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Screening of lipid degrading microorganisms for wastewater treatment

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ABSTRACT

Aims: Fats, oils and greases (FOG) are poorly removable materials in wastewater treatment systems. The aim of this work is to find the most suitable strain(s) for a biological treatment technology of FOGs polluted wastewaters.
Methodology and results: The 142 microorganisms from polluted environment were screened for lipase activity (LA) by sequentially using assays on agar-Tween 80, agar- fats, and turbidimetrically measuring the quantity of calcium salts with fatty acids. The isolates G23, G30, and Zb32 showed highest units of LA and were identified by sequence analysis of 16S rRNA genes. Lipid masses were determined gravimetrically after chloroform/ethyl alcohol extraction. In the model solutions with animal fats the strain *Pseudomonas aeruginosa* G23 reduced mass fractions of mutton fat, beef tallow, and lard by 79±5%, 88±4%, and 80±6% respectively. Under the same conditions *Aeromonas punctata* G30 reduced: 65±3%, 60±8%, and 75±4%, and *P. aeruginosa* Zb32 reduced: 47±5%, 52±6% and 73±7%. In the model solutions with FOGs trap specimens as a carbon source from the local cafeteria the strains *P. aeruginosa* G23*, A. punctata* G30, *and P. aeruginosa* Zb32 reduced a lipid mass fraction by 61.5±7%, 45.2±5%, and 37.5±3% respectively.
Conclusion, significance and impact of study: The strain *P. aeruginosa* G23 is the most effective lipid-degrading microorganism and the best candidate to use in biological treatment technology of FOGs polluted wastewater in Kazakhstan.

Keywords: lipid degradation, microorganism screening, molecular identification, *P. aeruginosa* G23, wastewater treatment

INTRODUCTION

Fats, oils and greases (FOG) are poorly removable materials in local wastewaters, particularly at food, wool and leather products processing factories. FOGs are substances of difficult biological treatment at cleaning systems and generate technological and environmental problems (Fadile *et al*., 2011). Isolation and identification of new effective strains with high lipase activity, a study of the mechanisms of lipase synthesis and secretion is particularly relevant for several reasons. First, lipases play a key role in the metabolism of strains that destroy FOGs. Second, a poor performance of indigenous microorganisms in FOGs polluted environment could be effectively intensified by using bioaugmentation strategy (El Fantroussi *et al*., 2005) which needs additions of new effective strains. Third, some inoculated microorganisms in modern biotechnological preparations produced for biological treatment of FOGs wastes, are not resistant in open systems and require periodic additions of inocula to achieve a high performance treatment (Loperena *et al*., 2007).

Analysis of the literature and patent data in Kazakhstan showed that studies on the biological treatment of industrial wastewater are directed mainly to develop microbial agents for treatment of oil pollution (Mukasheva *et al.*, 2004), and arsenite (Abdrashitova *et al*., 1990). Sewage treatment of fat wastes is only a few studies (Rozvaga *et al*., 1996). Therefore, this work represents one of the few studies on the biological treatment technology of FOGs contaminated wastewaters in Kazakhstan.

The aim of this work is to find the most suitable strain(s) for a biological treatment technology of FOGs polluted wastewaters. To reach the goal we had to obtain a working collection of microorganism cultures, screen and identify new local microorganisms with high lipase activity from local wastewaters and soils polluted by FOGs, and test the most active strains in model solutions with animal fats and FOGs trap specimens from the local cafeteria.

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MATERIALS AND METHODS

Research objects

The 142 isolates of microorganisms were sampled from polluted ecological niches. Table 1 lists the sources of isolates for analysis and number of isolates.

Table 1: Objects of research.

Source of isolates	Number of isolates
Soil of dairy plants in Almaty and Astana cities.	33
Wastewater of slaughterhouse.	27
Wastewater of urban sewage system.	39
Active sludge in aeration tanks of wastewater treatment facilities.	43
Total	142

Isolation of pure microorganisms

Water samples of a volume 10 mL were collected at a distance of 10-15 cm from the bottom, under aseptic conditions into a sterile glass container with cotton-gauze plugs from a wastewater. Soil samples (10 g) were collected preserving the rules of asepsis, and ground in a porcelain mortar to pasty state, and moistened with sterile saline. The resulting suspension was diluted 10 times and plated on a nutrient agar surface of solid medium in Petri dishes [meat-peptone agar, wort agar medium, and Sabouraud agar (Himedia), India]. Sowing was carried out by a bacterial loop according to the spread-plate method. Plates were incubated at 37 °C for 3-5 days. Isolates of microorganisms on solid media formed convex or flat, shiny or rough colonies from whitish-gray to brown colors. The surface was smooth or wrinkled, with regular or irregular roundness. Edges of colonies were smooth, rough, or rugged. The size of the colonies ranged from 0.1 to 10.0 mm of homogeneous structures with soft, thick and slimy consistency. Individual colonies were purified by repeated streaking on agar medium. The purity of colonies of microorganisms was monitored by microscopy. All isolates were stored at −20 °C in liquid cultures containing 15% glycerol (v/v).

Lipase activity on solid medium with Tween-80

The medium consisted of (g/L): 5 mL/L Tween-80 (Sigma-Aldrich), 10 g/L peptone, 5 g/L NaCl, 0.1 g/L $CaCl_2$, 20 g/L agar, pH 7.4. Basal medium was prepared without Tween-80 and sterilized at a pressure of 10,1325 Pa for 15 min. An aqueous solution of Tween-80 was sterilized separately at a pressure of 50,662.5 Pa for 15 min and added to the sterile basal medium. The medium was poured into sterile Petri dishes. Tween-80 is a nonionic surfactant and emulsifier derived from polyethoxylated sorbitan and oleic acid. To investigate a capacity of isolated microorganisms to hydrolyze Tween-80 which is the lipase activity (LA) they were plated through stab or plaques and cultivated from 2 to 10 days at 37 °C. Provided that isolates had LA the oleic acid was released out the Tween-80 as a result of hydrolysis of the bond between polyethoxylated sorbitan and oleic acid and insoluble calcium salt of oleic acid was formed. The salt created the opaque zone around the colony and indicated the presence of LA (Sierra, 1957). Isolates with LA were selected for the further experiments.

Lipase activity on solid medium with animal fats

Agar medium of a volume 20 mL consisting of 0.5 g/L KH_2PO_4, 0.5 g/L $(NH_4)_2SO_4$, 0.1 g/L $MgSO_4$ and 0.1 g/L $CaCl_2$ (pH 6.8), were melted and cooled to 45-50 °C, and afterwards 1 mL of substrate emulsion of mutton fat, beef tallow, and lark (Sigma-Aldrich) was added. Medium also contained 1% aqueous solution of the dye Nile Blue sulphate (Nile blue A, Sigma-Aldrich). The mixtures were well stirred and poured into sterile Petri dishes. To investigate the capacity of the isolates to degrade fatty substrates microorganisms were plated through stab or plaques and cultivated at 37 °C during 2 to 5 days. Formation of blue halo around colonies was considered as a positive reaction and the more intensive color the higher LA (Christen and Marshall, 1984). Isolates with capacity to degrade all three fats were selected for the further experiments.

Lipase activity in liquid medium with Tweens

Tween hydrolyzing capacity which is LA of isolates was measured quantitatively after some preparatory procedures. Erlenmeyer's flasks of 250 mL with 100 mL of a sterile (50,662.5 Pa for 15 min) cultivation medium consisting of: 0.01% $MgSO_4$, 0.1% KH_2PO_4, 1% Tween-80, 0.2% glucose, 0.5% peptone (pH 7.0) inoculated by 5% of the overnight cultures of isolates and cultivated at 37 °C for 24 h at 150 rpm on a shaker incubator (Innova-44, USA). Then cell-free culture liquid was prepared by cell sedimentation in Eppendorf 5810-R centrifuge with rotor A-4-62 at 4000 rpm for 15 min. LA was measured in the supernatant (Lonon *et al*., 1988). Briefly, the reaction mixture in 3 mL of total volume consisted of 0.1 mL of 10% of each of Tweens (Tween 20, 60, and 80), dissolved in Tris buffer (50 mM Tris hydrochloride, pH 7.6), 0.1 mL of 1 M $CaCl_2$, 0.5 mL of cell-free culture liquid, and 2.3 mL of Tris buffer. Triplicate samples were prepared for each isolate tested and incubated in a water bath at 37 °C for 2 h. Tweens were hydrolyzed to produce a fatty acids and polyethoxylated sorbitan. In the presence of calcium, insoluble calcium salts of fatty acids were formed giving precipitates which were measured turbidimetrically by spectrophotometer (PD-303 UV, Japan). The measurements of optical density were carried out at a wavelength of 400 nm. Control samples were similar except for 0.5 mL of deionized water was added instead of cell-free culture liquid. Activity was expressed by the number of units of enzyme activity per 1 mg of total protein. One unit of LA was defined as that amount of

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enzyme which, after 2 h under the conditions of the assay, resulted in the 0.01 increase of optical density.

Species affiliation of the isolates by sequencing of 16S rRNA gene

Preliminary identification of individual bacterial isolates was obtained by classical tests (Gerhardt *et al.*, 1981; Bergey *et al*., 1994). Such identification included the shape of cells, Gram stain and colony morphology on solid nutrient media, liquid media, and biochemical tests. Genetic identification of isolates was performed by determining nucleotide sequences of 16S rRNA genes. Extraction and purification of DNA from bacterial cells were carried out by the hexadecyltrimethyl ammonium bromide (CTAB) method (Wilson, 1997). Fragments of the 16S rRNA gene were amplified using universal oligonucleotides FD1 (AGAGTTTGATCCTGGCTCAG) and RD1 (AAGGAGGTGATCCAGCC) as forward and reverse primers, respectively (Weisburg *et al*., 1991). PCR was performed in a total volume of 50 µL of the reaction mixture containing approximately 10 ng of genomic DNA, 5x GoTaq reaction buffer, 0.2 mM of each deoxynucleoside triphosphate, 2 mM of each primer (fD1 and rD1) and 1.5 units of GoTaq DNA polymerase (Promega). Thermal cycling consisted of an initial denaturation at 95 °C in 5 min followed by 30 cycles of 1 min at 95 °C, 1 min at 50 °C, 2 min at 72 °C, and a final extension in 7 min at 72 °C. The PCR obtained products were purified with illustra GFX PCR DNA and gel band purification kit (Amersham Biosciences, GE Healthcare) according to the manufacturer's protocol. Partial 16S rRNA gene sequences were performed using a set of "BigDye Terminanor v 3.1 Cycle sequencing Kit on an automated genetic analyzer ABI 3730xl (Applied Biosystems, USA) and the sequencing primers fD1 and rD1 according to the protocol of the manufacturer. The resulting nucleotide sequence was compared to all bacterial sequences available in the Gene Bank database using the BLAST program (Altschul *et al*., 1997).

Measuring the amount of fats in the model solutions with animal fats

Animal fats processing capacity of active strains was measured in model solutions as follows. 0.1 g of each animal fats (mutton fat, beef tallow and lark) in triplicate was added as the sole carbon source in the 3 lots of Erlenmeyer's flasks of 250 mL with sterilized cultivation Medium A consisted of: 0.1 g NH_4NO_3, 0.1 g K_2HPO_4, 0.1 g KH_2PO_4, 0.02 g $MgSO_4$, 0.002 g $CaCl_2$, 0.0002 g $FeSO_4$, 0.1 g yeast extract, 0.1 L distilled water. Inoculum of 10% of overnight culture of each strain (*Pseudomonas aeruginosa* Zb32*, P. aeruginosa* G23 and *A. punctata* G30) was added in each lot to a final volume of 100 mL and cultivated at 37 °C for 7 days at a speed of 100 rpm on a shaker incubator (Innova-44, USA). Control lot was not inoculated and incubated in parallel under the same conditions. Then culture broths were centrifuged as described as above to obtain cell-free culture liquid and fat extracted from the latter according to the most popular procedure (Folch *et al.*, 1957) with a slight modification. Briefly extraction of fat carried out with a mixture of chloroform and ethyl alcohol in the filter funnel with subsequent determination of its mass in the resulting extract, after removal of the solvent. An average value of two parallel determinations of fat mass was taken as a final result. The amount of fat was expressed as fat mass fraction in %. In Table 2 animal fat hydrolysis is expressed according to the algorithm: "(1- Y/X) ·100 %" where X – is the residual fat mass fraction in the control flask, Y – is the residual fat mass fraction of the sample flask.

Table 2: Animal fats removal efficiency under the action of 3 screened microorganisms in the model solutions.

Strain	Hydrolysis, %		
	Mutton fat	Beef tallow	Lark
P. aeruginosa G23	79±5	88±4	80±6
A. punctata G30	65±3	60±8	75±4
P. aeruginosa Zb32	47±5	52±6	73±7

Measuring the amount of lipids in model solution with FOGs trap specimens

Wastewater FOGs removal efficiency under the action of active strains was measured in a model solution as follows. FOGs trap specimen from Astana cafeteria was taken and sterilized at a pressure of 101,325 Pa for 15 min. The specimen of a weight 10 g in triplicate was added as the sole carbon source in the 3 lots of Erlenmeyer's flasks of 250 mL filled with sterile cultivation Medium A (see above). Inoculate as 10% of overnight culture of each strain (*P. aeruginosa* Zb32, *P. aeruginosa* G23 and *A. punctata* G30) was added in each lot to a final volume of 100 mL and cultivated at 37 °C for 7 days at a speed of 100 rpm on a shaker incubator (Innova-44, USA). Control flasks were not inoculated and incubated in parallel under the same conditions. Then, amount of FOGs/lipids were determined in cell-free culture liquids in the same way as described in the previous paragraph. In Figure 1, FOGs removal efficiency is expressed according to the algorithm: "(1- Y/X) ·100 %" where X – is the residual FOGs mass fraction of the control flask, Y – is the residual FOGs mass fraction of the sample flask. FOGs or lipid mass fraction in control flasks was taken as 100% in the Figure 1.

In the separate experiments 10% of overnight culture of the strain *P. aeruginosa* G23 and 10 g of the FOGs trap specimen were added to the medium A in a final volume of 100 mL in Erlenmeyer's flasks of 250 mL and cultivated within 7 days under 2 different conditions both in triplicate. The variant 1 was designated as "normal" conditions and created in a thermostatic shaker (100 rpm and 37 °C). The variant 2 was designated as "stress" conditions and created in the thermostat without shaking (37 °C). Control flasks of both variants were not inoculated and incubated under the same conditions in parallel. Aliquots of the two variants of cultivation were daily carried out for cell

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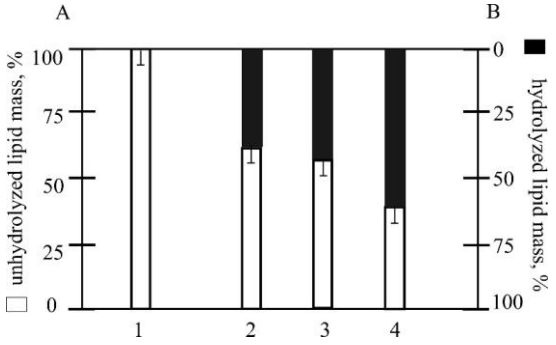
counting. Cell count was determined by plating serial dilutions of samples on nutrient agar plates and incubating at 30 °C for 24 h. Amount of FOGs/lipids were determined in cell-free culture liquids as described in the previous paragraph. In Figure 3 residual FOGs mass fractions in control flask and sample flasks in 2 cultivation conditions are presented. FOGs mixture in control flasks as we expected were not hydrolyzed (or less abiotically hydrolyzed) and its mass fraction was about 16%.

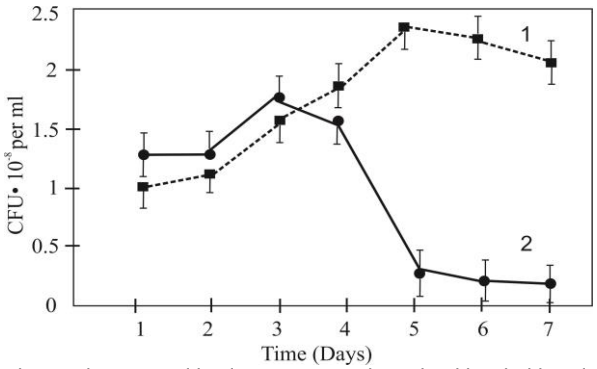
Statistical methods of data processing

We determined the average value of 3-5 measurements and standard deviation of the average values on the program Microsoft Office Excel.



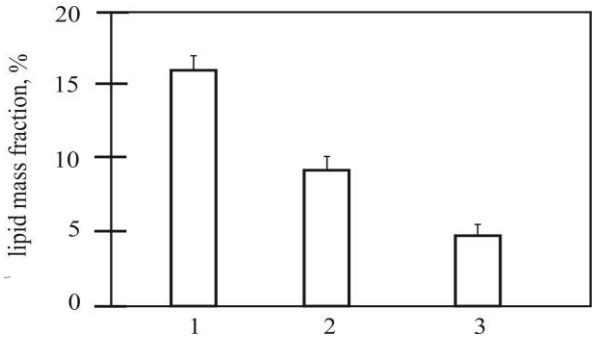
Vertical axis on the left, A: unhydrolyzed residual FOGs amount. Vertical axis on the right, B: removal efficiency of FOGs.
1: Control cultivation without inoculum; A: 100%, B: 0%
2: Cultivation with *P. aeruginosa* Zb32; A: 62.5%, B: 37.5%
3: Cultivation with *A. punctata* G30; A: 54.8%, B: 45.2%
4: Cultivation with *P. aeruginosa* G23; A: 38.5%, B: 61.5%

Figure 1: FOGs removal efficiency under the action of 3 screened microorganisms in specimen from the local cafeteria FOGs trap.



The variant 1 cultivation was conducted with shaking in a thermostatic shaker with operating mode of 100 rpm and temperature at 37 °C ("normal" conditions). The variant 2 cultivation was conducted in a thermostat without shaking and at 37 °C ("stress" conditions).

Figure 2: Dynamics of change in the number of cells of *P. aeruginosa* G23 in 2 variants of cultivation with FOGs trap specimens from the local cafeteria.



1: Control cultivation without inoculum (residual FOGs mass fraction was 16%).
2: In "stress" conditions (residual FOGs mass fraction was 8.4%).
3: In "normal" conditions (residual FOGs mass fraction was 5.5%).

Figure 3: FOGs removal efficiency under the action of *P. aeruginosa* G23 in specimen from the local cafeteria FOGs trap in 2 variants of cultivation.

RESULTS AND DISCUSSIONS

Creation of a working collection of microorganism cultures

We have chosen such niches in which it was expected to find indigenous microorganisms that destroy fat. There were 112 viable pure cultures of microorganisms which we had obtained among the 142 isolates from polluted environment. Index of viability in yeast cells was 10^8 CFU/mL, and in bacteria - ranged from 10^7 to 10^{10} CFU/mL. Based on the total culture-morphological traits we created the working collection of pure cultures of microorganisms, comprising of 112 isolates from different taxonomic groups (Gram positive, Gram negative bacteria and yeast cells). In order to identify strains with LA all working collection of microorganisms was screened on this activity.

Screening of strains on lipase activity

Only 75 isolates which correspond to 67% of the total number of microorganisms showed the LA on agar-Tween-80 plates. The remaining 37 isolates had no LA. It was interesting to test the strains with Tween-80 hydrolyzing activity on the ability to hydrolyze animal fats which is important for their survival in FOGs polluted environment. Out of 75 isolates the next ones digested all three fats on agar plates: isolates from the wastewater of slaughterhouses (Zb3, Zb4, Zb9, Zb13, Zb17, Zb22, Zb23, Zb25); soil of dairy plants (Zb30, Zb31, Zb32); wastewater of urban sewerage systems (G13, G15 , G17, G22, G23, G24, G25); and from active sludge in aeration tanks of wastewater treatment facilities (G30, G39, G40, G45, G48, G52, G57, G60, G61, G62, G63, G65, G66). The rest isolates gave a negative response in this assay. In the Table 3 the results of experiments for 6 isolates are presented.

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Table 3: Qualitative determination of lipase activity to animal fats.

Isolates	Lipase activity		
	Mutton fat	Beef tallow	Lard
G 9	–	–	+
G 30	+++	+++	+++
Zb32	+++	+++	+++
G 23	+++	+++	+++
G 25	+++	+++	+
G 29	–	+	+

–: no lipase activity; +: weak lipase activity; ++: average lipase activity; +++: high lipase activity.

Positive results in the two qualitative methods on plates with Tween-80 and animal fats have been obtained with 31 isolates. In the next step, the LAs of the 31 isolates were compared between themselves using the quantitative method (Lonon *et al.*, 1988). . The most active isolates with some others are presented in Table 4. We conventionally divided the isolates into three groups: "low LA" (below 150 U/mg of total protein), "medium LA" (up to 250 U/mg total protein), and "high LA" (above 250 U/mg total protein). Screening revealed that the most active cultures were the isolates G23, G30, and Zb32, which had the highest hydrolytic activity for Tweens as shown in Table 4.

Thus screening of microorganisms on LA allowed us to select the most active strains with LA. Selected strains were representing the following niches: G23 from wastewater of urban sewerage system, G30 from active sludge in aeration tanks of wastewater treatment facilities, and Zb32 from soil of dairy plants. Isolate G17 and others with "medium LA" were possible candidates for the future separate investigations.

Species identification

Species identification for the most active isolates was carried out through morphological biochemical tests and confirmed by sequencing the 16S rRNA genes (Table 5). The partial 16S rRNA gene sequences of G23, S30, and Zb32 strains were determined (data not shown). Then, we compared these sequences with existing sequences in the gene bank database using BLAST (Altschul *et al.*, 1997). The sequence comparison demonstrated the affiliation of the strains to *Proteobacteria phylum*. S30, and both G23, and Zb32 strains were closely related to *Aeromonas punctata* (*Aeromonas caviae*), and *Pseudomonas aeruginosa*, respectively. Bacteria of the genera *Aeromonas*, and *Pseudomonas* belonged to organisms, playing an important role in FOGs degradation in environment (Loperena *et al*., 2009).

Biodegradation in model solutions with animal fats

The growth of pure cultures of *P. aeruginosa* G23, *P. aeruginosa* Zb32, and *A. punctata* G30 in the model solutions with animal fats showed the ability of microorganisms to consume fats in these conditions. We observed relatively high levels of fat hydrolysis within 7 days of cultivation (Table 2). Comparison of the results of beef tallow consumption with the other publication (Matsumiya *et al*., 2003) showed similarity. Authors isolated the most active strain *Burkholderia* sp. DW2-1, which under the similar to our terms and conditions hydrolyzed 77.4% of beef tallow in a culture medium within 2 days. Strain *P. aeruginosa* G23 during 2 days consistently hydrolyzed 50% of beef tallow (data not shown). In order to reach 88% of the removal efficiency, as shown in Table 2, seven days were required for the *strain P. aeruginosa* G23. Apparently, our strain had the lower growth rate, which is due to incomplete adaptation (El Fantroussi *et al*., 2005). It is known that in order to reach more efficient removal of pollutant bacterium must be pre-adapted in the medium containing the contaminant, which makes the bacteria more competitive. These results (Table 2) were in a good agreement with literature data and allowed us to test the 3 strains further for their suitability in a cleaning technology of FOGs polluted wastewaters.

Biodegradation in model solutions with FOGs trap specimens from the local cafeteria

The aim of this work is to find the most suitable strain(s) for a cleaning technology of FOGs polluted wastewaters. The sites intended for biological treatment were FOGs contaminated wastewaters having its environmental background. The relationships of suitable strains with their new biotic and abiotic environment in terms of survival, activity and mobility should be characterized by key criteria to help in choosing the most suitable strains (El Fantroussi *et al*., 2005). In our work, the lipase activity was chosen as the key selection criterion for the isolates originated from contaminated sites. This enzyme is the first one which starts the process of transformation of lipids from FOGs specimens into intermediate organic compounds, such as fatty acids.

It is well known that oleic acid is one of the products of the reaction catalyzed by lipase. Oleic acid is a good activator of some enzyme ensemble of bacteria, and inductor of effective alternative metabolic pathways in utilization of carbon-containing molecules (Fadile *et al*., 2011). Under the induction bacteria synthesize specific enzymes involved in the biodegradation of FOGs in the favorable for bacteria conditions. For bacteria proliferation lipase must interact with its substrates, i.e. FOGs, whereas other specific enzymes should be activated too, which a whole leads to a high metabolic rate and high growth rate of cell mass. Several authors are inclined to the view that the induction of the metabolism of microorganisms plays a key role in biodegradation (El Fantroussi *et al*., 2005). That is why we took FOGs trap specimens from a local cafeteria as a testing material to the chosen 3 strains. The cafeteria of Astana at the crossing of Abai and Valikhanov streets produces FOGs contaminated wastewater for which there are currently no suitable biological treatment technologies. For the purity of

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Table 4: Screening of isolates on lipase activity.

Isolates	Lipase activity, U/mg protein		
	Tween-20	Tween-60	Tween-80
G12	127±12	185±16	118±7
G13	131±10	214±19	136±12
G15	179±13	201±17	149±13
G17	128±10	258±16	167±11
G18	86±7	143±17	77±12
G19	55±4	80±9	63±5
G20	74±6	130±12	62±4
G22	97±2	227±12	95±9
G23*	298±20	371±22	224±14
G30*	286±20	239±23	297±13
Zb32*	213±17	271±15	248±14
G25	202±10	225±10	60±7
G24	217±18	180±9	74±4
G66	94±6	47±7	73±4
G69	20±2	16±5	22±5
G70	24±4	9±3	15±2
G72	30±4	48±3	29±2
B16	80±5	56±2	64±6
B22	88±6	121±7	79±8

* indicates the most active isolates: G23, G30, and Zb32

Table 5: Identification of bacteria isolates on the basis of biochemical tests and 16S rDNA sequencing.

Strains	Relevant characteristics[a]	Identification[b]	% Similarity
G23	Gram (−), Catalase (+), Oxidase (+); VP (-)	*Pseudomonas aeruginosa*	98
G30	Gram (−), Catalase (+), Oxidase (+); VP (+)	*Aeromonas punctata*	98
Zb32	Gram (−), Catalase (+), Oxidase (+); VP (-)	*Pseudomonas aeruginosa*	98

VP: Voges - Proskauer test
[a] As determined by biochemical tests.
[b] As confirmed by 16S rDNA sequencing.

the experiments possible endogenous microflora were eliminated by sterilization of specimens since we were interested in the effects if any of abiotic impurities in the FOGs specimens.

The ability of the 3 cultures chosen in the previous tests to remove FOGs in the culture broth after 7 days of cultivation was observed via measuring the unhydrolyzed lipid mass which is residual FOGs mass and subsequent calculation of the hydrolyzed lipid mass which is the rate of FOGs biodegradation or in other words FOGs removal efficiency as shown in Figure 1. The strain *P. aeruginosa* G23 with the FOGs degradation rate of 61.5% was the most effective. *P. aeruginosa* Zb32 was less active with the FOGs degradation rate of 37.5% as shown in Figure 1. *A. punctata* G30 occupied an average position on this FOGs removal efficiency scale. If one looks at the origin of the studied strains, one might say that *P. aeruginosa* G23 originated from wastewater of urban sewerage system had found its habitat and *P. aeruginosa* Zb32 originated from a soil of dairy plants was the less efficient destructor of FOGs from cafeteria. *A punctata* G30 was isolated from active sludge in aeration tanks of wastewater treatment facilities. Apparently, there might be an effect of abiotic factors i.e. physicochemical properties of specimens sampled from cafeteria FOGs trap. One can assume that the less efficient strain *P. aeruginosa* Zb32 showed instability and was inferior to two other strains due to unfavorable conditions caused by the impurities in FOGs trap specimen from the cafeteria (pH, detergents, toxic substances, etc.). In the other work (Wakelin *et al*., 1997) a growth of a number of pure and mixed cultures was investigated in a medium with FOGs from a trap of the restaurant in a bioreactor. *Acinetobacter* sp. was the most effective among the pure cultures studied in the work, and hydrolyzed 60-65% of fatty material in the initial concentration of lipids equal to 8 g/L. The initial concentration of lipids in cultivation media presented in Figure 1 were at the same order and were about two times more than in cited paper, i.e. 16 g/L. Comparison of the results from the cited paper and in Figure 1 allowed us

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to say that the best FOGs hydrolysis rate showed by the strain *P. aeruginosa* G23 and equaled to 61.5% was close to the one obtained for *Acinetobacter* sp. and equaled to 60-65 % in the cited paper (Wakelin *et al*., 1997). It should be noted that 2 strains isolated and identified in the present work surpassed the rate of lipid hydrolysis of commercial inoculum studied in another work. The authors of the cited paper observed a lipid concentration reduction in the liquid food waste by 38% in the presence of biodestructor (Loperena *et al*., 2009).

Effect of "stress" conditions on the FOGs removal efficiency under the action of *P. aeruginosa* G23 in specimen from local cafeteria

Initially dynamics of change in the number of cells of *P. aeruginosa* G23 in 2 variants of cultivation with FOGs trap specimens from the Astana cafeteria was investigated. Curve 1 in Figure 2, represents the cultivation under the "normal" conditions. Inoculum biomass in the "normal" conditions increased to 2.37 × 10^8 CFU/mL on the fifth day of culture.

Biomass growth apparently associated with organic compounds in the FOGs trap specimen from the cafeteria. When the culture medium was depleted by sources of carbon compounds, the growth stopped and bacteria passed into the stationary phase followed by quiescence. This explains termination of curve rising on the fifth day of cultivation and the beginning of decline on the sixth and seventh day of cultivation caused by cells' transition to the quiescence. Alternatively one could assume that the cessation of growth came from impurities in the FOGs trap specimen. Cultivation under "stress" (without shaking) was accompanied with biomass increasing to 1.75 x 10^8 CFU/mL (curve 2), within 4 days, i.e. the time of growth was reduced by 1 day compared to cultivation in "normal" conditions. After 4 days of growth curve 2 started to decline and then dropped sharply to the fifth day and declined almost to zero in the sixth and seventh days. Different dynamics of growth in the "stress" apparently was due to several reasons. The absence of mixing could prevent the normal processes of diffusion slow interaction of poorly soluble and solid lipids, fats and greases with lipase and other enzymes and cells in culture medium. It is well known that the reaction of lipase catalysis occurs at the interface between the aqueous and lipid phases and the substrates of the enzyme are poorly soluble in aqueous solutions (Gupta *et al*., 2004). No mixing could limit the access of oxygen to the cells in the culture liquid. Depletion of organic matter on the fourth day could also possible, as will be discussed below. As a result of the above presented reasons lipid hydrolysis as the carbon and energy source, was restricted, this caused the subsequent inhibition of cell proliferation and cell death.

At the next stage, we investigated the ability of the strain to metabolize FOGs in specimens from the cafeteria under these two conditions considered. The strain *P. aeruginosa* G23, as expected, was more effective in "normal" conditions and removed the FOGs after 7 days of incubation up to 5.5% mass fraction (mass fraction before cultivation was 16%) as shown in Figure 3. "Stress" conditions, however not strongly hindered the strain to remove the FOGs and nearly half of all introduced FOGs from trap specimen was hydrolyzed, as shown by column 2 in Figure 3. This seemingly paradoxical result could be explained by the fact that *P. aeruginosa* G23 belongs to the class of "zymogen" microorganisms with r-strategy for survival (Thompson *et al.*, 2005). By definition, they are microorganisms that exist primarily in the resting phase, which is interrupted by short periods of activity stimulated by substrate appearing. At high concentrations of substrate, they have high metabolic rate, short cell cycle and rapid growth of cell mass, they are "r-strategists" with high Km and Vm. Bacteria return to the resting phase after exhausting of the substrate. In contrast the "autochthonous" forms show a low but constant activity as, for example, bacteria from genus *Arthrobacter* does (Thompson *et al*., 2005).

We return now to Figure 2, where it was suggested to substrate depletion in the case of "stress" conditions. Belonging of the strain *P. aeruginosa* G23 to the "r-strategists" with a high Km and Vm possibly enabled the strain in the "stress" conditions, within the first 4 days to grow fast on a par with the strain in the "normal" conditions. And, apparently, due to the high initial concentration of FOGs in trap specimen from cafeteria and high metabolic rate of bacteria about 50% of the lipids were hydrolyzed within 4 days, i.e. 50 % depletion of the substrate occurred. This level of depletion could be perceptible for cells under "stress" conditions and they stopped dividing, went into resting phase, and died within days from fourth to seventh as shown by curve 2 in Figure 2. Cells of the same strain in "normal" conditions at 50% depletion of the substrate continued to grow for another 1 day and metabolized within the period from fourth to seventh day an additional 16% of the lipids and the mass fraction of residual FOGs from the trap specimen dropped to 5.5% as shown in Figure 3.

FOGs removal efficiency under the action of *P. aeruginosa* G23 i.e. biodegradation rate of lipids of FOGs trap specimens from cafeteria is within region of 62%-66% (Figures 1 and 3), and does not seem to be the limit for the strain. We added about 1.6% FOGs in cultivation media and the level of FOGs degradation may be improved by increasing the amount of applied substrates. Currently, work is underway in our laboratory to test this hypothesis.

CONCLUSIONS

The aim of this work is to find the most suitable strain(s) for a biological treatment technology of FOGs polluted wastewaters. To reach the goal we have obtained a working collection of 112 microorganisms cultures from local wastewaters and soils polluted by FOGs. The new local microorganisms with high lipase activity were isolated, screened, identified, and tested among the rest 112 isolates. Testing the most active 3 strains in model solutions with animal fats and FOGs trap specimens from the local cafeteria showed the 3 strains have a good

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opportunity to be suitable strains in biological treatment technology of FOGs polluted wastewaters. Among them strain *P. aeruginosa* G23 is the most suitable strain. It is the new local microorganism with high lipase activity from local wastewater urban sewerage system. Thus the strain *P. aeruginosa* G23 is the most effective lipid-degrading microorganism and the best candidate to use in biological treatment technology of FOGs polluted wastewater in Kazakhstan.

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