCNWHX 13-160^T

GGGTGGCGGCATGCTTACCATGCAGTCGAACGATGAACCACTTCGGTGGG
GATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCT
GGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCCCTCCCTC
CTGGGTGGGGGTTGAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCTAT
CAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGC
CTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCA
GGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTAC
GTGCCACCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAGTTAT
TGGGCGTAAAGAGCGCGTATGCGGCTTGTCACATCTGGTGTGAAAGCCCG
GGGCGTTTACCCGGGGCTCCTTTTTATACAGGGGTTTATAGTGTGGGGGG
GGGGGAACACGAAATGTGGAGTTGTACGTGTTAATCCGCGAAAAACTAAG
GAGAAAAACGGGGAGAAAAAGGACTCCCCTGGGCTACGTGGGACGACCGA
GGGGAGATAATGCTGGGGGGACCACACCACTTTTTTTCTCC

Colonies of the present isolate showed positive reaction for sudan black B stain . The colonies stained bluish black with high colour intensity as shown in figure 1. The colonies of the this isolate showed bright fluorescence on nutrient agar plates containing the specific nile blue dye when exposed to uv light fig. 2. and the fluorescence intensity become orange with age of the colonies figure 2. The bright colour turn bright orange. In the present study, this confirm that the isolate is definitely PHA producer Fig. 3.

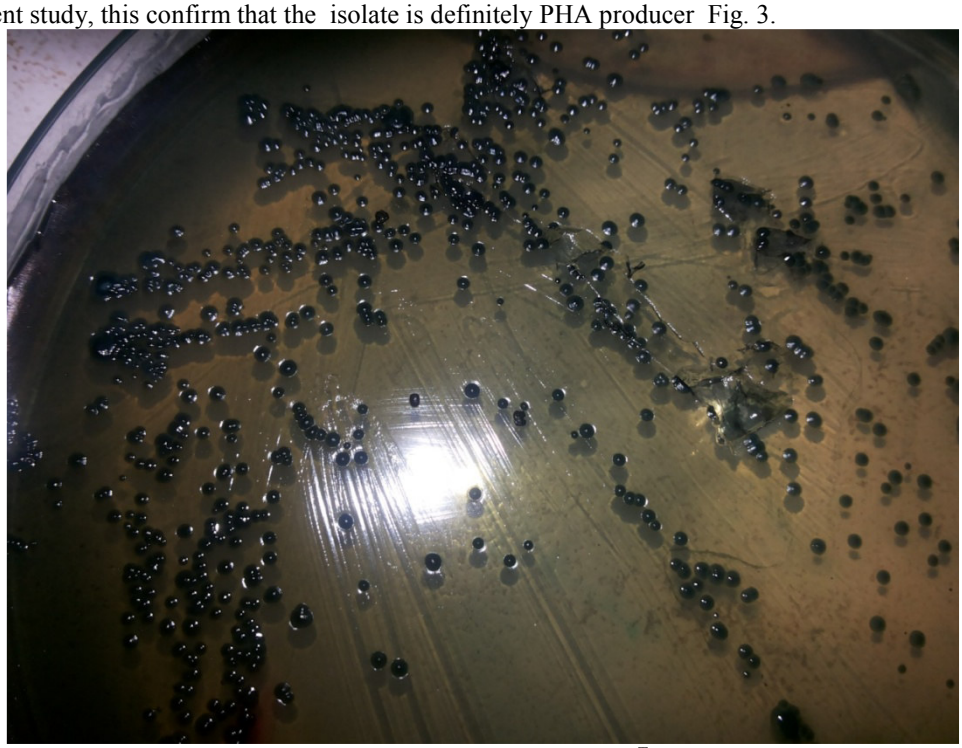

Fig. 1: Colonies of *Streptomyces plumbiresistens* CCNWHX 13-160^T on 1% glucose enriched nutrient agar showing positive reaction with sudan black B stain

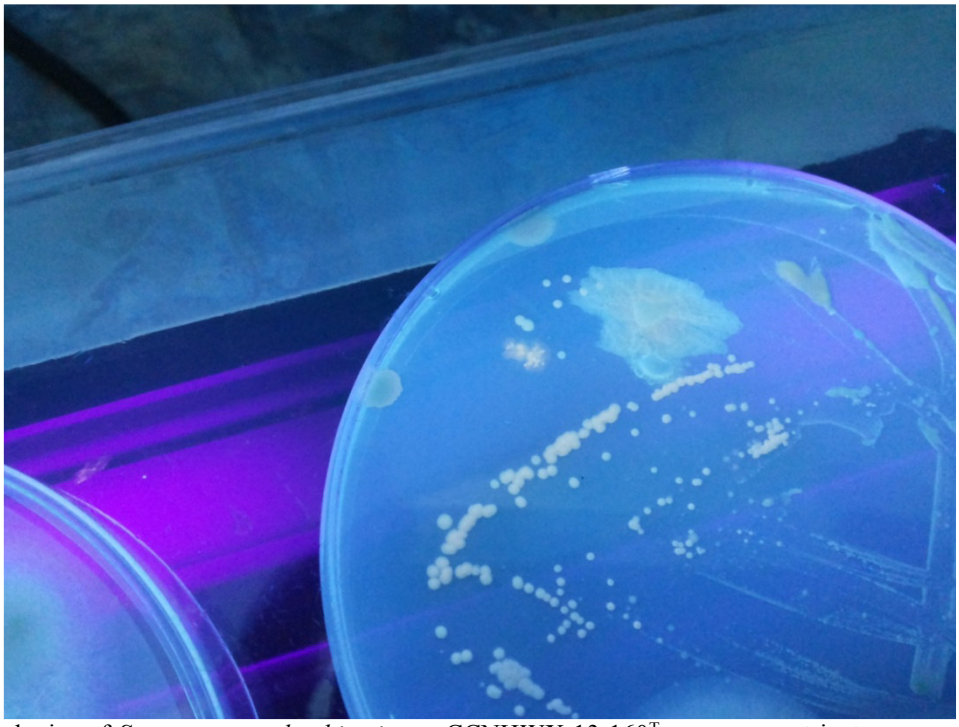

Figure 3: Colonies of *Streptomyces plumbiresistens* CCNHWX 13-160^{T} grown on nutrient agar containing nile blue A dye showed bright fluorescene when exposed to uv light

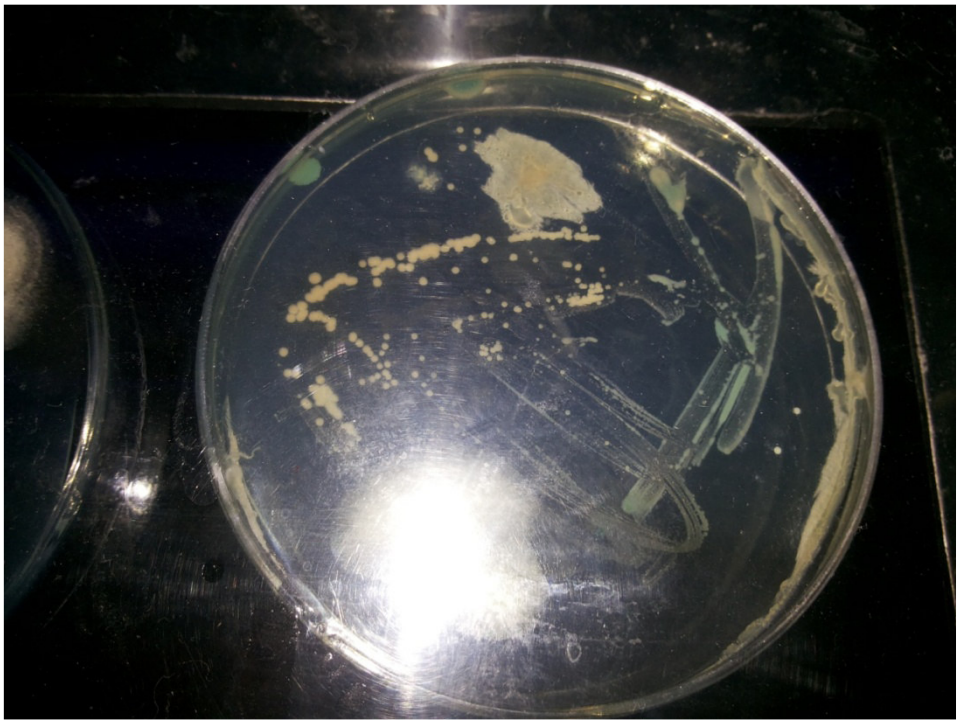

Figure 3: The same as in Fig. 2. The colonies showed bright orange colour with increasing age of the colonies .

The FTIR spectrum of extractedPHA powder was shown in Figure 3. The FTIR analysis revealed eight peaks at 3431 cm^{-1}, 2925 cm^{-1}, 2855 cm^{-1}, 1743 cm^{-1}, 1651 cm^{-1}, 1460 cm^{-1} and 1080 cm^{-1}. The peak 3431 cm-1 shows the presence of terminal OH group, at 2925 cm-1 shows the presence of methylene C-H group and at 1743 cm-1 shows the presence of estercarbonyl C=O group. The presence of these characteristic functional groups analysed by FTIR confirmed the presence of PHA in the extract.

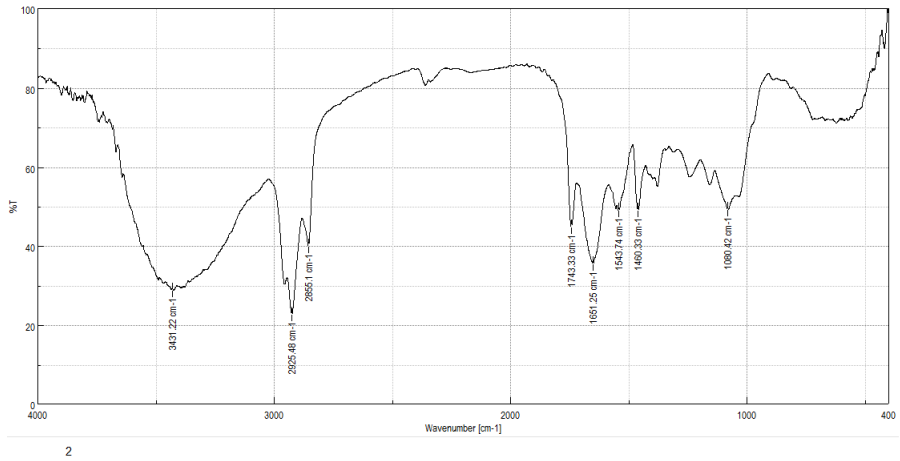

Figure 4: FTIR spectrum of powdered PHA extracted from *Streptomyces plumbiresistens* CCNHWX 13-160^{T}

Conclusion

An actinomycete was isolated from sandy soil inBasrah province, Iraq . This isolate was identified morphologically, by Gram-stained and by gene sequencing of 16S rRNA , designated as*Streptomyces plumbiresistens*CNNHWX 13-160^{T}. Polyhydroxyalkanoate production by this isolate was studied. Extracted powder of PHA from *Streptomyces plumbiresistens* in the present study was analysed by FTIR spectroscopy showed the characteristic functional groups of PHA.

References:

Chen GQ(2009). A microbial polyhydroxyalkanoates (PHA) based bio-and materials industry. ChemSoc Rev. 38(8): 2484-46.

Guo JK, Lin YB, Zhao ML, Sun R, Wang TT, Tang M and Wei GH. (2009). *Streptomyces plumbiresistens* sp. Nov., a lead-resistant actinomycete isolated from lead-polluted soil in north-west China. International Journal of Systematic and Evolutionary Microbiology 54: 1326-1330.

Juan ML, Gonzalez LW and Walker Gc. (1998). A novel screening method for isolating exopolysaccharide deficient mutants. Applied and Environmental Microbiology 64: 4600-4602.

Matias F, Bonatto D, Badilla G, Rodriques MF and Henriques JA. (2009). Polyhydroxyalkanoates production by actinobacteria isolated from soil. Can. J. Microbiol. 5(7): 790-800.

Ramsy JA, Berger E, Ramsy BA and Chavarie C. (1990). Recovery of poly -3- hydroxyalkanoic acid by a susfactant hypochlorite treatment. Bioooootechnol Tech. 4: 221-226.

Valappil SP, BoccacciniAR,Bucke C and Roy I. (2007). Polyhydroxyalkanoates in gram-positive bacteria. Insights from the genera *Bacillus* and *Streptomyces*. AntonieLeewenhoek 91(1): 1-17.

About the Author:

Assistant professor Dr. MaythamAyuob Al-Hamdani graduated from Dept. Biology, College of Science, university of Basrah, Iraq (1982), Awarded MSc in Mycology from Dept. Biology, College of Science, University of Basrah, Iraq(1985. Ph.D. from Dept. Biology, College of Education for pure Sciences, University of Basrah-Iraq. Lecturer of Microbiology in Dept. Biology, College of Education for pure Sciences since 1987