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Research Paper:
Single-cell Oil Production Using Low-Cost Carbon Sources by Newly Isolated *Kocuria Y205*

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ABSTRACT

Background & Aims of the Study: This study aimed to investigate, for the first time, the ability of single-cell oil production from low-cost carbon sources using *Kocuria Y205* native Iranian bacterial isolates from the soil.

Materials and Methods: Whey and lignocellulose compounds were used as carbon sources and yeast extract as a nitrogen source. Also, the isolated cultivation was done on Mineral Salts Medium (MSM) culture media. Molecular analysis based on 16s rRNA gene sequence was performed to identify isolated. Fourier-Transform Infrared Spectroscopy (FTIR) analysis was used to confirm the presence of carbon groups. GC analysis was also used to identify the fatty acids Sudan black. Finally, a Transmission Electron Microscopy (TEM) electron microscopy image was taken to view the stored lipid granule.

Results: The highest rate of lipid production among all carbon sources in different periods of cultivation by whey was 24.57% after 48 hours, and also, the highest rate of lipid production for lignocellulose compounds was 15.29% after 48 hours.

Conclusion: This study shows that the newly isolated *Kocuria Y 205* has excellent ability to use whey and lignocellulose compounds as waste containing carbon resources for the growth, production, and storage of microbial oil. It can be used in industrial applications, too.

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1. Introduction

Microbial oils are called single-cell oils [1] as oleaginous microorganisms produce them. Owing to their ability and specific characteristics, these microorganisms have attracted many researchers in the last few decades. Traditionally, microorganisms, including bacteria, yeast, mold, and microalgae that are capable of storing lipid greater than 20% of their dry weight, are considered oleaginous microorganisms [2]. Microbial oil production does not require land or other resources to produce food and is not affected by weather or seasons [3]. Lipid storage occurs when microorganisms are grown in an environment with excess carbon, which reduces the use of other nutrients, especially nitrogen. Therefore, the ratio of Carbon to Nitrogen (C/N) plays a vital role in stimulating lipid storage [4, 5].

In recent years, attention has been focused on bacteria for lipid production in biotechnology and industrial applications. Bacterial lipids include Triacylglycerol (TAG-long chain fatty acid) and Wax esters (WE-ester as long-chain primary fatty acid and long-chain primary alcohol). They are used to produce food additives, cosmetics, lubricants, chemical oils, candles, and biofuels [6, 7].

Most bacterial species can synthesize Polyhydroxyalkanoates (PHAs) as storage compounds [8, 9], while a few genera of bacteria can store triacylglycerol and wax ester [10]. The amount and structure of bacterial lipid compounds depend on several factors, including the bacterium itself, the carbon source structure, the time of cultivation, and the amount of carbon and nitrogen present in the culture medium [8, 11-13].

The triacylglycerol accumulation has been described by Actinomycetes, including Mycobacterium, Streptomyces, Acinetobacter, Nocardia, and Rhodococcus [10]. Among the bacterial genera of triacylglycerol storage, Rhodococcus is one of the most promising, as some species store more than 20% of their biomass weight in triacylglycerol form and are considered oleaginous bacteria. Members of this genus are found in various natural environments, from arid and tropical soils to cold ecosystems and marine sediments [14-16]. Besides, Rhodococcus can produce and store triacylglycerol in several types of substrates under low nitrogen conditions. These conditions include specific carbon sources such as sugar, organic acids, and hydrocarbons [8, 17, 18], as well as complex carbon sources present in the industrial wastes that show remarkable bacterial versatility during substrate degradation [19, 20].

The genus Kocuria is a member of the Micrococcus family of Actinobacteria. Members of the genus cells are coccoid, Gram stain positive, non-encapsulated, immobilized, chemoorganotrophic, highly aerobic, mesophilic, and catalase-positive metabolisms. Polar lipids included diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. They are present in one species. The major fatty acids are $C_{15:0}$ anteiso (above 50%), C_{15} iso, and $C_{16:1}$; each contains 10% fatty acid. The percentage of Gas Chromatography (GC) is between 60% and 75% of total DNA [21].

Numerous studies have focused on searching for and finding inexpensive and alternative raw materials for use as a substrate for microbial lipid production. These materials can be found in agricultural, forestry, and food waste [20, 22, 23]. Whey is a waste from the dairy industry, which is produced in bulk worldwide (production of 1 kg of cheese results in 9 kg of whey). The whey consists mainly of lactose (5%-7%), with lower amounts of glucose, cactus, and protein (0.8%-1.2%) and lipid (3%-0.06%) [24]. Final disposal of whey is a significant problem for the dairy industry because it produces a significant amount of contamination in the environment, and its cleaning is costly [25]. Bioconversion of whey to valuable microbial oil is an attractive and effective method that can significantly reduce the environmental impacts created by the release of waste. It simultaneously results in the low-cost production of high lipid used biodiesel, biological lubricants, chemical oils, cosmetics, and other quantities of biofuels [26-28].

The agricultural waste consists of cellulose, hemicellulose, lignin, protein, and ash. Many agricultural wastes are composed of lignocellulose, a complex carbohydrate polymer of cellulose, hemicellulose, and lignin. The percentages of cellulose, hemicellulose, lignin, and other compounds in lignocellulose are in the ranges of 35%-50%, 20%-35%, 15%-20%, and 15%-20%, respectively [29]. One of the essential agricultural wastes is corn stalks, which refer to the stems, leaves, and stalks that remain on the field after harvest. Corn stalks are one of the first biomass resources used to produce cellulose ethanol in the United States. It has already been shown that corn stalks containing lignin can be converted to lipids with using Rhodococcus [30]. The lignocellulose biomass, consisting of lignin, hemicellulose, and cellulose, is an abundant, sustainable source for large-scale, low-cost production [19, 31]. The bioconversion of lignocellulose to bacterial lipid involves several steps: pretreatment of lignocellulose biomass, hydrolysis of carbohydrate structure to usable sugar, microbial lipid production, product isolation, and purification [1, 32, 33].

The primary purpose of this study is to evaluate the production capacity of microbial oil from *Kocuria Y205* isolate to produce microbial lipid from cheap raw materials available in industrial and agricultural compounds that can be used in industrial applications for the first time.

2. Materials and Methods

Sample collection and culture medium

Soil samples were collected from agricultural fields around the city of Qom (Iran) and stored at ambient temperature until the experiment begins. Wheat stems were prepared as lignocellulose compounds from agricultural fields around Qom. Whey was produced as industrial waste from the dairy industrial Qom.

To investigate lipid production in bacteria from MSM synthetic medium containing glucose (40 g/L), we used SO_4 $(NH4)_2$ (2 g/L), KH_2PO_4 (7 g/L), NaH_2PO_4 (2 g/L), $MgSO_4.7H_2O$ (1.5 g/L), and yeast extract (1 g/L) (MERCK-Germany) [34, 35].

Isolation of bacteria

Nutrient Agar (NA) and Tryptic Soy Agar (TSA) medium were used for bacterial isolation. Serial dilutions (10^{-6} dilutions) of soil samples were performed aseptically. Aliquots of 0.1 M suspension were made from pure 10^{-4}, 10^{-5}, and 10-6 dilutions, respectively. The cultures were purified, and the media were incubated at 37°C. Purified cultures were obtained from the isolated bacteria colonies, and the isolates were obtained for subsequent steps [36].

Morphological and biochemical tests of isolates

Purified isolates were characterized by biochemical analysis using catalase tests, oxidase tests, urea tests, H_2S, and anaerobic growth tests (according to Bergey's Manual of Systematic Bacteriology). Morphological identification was performed with Gram staining, endospore staining, and motion test [36].

Primary evaluation of lipid production

The lipid production of isolates was evaluated by Sudan black staining. They were cultured in a TSA solid medium with 3% w/w glycerol as an additional carbon source; the plates were incubated for 7 days at 30˚C [37].

Preparation of seed cultures

After evaluation analysis by Sudan Black staining, the selected isolated was cultured in TSA broth agar medium for 24 hours in 250-mL flasks on a rotary shaker (150 rpm) at 30°C, and the growth was measured Optical Density (OD) at 600 nm wavelength with a spectrophotometer (CE-9500-England) [38].

Lipid accumulation experiments with the different carbon source

For cultivating with whey, first liquid pass the Whatman paper to remove suspended particles and then removed the microorganisms using sodium gluconate pH above 7.5, the solution was autoclaved, again it was passed through Whatman paper and added 50 mL solution to 100 mL flasks and then inoculate the prepared bacterium and culture on a rotary shaker 150 rpm at 30°C for 24, 48, 72, and 96 hours [38].

For the preparation of cultivating with lignocellulose compounds, the wheat stem crushed by 0.5 to 1 cm and boiled in a volume of 200 g on 1000 ml flask for 20 minutes, then removed the suspended particles with Whatman paper, the solution was autoclaved, Equal to the base medium added 50 mL solution to 100 mL flasks and then inoculate the prepared bacterium and culture on a rotary shaker 150 rpm at 30°C for 24, 48, 72, and 96 hours [39].

To produce lipids with pure carbon sources, the isolates were transferred to the MSM synthetic medium containing nitrogen. About 50 mL of this medium was cultured in 100-mL flasks on a rotary shaker at 150 rpm at 30°C for 24, 48, 72, and 96 hours. Using the same medium, glycerol with 3% (w/w) was used as a carbon source instead of glucose [40].

Lipid extraction method

We used the standard method of Fluch et al. (1957) to extract and calculate lipid weight [38]. To calculate the dry weight, we first centrifuge 10 mL of broth medium at 6000 rpm. Next, we extract the supernatant and then wash the precipitate 3 times with distilled water and dried at 80°C until constant weight (typically 24 h) [38].

Fourier Transform Infrared Spectroscopy (FTIR)

To confirm the presence of lipid in the samples, Fourier Transform Infrared Spectroscopy (FTIR) device analysis (Bruker-Tensor27-Germany) was performed according to the standard method with range spectrum analysis of the device from 400 cm^{-1} to 4000 cm^{-1} [37]. The experiment was conducted for all carbon sources.

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Gas Chromatography (GC) analysis

Gas chromatography [38] device analysis (Agilent-7890B-USA) was used to identify fatty acids. The experiment was performed according to the standard method and using an HP 5890 A GC equipped with an INNOWAX capillary column (30 m, 0.53 mm, 1 mm) and a flame ionization detector. The injection volume was 0.5 mL, and hydrogen was used as carrier gas (13 mL/min). A temperature program was used to efficiently separate the methyl esters (90°C for 5 min, temperature increase of 6°C/min, 220°C for 10 min). For quantitative analysis, tridecanoic acid was used as an internal standard [41]. GC analysis was performed on lignocellulose carbon source samples.

Transmission Electron Microscopy (TEM) analysis

Electron microscopy was used to observe the lipid granules in the isolate. Cells were washed, suspended in 0.1 M potassium phosphate buffer (pH 7.5), and fixed with glutaraldehyde for 24 hours. Then, the cells were washed with a solution of sucrose 0.32 M in phosphate buffer and embedded in low viscosity resin [42]. Electron imaging (Zeiss-EM900- Germany) was performed at Pasteur Institute of Iran.

Molecular identification based on 16S rRNA

Genomic DNA was extracted by boiling [43]. DNA extracted by NanoDrop was checked for accuracy and concentration [44]. Universal primers were used for the amplification of the 16s rRNA gene fragment with a length of 20 bp (total length 40 bp), 27F (Forward–5'-AGAGTTTGATCCTGGCTCAG-3'), and 1492R (Reverse-5'-GGGTTACCTTGTTACGACTT-3'). The rRNA amplification reaction (30 µL) consists of master mixes with DNA and primers. The PCR cycling conditions were as follows: an initial denaturation at 94˚C for 5 min, followed by 30 cycles of denaturation for 1 min at 94˚C, annealing for 1 min at 55˚C, extension for 2 min at 72˚C, and a final extension at 72˚C for 5 min [45]. The sequencing was performed at the Genetic Coding Institute (Tehran, Iran). The phylogenetic tree drawing was also plotted using the MAG7 software using the neighborhood attachment model with the highest similarity [46]. Sequence results were recorded on the NCBI site.

Statistical analysis

Significant differences between biological samples, cultivated on different carbon sources and different accumulation periods, were evaluated using a two-way Analysis of Variances (ANOVA) in SPSS (version 25). The significance level was set at less than 0.05.

3. Results

Isolation of bacteria

Seventeen bacteria were isolated from soil samples, and biochemical and morphological tests were performed on them. The results of these experiments can be seen for the selected *Kocuria Y205* isolate as follows, Gram-Staining, Catalase, Urea, Oxidase are Positive, and H2S, Anaerobic Growth, Endospore, Motion are Negative Respectively.

The bacteria Cells are coccus-shaped, Colonies on TSA agar are circular, smooth, and entire, orange in color. The Substrate mycelium and aerial mycelia are not observed, diffusible colored pigments are not produced on any tested media.

Lipid accumulation

After bacterial culture on TSA medium and addition of carbon source for 7 days, the isolates were tested using Sudan black. Then, the isolate with the ability to store lipid was selected to be evaluated. The microscopic examination images can be seen in Figure 1.

Cultivation in different carbon sources

The performance of the *Kocuria Y205* isolate at 4 different carbon sources indicates higher lipid production in the glycerol carbon source than glucose in pure carbon sources and higher whey lipid production than lignocellulose compounds in the waste carbon sources. The results of which are visible in Table 1.

Comparing lipid production by an isolate from all carbon source

Comparing lipid percentage in isolates indicates higher production in whey than all carbon sources (Figure 2).

Quantitative analysis of lipid production by FTIR spectrophotometry

FTIR analy sis was used in three different carbon sources to prove lipid production, and all the analyzed samples showed the presence of carbon and functional groups (lipids) and confirmed the presence of aliphatic carbon chains (lipids) in the samples. All carbon and hydrogen bonding results were found to be the precursor of the primary lipid structure and the methyl group, which

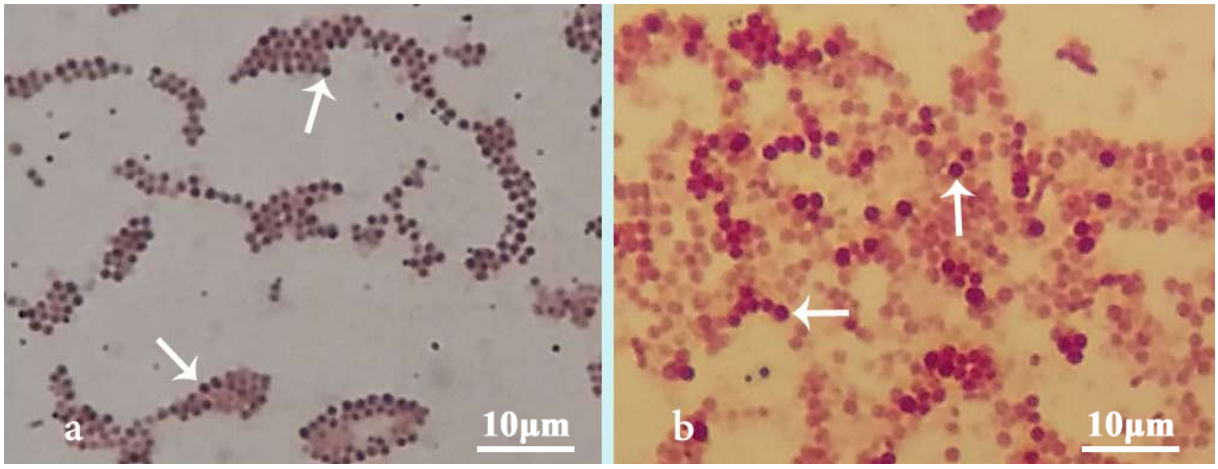

Figure 1. The results of the *Kocuria Y205* ability to produce lipid, the white arrows in the image represent the lipid granules stored in the isolate a) 7-day isolate culture and b) 7-day isolate culture with the addition of carbon source of glycerol

is a hydro phobic functional group and was observed from a methane molecule (CH_4) by removal of hydrogen and other lipid groups in the sample. These compounds include ca rbonyl groups, alkenes, alkyls, and glycerol. The above groups indicate unsaturated fatty acid in the samples. This finding confirms the presence of triacylglycerol in the composition obtained from lipolysis. The interpreta tion is presented in Table 2 for each carbon source, respectively, and microbial lipid graphs obtained from FTIR analysis are shown in Figure 3.

GC analysis

The lipid samples obtained from isolate culture on lignocellulose compounds for fatty acid identification were tested with GC. The results of which based on the percentage of carbon in the sample in Table 3 indicate the pres-

Table 1. *Kocuria Y205* isolate performance in different carbon sources based on mg/mL

No.	*Kocuria Y205*		24 hours	48 hours	72 hours	96 hours
	Carbon Source	Performance in mg/mL				
1	Whey	Dry weight	15.5	17.5	19.0	20.6
		Amount of lipid	3.5	4.3	4.6	5.0
		Lipid %	22.58	24.57	24.21	24.27
2	Lignocellulose compounds	Dry weight	12.0	17.0	24.0	30.0
		Amount of lipid	1.0	2.6	3.6	3.9
		Lipid %	8.3	15.29	15.0	13.0
3	Glucose	Dry weight	10.0	14.0	18.0	22.0
		Amount of lipid	0.7	1.6	2.2	2.5
		Lipid %	7.0	11.42	12.22	11.36
4	Glycerol	Dry weight	24.0	28.0	34.0	38.0
		Amount of lipid	3.8	4.1	4.7	5.1
		Lipid %	15.83	14.64	13.82	13.42

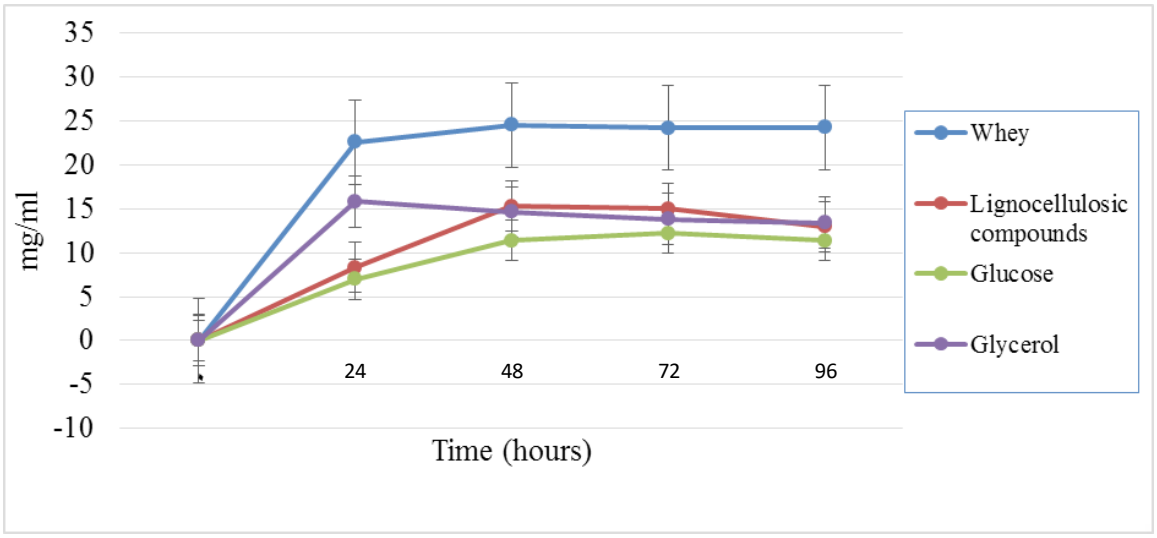

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Figure 2. Comparison chart of the lipid percentage of the isolate *Kocuria Y205* in different carbon sources

ence of fatty acid in the lipid composition obtained from isolate culture in lignocellulose compounds, which proves the conversion of lignocellulose compounds into lipid during the decrease of nitrogen by bacteria, the carbon structure of the fatty acid produced and investigated, 14 and 15-hydroxy pentadecyl glycerol, the carbon structure is 16 to 19-dihydroxyl glycerol, and 20 to 24 is orchols.

Transmission Electron Microscopy (TEM) analysis

Transmission Electron Microscopy (TEM) was used to observe the lipid storage granules. The images are shown in Figure 4. The storage granules of lipid produced in these images are seen in bright color.

Molecular analysis

The 16s rRNA sequence results showed more than 96% similarity with Kocuria sp. strain JSM 1684076 (accession number: MG893104.1) is found in the Gene Bank, demonstrating the close kinship of both strains. The native *Kocuria Y205* strain number MN818672.1 is registered at NCBI. The result of drawing a phylogeny tree is shown in Figure 5.

The evolutionary history is plotted using the neighborhood attachment method with the most similarity

4. Discussion

This study aimed to investigate the potential of a native and novel species of genus Kocuria isolate to produce single-cell oil from low-carbon sources. This study showed the ability of this strain to produce single-cell oil from pure carbon sources of glucose and glycerol as well as carbon sources of whey and lignocellulose compounds under low nitrogen conditions. Also, the amount of lipid produced in whey sources was higher than pure carbon sources. FTIR analysis was used to quantify single-cell oil production. The results of all samples showed the presence of carbon and functional groups, which meant the formation of lipids. In the sample, it was shown that transmission electron imaging was also used to view the storage granules, which is visible in the images of the storage granules. Carbon sources are the essential factor in determining the type of fatty acid produced in strains. The difference in the produced fatty acid in the samples is visible in the FTIR analysis of the samples of different carbon sources. Bacterial compatibility with early life environment and genetic variation and type of carbon affect the diversity of fatty acids.

Genus Kocuria is part of the family of Actinomycetes, and they are widely distributed in nature and can produce many biologically active substances [47]. Li Tuo et al. isolated Kocuria from the soil with the characteristics of being aerobic, Gram-stain positive, coccus-shaped, and approximately 1.1-1.8 μm in diameter, and pale yellow in color. Substrate mycelia and aerial mycelia were not observed; diffusible pigments were not produced on any tested media. They grow well on ISP 2 agar, ISP 4 agar, TSA, NA, LB agar, MA, and R2A agar. Growth occurs at 10˚C-37˚C (optimum 37˚C), pH 6.0-11.0 (pH 6.0-7.0) and with NaCl concentration of 0%-7% (w/w) (0%). No growth at 45˚C and no growth at pH 5.0. Cells reactions were positive for catalase, nitrate reduction and hydrolysis of gelatin, H_2S production, oxidase activity,

Table 2. Results of FTIR analysis with a different carbon source

No.	Carbon Source	Wave Number (cm^{-1})	Functional Group
1		3383.5	= C – H stretch
2		2924.52 - 2853.17	$-CH_3$ (Methyl groups)
3	Whey	1745.26	Carbonyl groups
4		1594.84 - 1421.28 - 1375.96	CH_2 binding
5		1117.55 - 1073.19 - 1035.59	C – O – C stretching in esters
6		3546. 45 - 3463.53 - 3415.31	= C – H stretch
7		2919.7 - 2848.35	$-CH_3$ (Methyl groups)
8	Lignocellulose compounds	1723.09 - 163.3 - 1614.13	Carbonyl groups
9		1447.31 - 1374. 03	CH_2 binding
10		1057.76	C – O – C stretching in esters
11		3450.23 - 3415.31	= C – H stretch
12		2934.78- 2839.78	$-CH_3$ (Methyl groups)
13	Glucose	1723.09 - 1636.3- 1614.13	Carbonyl groups
14		1447.31 - 1374.03	CH_2 binding
15		1667.76	C – O – C stretching in esters
16		3412.09	= C – H stretch
17		2950.13 – 2881.36	$-CH_3$ (Methyl groups)
18	Glycerol	1717.3 , 1631.48 , 1631.48	Carbonyl groups
19		1406.82	CH_2 binding
20		1226.5 , 1046.09	C – O – C stretching in esters

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urease, and methyl red test [48]. Hansda et al. isolated bacteria from dry tailing copper mines with the potential of metal resistance. The isolated bacteria were cocci shape, gram stain positive, pink and translucent in color, catalase positive, and H_2S production positive. Their morphological, physiological, biochemical, and molecular analysis characteristics showed that the isolated strain belonged to genus Kocuria [49].

The ability of lipid production and accumulation by *Kocuria Y205* was compared with Rhodococcus, the best known bacterial producer of single-cell oils [20]. To investigate microbial oil production, Ana Rito Castro et al., two different strains of *Rhodococcus opacus* on three carbon sources of glucose, acetate, and hexadegan and yeast and peptone extracts as nitrogen limiters cultivated, and both strains were able to store the highest amount of lipid within 72 hours. Thin Layer Chromatography (TLC) method was used to quantify the lipid production in the samples, indicating the presence of oil in the samples and qualitatively evaluating the oil produced by GC analysis. It was used to show triacylglycerol production in both strains that are closely related [50]. The results of the Ana Rito study and the results of the present study demonstrate the ability of the native *Kocuria Y205* isolate to store and produce triacylglycerol using low-cost sources.

Marcia and Alvarez investigated the production of microbial oil and biomass. Five strains of Rhodococcus were cultured on whey, and the results showed that *Rhodococcus opacus* produced more than 45% lipid and the

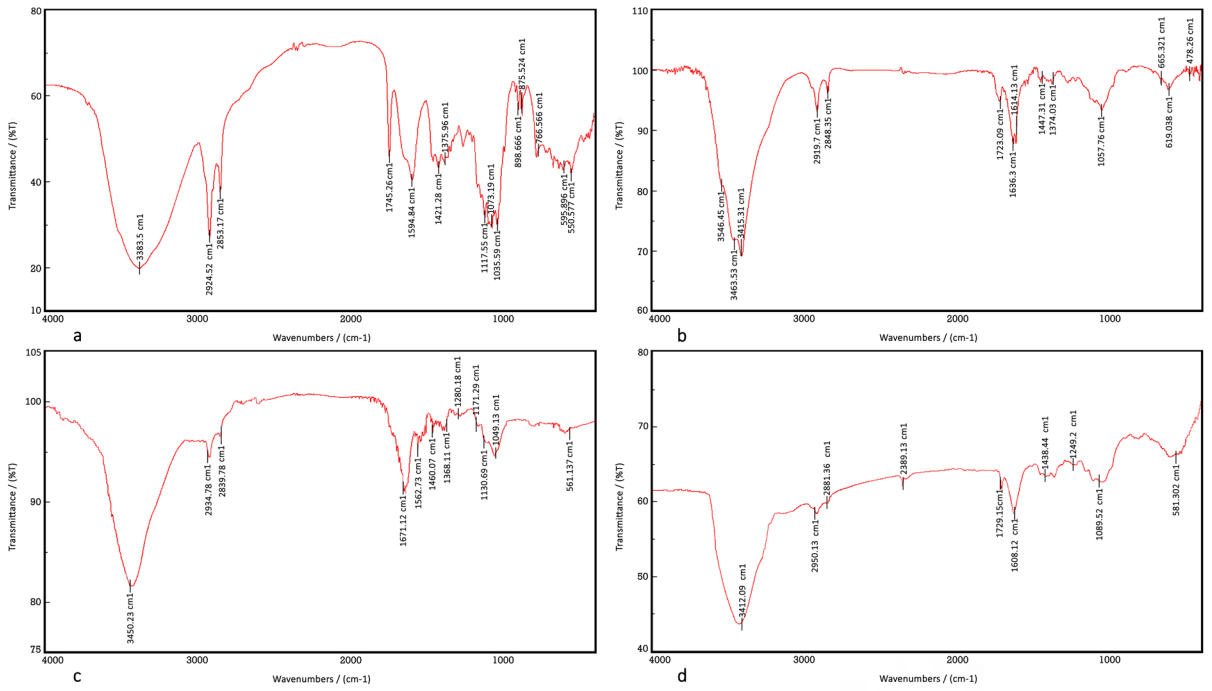

Figure 3. FTIR analysis image for the isolates of Kocuria Y205 in different carbon sources

A: The whey source; B: Lignocellulose compounds; C: Glucose; and D: Glycerol.

remaining bacteria produced less than 5% lipid [41]. The results of the Marcia and Alvarez study indicate that all Rhodococcus bacteria are not capable of using whey for lipid production, but in the present study, the native isolate of *Kocuria Y205* could produce the highest amount of lipid production from the whey substrate, indicating bacterial adaptation for lipid production from whey.

Herrero et al. used several Rhodococcus strains on lignocellulose olive waste. They could produce lipid with varying percentages, indicating the ability of these bacteria to convert this type of carbon source into lipid. However, examination of the produced lipid showed the formation of triacylglycerol [51]. Due to the similar structure of lignocellulose biomass, plant resources, including cellulose, lignin, and hemicellulose, have different percentages depending on the growing conditions and type of plant. Comparison of the present study and the study of olive plant waste showed the enzymatic ability of newly isolate *Kocuria Y205* to use lignocellulose compounds for lipid production.

Rasouli et al. examined microbial oil production from the genus *Rhodococcus erythropolis*, using MSM medium and glycerol, glucose, wheat straw, and whey, for the carbon sources, they could produce microbial oil under the same conditions. The FTIR test proved the production of oily groups and also used the GC test to examine the structure of the fatty acid produced [52]. In many studies, the genus Rhodococcus is a species suitable for microbial oil production. In this study, we could produce microbial oil for the first time with a new species using the bacterial isolate of *Kocuria Y205* with similar conditions in the above research and prove the production of carbon groups by FTIR test. Also, we tested the structure of the fatty acid produced by the GC test.

Table 3. The fatty acid composition produced during culture on lignocellulose compounds

Bacterial Isolate	The Total Amount of Fatty Acids	The Relative Percentage of Fatty Acids (%, w/w)										
		C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
Kocuria Y 205	15.29%	2.30	1.87	0.63	4.27	2.66	3	3.84	7.05	7.86	0.18	21.45

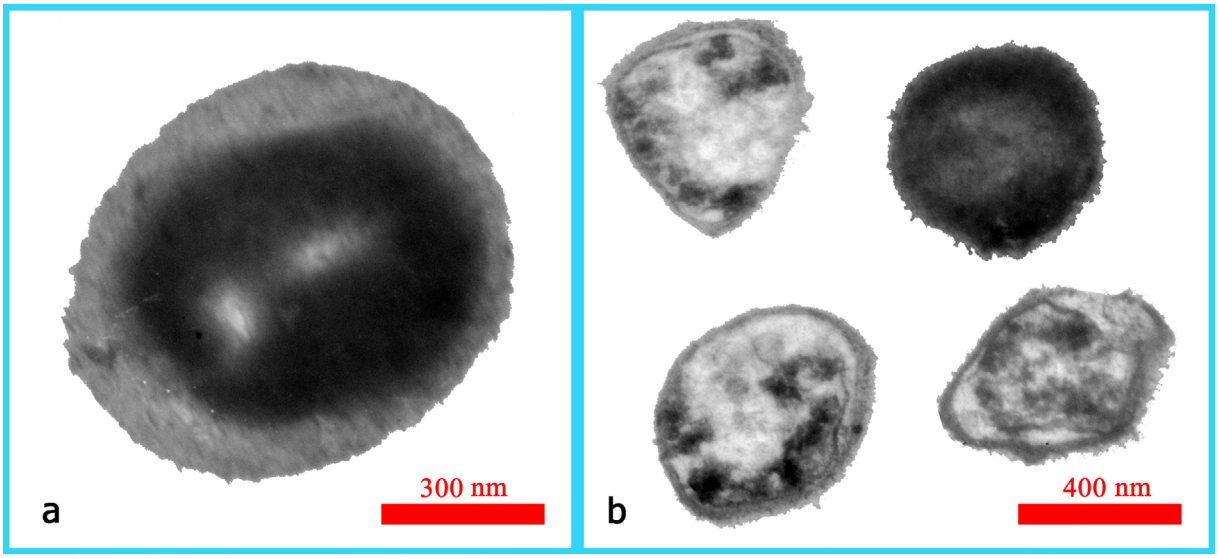

Figure 4. Electron image of the Kocuria Y205 strain cultured on TSA medium with glycerol as an additional carbon source, a) image with a magnification of 300 nm, and b) image with a magnification of 400 nm

5. Conclusion

Microbial oil has many applications, including biodiesel use as an environmentally friendly alternative to organic hydrocarbon sources. Microbial oil also can be used as dietary supplements due to the type of fatty acid produced in it and used as chemical oils in the pharmaceutical and cosmetic industries. The results of this study, the first kind on the country, showed that the native Iranian isolate *Kocuria Y205* which belongs to the family of Actinobacteria, Micrococcales, and Micrococcaceae, can use pure carbon sources and bio-conversion of industrial and agricultural waste as a cheap raw material for lipid production and in particular, it is used as a biodiesel. The results of this study will increase the theoretical knowledge about this bacterium and the production of microbial oil. The above bacterial isolate is available in the Microorganisms Storage Complex of Azad University of Qom with *Kocuria Y205* (accession number: MN818672.1) specifications.

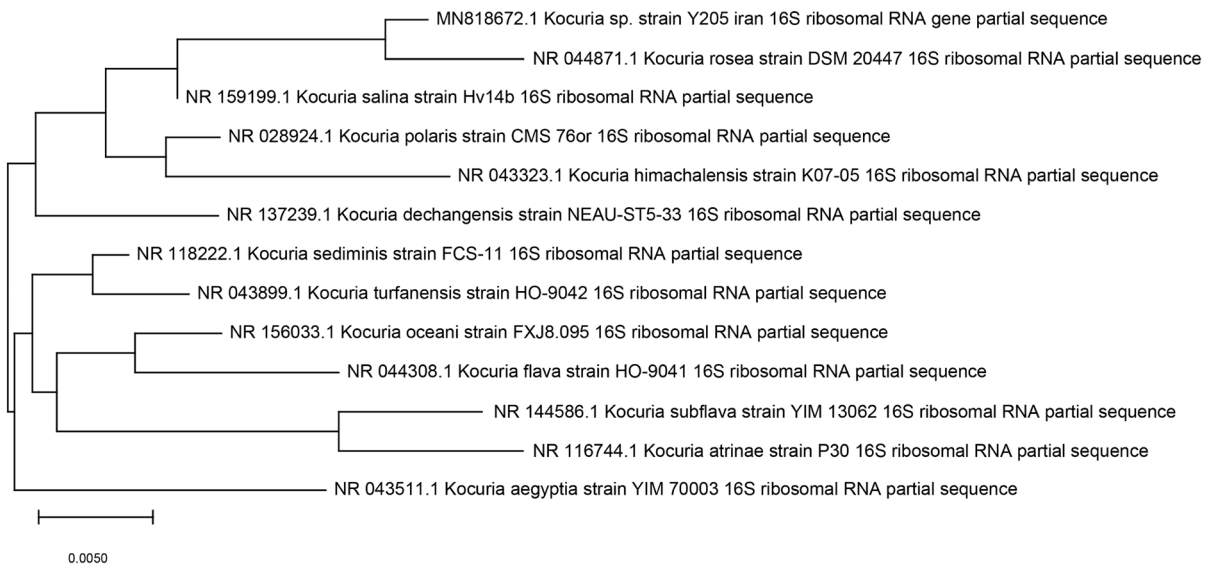

Figure 5. The result of drawing a phylogenetic tree with Mega 7 softwar

Ethical Considerations

Compliance with ethical guidelines

This article is a meta-analysis with no human or animal sample.

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Authors' contributions

Conceptualization, methodology, investigation, resources, project administration: Seyyed Soheil Aghaee and Alireza Rasouli; Software, validation, formal analysis, data curation, writing – original draft preparation, writing, visualization, supervision, project administration, and funding acquisition: Alireza Rasouli; Review & editing: All authors.

Conflict of interest

The authors declared no conflict of interest regarding the publication of the current article.

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