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Spectroscopic characterization of extracellular polymeric substances (EPS) extracted from *Pseudomonas putida*ATCC 11172 produced under different carbon concentrations

Abubaker K. Elayatt[1],Abdelqader Imragaa[2]

[1]A(*Chemical Engineering Department Faculty of Engineering, the University of Sirte P.O. Box 674 Sirte - Libya),*
[2]University of Benghazi, faculty of science, Department of Chemistry

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Corresponding author :
abuknish@hotmail.com

ABSTRACT

The extracellular polymeric substances (EPS) extracted from *Pseudomonas putida*
ATCC 11172, were used as model to quantify the effect of carbon concentration on the chemical composition of EPS.*P. putida* was grown using Luria broth (LB) medium with and without glucose in order to promote different EPS composition. The influence of glucose amount was studied by supplementing the LB medium with glucose at 0.5% w/v (LBG 0.5%) and 1.0% w/v (LBG 1%). The produced EPS was characterized by Fourier transform infrared (FTIR) and X-ray photoelectron spectroscopy (XPS). FTIR were used to describe the difference in functional groups on free and bound EPS surfaces. XPS analysis was performed to quantify the element surface composition, to evaluate the local chemical environment of carbon and oxygen at the free and bound EPS, and to calculate the overall concentration of polysaccharides, peptide and hydrocarbon compounds of free and bound EPS. The combination of XPS and FTIR spectroscopy allows a more comprehensive characterization of free and bound EPS surfaces.

INTRUDOCTION

Extra cellular polymeric substances (EPS) secreted by bacteria are essential for bacteria community grow and survival. EPS are excreted by bacteria and accumulate outside the bacterial cell [1]. These substances form a protective layer for the cell against the harsh external environment and also serve as carbon and energy reserves during starvation [2]. There are two types of EPS found in flocs or biofilm: free and bound EPS. The free EPS include microbial produced soluble polymers with no direct contact with the cells, whereas the bound EPS is closely associated with the cells [3]. To classify and differentiate the two types of EPS a theory is suggested based on metabolism of bacterial cells and electron flux [4]. EPS are mainly responsible for the structural and functional integrity of biofilms and are considered as the key components that determine the physicochemical and biological properties of biofilms.The main possible purposes that have been suggested for EPS are adhesion to surfaces. This seems to be a primal mechanism of aggregation of bacterial cells, function of flocs and biofilms, protection barrier, retention of water, sorption of exogenous organic compounds and sorption of organic compounds [5]. The importance of EPS in the environment is well known. There is great interest in a wide range of applications. e.g. protection of drinking water supply from bacterial contamination; bioremediation of oil-contaminated environments; riverbank filtration; in situ bioremediation of contaminated soil; in wastewater treatment; biotechnology used in the food and bioleaching fields due to their wide structural variety and biopharmaceutical industries [6].

Fourier-transform infrared spectroscopy (FT-IR) is a perfect tool to characterize the very complex chemical composition of microorganisms. The technique has been applied successfully in various fields of quality control and identification of bacteria and yeasts [7,8]. It has also been used to characterize the effect on chemical cell wall composition due to changes in nutrient conditions during the formation of microbial biofilms [9] and study changes in the surface reactivity of bacterial cells intact EPS or stripped of EPS and

purified EPS [10]. IR spectroscopy has been used to identify microorganisms based on the observation that different bacteria display different IR spectra. In recent years, FT-IR spectroscopy has been shown to be a powerful technique for the study of biological macro-molecules[11,12,13,14]. And rapid, simple and cost efficient identification of bacteria [15]. Eboigbodin et al [16] investigated the changes of bacterial extracellular composition using different growth media at different growth phase. This study showed that the IR spectra are different indicating variations in surface functional groups and outer membrane proteins of microbial cells. Jiao et al [17] examined the chemical composition of EPS extracted from two natural microbial pellicle biofilms growing on acid mine drainage. The FTIR spectra showed the presence of carbohydrates, proteins and minor quantities of DNA and lipids, although the relative concentrations of these components were different depending on their origin. ATR-FTIR was used to investigate the chemical interactions between *Pseudomonas putida* and hematite. ATR-FTIR spectra of bacteria growing on hematite showed a shift in the carboxylate signal when compared to the samples obtained from free cell [18]. XPS is frequently employed to determine element composition (C, O, and N molar ratio) and associated functionalities of biomolecules on cell surfaces and interactions of macromolecules of biological origin with range of surfaces [12, 14, 19, 20, 21, 22]. XPS and FT-IR combined to comprehensively characterize EPS functionalities, protein secondary structures, and element and chemical composition [23, 24]. We present here the characterization of EPS extracted from *Pseudomonas putida*ATCC 11172 grown under different carbon concentration using FT-IR and XPS. The influence of carbon source on the abundance of surface functional groups and the implications on bacteria attachment are also discussed.

MATERIALS AND METHODS

Preparation of Bacterial Inoculum

An inoculum of *P. putida*ATCC11172 was prepared from overnight culture cultivated in LB medium containing different concentrations of 0.5% w/v and 1.0% w/v glucose. Cells were washed three times with 0.9% NaCl, by centrifugation at 4000 g (Hermle Z 300 K centrifuge, HERMLE Labortechnik, Germany) for 10 minutes and re-suspended using 50 mL plastic tubes (Sarstedt, Germany). The inoculum was adjusted to OD 600nm 0.5 and 1 mL of inoculum was applied per 100 mL LB medium in a 500 mL conical flask. In addition, a working stock of *P. putida ATCC* 11172 was maintained in nutrient agar plates at 4ºC. However, long-term maintenance of bacteria was performed by cultivating *P. putida ATCC* 11172 overnight on LB medium containing different concentrations of glucose at 30ºC. 1 mL aliquots were distributed into 1.5 mL microtubes (Sarstedt AG & Co, Nümbrecht, Germany) in sterile conditions and stored at -20ºC.

Extractionandpurificationof EPS

Free and bound EPS was extracted using the method showed by Elayatt A. and Romero-Gonzalez ME [6], where they used ethylene diamine tetra acetic acid (EDTA) to precipitate EPS. Centrifugation 12,000 rpm at 4^0C for 10 minutes was used to separate cells from their solution. The cell pellets were transferred to 10 ml double-distilled water in 25ml tubes, followed by the addition of EDTA at 4°C, the extraction time was 3 hours (2% EDTA produced approximately 3.2 g g−1 dry cells). The supernatant was then recovered after filtration through a 0.22µm cellulose membrane filter. To remove residual cells the supernatant was centrifuged at 12000g for 30 minutes at 4°C. The “free EPS” (from supernatant) were precipitated by adding three volumes of cold reagent-grade ethanol, and stored at -20°C for 18 hours. Crude EPS was collected by centrifugation at 12000 g for 30 minutes at 4°C. To remove ethanol and entrained media residue, the pellet was dissolved in ultra-pure water dialyzed against the ultra-pure water using regenerated cellulose membranes (3500 MWCO). Dialysis was carried out for 72 hours with two changes of ultra-pure water per day then the dialysed EPS was freeze-dried for storage.

Fourier transform infrared spectroscopy (FTIR)

Free and bound EPS extracted from *Pseudomonas putida*ATCC 11172 at stationary phase and different medium growth (LB, LBG 0.5% and LBG 1%) were characterized using a Perkin Elmer Spectrum One Fourier Transformation Infrared Spectrometer (Perkin Elmer, UK). An aliquote of twenty microlitres of EPS was allowed to dry on a demountable liquid-cell kit of CaF_2 at room temperature for 45 minutes (Sigma, UK). The FTIR spectrum was collected from 4000 cm^{-1} to 400. Each sample was analysed by collecting 100 scans at a resolution of 4cm^{-1}. Background spectra of

water and of the CaF_2 windows were collected prior to measurement of EPS samples.

X-ray Photoelectronic spectroscopy (XPS)

XPS measurements were made on a KRATOS AXIS 156 ultra photoelectron spectrometer at 10 kv and 20 mA using Al Kα X-ray source (1486.6 eV). The take off angle was fixed at 90º, where the angle is known between the substrate and the detector. The data was collected from three randomly selected locations, and the area corresponding to each acquisition was 400 µm in diameter. All experiments were performed in triplicate. C (1s) peak (284.6 eV) was used as calibrated peak for binding energy. Analysis consisted of a broad survey scan (20.0 eV pass energy). The high-resolution scan for major elements composition was (80.0 eV pass energy). To fit the XPS spectra peaks the software Casa XPS 2.3.12 was used.

Results and discussion

Yield of Extracellular Polymeric Substances (EPS)

The amount of EPS produced by *P. putida*ATCC 11172 depend on the amount of glucose added to the medium and due to the effect of the cells growth with an increase for glucose by decreasing the pH value, which in turn reduces the rate of yield of EPS. Figure 1 shows the yield of EPS depending on the carbon concentration added to LB media, where the amount of free EPS found was 120.39±2.20, 111.16±1.75 and 100.38±2.0 mg g-1 dry cell for LB, LBG 0.5 and LBG 1 respectively. The amount of bound EPS was 29.22±1.40, 21.18±0.90 and 17.10±1.10 mg g-1 dry cell for LB, LBG 0.5 and LBG 1 respectively. More details are discussed in our previous paper [6].

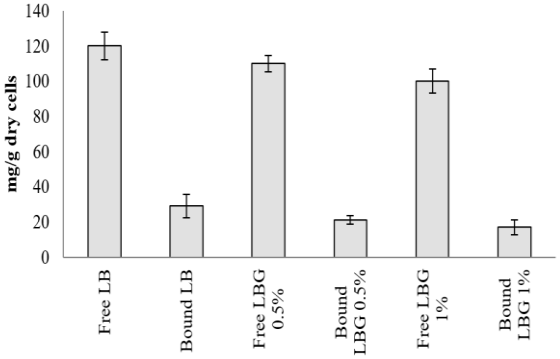


Figure 1: Yield of EPS (free and bound) at different media composition.

Fourier transformation infrared spectroscopy (FTIR)

Bound and free EPS extracted from *Pseudomonas putida* ATCC 11172 at stationary phase at different carbon concentrations (LB, LBG 0.5 and LBG 1) were characterized using FTIR spectroscopy. The spectra analyzed showed that the functional groups present are typical of bacteria EPS dominated by carbohydrate, protein and uronic acid. Figure 2 and 3 shows the FTIR spectra of free EPS and bound EPS respectively; extracted from *Pseudomonas putida*ATCC11172 at different carbon concentrations (LB, LBG 0.5 and LBG 1). The widest peak at 3400 cm^{-1} corresponds to the O-H bond in water [25]. The region between 3000 and 2800 cm^{-1} was assigned to fatty acids and lipids and exhibited the C-H stretching vibration corresponding to functional groups CH_3 and > CH_2[18].

Table 1. List of band assignments from FTIR analysis of EPS

Wave number (cm^{-1})	Function groups
~ 3400	Stretching vibration O-H of water
~ 2960	Asymmetric stretch C-H of methyl groups
~ 1740	ν>C=O stretching (esters) from membrane lipids and fatty acids
~ 1647	νC=O of amide associated with protein (amide I)
~ 1550	δN-H bending and C-N stretching in amide (amide II)
~ 1447	Bending $\delta_{ac}CH_2/\delta_{ac}CH_3$ possibly associated with protein
~ 1405	For deprotonated COO^- group, associated with amino acids
~ 1326	C-H stretches associated with amines and lipids
~ 1240	ν_sP=O asymmetric stretch of phosphodiester background of nucleic acids.
~1124	ν_sC-O-C of the glycosidic linkage
~1200 – 900	νC-O-C of polysaccharides
~ 1080	ν_{as}P=O symmetric stretch of the phosphodiester backbone of nucleic acids
~ 916	νC-O-C associated with phosphodiester

The absorbance observed at 1645 & 1550 $cm^{-1}$was attributed to amid I and II bands in protein respectively [26]. The band at 1045 cm^{-1} corresponds to the C-O bond in polysaccharides [27]. Focusing on the EPS fingerprint region between 1800cm^{-1} and 800 cm^{-1}, the peak at approximately 1740 cm^{-1}, appears in LBG0.5 and LBG 1 in free EPS, while it disappears in LB EPS, this is indicating a possible effect of the glucose in the concentration of carbonyl groups. Table 1, shows a list of absorption bands assignments corresponding to functional groups of these macromolecules in the region between 4000 and 400 cm^{-1}. contains similar information about functional groups arising from dominantly as listed in This is based on previous reports

[10, 14, 15, 21, 28, 29, 30] of the vibration patterns. Dominate peaks were observed in figures (2, 3) for full scan of FTIR spectra from 400 – 4000 cm^{-1} with widest peak at 3400 cm^{-1} corresponding to the O-H bond in water [25]. The region between 3000 and 2800 cm^{-1} presented in fatty acids and lipids exhibited the C-H stretching vibration corresponding to functional groups CH_3 and > CH_2[18]. The greatest absorbance was observed at 1645 & 1550 cm^{-1} which attributed to amid I and II bands in protein respectively [26]. The band at 1045 cm^{-1} corresponding to the C-O bond in polysaccharides [27].

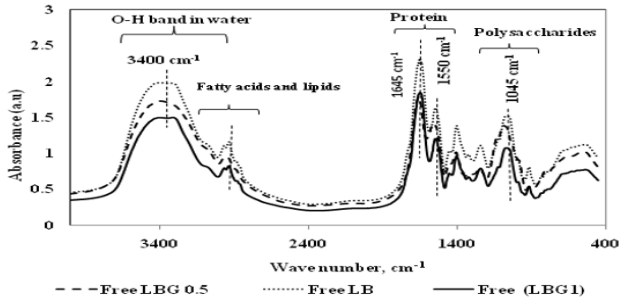


Figure 2: FTIR spectra of free EPS extracted from *P. Putida*ATCC11172 harvested during stationary phase from different carbon concentrations (LB, LBG 0.5, and LBG 1).

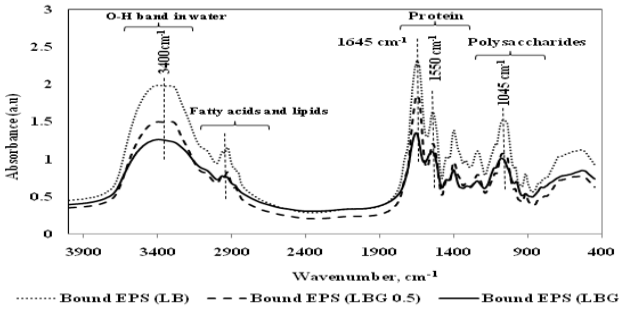


Figure 3: FTIR spectra of bound EPS extracted from *P. putida*ATCC11172 harvested during stationary phase from different carbon concentrations (LB, LBG 0.5, and LBG 1).

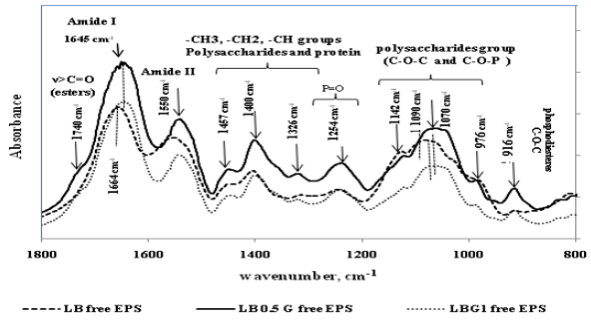
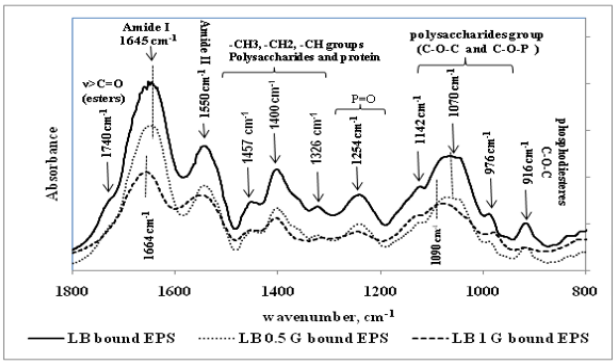


Figure 4: FTIR spectra of free EPS extracted from Pseudomonas putidaATCC11172 harvested during stationary phase from different carbon concentrations (LB, LBG 0.5, and LBG1).

Figure (4) for the free EPS extracted from *Pseudomonas putida*ATCC11172 at different carbon concentrations demonstrate a difference in spectra intensity. The polysaccharides group appeared at bands between 1200 – 900 cm^{-1}, free LBG 1 EPS and LBG 0.5 EPS are relatively low intensity as compared to free LB EPS. The peak at approximately 1740 cm^{-1}, appears in LBG0.5 and LBG 1 in free EPS, while it disappears in LB EPS, this is indicating a possible effect of the



glucose in the concentration of carbonyl groups.

Figure 5: FTIR spectra of bound EPS extracted from *P. putida*ATCC11172harvested during stationary phase from different carbon concentrations (LB, LBG 0.5, and LBG 1).

Figure 5 for bound EPSs extracted from *Pseudomonas putida*ATCC11172 at different carbon concentrations (LB, LBG 0.5, and LBG 1) demonstrate a difference in spectra intensity. The polysaccharides group appearing at bands between 1800 – 900 cm^{-1} for bound (LBG 0.5) EPS and free (LBG1) EPS are relatively low as compared to bound (LB) EPS. Amide bands arising mainly from protein were intensity spectra relatively high for free LB EPS when compared to other two EPSs (LBG 0.5 and LBG 1). The major difference between bound EPS extracted from *Pseudomonas putida*ATCC11172 at different carbon concentrations (LB, LBG 0.5 and LBG 1) is intensity of a peak at ~ 1240 cm^{-1}, corresponding to the symmetry stretching P=O asymmetric stretch of phosphodiester background of nucleic acids; its primarily low in bound LBG0.5 and free LBG 1 EPS compared with bound LB EPS. The

intensity of a peak at ~ 1326 $cm^{-1}$is resultant to the bending of C-H stretches associated with amines and lipids; this peak is more dominant in bound LB. The peak at 916 cm^{-1} corresponding to the C-O-C associated with phosphodiesters is disappearing in LBG 1 EPS. At the peak 1645 cm^{-1} (Amide I) no shift occurred in LB and LBG 0.5 EPS and some shift takes place in LBG 1 EPS to 1664 cm^{-1}. These shifts are mainly due to the difference of carbon concentrations that have direct effect on the composition of bound EPS.

Amide bands arising mainly from protein were intensity spectra relatively high for free LB EPS when compared to other two EPSs (LBG0.5 and LBG 1). The major difference between free EPS extracted at different medium growth (LB, LBG 0.5 and LBG 1) is intensity of a peak at ~ 1240 cm^{-1}, corresponding to the symmetry stretching P=O asymmetric stretch of phosphodiester background of nucleic acids; its primarily low in free LB0.5 and free LBG 1 EPS compared with free LB EPS. In addition, the difference in intensity of a peak at ~ 1326 $cm^{-1}$is resultant to the bending of C-H stretches associated with amines and lipids; this is mainly low in free LB and LBG 1 EPS. Moreover, the peak at 916 cm^{-1} corresponding to the C-O-C associated with phosphodiesters is low intensity in free LBG1 EPS and LBG 0.5 EPS. These insignificant changes in intensity of peaks and some shift of peaks, which occur, suggest different carbon concentration effects on the composition of free EPSs.

These results show that the content of bound and free extracted from *Pseudomonas putida*ATCC11172 at different carbon concentrations are controlled by proteins and carbohydrates. Both types of EPS (free and bound) which are extracted from *Pseudomonas putida*ATCC11172 at different carbon concentrations (LB, LBG 0.5, and LBG 1) show relative intensity of the peaks in the proteins and polysaccharides region. The amine I and II ratio was varied between all types of EPS; figures (4, 5) indicate that the difference in types of proteins gives a clear figure that the amount of proteins are more than polysaccharides in both EPS types.

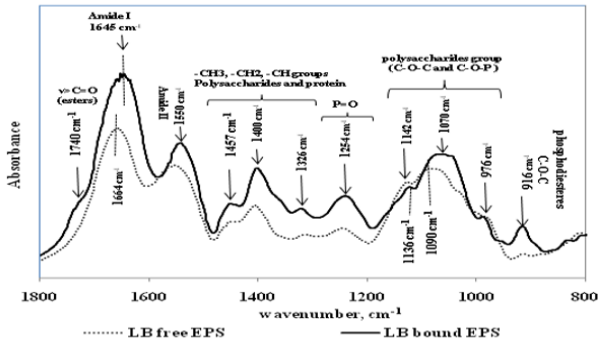


Figure 6: FTIR spectra of free and bound EPS extracted from *P. putida*ATCC11172 harvested during stationary phase from growth medium (LB).

Figure 6 showing free and bound EPS extracted from *Pseudomonas putida*ATCC11172 at (LB) demonstrates some difference in spectra. The peak at 1740 cm^{-1} in free EPS disappears. The peak at 916 $cm^{-1}$is more dominate in bound than free EPS. Also, in bound EPS some shift occurred in peak at 1645 $cm^{-1}$to 1661 cm^{-1} in free EPS, peak at 1136 cm^{-1} in bound EPS shifted to 1142 in free EPS, and peak at 1080cm^{-1} in bound EPS shifted to1890 cm^{-1}in free EPS. These changes suggest there is some difference in the composition of free and bound EPS.

Figure 7 of the free and bound EPS extracted from *Pseudomonas putida*ATCC11172 at (LBG 0.5) growth medium demonstrates no difference in spectra intensity. These changes suggest there is no difference in the composition and the amount of free and bound EPS.

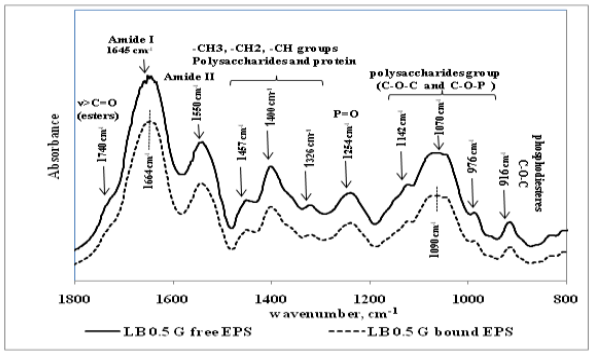


Figure 7: FTIR spectra of free and bound EPS extracted from *P. putida*ATCC111 72 harvested during stationary phase from growth medium (LBG 0.5).

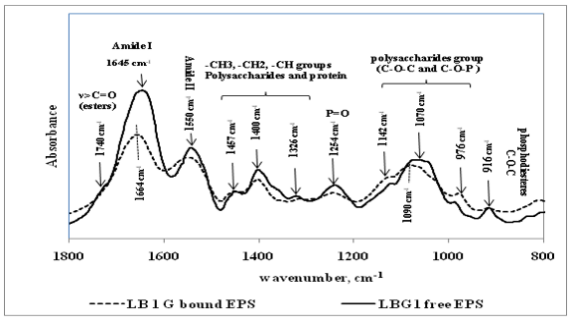


Figure 8: FTIR spectra of free and bound EPS extracted from *P. putida*ATCC11172 harvested during stationary phase from growth medium (LBG 1).

Figure 8 of the free and bound EPS extracted from *Pseudomonas putida*ATCC11172 at (LBG 1) medium growth demonstrates difference in spectra intensity. These insignificant changes in intensity of peaks suggest that the functional groups and the amount of free and bound EPS are nearly the same with difference in the amount of protein appearing in the peak in free (LBG 1) and with more intensity in bound (LBG 1). This maybe elucidates that the amount of protein in free (LBG 1) is more in bound (LBG1).

X-ray Photoelectronic spectroscopy (XPS)

The X-ray photoelectronic spectroscopy (XPS) wide scan figure 9 at different medium extracted from *P. putida*ATCC11172, high-resolution C 1s, O 1s spectra figure 10, 11 respectively, collected from free and bound EPS (free LBG 0.5 and Bound LBG 0.5). Different types of EPS (free and bound) extracted from growth medium supplemented by different carbon concentrations include comparable element composition (Table, II, III) it's included in file of supplementary information. EPS was mainly composed of C, O, N and P. To evaluate the amount of carbon in both types of EPS (free and bound) at different growth medium by high temperature combustion indicates around 36 - 37 % C free EPS and 36 - 44 % C for bound EPS. The overestimate of carbon in XPS attributable to adventitious hydrocarbon moieties [C(C, H)] is due to pre-exposure of the sample to the air and the XPS vacuum chamber [33]. Omoike et al [34], reported possibly that XPS, a surface sensitive technique, is detecting a higher carbon concentration at the surfaces, relative to bulk, of EPS molecules in the chamber**.** Rouxhet et al. [35] reported that the Cls peak can be decomposed into four components: (i) a component due to carbon, bound only to carbon and hydrogen (C-(C,H)); this peak is fixed at 284.8 eV (ii) a component due to carbon, creating a single bond with oxygen or nitrogen (C-(O.N)); this peak is at 286.3 $\pm$ 0.1 eV and including alcohol, ether, amine and amide (iii) a component due to carbon, making two bonds with oxygen (C=O); this peak is at 288.0 $\pm$ 0.1 eV, either two single bonds or one double bond, and including hemiacetal, acetal, amide, carboxylate and carbonyl (iv) a weak component or shoulder attributed to carboxyl O=C-OH near 289.0 eV.While the O ls peak can be decomposed into two components: (i) a component due to oxygen making a double bond with carbon (O=C) at 531.3 $\pm$ 0.2 eV, and including carboxylic acid, carboxylate, ester, carbonyl or amide (ii) a component attributed to alcohol (C-OH) at 532.6 eV, hemiacetal and acetal (C-O-C-O-C), which may include the hydroxyl of carboxylic acid, expected near 533.4 eV.

The fraction of carbon bound to oxygen or nitrogen, obtained from the decomposition of the carbon peak, can be related to the sum of atomic concentration ratios of oxygen and nitrogen with respect to carbon (O/C + N/C). A good correlation between these two independently obtained values indicates that nitrogen or oxygen is mainly bound to carbon in a 1:1 ratio [20, 35]. The agreement obtained from the (O/C + O/N) compared to C-(O,N)/C found in free EPS (Table 2) suggested that the functional groups on the free EPS corresponded mainly to amides, alcohols, amines, esters, or groups in which oxygen or nitrogen is bound to carbon in a 1:1 ratio. While in bound EPS the (O/C + O/N) compared to C-(O,N)/C (Table III and V, it's included in file of supplementary information) are in less agreement, found in bound EPS 0.86, 0.87, and 0.86 :1 for Bound LB, LBG0.5 and LBG1 respectively.

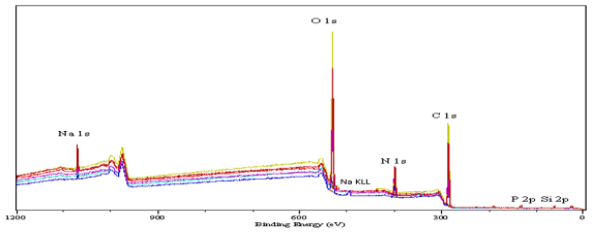


Figure 9: XPS wide survey scans of different types of EPS (free and bound) extracted from different bacterium growth medium.

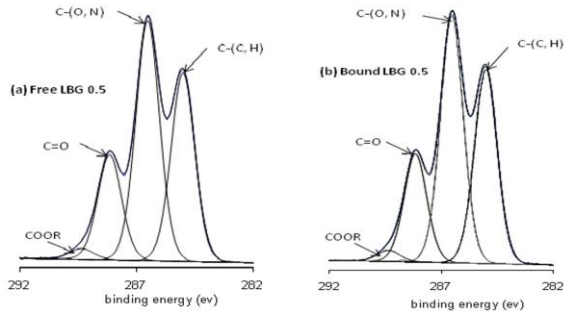


***Figure 10**: X-ray photoelectron high-resolution C 1s spectra of free and bound LBG 0.5 EPS.*

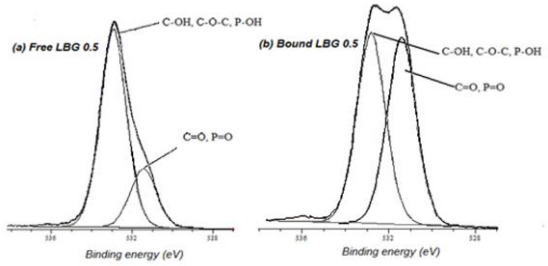


Figure 11: X-ray photoelectron high-resolution O 1s spectra of free and bound LBG EPS.

Table 2: O and N atomic ratios and functional groups with respect to total C obtained from high resolution of XPS of EPS extracted from different growth medium.

EPS	(O+N)/C	(C=O)/C + (O-C-O)/C	O/C + O/N	C-(O,N)/C
free LB	0.71	0.84	0.71	0.71
Bound LB	0.61	0.63	0.61	0.71
free LBG 0.5	0.65	0.76	0.65	0.67
Bound LB 0.5	0.61	0.65	0.61	0.70
free LBG 1	0.70	0.80	0.70	0.72
Bound LBG1	0.62	0.59	0.62	0.72

For amide functions (O=C-N), when comparing the atomic concentration ratio of nitrogen with respect to carbon (N/C) and the oxygen doubly bounded to carbon (O=C)/C, there was large excess of C=O with respect to N/C, this is indicating there are presences of other C=O groups different from amides. Such as, the oxygen double bounded with carbon (O=C)/C was what could be attributed to amide, thus indicating the presence of other function groups. The result combined with the FTIR spectra (bands at 1739, 1725 and 1402 cm^{-1} for carboxylic acid, ester, and carboxylate anions)

Molecular Composition

XPS analysis been used to estimate the concentration of the main ingredients of the EPS, concentration of total protein (C_{PR}), polysaccharides (C_{PS}), uronic acid (C_{UA}) and hydrocarbon-like compounds (C_{HC}) with respect to total carbon. A simple approach to compute the molecular composition of the bacteria surfaces was made by Rouxhet et al [35], and also applied by many other researches [14,36,37,38]. These calculations are dependent on comparing the measured concentration ratios of N/C, O/C and P/C with the carbon concentration and the atomic concentration ratio of nitrogen and oxygen with respect to carbon. Using C_{PR} = 3.57 (N/C); ≈ $C_{289.2}$ represented uronic acids; C_{PS} = $C_{286.2}$ – (N/C) - C_{AU} and C_{HC} = 1-C_{PS} - C_{PR} - C_{UA}. The results of these calculations summarized in (Table III and IV included in supplementary information file). These results confirmed calorimetric results at our previous study [6].

CONCLUSIONS

Under the EPS type (free and bound) and difference of medium conditions employed in the present study, FTIR confirmed the presence of polysaccharides and protein in EPS (Fig. 2 and 3). Moreover, in the functional groups, it is most likely that the carboxyl groups are due to the polysaccharide component of EPS. Phosphoryl groups are likely to be present on sugars and nucleic acids. Amino groups are most likely due to presence of protein in EPS. Previous studies of EPS have found the most common functional groups to be carboxyl, phosphoryl and amine groups [2, 14,39,40,41]. FTIR have shown that EPS of the same strain can change the nutrients surrounding it [16]. Slight difference in EPS composition at the molecular level observed between

free and bound (uronic acid and carboxyl groups were predominant in the bound EPS). The peak at approximately 1740 cm^{-1}, appears in LBG0.5 and LBG 1 in free and bound EPS while it disappears in LB EPS and there are shifts in peaks at 1645 $cm^{-1}$and 1080 cm^{-1} in both types; these are indicating a possible effect of the glucose in the structures of free and bound EPS.

The calculated molecular composition of the EPS extracted from *P. putida* ATCC 11172 at different medium growth as analyzed by XPS was as follows: uronic acids (2.0 – 2.8%) and (1.5 – 2.3%) for bound and free EPS respectively. The amount of proteins were found more in free than bound EPS, and the ratio (59.3 - 68.7%) and (36.0 – 48.9%) for free and bound respectively. Polysaccharides were (20.2 – 21.7%) and (27.2 – 33.9%) for bound and free EPS respectively. The hydrocarbons-like compounds for free and bound EPS were (9.6 – 17.5%), (21.5 – 27.3%) respectively. These results are confirmed as the results obtained from colorimetric experiments except that the hydrocarbons-like compounds were not performance with colorimetric technique. XPS spectroscopy technique can be used to detect the effect of the change for glucose by analysing the chemical composition of EPS in terms of elemental and, to a certain extent, of functional group and molecular compositions.

SUPPLEMENTARY MATERIAL

Details about sample collection and preparation are available electronically at the pages of journal website: xxxxxxxxxx, or from the corresponding author on request.

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