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ANALYSIS OF BACTERIAL COMMUNITY AND ITS BIODEGRADATION ABILITY OF 17-B ESTRADIOL IN WASTEWATER PLANT IN ASSIUT, UPPER EGYPT

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Wastewater treatment is essential for environmental sanitation in urban environments. Nevertheless, chemical, and organic substances, and microorganisms accumulate in wastewater treatment plants (WWTPs), which may be liberated into the environment via their effluents. This study aimed to analyze the bacterial community at the sewage treatment plant entrance in Assiut, Upper Egypt as well as their degradation ability of 17-β estradiol (E2) as an Isolates were identified by conventional methods and, 16S rRNA gene sequencing. E2 degradation by different strains was assessed using HPLC/UV. Conventional methods and 16S rRNA based amplicon sequencing revealed three bacteria including Bordetella sputigena (ASc1), Pigmentiphaga daeguensis (ASc3), and Stenotrophomonas pavanii (ASc4), and when tested for E2 degradation, they degraded 65.5%, 59%, and 66.4% of E2 (50 mg. L^{-1}) respectively. Other bacterial strains were identified using 16S rRNA based amplicon sequencing. Bacteroidetes, Firmicutes and Proteobacteria were the prominent phyla. However, Actinobacteria, Cyanobacteria, Chloroflexi, Caldithrix, and Chlamydiae were detected in lower proportions.

Keywords: *Bacterial community composition; Wastewater treatment plants (WWTPs); 17-β estradiol (E2); Biodegradation*

INTRODUCTION

All communities of bacteria which are responsible for the degradation of carbon and the elimination of nutrients from the wastewater are unknown. These nutrients are the core elements of any biological WWTP[1,2]. Most waterborne microorganisms spread via the fecal-oral route and enter sewage treatment plants through human excretion of feces[2].

Endocrine-disrupting chemicals (EDCs) involve various kinds of synthetic and natural chemicals which stimulate or inhibit the reproductive effects of the endocrine system in humans and animals. Several important EDCs have been classified into two main classes: natural estrogens like estrone (E1), E2, and estriol (E3) and synthetic estrogens like ethinyl estradiol (EE2)[3]. The impacts of these compounds in humans include Infertility, defined cancers, decreased sperm number, changed testosterone levels, intrauterine growth restriction and preterm labor, decreased fetal testosterone, decreased male fertility, urogenital disorders such as cryptorchidism and hypospadias, recurrent miscarriages, testicular cancer, birth weight loss, polycystic ovary syndrome, genital abnormalities, breast and prostate cancer, endometriosis, early puberty, infertility, menstrual disorders, and obesity[4,5].

E2 is one of the natural estrogens excreted by humans and cattle, which in very low concentrations (nanogram/litre) has an endocrine effect and causes serious problems in the ecosystem[6,8]. Women excrete estrogen from their bodies without using external hormonal

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drugs[7,10]. Ying et al. found that women and men excreted the hormone estradiol at a rate of 1.6 µg/day[8]. Therefore, the presence of this hormone in wastewater has become normal and should be treated as a problem to be solved.

Many processes are involved in the elimination of estrogens from wastewater, involving biological, physical and chemical removal[9,13]. In plants of sewage treatment, the elimination of estrogens by microorganisms under aerobic conditions through conversion reactions and biological metabolism plays an important role, and by which the bacteria use estrogens in biochemical reactions as a source of carbon[10,11] and[12,17].

The microbial communities in the sewage treatment plants were determined by traditional methods (culture-dependent methods) such as observation under an optical microscope[13,19], or by performing various biochemical tests on isolated strains[14,21]. Current methods for the detection of microorganisms (culture-independent methods), like Next-generation sequencing, were used for identification purposes by extraction of genomic DNA from wastewater samples and 16S rRNA gene sequencing[15].

In this work, bacterial populations in wastewater were identified using culture-dependent or culture-independent techniques and the ability of each isolate to degrade E2 alone was evaluated.

MATERIAL AND METHODS

Materials

Estradiol (E2; >98% purity) was obtained from Sigma-Aldrich. (USA). Acetonitrile and methanol was bought from TEDIA that were pure enough for high-performance liquid chromatography (HPLC). The E2 was made as a stock solution with a high concentration of methanol (1000 mgL^{-1}).

The medium of mineral salt (MSM)[16] consisted of 0.50 g KH2PO4, 1.50 g (NH4)2SO4, 1.91 g K2HPO4_3H2O, 0.20 g MgSO4 -7H2O, 0.5 g NaCl, and 1 mL trace element solution in 1 L ultra-pure water. The trace element solution comprised the following amounts of each element: 0.10 g/L CoCl2 -6H2O, 0.015 g/L CuSO4-5H2O, 0.50 g/L ZnCl2, 0.425 g/L MnCl2 -4H2O, 0.01 g/L NiCl2- 6H2O, and 0.01 g/L Na2MoO4-2H2O. The estrogen mineral salt medium (EMM) contained MSM augmented with E2 at the test doses and had a pH of 7.0 ± 0.2. E2 was placed into sterilized flasks with the required concentrations, and the methanol was permitted to volatilize. Following that, the MSM was appended to generate the liquid EMM. For the solid EMM plates, the melted MSM was combined with E2, the medium was put onto the plates, and the methanol was then volatilized with a stream of sterile air. Agar at a concentration of 18.0 g/L was added to the liquid medium to create solid media plates. Mannitol salt agar (Oxoid, UK) was used for the isolation of *Staphylococci*[17]. While MacConkey agar (Oxoid, UK) was used for isolation of Gram negative bacteria[18] and Cetrimide agar (Becton Dickinson, U.S.A) was used for isolation of *pseudomonas aeruginosa*[19].

METHODS

Enrichment procedure for bacterial isolation

The enrichment procedure was done in a 250-mL Erlenmeyer flask involving 100 mg. - L^{-1} E2 in 100 mL of liquid MSM[20]. One hundred water specimens were collected over six months (from December 2020 to May 2021), from the main sewage water drain (Sewage treatment plant, Arab El- Madabegh, Assiut, Upper Egypt). Wastewater specimens were collected from a depth of 0.5 m. Ten samples (each one is 0.5 liter) were obtained from different positions at one time were collected in sterile, nonreactive borosilicate glass bottle to form 5 liter pooled together, and prefiltered through cheesecloth to remove debris, then filtered on a sterile membrane filter with a pore size of 0.22 µm. Filter papers were cultured in a mineral salt medium (MSM), and the mixture was grown in an aerobic incubator shaking at 150 $r.min^{-1}$ and at 30 °C. Five percent (v/v) of this combination was put into new liquid MSM at 100 mg. - L^{-1} E2, every 2 days, and this procedure was carried out three times. The bacterial isolates were cultured for 48 hours at 30 °C after being seeded into independent EMM plates with 100 mg.- L^{-1}. Repetitive streaking was used to choose morphologically different colonies for purification before a single purified colony was

produced and transferred to maintenance slant cultures[20].

Harvesting of bacterial cells. To create cell suspension, every purified unique colony was increased by culturing it in Luria Bertani (LB) medium on a rotary shaker at 150 r.min^{-1} and at 30 °C. The cell suspension was centrifuged at 8000 rpm min^{-1} for 4 min, after which the pellet containing bacteria was gathered and twice rinsed with phosphate buffer saline (PBS). It was then put back together in sterile MSM and given an optical density of 1 at 600 nm[20].

Identification of isolated strains

Three bacterial isolates named as ASc1, ASc3 and ASc4 were isolated from all samples, and subjected to Gram stain, culture on Cetrimide agar, Mannitol salt agar, and MacConkey agar and biochemical tests including citrate utilization, catalase, DNase, oxidase and indole production for characterization and identification[21]. The genomic DNA was extracted by utilizing Gene JET Genomic DNA Purification Kit #K0721 (thermo scientific, Lithuania). The following universal primer pairs[22,23] (sequence5'-3') 27 forward (F) 5'-AGA GTT TGA TCC TGG CTC AC-3' and 1492 reverse (R) 5'-GGT TAC CTT GTT ACG ACT T-3' were utilized for amplification of 16S rRNA gene by PCR. The following profile was used for the amplification: initial denaturation for 5 min at 95°C, followed by 30 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 57°C, extension for 1 min at 72°C, and final extension for 10 min at 72°C[24]. Amplification products were sequenced at GATC Company utilizing ABI 3730xl DNA sequencer utilizing forward and reverse primers. 16S rRNA gene sequences from isolates were submitted and aligned to the gene sequences available in the NCBI database utilizing the BLAST server **(http://blast.ncbi.nlm.nih.gov/)**. The tree built from aligned 16S sequences of stems that closely match the searched database sequences[25].

Screening the ability of ASc1, ASc3 and ASc4 strains for E2 biodegradation

The bacterial capacity for biodegradation of E2 was evaluated by growing it aerobically in an EMM medium. The degradation system consisted of (50 mg.- L^{-1} E2) a 5% (v/v) cell suspension in 20 ml of EMM in 50 ml Erlenmeyer flasks. The combination was cultivated on a rotary shaker for 48 hrs at pH 3 and 150 rpm.- (7.00). Residual E2 in bacterial solutions was identified by HPLC/UV every 12 hours as follows; 20 mL of methanol was appended to flasks containing bacterial cultures to lyse the rest of E2, and the combination was sonicated for 30 min and then filtered via 0.22 µm polytetrafluoroethylene membrane filters. These settings were utilized for HPLC/UV identification; column C_{18}, a motile stage of acetonitrile and water (70/30 v/v) at a flow rate of 1 mL/min and an injection volume of 20 µL[26].

The degradation efficiency (%) of E2 in batch culture was calculated as follows:

$$\text{Degradation efficiency (\%)} = (C_{\text{Initial}} - C_{\text{Residual}} / C_{\text{Initial}}) \times 100\%.$$

Where C_{Initial} and C_{Residual} represent the primary and last concentrations of estradiol in culture solution.

Microbial community characterization

Genomic DNA extraction and 16S rRNA based amplicon sequencing

The samples of wastewater were pre-filtered with a cheesecloth before extraction to remove debris and then filtered through a sterile membrane filter (0.2 µm). Total genomic DNA was isolated from membrane filters by utilizing the DNeasy PowerSoil Kit Cat No. 12888-100 (Qiagen, USA) as per the manufacturer's instructions.

PCR was used to amplify up the isolated DNA utilizing primers specific for the V1–V3 regions. Primer-attached Illumina adaptor (underlined),

Forward Primer 5' AATGATACGGCGACCACCGAGATCTAC AC ATCGTACGTAT GGTAATTCAATTACCGCGGCTGCTGG

Reverse Primer 5' CAAGCAGAAGACGGCATACGAGAT AACTCTC GAGTCAGTCAGCCGAGTTTGAT CMTGGCTCAG[27]. An initial denaturation for 2 min at 95°C, followed by 22 cycles of denaturation for 20 seconds at 95°C, annealing for 15 seconds at 55°C, extension for 1 min at

72°C, and the last extension for 10 min at 72°C, formed the PCR amplification protocol[28]. Utilizing Agencourt XP Ampure Beads (Agencourt Bioscience Corp., Beverly, MA), amplification materials were purified and sequenced with the Illumina MiSeq sequencing system at IGA Technology Service (Udine, Italy).

Sequence processing and analysis

The Illumina MiSeq platform generated data in fastq file format for forward and reverse paired-end reads without barcode sequences. The Quantitative Insights Into Microbial Ecology (QIIME2)[29,30] bioinformatics pipeline was used to analyze the 16S rRNA gene readouts. Row sequence preparation depended on ribosomal sequence variants (RSVs), which are sequences with differences of more than one nucleotide. Quality control was performed using the DADA2 plugin of the Qiime2 platform[31]. In summary, demultiplexed coupled reads from the research specimens and the negative controls were used as input for filtering and clipping reads with an absolute peak of dual predicted faults per read (maxEE= 2) and a diminished length for reverse and forward reads of 210 and 270, respectively. At the final stage of the readings, discard low-quality bases, and demand an average Phred quality of at least 25. Then the filtered reads were dereplicated into distinguishable sequences, and the DADA2 inference algorithm used the 1000000 parameters of the DADA2 error model to denoise each sequence. When merging denoised reverse and forward sequences, a minimal overlapping of 20 base pairs and zero mismatches were used. Potential chimeric sequences were eliminated after ribosomal sequence variants (RSVs) were developed to create high-resolution RSVs. Representative of RSVs Sequences differed by >3% from each other. The resulting representative sequences were used for subsequent taxonomic assignments and diversity metrics.

Representative sequences of every RSV were utilized for taxonomy categorization versus the SILVA SSU Ref NR dataset v.132[32] utilizing trained RDP's naive Bayesian classifier at 99% sequence similarity[33,37]. Instead, ESPRIT-Tree software used taxon-independent clustering[34,34] to group sequences into operational taxonomic units (OTUs) by calculating the k-mer distance across sequences at similar values ranging from 99% (species/strain stage) to 80%. (phylum stage).

RESULTS AND DISCUSSION

Results

Identification of isolated strains

Three strains of bacteria; ASc1, ASc3 and ASc4 were isolated from wastewater. All three isolates were grown on MaCconkey agar and pure gram-negative bacilli. Analysis of the biochemical properties of the isolated strains showed that; The ASc4 isolate lacked the ability to grow on Cetrimide agar, Mannitol salt agar and was negative for oxidase, indole, ornithine decarboxylase and citrate, and positive for lactose fermentation and catalase. Despite ASc1, ASc3 isolates lacked the ability to grow on Cetrimide agar, Mannitol salt agar and were negative for indole, lactose fermentation, ornithine decarboxylase and citrate, yet were - positive for catalase and oxidase.

16S rRNA gene sequences from isolates were submitted and aligned to gene sequences available in the NCBI database utilising the BLAST server. A BLAST analysis revealed that the strains ASc1, ASc3 and ASc4 were 95.43%, 87.96% and 99.43% identical to *Bordetella sputigena*, *Pigmentiphaga daeguensis* and *Stenotrophomonas pavaini* respectively. **Fig.s (1-3)** show a phylogenetic tree of strains ASc1, ASc3 and ASc4 and related strains.

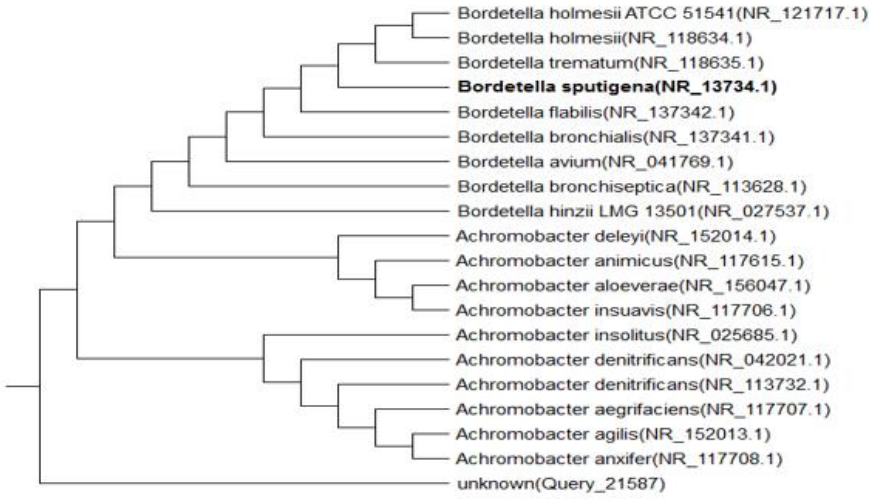

Fig. 1: Phylogenetic tree of *Bordetella sputigena* and closely linked species.

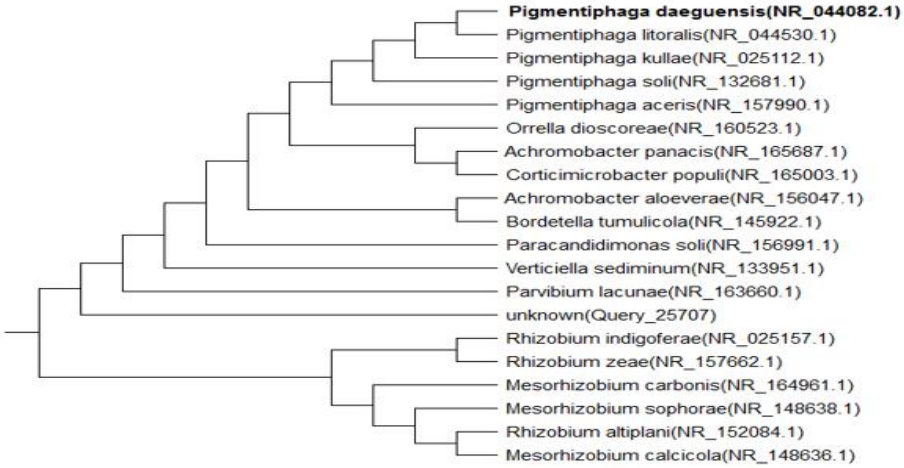

Fig. 2: Phylogenetic tree of *Pigmentiphaga daeguensis* and closely linked species.

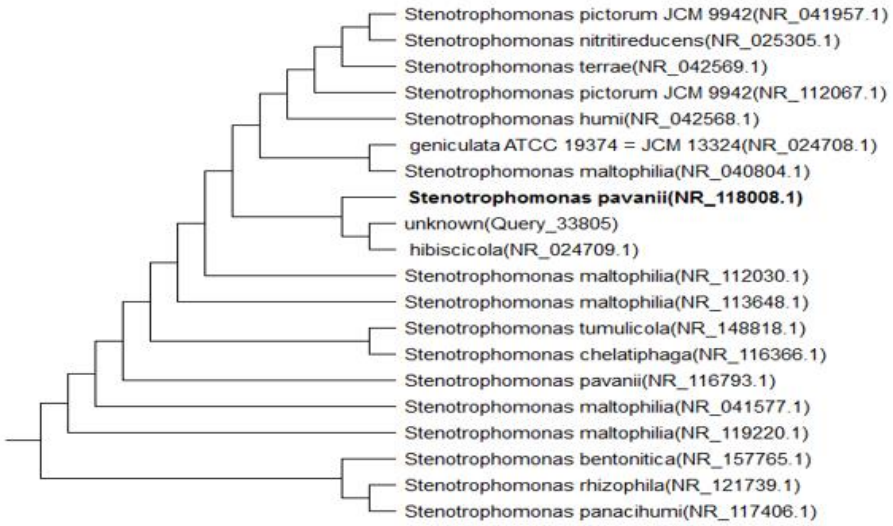

Fig. 3: Phylogenetic tree of *Stenotrophomonas pavanii* and closely linked species.

Degradation of E2 by ASc1, ASc3 and ASc4 strains.

The rate of E2 degradation by strains ASc1, ASc3 and ASc4 strains was evaluated separately for each strain. ASc1, ASc3 and ASc4 strains showed E2 biodegradation efficiencies of 65.5%, 59% and 66.4%, respectively, with an initial E2 concentration of 50 mg.- L^{-1} after 48 hrs, as shown in **Fig.(4).**

Bacterial diversity analysis at WWTP

Bacterial diversity was analyzed depending on 16S rRNA gene sequence analysis, leading to a total of 11, 979, 250 reads throughout the ten specimens. After quality filtering, removal of reads 420 ≤ and ≥470, removal of duplicate sequences and removal of chimeric sequences, and there were 1, 669, 510 reads as representative sequences that were unique sequences in our dataset, as shown in **Table (1).**

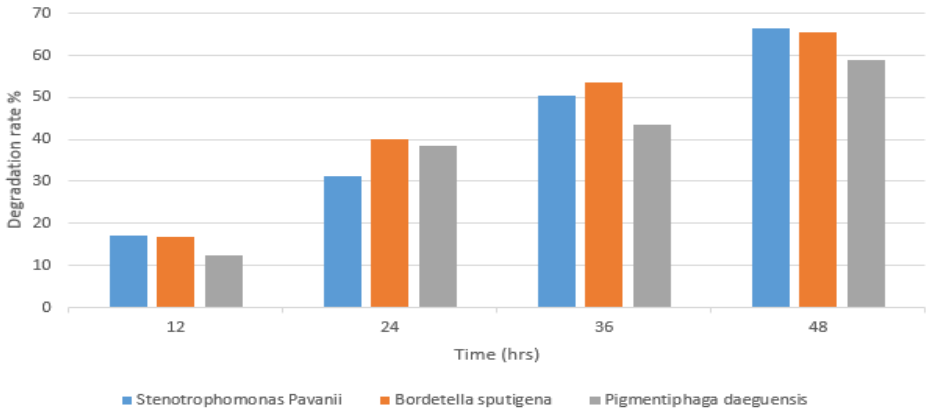

Fig. 4: Degradation of E2 by *Bordetella sputigena*, *Pigmentiphaga daeguensis* and *Stenotrophomonas pavaini* in MSM with E2 at concentration of (50 mg.- L^{-1}) during 48 hours of incubation.

Table 1: Sequence analysis for the generated reads and the remaining reads after preprocessing and filtration.

Sample Code	Number of reads	No. of reads after quality and length filtering	No. of representative sequences	No. of reads after chimeras elimination	Percentage of chimeras sequences
ASu1	784,721	731,502	133,342	125,341	6 %
ASu2	690,162	643,318	109,339	104,474	4.2 %
ASu3	820,266	769,499	131,241	122,447	6.7 %
ASu4	339,702	316,396	60,394	58,038	3.9 %
ASu5	1,577,319	1,428,655	257,121	238,351	7.3 %
ASu6	1,753,896	1,607,497	258,996	242,420	6.4 %
ASu7	440,319	410,107	61,493	58,787	4.4 %
ASu8	1,406,148	1,282,586	179,537	168,944	5.9 %
ASu9	1,915,402	1,752,631	262,867	242,889	7.6 %
ASu10	2,251,315	2,049,063	327,816	307,819	6.1 %

Bacterial community structure at WWTP

At the phylum, class, order, family, and genus stages, analyses of the relative abundances of bacterial groupings were conducted. Ten samples yielded 28 bacterial phyla, 53 classes, 104 orders, 226 families, and 559 genera. **Fig. (5)** displays the bacterial community's architecture and relative abundance at the phylum level. As indicated in **Fig. (5)**, the general sewage sequences have been classified into three dominant phyla, namely Bacteroidetes, Firmicutes and Proteobacteria. Samples ASu6, ASu7, ASu8 and ASu10 had the highest frequency of Bacteroidetes, representing (49%), (50%), (55%) and (56%) respectively, while samples ASu1, ASu2, ASu3 and ASu4 had the highest frequency of Firmicutes accounting for (56%), (49%), (45%) and (51%) respectively. Proteobacteria represented approximately (27%), (26%), (25%), (24%) and (18%) in ASu7, ASu8, ASu6, ASu9 and ASu10 samples, respectively.

At the class level, the dominant classes among Bacteroidetes were Bacteroidia, Flavobacteria and Sphingobacteria. Bacteroidia class represents (49.7%) of all sequences, most Bacteroidetes sequences are closely related to Genera Prevotella (39.8%), Bacteroides (6.3%), Parabacteroides (2.1%), Flavobacterium (2.8%) and Sphingobacterium (0.61%) as shown in **Fig. (6)**. Regarding the Firmicutes phylum, three main classes have been found within this phylum, Clostridia, Bacilli and Erysipelotrichi. The Clostridia class represents (50.1%) of all sequences that were considered the highest dominant class, while sequences assigned to Bacilli in the lowest dominant class averaged (5.1%). At the genus level, most Firmicutes sequences were related to Clostridium (13.46%), Blautia (5.3%) and lactobacillus (3.9%), as shown in **Fig. (7)**.

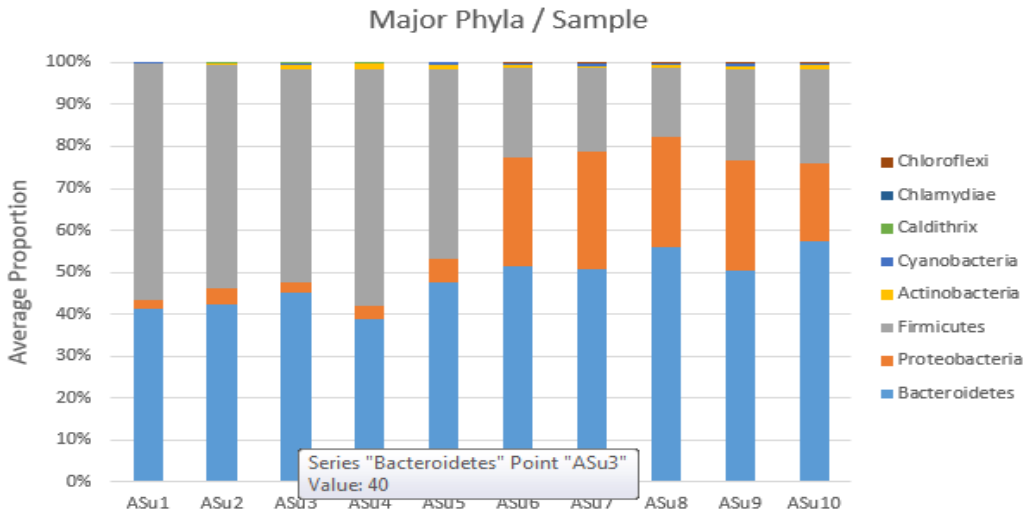

Fig. 5: Distribution of most predominant phyla in wastewater samples.

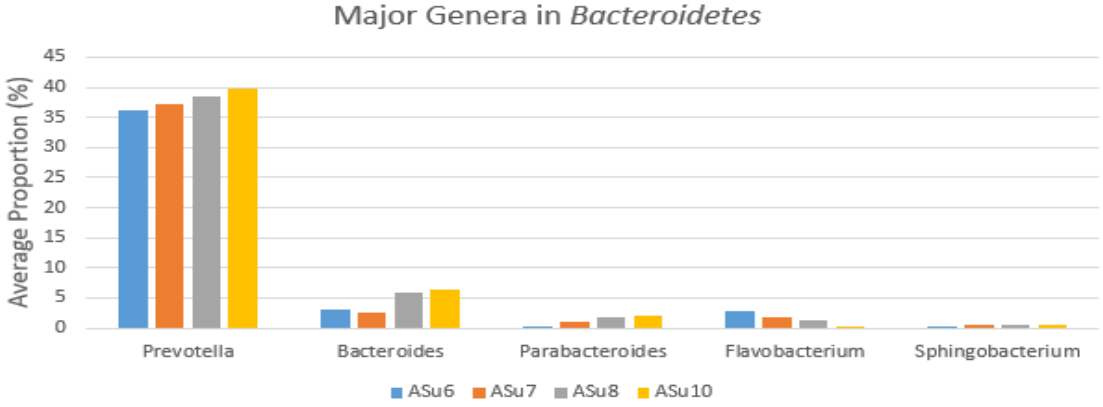

Fig. 6 : Distribution of most abundant genera in Bacteroidetes phylum.

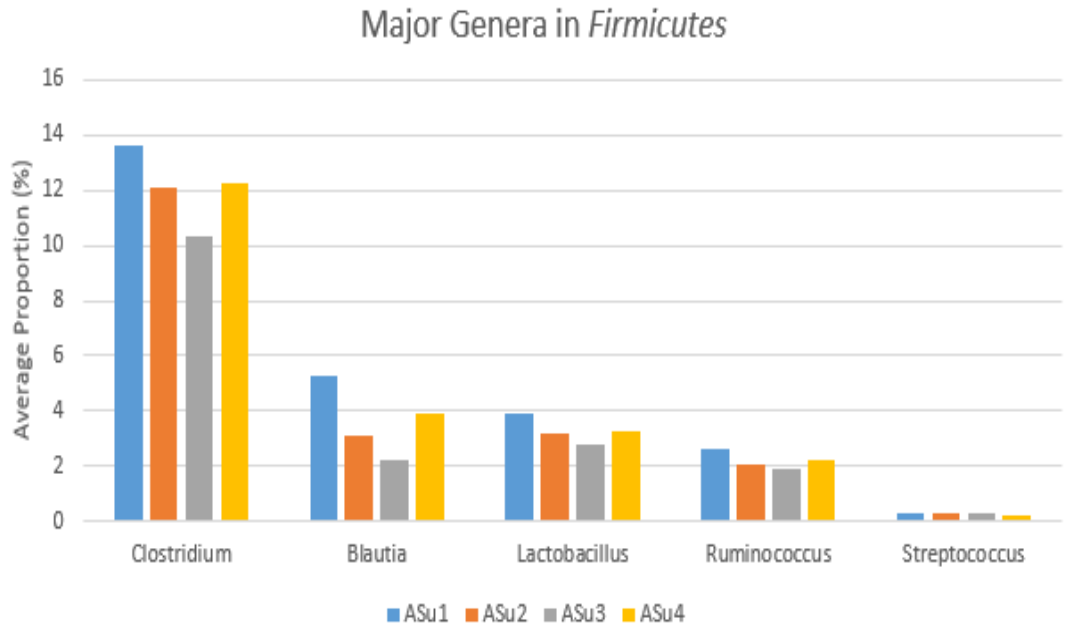

Fig. 7: Distribution of most abundant genera in Firmicutes phylum.

Regarding the Proteobacteria phylum, most of the bacterial sequences belong to the Gammaproteobacteria, Epsilonproteobacteria, Betaproteobacteria, Deltaproteobacteria, and Alphaproteobacteria classes, as shown in **Fig. (8)**. At the species level, *Arcobacter Cryaerophilus*, *Arcobacter Defluvii*, *Escherichia Albertii*, *Succinivibrio Dextrinosolvens* and *Acinetobacter Johnsonii* were the predominant species under the *Proteobacteria* phylum as shown in **Fig. (9)**.

Additionally, five phyla; Actinobacteria, Cyanobacteria, Chloroflexi, Caldithrix and Chlamydiae were detected in low proportions in all samples (0.2% to 1.1%), (0.1% to 0.5 %), (0.005% to 0.3%), (0.001% to 0.1%) and (0.001% to 0.01%) respectively.

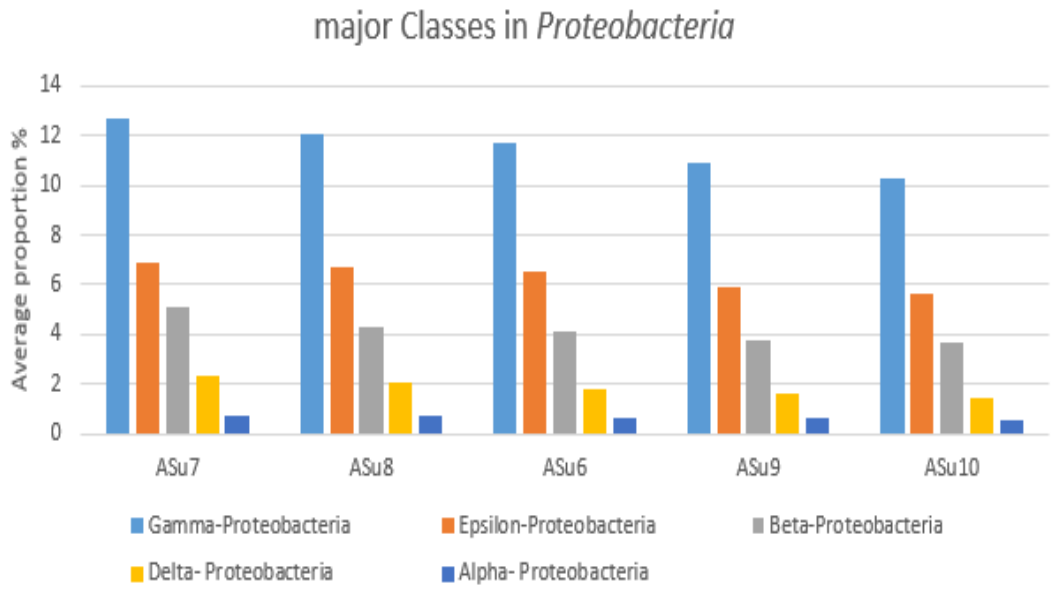

Fig. 8 : Distribution of classes in Proteobacteria phylum.

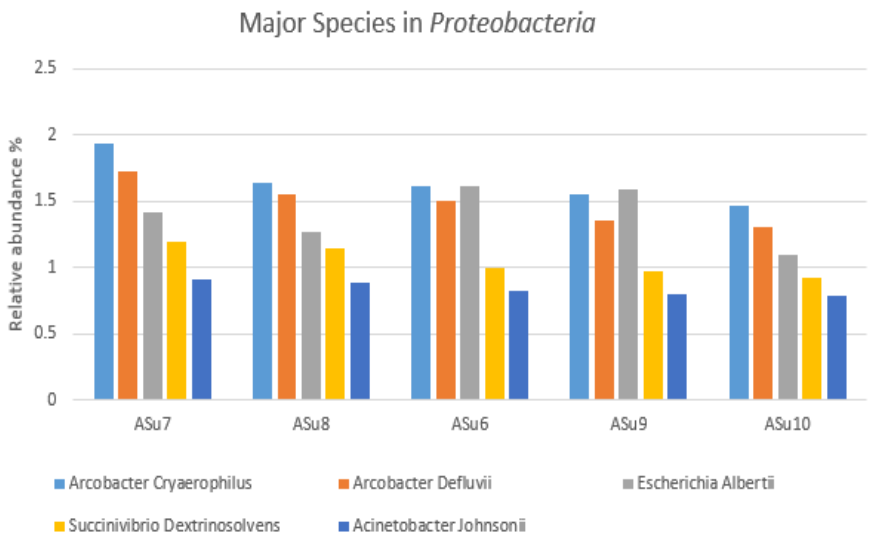

Fig. 9 : Relative abundance of major Species in Phylum Proteobacteria.

Unclassified sequences

At various taxonomic stages, there were sequences which could not be allocated to the reference sequence based on comparison with the SILVA reference database at 0.97 and 0.99% similarity. The number of unclassified sequences increased with the focus on more informative taxonomic levels such as species or genus stages.

At the phylum level, unassigned sequences accounted for 0.78% of all reads, while at the class level, they accounted for 1.60 %. The number of unclassified sequences increased steadily with the taxonomic classification by 1.94 %, 5.36 % and 7.45 % at the order, family and genus stages, respectively. At the species level, unclassified sequences accounted for 39.1 % of all sequences.

Discussion

Treatment of wastewater is one of the most important biotechnological approaches applied to treat domestic and industrial wastewater throughout the world. WWTPs are intended to lower overall bacterial loads in addition to sediments and nutrients[36,37]. Many toxic compounds (chemicals and organics) and bacteria that have been collected in sewage treatment facilities may be discharged into the environment through the effluent from WWTPs. As a result of improving microbial populations in wastewater treatment systems, numerous hazardous chemicals and diseases are effectively removed[38,43].

In developing countries, knowledge of the overall microbial communities present in WWTPS is very limited. The investigation of the function and structure of the bacterial community of these populations has become a critical component of the biotechnological procedures employed in sewage treatment plants[39].

To quantify the bacterial diversity in wastewater specimens, we employed both culture-independent and culture-dependent techniques in this research. In terms of culture-dependent approaches, three strains; *Bordetella sputigena*, *Pigmentiphaga daeguensis* and *Stenotrophomonas pavaini* were identified in a culture medium using E2 as the sole source of carbon. These isolates showed E2 biodegradation efficiencies of 65.5%, 59% and 66.4%, respectively, at an initial E2 concentration of 50 mg. L^{-1} after 48 hrs. This finding is similar to that of Zhou et al., who identified three bacterial isolates (*Isoptericola* spp. (EsD8), *Nubsella* spp. (EsD18), and *Rhodococcus* spp. (EsD20)) from wastewater samples that could decompose E2[40] and by Fernandez et al., who isolated five bacteria from sediment enrichment cultures containing E2 as the only carbon source. Three of these were identified as *Bacillus flexus*, *Virgibacillus halotolerans*, and *Bacillus licheniformis*[41]. A prior research by Mainetti et al. noted that 26 isolates (including *Rhodococcus* spp. *Pseudomonas* spp., and *Alcaligenes* spp) were isolated from plant effluents in Switzerland by

growing them in a liquid medium containing E2 as the sole source of carbon[42]. In China, the species *Orchobactrum* (strain FJ1) was isolated from WWTP and demonstrated its ability to remove 98 ± 1% of the initial E2 concentration after 10 days[43].

Previous Egyptian studies showed diverse results. For example, khatab *et al.*, who performed their study on sewage water at Saft El-Henna (Northern Egypt), demonstrated six bacterial strains that degraded E2 involving *Klebsiella* sp., *Bacillus* sp., *Enterobacter* sp., *Enterobacter* sp II., *Aeromonas veronii*, and *Aeromonas punctata*[44]. In another study, Sewage water from the Industrial Water Works in the Tenth of Ramadan City (El-Sharkia Governorate) tested positive for *Pseudomonas* sp[45]. Also, *Bacillus subtilis* was found in association with *Lactobacillus plantarum*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus* in Quhafa Wastewater Treatment Plant (QWWTP), Fayuom City[46].

In the culture-independent methods, 28 bacterial phyla were found in ten specimens. Firmicutes. Bacteroidetes and Proteobacteria were the most common phyla. Bacteroidetes was the most dominant phylum in ASu6, ASu7, ASu8 and ASu10 samples representing (49%), (50%), (55%) and (56%) of the classified sequences, respectively. Firmicutes was the mostly second abundant phylum, comprising (56%), (49%), (45%) and (51%) of the classified sequences in ASu1, ASu2, ASu3 and ASu4 samples, respectively, while Proteobacteria was the mostly third abundant phylum representing about (27%), (26%), (25%), (24%) and (18%) in samples ASu7, ASu8, ASu6, ASu9 and ASu10 respectively. This is in agreement with the observations mentioned by Silva-Bedoya et al., who reported that the commonest microorganisms in WWTPs were Firmicutes, Proteobacteria and Bacteroidetes phyla[47]. Previous research by Xu et al. reported a total of 36 phyla 8 specimens. Bacteroidetes, Actinobacteria, Proteobacteria, and Chloroflexi were the most predominant phyla, which accounted for (19.3 – 37.3%), (1.5 – 13.8%) (26.7 – 48.9%), and (2.9 – 17.1%) of the total sequences in every specimen, respectively[48]. Also, Shu et al. noted that Bacteroidetes, Firmicutes, Proteobacteria, *and* Chloroflexi were the most common phyla in six specimens from WWTPs[49].

However, on the other hand to the current work, some investigators reported that Proteobacteria was the phylum that was detected in three WWTPs to be the most frequently occurring, accounting for (11-85%), followed by Firmicutes (0.8-82.9%), Actinobacteria (3.9-16.1%), Bacteroidetes (0.1-5%) and Chloroflexi (0.1-2.5%) in South Africa[50]. In Germany, Firmicutes was the phylum with the highest average abundance among sequences found in samples from WWTPs with an average (52.2 ± 4.4%) followed by Proteobacteria (37.8 ± 4.7%), Bacteroidetes (4.9 ± 1.9%) and Actinobacteria (2.2 ± 0.2%)[51]. Proteobacteria was also the most common phylum in China, representing (59.63%) of total sequences discovered in WWTPs, followed by Bacteroidetes, Acidobacteria, Firmicutes, and Nitrospirae[52]. In addition, water samples taken from Lake Qarun in Egypt included five different bacterial phyla in varying proportions: Firmicutes (53%), Proteobacteria (33%), Bacteroidetes (7%), Actinobacteria (5%) and Thermi (1%)[53].

In this study, from winter (43% relative abundance) to spring (56% relative abundance), the relative abundance of Bacteroidetes rose steadily. A rise from winter's 5% to spring's 27% relative abundance of Proteobacteria was also observed. While the relative abundance of Firmicutes grew dramatically from 22% in the spring to 56% in the winter. The results of this study are in line with those of a previous one[54], which found that seasonal shifts had an effect on the composition of bacterial communities. The results of the wastewater analysis also showed that the total bacterial counts were higher in El Mehala Elkobra throughout the summer. Isolated microorganisms include *Aeromonas sobria*, *Staphylococcus aureus*, *Salmonella sp*., *Proteus mirabilis*, *Serratia plymuthica*, and *Pantoea agglomerans*[55].

In this study, the dominant classes at ten wastewater specimens were Bacteroidia, Flavobacteria and Sphingobacteria, Clostridia, Bacilli, Erysipelotrichi, Gammaproteobacteria, Epsilonproteobacteria, Betaproteobacteria, Deltaproteobacteria and Alphaproteobacteria. These findings are consistent with a prior study[50] stating that the dominant classes at the 6 wastewater specimens were Actinobacteria, Alphaproteobacteria, Bacilli,

Gammaproteobacteria, Clostridia, Deltaproteobacteria, Bacteroidia, Flavobacteria, and Betaproteobacteria. In addition, Tian et al. (2017) from China claimed that the key efficient classes were Alphaproteobacteria, Anaerolineae, Gammaproteobacteria, Betaproteobacteria, and Flavobacteria[56]. In contrast, Oyedara et al. (2022) in Mexico noted the distribution of classes Epsilonproteobacteria, Bacteroidia, Fusobacteria, Erysipelotrichi, Planctomycetacia, Sphingobacteria, and Nitrospirae in wastewater samples[57]. Observed microbial diversity between this study and other studies may be attributed to geographical differences and different climate conditions.

Conclusion

Three different gram-negative bacterial strains (ASc1, ASc3 and ASc4) isolated from wastewater were identified as E2-degrading bacteria by a culture-dependent method. Analysis of the 16S rRNA gene sequences of the extracted strains identified them as belonging to the genus *Bordetella sputigena*, *Pigmentiphaga daeguensis* and *Stenotrophomonas pavaini*, respectively. ASc1, ASc3 and ASc4 strains exhibited E2 biodegradation efficiency of 65.5%, 59% and 66.4%, respectively, at an initial E2 concentration of 50 mg. L^{-1} after 48 hrs. according to the culture-independent method, 28 bacterial phyla, 53 classes, 104 orders, 226 families and 559 genera were detected from ten samples. The most common phyla in all sequences were Bacteroidetes, Firmicutes and Proteobacteria. Bacteroidia was the dominant class among Bacteroidetes and Clostridia was the major class among Firmicutes, while most bacterial sequences in the phylum Proteobacteria were the classes of Gammaproteobacteria, Epsilonproteobacteria, Betaproteobacteria, Deltaproteobacteria and Alphaproteobacteria.

Recommendations

Because E2-degrading strains have demonstrated their ability to reduce the potential environmental risks posed by E2, the removal and biodegradation of ecosystem pollutants should be investigated further.

· More knowledge of the entire microbial communities found in sewage water will aid researchers in improving the efficacy of biodegradation of environmental contaminants.

Abbreviations

ASc1	*Bordetella sputigena*
ASc3	*Pigmentiphaga daeguensis*
ASc4	*Stenotrophomonas pavanii*
$C_{Initial}$	the initial concentrations of estradiol in culture solution
$C_{Residual}$	the final concentrations of estradiol in culture solution.
E1	estrone
E2	17 β-estradiol
E3	Estriol
EDCs	Endocrine disrupting chemicals
EE2	ethinyl estradiol
EMM	estrogen mineral salts medium
LB	Luria Bertani
MSM	mineral salt medium
OTUs	operational taxonomic units
PBS	phosphate-buffered saline
QIIME2	The Quantitative Insights Into Microbial Ecology
RSVs	ribosomal sequence variants
WWTPs	wastewater treatment plants

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نشرة العلوم الصيدليـــــة
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تحليل المجتمع البكتيري وقدرته على التحلل الحيوي لمادة ١٧- بيتا استراديول في محطة مياه الصرف الصحي في أسيوط ، صعيد مصر

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تتم إزالة هرمون الإسترجين فى البول والبراز كمركبات هرمونية قطبية غير فعالة، سواء تم إنتاجها بشكل طبيعى من قبل البشر والحيوانات أو تم إستخدامها كوسيلة للنظافة الشخصية. أثناء التجميع الأولى وخلال مرحلة معالجة مياه الصرف الصحى، يمكن للإنزيمات البكتيرية تعديل تلك المركبات الى الأشكال النشطة لهرمون الإستروجين .

على الرغم من أنه قد أقترح أن يتم التخلص من هرمون الإستروجين أثناء معالجة مياه الصرف الصحى فى أغلب الأحيان بواسطة التحلل الحيوى، إلا أن هناك القليل من المعلومات حول البكتريا المسؤولة عن تكسير هرمون الإستروجين. وقد أظهرت التجارب الأولية ان العديد من البكتريا الموجودة فى الأمعاء البشرية والكائنات الحية الدقيقة الموجودة فى الفم قادرة على استخدام ١٧-بيتا إستراديول كمصدر وحيد للكربون. لذا فإن الهدف من هذة الدراسة هو عزل وفحص البكتريا القادرة على تكسير مادة ال١٧-بيتا إستراديول من مياه الصرف الصحى بإستخدام طرق العزل التقليدية (الوسط الغذائى المناسب لنمو للبكتريا). وكذلك تحديد التوصيف الكيميائى الحيوى، التوصيف الجزيئى وبناء شجرة النشوء للبكتريا من الجينوم المعزول. علاوة على ذلك، تحديد البكتريا غير المزروعة التى قد تكون موجودة فى عينات مياه الصرف الصحى التى تم جمعها بإستخدام تحليل التنوع البكتيرى عن طريق تحليل تسلسل الجينات من خلال الكشف عن جين (16S rRNA) الريبوسومى. وأخيرا، تقييم الكفاءة التكسيرية للسلالات المعزولة لمادة ال١٧-بيتا إستراديول .

وقد أجريت هذة الدراسة على عينات من مياه الصرف الصحى لتحليل وتحديد المجتمعات البكتيرية. وأظهرت النتائج وجود ثلاث سلالات بكتيرية سالبة الغرام (ASc1, ASc3, and ASc4) تم عزلها من مياه الصرف الصحى كسلالات قادرة على تكسير مادة ال١٧-بيتا إستراديول واستخدامها كمصدر وحيد للكربون. تم التعرف على السلالات المعزولة عن طريق تحليل تسلسل الجينات من خلال الكشف عن جين (16S rRNA) الريبوسومى ووجد أنها تنتمى الى بورديتيلا سبوتيجينا (Bordetella sputigena)، بيجمنتيفاجا ديجنسس (Pigmentiphaga daeguensis) ، ستينوتروفوموناس بافيانى (Stenotrophomonas pavaini)على التوالى.

وبإستخدام تحليل التنوع البكتيرى، تم إكتشاف ٢٨ فئة بكتيرية (bacterial phylum) ، ٥٣ طبقة بكتيرية (bacterial class) ، ١٠٤ ترتيب بكتيرى (bacterial order) ، ٢٢٦ عائلة بكتيرية (bacterial family)و ٥٥٩ جنس بكتيرى .(bacterial genus) كانت الفئات البكتيرية التالية (Bacteroidetes, Firmicutes and Proteobacteria)والتى تمثل (٥٦ – ١٨%) من التسلسلات المصنفة هى الأكثر فئات وفرة فى جميع التسلسلات. وايضا، تم إكتشاف الفئات البكتيرية التالية (Actinobacteria, Cyanobacteria, Chloroflexi, Caldithrix and Chlamydiae)والتى تمثل (٠,٠٠١ – ١,١%) من التسلسلات المصنفة هى الأقل فئات وفرة فى جميع التسلسلات. فيما يخص الطبقات البكتيرية، أظهرت النتائج ان الطبقات البكتيرية التالية (Bacteroidia, Flavobacteria and Sphingobacteria) هى الأكثرالطبقات وفرة فى الفئة البكتيرية (Bacteroidetes phylum) حيث أن (Bacteroidia) تمثل (٤٩,٧%) من جميع التسلسلات المصنفة. فيما يتعلق بالفئة البكتيرية (Firmicutes phylum) ، أظهرت النتائج وجود ثلاث طبقات رئيسية داخل هذة الفئة وهى (Clostridia, Bacilli and Erysipelotrichi) حيث تمثل ال 50.1% (Clostridia) من جميع التسلسلات المصنفة والتى تعتبر الأعلى وفرة، فى حين تمثل ال 5.1% (Bacilli) من جميع التسلسلات المصنفة. فيما يتعلق بالفئة البكتيرية (Proteobacteria) ، تنتمى غالبية التسلسلات البكتيرية المصنفة الى الطبقات البكتيرية التالية (Gammaproteobacteria, Epsilonproteobacteria, Betaproteobacteria, Deltaproteobacteria and Alphaproteobacteria).

وفيما يتعلق بكفاءة السلالات المعزولة على تكسير مادة ال١٧–بيتا إستراديول واستخدامها كمصدر وحيد للكربون، كان معدل التكسير للسلالات ستينوتروفوموناس بافيانى، بورديتيلا سبوتيجينا و بيجمنتيفاجا ديجنسس هو ٦٦,٤%، ٦٥,٥% و٥٩% على التوالى.