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BIOABSORBENT PRODUCTION BY CMG646: A MARINE ISOLATE

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ABSTRACT

Microbial products have been recognized as an integral component of natural products chemistry and microbes they are significant resource for environment friendly compounds, which have multiple industrial applications. One of the eco-friendly products of bacteria is bioabsorbent polymer. Biopolymer from bacteria offers a number of novel properties and commercial opportunities. CMG646, a marine isolate identified as *Pseudomonas aeruginosa* was found to produce two types of polymers. One of which is water-absorbing EPS that could absorb and retain water significantly more then 47 times of their own weight.

Key Words: *Pseudomonas aeruginosa*, biopolymer, bioabsorbent, EPS, marine isolate, eco-friendly.

INTRODUCTION

The accelerated expansion of desert (desertification) is currently a global ecological concern (FAO, UNEP, 1984). Japan is making a contribution to the greening of deserts by supplying Egypt and other desert countries with synthetic high polymer water absorbents that are to be used to retain water for irrigating seedlings (Ishii, 1990; Tooyama, 1990). If such water absorbents were organism derived and readily biodegradable, the adverse effects on the environment after the growth of the seedlings would be negligible. Thus the development of biodegradable alternatives is strongly desired. (Ryuichiro and Nohata, 1995). Bioabsorbent biopolymers are polysaccharides derived from bacterial sources, having high water absorbing capacity and can retain water for long periods of time, hence enhance the water holding capacity of soil. They are easily biodegradable and safe to environment.

Bacterial polysaccharides are usually associated with the outer surface of the bacterium. In crude terms, these molecules can be divided into two groups. Either they form an amorphous layer of extracellular polysaccharide surrounding the cell which may be organized into a distinct structure termed a capsule, or alternatively, the polysaccharide molecule may be more intimately associated with the cell surface either through linkage to a lipid-A moiety, as in the case of the lipopolysaccharide (LPS) molecules in Gram-negative bacteria, or linked to cell-wall techoic acids as in Gram-positive bacteria. Polysaccharides are highly hydrated polymers composed of repeating single units (The monosaccharide) joined by glycosidic linkages.

The synthesis of extra cellular polysaccharides has been recognized in certain bacterial cultures since 1880s. It is now known that a wide range of bacteria produces these polymers.

Most of the synthetic water absorbents used in sanitary articles and paper diapers are based on synthetic high polymer materials when they are discharged into the environment as waste they are not biodegradable and remain in the environment for long periods (Vonhorick and Moens, 1983; Dearfield and Abermathy, 1988).
Polysaccharide of *Alcaligenes latus* B-16 is expected to be used as sanitary products and diapers and even humectants, used during irrigation of seedlings for promoting greenings of deserts (Yoshinaga *et al.*, 1997).

The present paper describes the production of two types of bioabsorbent compounds by a marine isolate *Pseudomonas aeruginosa* CMG646. The compounds have been extracted and found to absorb and retain water more then their own weight.

MATERIALS AND METHODS

Screening of bacterial isolates for bioabsorbent production

Total eight isolates were screened for the production of bioabsorbent polymer i.e. CMG641, CMG642, CMG643, CMG644, CMG645, CMG646, CMG647, CMG648. Broths of all bacterial isolates were treated with ethanol. All strains were grown in YM (Yeast extract and Malt extract) medium containing Yeast Extract 3gm, Malt Extract 3gm, Bactopeptone 5gm, and Glucose 10g/l. medium. Cultures were grown at 37ºC at 180 rpm at shaker in an incubator for 24 h. After 24 h these cultures were treated with 70% ethanol to detect the presence or absence of the bioabsorbent polysaccharide production.

Selecting suitable medium for the production of Bioabsorbent

Selection suitable media was decided by inoculating the selected strains in NB (Nutrient broth), LB (Luria broth), YM (Yeast extract and Malt extract) medium. After 24 h these fermented broth were treated with ethanol to test the precipitates production. Out of eight isolates CMG646 gave positive results for bioabsorbent production by ethanol precipitation method. CMG646 was isolated from Arabian Sea, later identified as *Pseudomonas aeruginosa* therefore for further analysis and extraction of bacterial bioabsorbent polysaccharide CMG646 was selected. YM medium was found to be the most suitable medium for the production of bioabsorbent polymer.

Water Absorbance Capacity

In order to study the water absorbance capacity of the bioabsorbent polymer a filter paper was taken and weighed, the bioabsorbent polysaccharide was placed on the filter paper and it was kept in the hot air oven at 50ºC for 72 h. During this 72 h period the bioabsorbent was weighed 2-3 times at intervals and when the weight was found to be constant it was recorded as dry weight (W1) of bioabsorbent. This dry bioabsorbent was placed in a weighed petri plate and calculated amount of water was poured into it now it was left for 24 hours and next day the excessive water was dried and the gelatin (bioabsorbent polysaccharide) was again weighed (W_2) and then the weight of the petri plate, filter paper, and dry bioabsorbent was subtracted from the W_2 the resultant was the regain of water in grams (g) i.e. W_3. The total weight of the water regained, wet gelatin and dry gelatin was calculated by the following formula,

$$\mathbf{W2 - W1 = W3}$$

Where, W1= weight of dry gelatin

W2= weight of gelatin having absorbed water

W3= weight of water regained

RESULTS AND DISCUSSION

The exopolysaccharides were precipitated out from culture media of CMG646 when an adequate volume of 70 % ethyl alcohol (about 2 volumes) was added to culture media. These precipitates were somewhat off- white, creamy in color and formed long thread like bunched structures at the top of the liquid nutrient broth along with these fine threads like precipitates CMG646 also produce gel like material, which was later named as gelatin (Fig. **1**). CMG646 showed good growth in nutrient broth it entered in the log phase after about 10 h of transfer in the fresh medium.

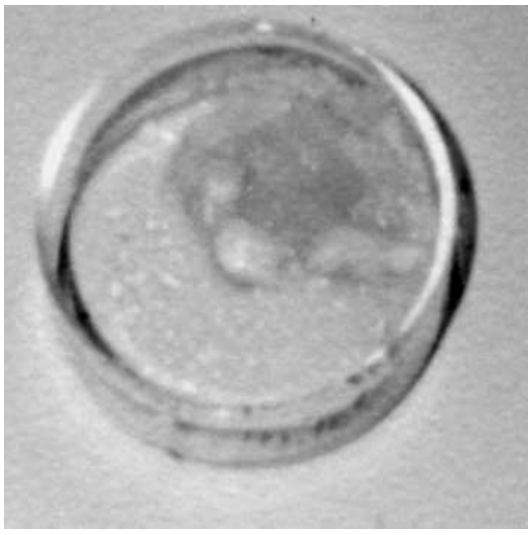

Fig. 1. Bioabsorbent Polymer named as Gelatin Produced by CMG646.

For extraction of bioabsorbent polysaccharide CMG646 was grown in YM medium that was proved to be the best-suited medium for the production of bioabsorbent polysaccharide in this study. Growth curve was plotted, by

taking O.D at 600nm (at Y-axis) against time (at x-axis). First day O.D was taken at two hours intervals and from second day onwards after 24 h readings were observed.

In the first 5-6 h CMG646 showed lag phase that may be acclimatization phase, after which it entered into the log phase and remained there till 14 h after which it entered into the stationary phase, it is the phase when most of the bacteria start producing secondary metabolites so as to prevent them selves from dying that is when the nutrients deplete in the Medium, bacteria it self uses the secondary metabolites after synthesizing them up to a certain amount. So after maximum production of secondary metabolites they remain in the Medium instead of getting disappear (Jawetz *et al.*, 1998).

Stationary phase continued till 29 hours followed by decline phase, after decline phase CMG646 regained the O.D and another stationary phase was observed, which could be due to the reason that when nutrients were depleted in the Medium, it was observed that CMG646 started producing the secondary metabolites from the 33rd h. To check whether estimated bioabsorbent had water absorbance capacity or not biomass was also studied for water absorbance so biomass was taken by centrifugation of the culture. Supernatant was drawn out and used for precipitate production.

Biomass was taken in a pre-weighed falcon tube and weighed then dried and weighed again until a constant weight was obtained then the loss of water was measured. With increasing time weight of biomass increased while in growth curve the O.D decreased or remained same as the time increased (Fig **4**). The reason behind this could be that the biomass may contained some amount of gelatin and precipitates due to which it absorbed more water and weight of biomass increased as the time increased and that is also why there is difference between the wet biomass (state before the water was dried) and dry biomass (state after the water was dried completely) about two times (Ahmed *et al.*, 2004).

Bioabsorbent production was also measured in grams and its weight was plotted against time. Water absorbance capacity was tested by taking the weight of wet gelatin and dry gelatin and weight of water regained and plotted in a graph against time (Fig. **2**). It was observed that weight of wet gelatin increased with time but dry gelatin did not show such pattern and also water regained which may be due to the reason that in the beginning of gelatin production the gelatin contained less water and more gelatin so the difference between wet and dry gelatin was not very much but the water regained was approximately 2 times its weight, while with increase in time the difference between wet and dry gelatin increased and water regain decreased, it may be due to the reason that the bacterial strain itself started using the gelatin and also some gelatin got dissolved in water. As the water regained, by the gelatin, was two times more than its weight while the biomass could not absorbed as much water so it is suggested that the gelatin was a water absorbing bioabsorbent polysaccharide. Bacteria especially of marine and soil origin have been reported for bioabsorbent polymers. These have the ability to absorb water significantly more than its own weight (Weiner, 1997). The supernatant drawn out from the culture was treated with 70% ethanol, off-white precipitates were obtained, these precipitates were collected and weight of the wet and dry precipitates were measured and water regained was also measured and it was found that it could also absorb water up to one and a half times more an its weight (Fig. **3**).

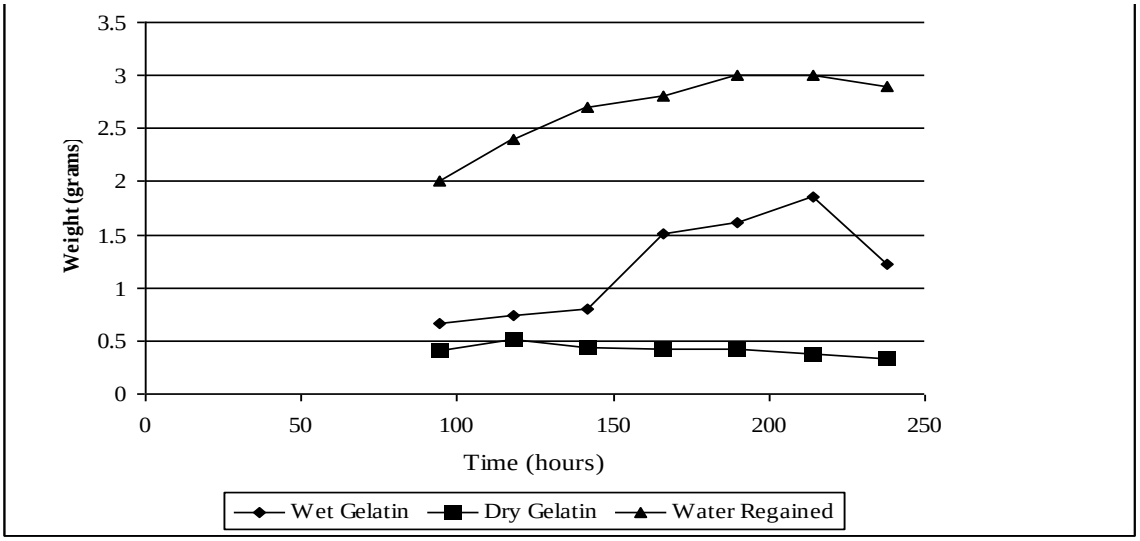

Fig.2. Bioabsorbent production as a function of time.

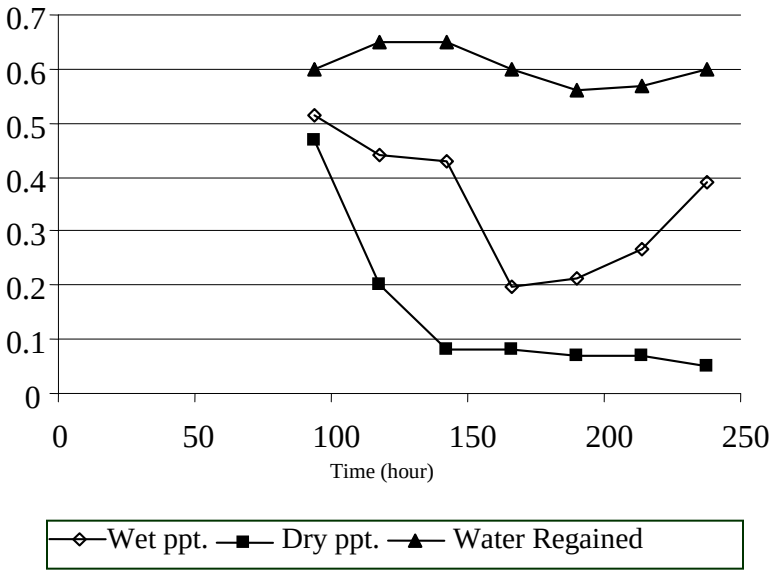

Fig. 3. Precipitates production as a function of time.

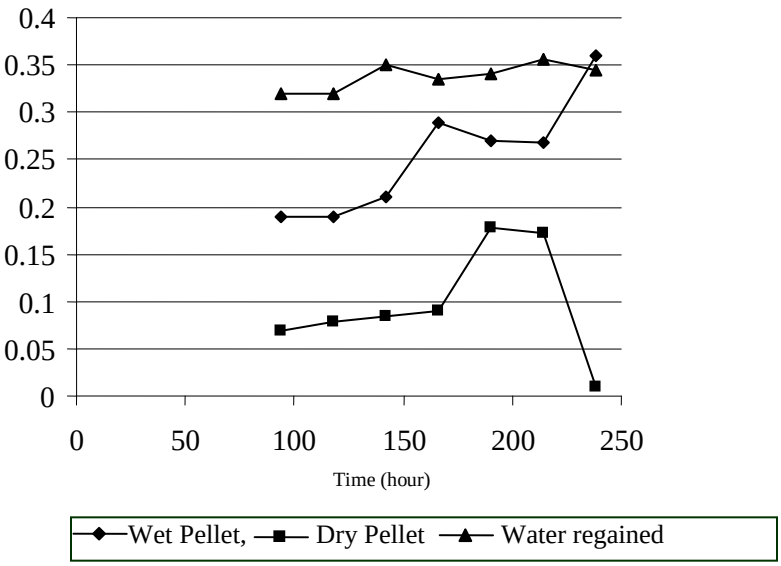

Fig. 4. Biomass of CMG 646 as a function of time.

The difference between the water absorbance of gelatin and precipitates could be due to their structures i.e. the precipitate are not loosely bound molecular material like gelatin so the rate of water absorbance was low while as the gelatin was loosely bound so it could absorb and retain more water.

CMG646 was grown in 50ml broth to produce comparatively more amount of bioabsorbent (gelatin). This bioabsorbent was similarly checked for the water absorbance capacity it was observed that from 50ml of broth approximately 6.365gms of bioabsorbent was produced and on drying it gave 0.13gm hence a loss of 6.235gms of water i.e. 47 times more then its own weight (Fig. **2**).

CONCLUSION

CMG646 produces a water absorbing polysaccharide that can absorb water up to 47 times more then its own volume so it is suggested that Bioabsorbent polymer from CMG646 can be used for industrial and commercial purposes in future if it is produced in bulk amount.

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