Isolation and characterization of Roseomonas mucosa from different clinical specimens.

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Abstract

Objective: This study is focused on isolation and identification of *Rosomonas mucosa* from different clinical specimens and extracted and partial purification of its pigment.

Methods: In this study, different specimens were collected from different organs such as skin, teeth root canal, urine catheter swab and blood from Al-Yarmouk hospital and some specific clinics, through a period from October 2019 till February 2020. The collected specimens were used to isolate the *Roseomonas mucosa* specifically. The bacterial colonies found in different specimens collected were isolated using blood and luria bertani media agar. Then the selected specific isolate is used for pigment extraction by ethanol. The potential pigment was characterized by ultraviolet spectroscopy, Fourier transform infrared (FT-IR) and thin layer chromatography (TLC). All these techniques were used to analyze the functional group of the extracted pigments.

Results: Primary screening shows a suspected Roseomonase sp. are 53/173 specimens. Whereas secondary screening concluded only 3 isolates showed a characteristic phenotype of *Roseomonase* sp. The color of the bacterial colonies is pink, mucoid and almost runny texture. The isolated colonies were examined microscopically, *Roseomonas* sp. was appeared as coccobacilli that form mostly pairs and short chains and identified using biochemical tests. The pigment-producing bacterial isolate was selected and propagated using suitable culture media. The extracted pigment showed the maximum spectrophotometric absorbance at 595 nm and their functional groups were identified using FT-IR analysis, containing alcohol, alkenes, alkynes phenols, alkanes, and primary amines functional groups, in addition may be included halogen compound mainly iodine. TLC result of this experiment showed that, the suspected spot that referred to an extracted pigment which migrate as brown component in the TLC sheet with RF. equal 0.87.

Conclusion: Based on the recent results, *Roseomonas mucosa* may adhere different surfaces because of mucosal texture, and can be isolated locally and characterized using ordinary biochemical tests, and its pigment RF. closely like *R. gilardii* pigment.

Keywords: Pigment, *Roseomonas mucosa*, Extraction, Characterization.

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Introduction

The genus Roseomonas is pink-pigmented, oxidative, mucosal Gram-negative coccobacilli, which is mostly isolated from environmental specimens, such as soil, water, air and plants [1] Roseomonas species are infrequently but increasingly involved in human infectious diseases, about 40 reports in 2016 are described clinical infections caused by Roseomonas sp., leading to a rather complete indication about susceptibilities to infection, clinical demonstrations and treatments [2]. Infections in humans have mainly been recognized during healthcare-associated infections in patients with primary diseases and/or indwelling devices [3]. Indeed, beside *R. mucosa*, *R. gilardii* and *R. cervicalis*, the specimens that were initially described in humans [4]. A 24 new species have been described (2006-2016) in environmental specimens, mostly in technological water, soil, air, plants and on diverse surfaces associated to

humans [3]. *Roseomonas mucosa* was initially grouped with *Roseomonas gilardii*. However, due to the significant difference in genotype and phenotype, the isolated *R. mucosa* from a patient blood specimen in 2003 was re-established as a distinct species [5]. It is reported that *R. mucosa* can be isolated from water [3], skin [6] and teeth root canal [7]. Although *R. mucosa* shows low human pathogenicity, it can lead to systemic infection with underlying diseases or immunocompromised patients, including patients with infectious spondylitis, peritonitis with HIV, and acute lymphoblastic leukemia [8]. As well as some species of this genus seemed possible transmission by an arthropod through a healthy woman was reported to be infected by *R. gilardii* after being bitten by a spider [9] and from ticks in china [10]. Indra and his colleagues reported that Roseomonas sp. isolated from catheter-related infection (bacteremia) of dialysis patients [11]. Also in 2010, J. Dien et al., documented a case of catheter-related bacteremia related with *R. mucosa* isolated from an immunosuppressed pediatric patient with a case history of multiple occurrences of urinary tract infection and bacteremia [12]. The genus *Roseomonas* constitutes 15 valid species, including *R. aquatica, R. aerilata, R. cervicalis, R. gilardii, R. lacus, R. mucosa, R. terrae, R. stagni, R. vinacea, R. fauriae,* and other unnamed genomospecies [1].

Methods

Collection of specimens and bacterial isolates culture

A 173 clinical specimens were collected from different human sources such as skin, tooth root canal, urine catheter swab and blood from Al-Yarmouk hospital and some of specific clinics during a period from October 2019 till February 2020. These specimens were cultured on different media agar included; Blood, Brain heart infusion, Nutrient and Luria bertani (LB) (Himedia/India), plates are incubated at 37 °C for 48 hours. After incubation the colony was isolated and identified by Gram's staining, Colony morphology (size, shape, color, margins, opacity, consistency, and elevation) were done. The biochemical characteristics were determined by various biochemical tests included; oxidase, catalase, indole, methyl red, Voges–Proskauer, citrate utilization, urease, gelatin hydrolysis and some sugar fermentation test [1]. The pure characterized isolates were selected and a single colony was transferred to sterile Luria bertani broth with 20% glycerol and stored at freezing temperature for long term preservation.

Extraction of a pigment from pigment-producing bacterial isolate [13]

After the culture and incubation for 48 hr. agar plates were monitored for growing a pink pigmented production, increasing the intensity of pigment was observed when further maintaining of cultured plates for 10-14 days in refrigerator (4˚C). The pigment-producing bacterial isolate was harvested and collected the bacterial suspension. Centrifugation at 6000 rpm for 10 min. Then, the supernatants were discarded and the pellets were suspended in ethanol absolute. The mixture was vortex and the suspension was centrifuged at 6000 rpm for 10 min and the supernatant was collected. The ethanol washing and centrifugation were repeated till the pellet changes to colorless. All collected supernatants were filtered through Millipore filter 0.22µm. Ethanol evaporated at room temperature, and then dried pigment was collected for further uses. This method of extraction is considered as partial purification of bacterial pigment using organic solvent and Millipore filtration.

Characterization of extracted bacterial pigment

1- **Ultraviolet (UV) spectroscopy analysis of partial purified pigment**

Maximum absorption spectra of the obtained bacterial pigment were performed using UV spectrophotometer. UV spectrum analysis. The obtained bacterial pigment was suspended in ethanol absolute and measured the wavelength at a range 350 - 750 nm to find out the maximum absorption spectra *vs* ethanol absolute used as blank [14].

2- **Fourier-transform infrared (FT-IR) spectroscopy analysis [15]**

The pigment was analyzed using FT-IR spectroscopy. The IR spectra were collected using a Shimadzu spectrometer within the range of 4000–400 cm−1. The FT-IR spectroscopy is used to analyze the functional group of the extracted pigments.

3- **Thin Layer Chromatography [15]**

Partial purified pigment was analyzed by TLC with silica gel. A 20µl of pigment solution were loaded in a spot on TLC plates at 1.0 cm interval and then allowed to dry at room temperature. The

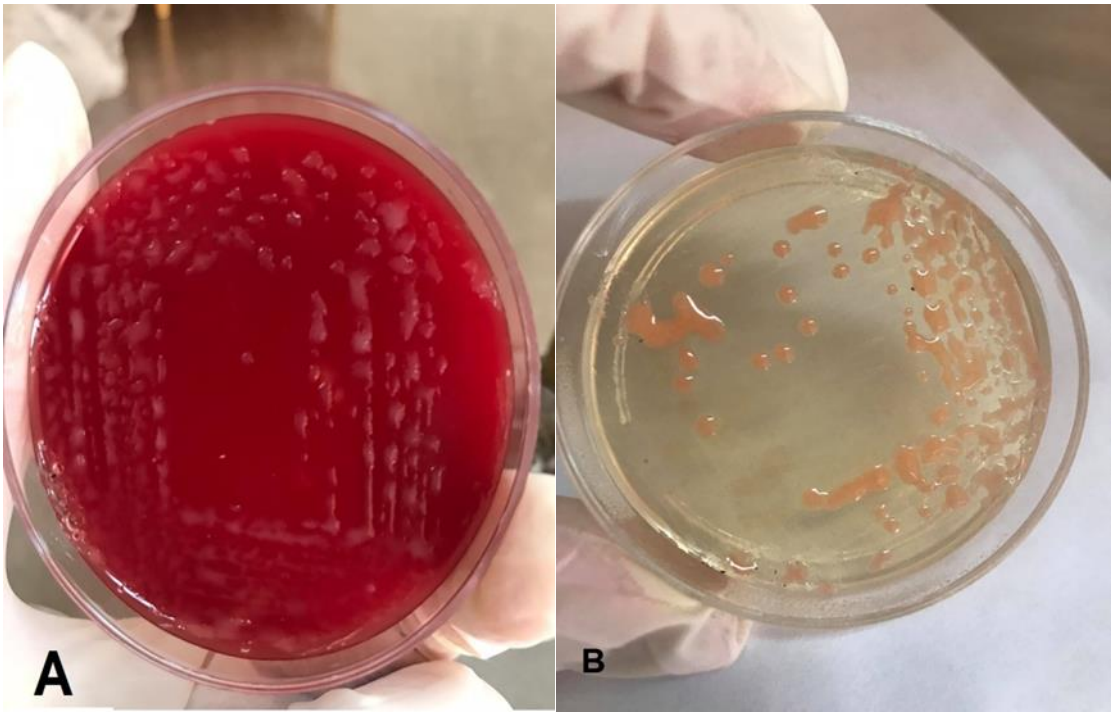
pigment sample applied on TLC plates was placed in a pre-saturated TLC chamber containing mobile phase (chloroform: methanol in the ratio 9:1; v/v). After suitable time, the silica gel plate was taken out and dried. After 45 min, the TLC sheet was carefully removed and under UV. Light, the Retention factor (Rf) value was calculated using the following formula.

Rf value = Distance travelled by the solute/ Distance travelled by the solvent.

Results and discussion

Isolation and identification of bacteria

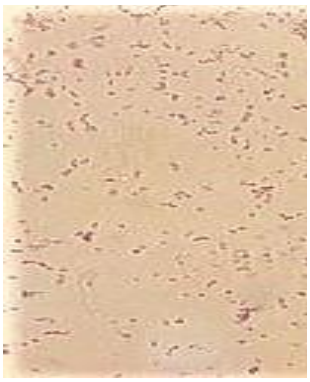
The most noticeable feature of the selected isolate colony was slight pink color, mucoid, almost runny, sometimes teardrop-shaped colonies, with large size and round. Luria bertani media elucidated as a suitable media for bacterial growing with big mucoid colony (fig.1). Primary screening shows a suspected Roseomonase sp. are 53/173 specimens. Secondary screening concluded only 3 isolates showed a characteristic phenotype of *Roseomonase* sp. as Rhis et al., (1993) documentation, the identification of *Roseomonase* sp. is done by using different biochemical tests to confirm the bacterial isolates are belonging to the genus *Roseomonas* (table-1). Moreover, the results of microscopic examination showed that the isolates were gram negative and coccobacilli that form mostly in pairs and short chains (fig.2**).** Biochemical tests are providing a fact that, these isolated bacteria are identified as *R. mucosa.*



(Figure-1): A- Colony morphology of Roseomonas sp. on blood agar incubated at 37°C for 24 hr. B- Colony morphology of Roseomonas sp. on Luria bertani agar incubated at 37° C for 48 hr.

(Table-1): Biochemical characteristics of bacterial isolates.

Biochemical characterization	
Catalase	positive
Oxidase	positive
Coagulase	positive
Urease	positive
Indole	negative



Voges–Proskauer	positive
Methyl red	negative
Citrate utilization	positive
Gelatin hydrolysis	negative
Starch hydrolysis	negative
Glucose	positive
Maltose	negative
Sucrose	positive
Lactose	negative
Mannitol	positive

(Figure-2): Illustrated the microscopic conformation of *R. mucosa* (100x).

All these *R. mucosa* are isolated from catheter of dialysis patients. Thomas et al., reported two cases of central venous catheter-related bacteremia associated with Roseomonas sp.[16]. As well as Kimura and his colleagues reported a case of *R. mucosa* bacteremia that involved a child, Japanese boy who was in a condition of pyretic neutropenia caused by chemotherapy[17]. Therefore, the recent result is another evidence improved that *R. mucosa* may have isolated from immunocompromised patients.

Extraction of bacterial pigment

Dependent on Rhis et al (1993), the bacterial pigment was extracted from pigment-producing bacterial isolate after 2 weeks of culturing and maintaining, the pigment yield was 4.12 g/ 25.33 g of bacterial pallets and the pigment is pink in color, figure 3. As well as this study improved that, ethanol as a good organic solvent for extraction and partial purification of intracellular pigment. This result is compatible with Vora et al. found that the intracellular pigment was extracted by various methods, but the addition of ethanol gets the cell lysed and intracellular pigment can be extracted [20]. Also Krishna and coworkers noted that**,** a pigment with hydrophobic nature so that maximum extraction of the pigment was extracted using different solvents such as ethanol, methanol, chloroform, ethyl acetate, petroleum ether, acetone, and distilled water which also have been considered to find the suitable solvent for effective extraction [21].

Figure-3: Partial purified pink pigment extracted from *R. mucosa* isolate.

UV–visible spectroscopy analysis of crude pigment [14]

Absorption spectra of pigment were carried out using a visible range between the wavelength range of 350 - 750 nm. The spectrophotometric analysis at the respective wavelength and the maximum absorbance (λ_{max}) was observed at 595 nm (Fig.3)

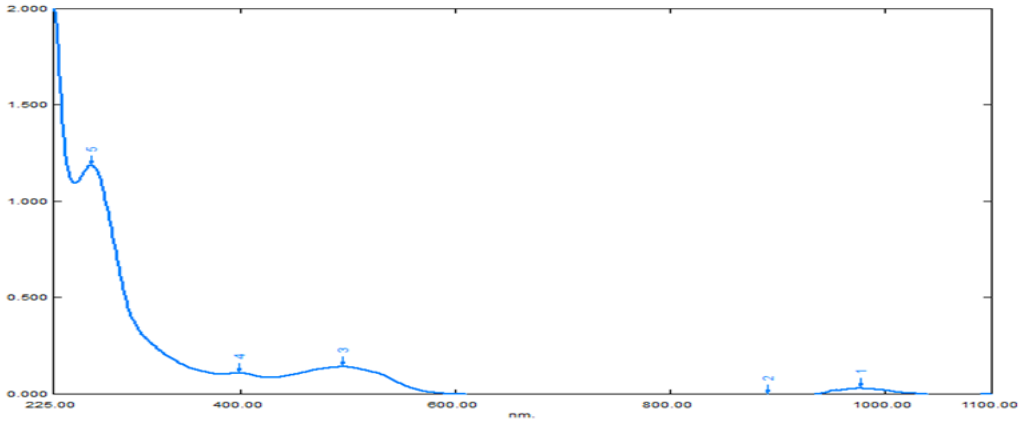


Figure-4: Ultraviolet spectra absorption of pigment.

According to the spectrophotometric analysis, the results of λ_{max} was improved that, the pigment is a pink in color, and there are 2 main peaks, these peaks explained the existence of double bonds may be one or more, in addition to a C= O in structure of extracted pigment. In earlier, *Roseomonas gilardii* producing pigment was observed in maximum spectrum that was 450 nm in UV-visible spectrometry [14]. Similarly, the prodigiosin pigment from *Serratia* sp. was showed the strong absorbance in the UV region [22].

FT-IR analysis of partial purified pigment [15]

The partial purified pigment was characterized by FT-IR spectrum. Based on the FT-IR spectra, the pigment had containing alcohol, alkenes, alkynes phenols, alkanes, and primary amines functional groups, in addition may be included halogen compound mainly iodine that make a pigment with pink color (Fig. 5). These results is nearly compatible with Siddharthan et al. (2020) documentation that, the pigment extracted from *R. gilardii* had containing the alkenes, phenols, carboxylic acids, alkanes, and primary amines functional groups. FT-IR analysis is in agreement with spectrophotometric analysis results with [14].

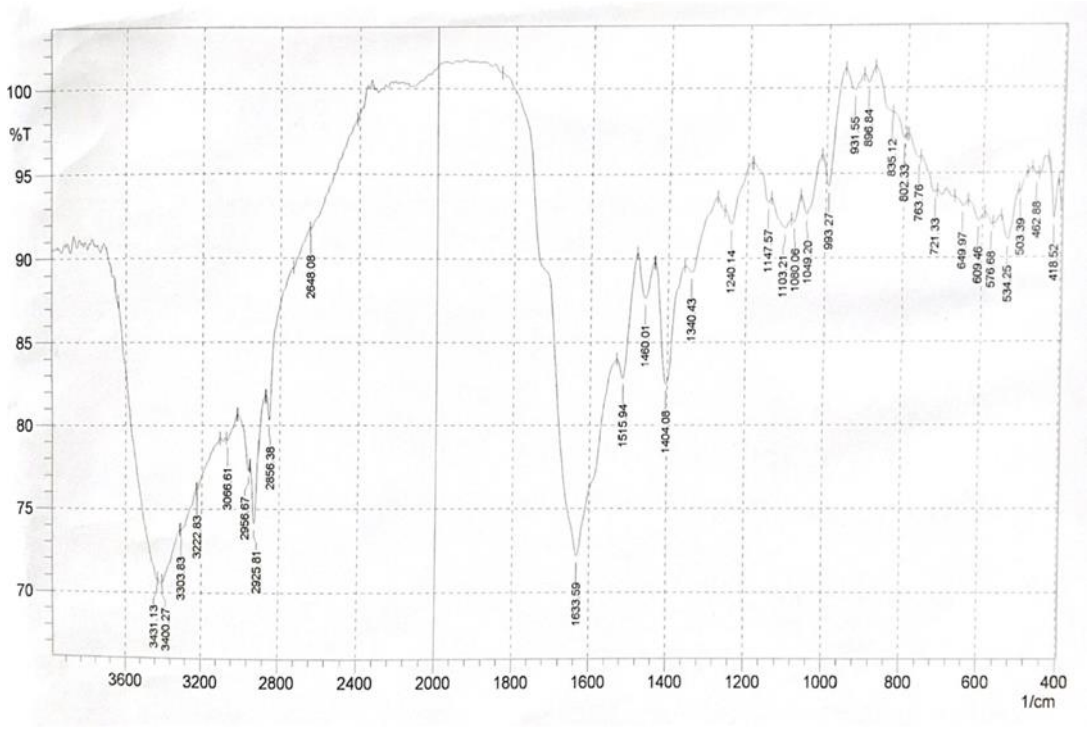
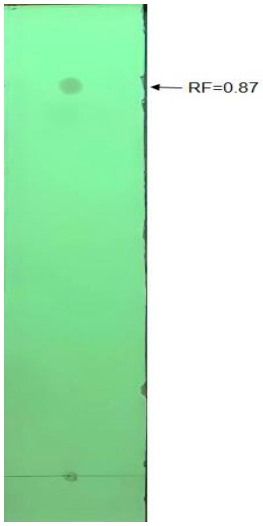


Figure-5: Fourier transforms infrared characterization of pigment.

TLC analysis of partial purified pigment [14]

Thin Layer Chromatography, is a technique for analyzing combinations by separating the compounds in the mixture. This technique can be used to help establish the number of constituents in a mixture, the identity of composites, and the purity of a compound. Through observing the attendance of a product or the disappearance of a reactant, also can be used to display the progress of a reaction. Thus, it is a sensitive technique - microgram quantities can be analyzed, and it needs little time for an analysis (about 5-10 minutes).

The partial purified pigment was separated by TLC silica-coated plate. The solvent system of chloroform: methanol (9:1) was used for the separation of pigment. The result of this experiment showed that, there is one component in the mixture of pigment solution is seen in the TLC plate, this compound according to RF value is 0.87. Dependent on a global literature [14], a pink pigment of *Roseomonas gilardii* was 0.82. Thus it can be suggesting that the suspected spot that referred to an extracted pigment which migrate as brown component in the TLC sheet with RF. equal 0.87 (fig.6). On another hand, some constituents may have such analogous polarities that they seem under one spot after expansion.



(Figure-6): TLC analysis of crude pigment.

The balance among different partners in TLC is depends upon (1) the polarity of the TLC plate, (2) the polarity of the progress solvent (may be diverse via different solvents), and (3) the polarity of the composites in the spot (variation depending upon what composites in the spot [23]. Therefore, pigment sample may consists one or more components, and this pigmented spot is less polar which tend to move along more freely with the solvent in spite of using a more polar development solvent *vis versa* the more polar will tend to adhere more tightly to the plate as Harry and Christopher documented [23].

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