

DYNAMICS OF SODIUM DODECYL SULFATE UTILIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF STRAIN *PSEUDOMONAS* SP. ATCC19151

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Abstract — *Pseudomonas* sp. ATCC19151 harbors a gene encoding a putative alkylsulfatase (*sdsA*). Here we report a growth ability of this strain in minimal media containing 0.5, 0.75, and 1% sodium dodecyl sulfate as the sole carbon source. The most prominent growth was detected for the minimal medium with 0.5% SDS, so this concentration of SDS was used to monitor *Pseudomonas* sp. ATCC19151 SDS biodegradation dynamics. Bacterial growth coincided with the disappearance of SDS. Antibiotic susceptibility was tested as well. *Pseudomonas* sp. ATCC19151 was resistant to six out of nine tested antibiotics, including ampicillin, tetracycline, chloramphenicol, tobramycin, nalidixic acid, and gentamycin.

Key words: *Pseudomonas*, sodium dodecyl sulfate, biodegradation, antibiotic susceptibility

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INTRODUCTION

Synthetic surfactants are components of a variety of household and industrial detergent formulations. Other industrial applications include paints, textiles and fabrics, oil-spill dispersants, concrete, paper, lubricants, and many others (Karsa, 1992). Due to their amphiphilic properties, long-chain aliphatic sulfate esters such as sodium dodecyl sulfate (SDS) are in common use as components of surfactant formulations and are consequently discharged into wastewater. The rapid removal of surfactants from the environment to avoid secondary pollution will make their application safer and more widespread (Zeng et al., 2007). Using microorganisms to degrade surfactants is one promising method (Zeng et al., 2007). A range of bacterial strains able to degrade aliphatic sulfate esters have been isolated from contaminated sources such as sewage sludge on the basis of their ability to utilize aliphatic sulfate esters as carbon sources for growth. In most cases, degradation of alkyl sulfate esters was found to be initiated by alkylsulfatase enzymes that catalyze hydrolytic cleavage of the ester bond to liberate inorganic sulfate. The resulting parent alcohol is further degraded (Dodgson et al., 1982) or incorporated into cellular lipids (Thomas and White, 1989).

The genus *Pseudomonas* is ubiquitous and well known for versatility in the biodegradation of industrial compounds. Alkylsulfatase activity has been reported for many *Pseudomonas* strains, for example *Pseudomonas* C12B (Cloves et al., 1977; Bateman et al., 1986), *Pseudomonas aeruginosa* (Harada, 1964), *Pseudomonas putida* FLA (Lillis et al., 1983), *Pseudomonas* sp. AE-A, and *Pseudomonas* sp. AE-D (Ellis et al., 2002). It was shown previously that *Pseudomonas* sp. ATCC19151 also has the ability to grow on SDS as the sole carbon source. Genes encoding a putative alkyl sulfatase enzyme (*sdsA*) and a putative alkyl sulfatase regulator of the LysR family (*sdsB*) from this strain were cloned and sequenced (Davison et al., 1992). Homologs of the *sdsA* gene can be found in the genome sequences of *P. aeruginosa* PAO1 and *P. putida* KT2440, although it is not yet known how their expression is regulated in these species or whether the SDS sulfatase is synthesized in response to sulfate limitation or as part of the carbon cycle. There are no data on the dynamics of *Pseudomonas* sp. ATCC19151 growth in minimal media containing SDS as the sole carbon source. Moreover, data dealing with the ability of this strain to degrade SDS during growth are also lacking.

Here we report the dynamics of growth of *Pseudomonas* sp. ATCC19151 in a rich medium as well as in a minimal medium containing three different concentrations of SDS as the only carbon source. In addition, the ability of *Pseudomonas* sp. ATCC19151 to utilize SDS during various periods of growth is analyzed for the first time. For further characterization of the strain, sequencing of the 16S rRNA gene and antibiotic susceptibility tests were also carried out.

MATERIALS AND METHODS

Bacterial strains and growth conditions

The bacterial strain used in this study was *Pseudomonas* sp. ATCC19151 from the American Type Culture Collection (Hsu, 1963, 1965). The strain was grown aerobically at 30°C in Luria broth medium (LB) and in M9 minimal medium containing various concentrations of SDS (0.5, 0.75, and 1%) or 0.2% glucose as the sole carbon source (Davis et al., 1980). Luria broth agar plates were prepared by adding 15 g of agar (Torlak, Belgrade, Serbia) to 1 l of LB medium.

Strain identification

To identify strain *Pseudomonas* sp. ATCC19151, primers UNI16SF 5'-GAG AGT TTG ATC CTG GC-3' and UNI16SR 5'-AGG AGG TGA TCC AGC CG-3' were used for amplification of the variable region of the 16S rRNA gene. Total DNA from *Pseudomonas* sp. ATCC19151 was isolated by Sarkosyl-pronase lysis as previously described (Better et al., 1983) and mixed with 2.5 µl of 10X PCR buffer (0.5 M KCl, 0.1 M Tris-HCl, pH 8.8 at 25°C, and 0.8% Nonidet P40), 1.5 µl of MgCl₂ (25 mM), 18.25 µl of bidistilled water, and 0.25 µl of *Taq* polymerase (Fermentas, Lithuania). Performed using the GeneAmp 2700 PCR Cyclyer (Applied Biosystems, Foster City, California, USA), the PCR program consisted of 5 min at 96°C; 30 cycles of 96°C for 30 s, 55°C for 30 s and 72°C for 30 s, and an additional extension step of 5 min at 72°C. Resulting PCR amplicons were sequenced at Macrogen in Seoul, South Korea.

Measurement of growth characteristics

Investigation of the ability of *Pseudomonas* sp. ATCC19151 to grow in the presence of SDS was done as follows. A series of Erlenmeyer flasks (500 ml) containing 100 ml of LB medium, M9 minimal medium with 0.2% glucose, and M9 minimal medium containing SDS (0.5, 0.75, or 1%) were inoculated with an overnight culture (1% v/v). The overnight cultures used for inoculation were prepared in the corresponding medium. Growth was monitored using the microdilution test technique by plating various dilutions of each bacterial culture onto LA agar plates at two-hour intervals during the analyzed growth period. The LA agar plates were incubated overnight at 30°C. The number of bacteria was determined by counting the number of colony-forming units (CFU). This experiment was done in triplicate.

MBAS assay

Concentrations of SDS were determined by the methylene blue active substance (MBAS) assay as described previously (Hayashi, 1975) with minor modifications. The method is based on the formation of a complex between the anionic surfactant and an excess of the cationic dye methylene blue, followed by extraction of the complex (but not excess dye) into chloroform and measurement of absorbance of the blue chloroform layer. A stock solution of methylene blue (0.5% in distilled water) was underlaid with a 1/20 volume of chloroform to remove any chloroform-extractable impurities and stored in a dark bottle to minimize photochemical degradation. Methylene blue stock (0.5%) was diluted 100-fold in 0.7 mM Na-phosphate buffer, pH 7.2. Aliquots of such stock (0.5 ml) and an appropriate volume of the sample (0.03 ml) were mixed in a glass tube and vigorously mixed intermittently five times for 3 sec each time. Chloroform was added to each tube, and the contents were vigorously shaken for 5 sec and then centrifuged in a 5804 centrifuge (Eppendorf, Hamburg, Germany) at 2000 rpm for 3 min. The tubes were allowed to warm up to room temperature, and absorbance of the chloroform layer was measured at 655 nm against an appropriate blank using an UltraSpec 3300 pro

instrument (Amersham Biosciences, Biochrom Ltd., Cambridge, England). Calibration curves were prepared using standard solutions based on the weight of pure SDS.

Broth microdilution test for antibiotic susceptibility

For determination of the MIC (minimum inhibitory concentration), the broth microdilution method was performed in a 96-well flat-bottom microtiter plate (Sarstedt Inc., Newton, USA). The panel contained a two-fold dilution series of the following antibiotics: ampicillin (500-4000 µg/ml), gentamycin (2-16 µg/ml), chloramphenicol (8-32 µg/ml), vancomycin (4-32 µg/ml), tobramycin (2-16 µg/ml), amikacin (8-64 µg/ml), kanamycin (12.5-100 µg/µl), tetracycline (1-16 µg/ml), and nalidixic acid (25-200 µg/ml). A sample (100 µL) of *Pseudomonas* sp. ATCC19151 overnight culture was inoculated in 20 ml of fresh LB and incubated at 37°C until it reached turbidity of the 0.5 McFarland standard ($OD_{600nm} = 0.132,5$ h). Within 15 minutes of adjusting the inoculum to the 0.5 McFarland turbidity standard, the suspension was diluted so that the final concentration in each well was 5×10^5 CFU/mL. Each well was given 100 µL of a two-fold dilution series of the antibiotic solution and 100 µL of bacterial suspension. One well served as a positive growth control (broth plus inoculum), and one served as a negative control (broth only). Plates were incubated at 37°C and culture turbidity was read after 24 h. The MIC was considered as the lowest concentration of an antimicrobial agent that after 24 h completely inhibited growth of the tested strain as detected by measuring OD_{600nm} in a plate reader (Labsystems Multiscan RC, MTX Labsystems, Inc., Vienna, Wyoming, USA).

Nucleotide sequence accession number

The nucleotide sequence of the variable region of the *Pseudomonas* sp. ATCC19151 16S rRNA gene was submitted to EMBL GeneBank under accession number FM958510.

RESULTS AND DISCUSSION

Analysis of the 16S rRNA gene sequence of strain *Pseudomonas* sp. ATCC19151 showed 98% sequence similarity at 89% sequence coverage to the 16S

rRNA gene of seven *Pseudomonas* sp. strains (strains 7.5, RW2P3, RW2P1, AHL, m11, 6.14, and 8.2), as well as two *P. otitidis* species and *P. guezzennei* RA26T from the NCBI database (<http://www.ncbi.nlm.nih.gov/blast/>). Further, we found 96% sequence similarity to the 16S rRNA genes of *P. aeruginosa* ZFJ-1 and *P. alcaligenes* LB19, at the same sequence coverage. Of 100 BLAST sequences that show between 94 and 99% sequence similarity, 94 of them belonged to the genus *Pseudomonas* (mostly *P. aeruginosa* species) and six were uncultured bacterial clones. This result suggests that strain *Pseudomonas* sp. ATCC19151 belongs to the *P. aeruginosa* group, whose species harbor highly homologous 16S rRNA gene sequences and exhibit similar phenotypic and biochemical properties (Garrity et al., 2005). Since the 16S rRNA gene sequence of *Pseudomonas* sp. ATCC19151 shares the highest homology with *P. otitidis* WL15, which was recently described as a new species (Clark et al., 2006), it could be supposed that they are probably the same species. This supposition is supported by a biochemical property of ATCC19151, namely its inability to grow on glycerol as the sole carbon source, which was also described for *P. otitidis*. In contrast, *P. aeruginosa* utilizes glycerol as the only sole carbon source for growth.

The ability of the ATCC19151 strain to grow in various media (see Materials and Methods) was tested over 48 h. The obtained results showed that cultures grown in M9 minimal media with SDS (0.5, 0.75, and 1%, respectively) entered the logarithmic phase of growth later (after 6 h) than those grown in LB or M9 containing glucose (after 4 h) (Fig. 1). After 24 h of growth in LB medium, the ATCC19151 strain entered the stationary phase, reaching 5.12×10^9 CFU per ml (Fig. 1). During the same time of growth in M9 minimal media with 0.2% glucose, the number of cells was something lower upon arrival at the stationary phase (1.06×10^9 CFU per ml) (Fig. 1). When *Pseudomonas* sp. ATCC19151 grew in M9 with SDS, it reached the highest number of cells after 12 h of growth (Fig. 1). However, the number of cells was two orders of magnitude lower compared to the number of cells when it grew in LB medium or M9 with glucose. In addition, the number of cells in M9 with 0.75 and 1% SDS significantly decreased

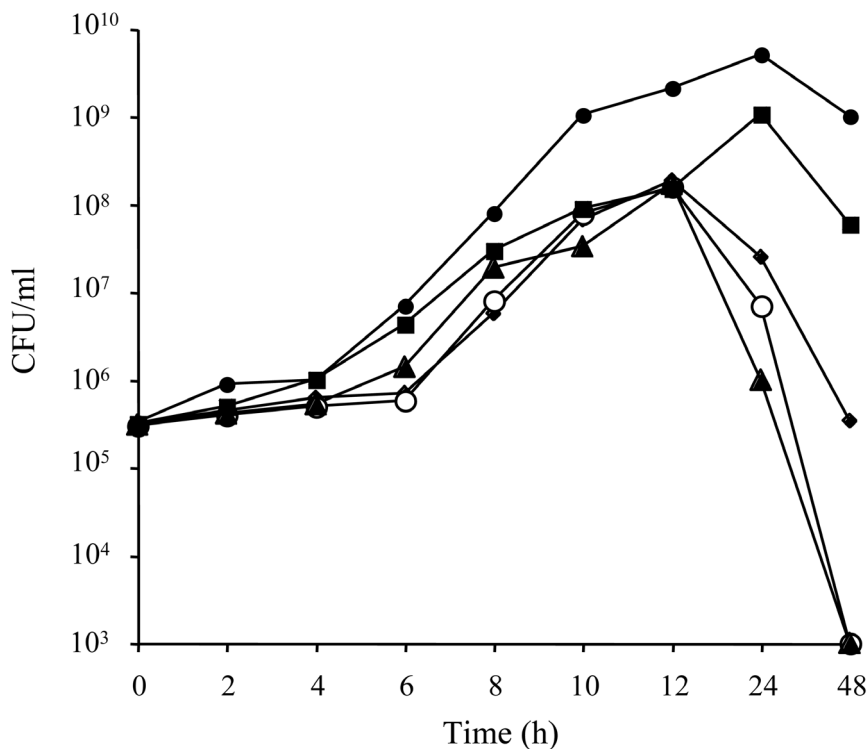


Fig. 1. Growth curves of *Pseudomonas* sp. ATCC19151 in rich LB medium and M9 minimal medium with glucose or three different concentrations of SDS as the sole carbon source. ● - LB medium; ■ - M9 + 0.2% glucose; ◆ - M9 + 0.5% SDS; ○ - M9 + 0.75% SDS; ▲ - M9 + 1% SDS.

after 24 h of growth, while this was not noticed in M9 with 0.5%, or at least it happened to a lesser extent. This phenomenon is probably a consequence of SDS concentrations in the growing medium (0.75 and 1%) that were too high for optimal growth of the culture, so when cultures reached the stationary phase of growth cell lysis occurred faster than in M9 medium with 0.5% SDS. Taking all these data together, we conclude that the optimal concentration of SDS for *Pseudomonas* sp. ATCC19151 growth in M9 minimal medium is 0.5%.

Based on the results obtained in this study, we selected M9 minimal medium with 0.5% SDS for testing of sodium dodecyl sulfate biodegradation by *Pseudomonas* sp. ATCC19151. The dynamics of SDS deprivation was followed over 48 h (see Materials and Methods). Bacterial growth coincided with disappearance of the surfactant (Fig. 2). After the culture entered the logarithmic phase of growth, a

two-fold decrease of SDS concentration occurred. A tendency of significant SDS concentration decrease in the medium was observed all along the logarithmic phase of growth, and at 12 h of growth it reached 0.1 $\mu\text{g}/\mu\text{l}$, which represents a decrease of 98% compared to the starting concentration of SDS. This pattern of SDS degradation by *Pseudomonas* sp. ATCC19151 is similar to patterns of alkyl sulfate utilization described for some other *Pseudomonas* species, for example *Pseudomonas* sp. AE-A (utilization of SDS; Ellis et al., 2002) and *Pseudomonas* sp. AE-A (utilization of both SDS and 2-butyloctyl sulfate; Ellis et al., 2002).

The genus *Pseudomonas* encompasses a number of species that are often clinically significant pathogens characterized by intrinsic resistance to a number of antimicrobial agents (Pitt et al., 2003). Results of the microdilution antibiotic susceptibility test showed *Pseudomonas* sp. ATCC19151 to

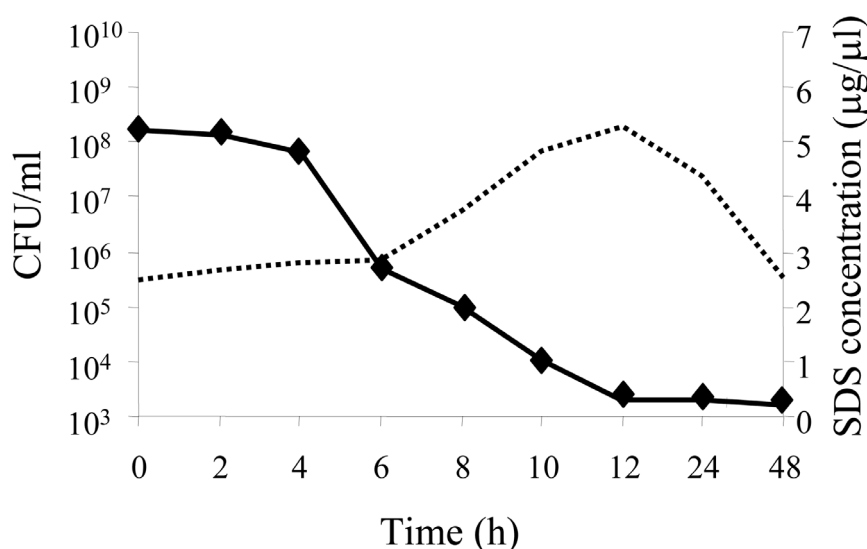


Fig. 2. Biodegradation of SDS by *Pseudomonas* sp. ATCC19151 in M9 minimal medium (supplemented with 5 g of SDS per liter in batch culture). ♦ - residual surfactant concentration during growth; ••••• - growth curve of *Pseudomonas* sp. ATCC19151 in M9 + 0.5% SDS.

be susceptible to the aminoglycosides kanamycin and amikacin. On the other hand, this strain was resistant to six out of nine tested antibiotics, including ampicillin, tetracycline, chloramphenicol, tobramycin, nalidixic acid, and gentamycin (Table 1). Interestingly, this strain exhibited very high resistance to ampicillin (MIC > 4,000 mg/L). Antibiotic resistance represents an important characteristic of this species, since it is the most common organism isolated from patients with cystic fibrosis. On the other hand, unlike *Pseudomonas* sp. ATCC19151, *P. aeruginosa* isolated from the natural environment and from non-CF patients is generally susceptible to widely used antipseudomonal agents such as gentamycin, tobramycin, and others. The resistance of *P. aeruginosa* to antimicrobials depends on various broad mechanisms, including low cell wall permeability, the capacity of their large genome (6.26 Mbp) to express a wide range of resistance mechanisms, and the potential to acquire resistance genes from other organisms via plasmids, transposons, and bacteriophages (Lambert, 2002). In addition, biofilms formed by *P. aeruginosa* participate in the development of antibiotic resistance that can be up to 1,000-fold greater than that of planktonic cells (Mulcahy, 2008). Further work will be focused on elucidation

of the molecular mechanism(s) responsible for the detected phenotypic resistance of *Pseudomonas* sp. ATCC19151.

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ДИНАМИКА КОРИШЋЕЊА НАТРИЈУМ ДОДЕЦИЛ СУЛФАТА И ОСЕТЉИВОСТ НА АНТИБИОТИКЕ СОЈА *PSEUDOMONAS* SP. ATCC19151

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Познато је да сој *Pseudomonas* sp. ATCC19151 поседује ген који кодира потенцијалну алкил сулфатазу. У овом раду анализирана је способност раста овог соја у минималном медијуму са различитим концентрацијама натријум додецил сулфата (0.5, 0.75 и 1 %) као јединим извором угљеника. Показано је да *Pseudomonas* sp. ATCC19151 испољава најбољи раст у минималном медијуму са 0.5 % натријум додецил сулфата, те је стога ова концентрација узета као оптимална за тестирање

динамике коришћења натријум додецил сулфата током различитих фаза раста. Динамика коришћења натријум додецил сулфата подударала се са растом културе. Поред тога у циљу детаљније карактеризације соја, анализирана је и осетљивост *Pseudomonas* sp. ATCC19151 на антибиотици. Показано је да је анализирани сој резистентан на шест (ампицилин, тетрациклин, хлорамфеникол, тобрамицин, налидиксичну киселину и гентамицин) од девет анализираних антибиотика.