Diversity and Biotechnological Potential of Xylan-Degrading Microorganisms from Orange Juice Processing Waste

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Abstract: The orange juice processing sector produces worldwide massive amounts of waste, which is characterized by high lignin, cellulose and hemicellulose content, and which exceeds 40% of the fruit’s dry weight (d.w.). In this work, the diversity and the biotechnological potential of xylan-degrading microbiota in orange juice processing waste were investigated through the implementation of an enrichment isolation strategy followed by enzyme assays for the determination of xylanolytic activities, and via next generation sequencing for microbial diversity identification. Intracellular rather than extracellular endo-1,4-β-xylanase activities were detected, indicating that peripheral cell-bound (surface) xylanases are involved in xylan hydrolysis by the examined microbial strains. Among the isolated microbial strains, bacterial isolates belonging to Pseudomonas psychrotolerans/P. oryzihabitans spectrum (99.9%/99.8% similarity, respectively) exhibited activities of 280 U/mg protein. In contrast, almost all microbial strains isolated exerted low extracellular 1,4-β-xylosidase activities (<5 U/mg protein), whereas no intracellular 1,4-β-xylosidase activities were detected for any of them. Illumina data showed the dominance of lactic and acetic acid bacteria and of the yeasts Hanseniaspora and Zygosaccharomyces. This is the first report on indigenous xylanolytic microbiota isolated from orange juice processing waste, possessing the biotechnological potential to serve as biocatalysts for citrus biomass valorization through the production of high-added value products and energy recovery.

Keywords: orange juice processing waste; biomass valorization; xylanolytic bacteria and yeasts; endo-1,4-β-xylanase activity; 1,4-β-xylosidase activity; hemicellulose hydrolysis; lactic acid bacteria (LAB); acetic acid bacteria (AAB)

1. Introduction

The cultivation of citrus is one of the major global agricultural sectors, which resulted in the production in 2015 of approximately 130 million tons worldwide, with China, the Mediterranean countries, Brazil, India, USA and Mexico being responsible for more than 80% of the world’s citrus harvest [1]. Citrus utilization for processing reached globally 25 million tons, with 80% of the processed citrus being oranges [1]. During orange juice processing, more than 60% of the fruit is discarded as waste, used either for animal feed or depurated in conventional wastewater treatment plants in the cases of the solid and liquid fraction of the waste produced, respectively [2].

On the other hand, orange juice processing waste consists of high levels of lignin, cellulose and hemicellulose, which has been reported to be equal to 40% of the dry weight [3]. A range of biotechnological applications have recently been proposed for the valorization of orange juice
processing waste, including the recovery of high added-value products like natural antioxidants, essential oils and pectin [4,5], the manufacturing of absorbent material [6] and the production of bioethanol and biomethane [7–9]. Regarding enzyme technology, the use of citrus processing waste as bioresource for cellulases and pectinases has also been explored [10] for potential applications in food technology, solid-state fermentation and energy recovery [11,12]. In addition, the production of xylanases and the investigation of their potential uses in food and feed technologies, the pulp and paper industries, and biofuel production are of high interest [13].

Endo-1,4-β-xylanases (EC 3.2.1.8) belong to a class of catabolic enzymes which degrade xylan, a biopolymer containing β-1,4-glucosidic bonds, to D-xylose and xylooligosaccharides [14]. Despite the fact that xylan requires a range of enzymes for its complete depolymerization, endo-1,4-β-xylanase is considered as the key enzyme. 1,4-β-xylosidases (EC 3.2.1.37) catalyze the release of D-xylose from the non-reducing ends of xylooligosaccharides [15]. Indeed, hemicelluloses including xylan, together with celluloses, are considered as the most copious renewable biomass reserves on the globe [13]. Taking into account the high hemicellulose content of citrus waste, orange juice processing waste appears to be a suitable source for novel xylanolytic microorganisms and for the production of high-performance xylanases.

However, the study of xylanolytic microorganisms and their secreted xylanases in xylan-rich citrus processing wastes is limited to a few studies. In most cases, a single allochthonous fungal strain is applied to ferment orange wastes for solid-state fermentation purposes. For instance, a Eupenicillium javanicum strain that was isolated from forest rotten wood exhibited high xylanase activity when grown on such residues [16]. Moreover, the soil-isolated fungus Aspergillus japonicus PJ01 growing on orange waste powder exerted important xylanolytic activities [17]. To the best of our knowledge, there is no previous report on the isolation and molecular characterization of the main autochthonous xylanase-producing microbiota in citrus processing wastes.

Thus, this study attempts for the first time to reveal the xylanolytic potential of microorganisms obtained from orange processing waste by determining their endo-1,4-β-xylanase and 1,4-β-xylosidase activities and to proportionally assess the indigenous xylanolytic diversity identified in such waste by employing both conventional molecular and next-generation sequencing techniques.

2. Materials and Methods

2.1. Physicochemical Characterization of Orange Juice Processing Waste

Electrical conductivity (EC) and pH were recorded by a Crison CM35 and a Metrohm 632 meter respectively after 1:10 v/v sample: water dilution. A measurement of total organic matter and ash content was carried out after ashing at 550 °C for 4 h, whereas carbon content determination was based on the van Bemmelen transformation factor. Total nitrogen content was estimated by sulphuric acid Kjeldahl digestion of fine-grained sample and subsequent alkaline steam distillation in boric acid solution [18]. Total phosphorus was calculated spectrophotometrically according to Anderson and Ingram procedure [19]. Total soluble carbohydrate content was determined after potassium dichromate digestion in concentrated sulfuric acid and titration with acidified ferrous ammonium sulphate in the presence of o-phenantholine indicator [20]. Lastly, total soluble phenolics and anthrone-reactive carbon were measured according to the Singleton and Rossi [21] and Brink et al. [22] protocols, respectively.

2.2. Enrichment and Isolation of Xylan-Degraders

To isolate xylanolytic microorganisms from orange juice processing waste, an enrichment procedure was followed, where the orange processing waste was initially subjected to 1/10 dilution using a solution consisting of 2 g/L xylan from corn core (TCI Chemicals, Tokyo, Japan), 13 mM Na₂HPO₄, 87 mM KH₂PO₄, 10 mM (NH₄)₂SO₄ and 0.4 mM MgSO₄. After a 1-week incubation period at 28 °C, 1/100 of the exhausted medium was transferred to the sterile solution described above and was incubated for a period of 1 week. A third enrichment step was also included, transferring 1/100 of the second exhausted medium to fresh
sterile solution, following the above-mentioned incubation conditions. Growth plates for xylanolytic bacteria were made by mixing the above-mentioned solution with 1.7% w/v agar.

2.3. Determination of Endo-1,4-β-xylanase and 1,4-β-xylosidase Activities of Xylanolytic Isolates and Fermented Orange Juice Processing Waste

All xylan-degrading bacteria isolated from the orange juice processing waste examined were grown for a period of 1 week in the above described liquid medium, where xylan served as the sole carbon and energy source. The microbial biomass was obtained by centrifugation at 12,000 g for 5 min and the collected supernatant was transferred to a sterile tube to estimate extracellular activities. The pelleted biomass was rinsed twice in 0.02 M acetate buffer and then sonicated in an ice bath for 15 min under 0.6 s pulse/0.4 s interval (50% duty level) through the use of a UP200S sonicator (Hielscher, Germany) [23]. Cell debris was removed from the lysate through centrifugation at 4 °C and 15,000 g for 15 min and the clean lysate was used for the determination of the endocellular activities. In the case of the fermented orange juice processing waste, the waste was mixed thoroughly with 1/10 v/v 0.02 M acetate buffer for 30 min and subjected to centrifugation at 4000 g for 5 min to obtain the extract for determination of extracellular activities. Fermented orange juice processing waste mixed with 1/10 v/v 0.02 M acetate buffer and sonicated under the aforementioned conditions was used for the estimation of waste endocellular activities. The protein content of the collected supernatants and the cell-free lysates were measured by the Bradford method [24].

A mixture of 1.6 mL 4 mM p-nitrophenyl-β-D-xylopyranoside (Apollo Scientific Ltd., Manchester, UK) in 0.02 M acetate buffer and 0.4 mL cell-free lysate or culture supernatant/waste extract, as appropriate, was prepared for the determination of endocellular and extracellular 1,4-β-xylosidase activity, respectively. The mixture was incubated at 40 °C and the 1,4-β-xylosidase activity was measured spectrometrically at 410 nm by estimating the color change against blank at successive time intervals (up to 8 h) [25]. A standard curve was constructed by preparing dilutions from a 1 g/L p-nitrophenol solution in acetate buffer.

Endo-1,4-β-xylanase was estimated by mixing 1.6 mL 0.5% w/v xylan from corn core (TCI Chemicals, Tokyo, Japan) in 0.02 M acetate buffer and 0.4 mL cell-free lysate or culture supernatant/waste extract, as appropriate, and incubation of the mixture at 50 °C for a period of 12 h [26]. A color reaction was made by adding 2 mL dinitrosalicylic acid (DNS) solution, consisting of 1% w/v dinitrosalicylic acid, 0.2% w/v phenol, 0.05% w/v Na$_2$SO$_3$ and 1% w/v NaOH [27], and placing the mixture at 65 °C for a period of 6 min. Color stabilization was achieved by the addition of 0.67 mL 40% w/v potassium sodium tartate solution. The color developed against a blank was determined spectrometrically at 540 nm. The standard curve was constructed by preparing dilutions from 0.4% w/v xylose solution and determining the color developed after reaction of the mixture with DNS reagent.

In all enzymatic determinations, estimation of the incubation period was made in preliminary assays. Xylanolytic activities are expressed as U (Units)/mg protein, where 1 enzyme unit is defined as the amount of endo-1,4-β-xylanase or 1,4-β-xylosidase needed for the cleavage of 1 mole/min of the appropriate substrate.

2.4. Molecular Characterization of Xylanolytic Bacteria Isolated from Orange Juice Processing Waste

Genomic DNA from the xylan-degraders exhibiting the highest xylanolytic activities was extracted by scraping cells from agar plates and further processing the biomass through the “NucleoSpin Tissue” DNA extraction kit (Macherey-Nagel, Düren, Germany). PCR 16S rRNA gene amplicons were obtained in a TP600 PCR apparatus (TaKaRa, Shiga, Japan) by using the primer set 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). A 50 μL-containing mixture was prepared for each bacterial strain, which consisted of 30 ng DNA, 10× PCR buffer, 2 mM MgCl$_2$, 0.2 mM dNTP (Jena Bioscience, Jena, Germany) and 0.5 μM primer (both 27F and 1492R) as well as 2.5 U Taq polymerase (Minotech Biotechnology, Heraklion, Greece). The PCR program comprised of 2 min initial denaturation at 94 °C, and 35 thermocycles of 30 s denaturation at
94 °C, 30 s primers’ annealing at 52 °C and 75 s at 72 °C. The thermocycling program was completed by a DNA extension period of 5 min at 72 °C. The 16S rRNA gene amplicons were ligated into T-Vector pMD20 (TaKaRa, Shiga, Japan) using T4 ligase (TaKaRa, Shiga, Japan) and the recombinant plasmids were TA-cloned into Escherichia coli DH5α cells (TaKaRa, Shiga, Japan). Plasmid DNA from cloned DH5α cells was obtained by using the “NucleoSpin Plasmid kit” (Macherey-Nagel, Düren, Germany) and sequenced at Eurofins Genomics (Germany). The amplified 16S rRNA gene products were merged in “CAP3 Sequence Assembly Program” [28]. The sequences of the most effective xylanolytic strains were deposited in GenBank under the code numbers MK212383-MK212384. The 16S rRNA gene amplicon and its closest relatives identified by using blastn were aligned through the use of “Clustal Omega” platform [29]. MEGA X was used for the construction of the phylogenetic tree [30] using the Jukes and Cantor algorithm [31], whereas the tree topology was based on the “neighbor-joining” method [32] and bootstrapping of 1000 trees.

2.5. Identification of Microbial Community Structure in Orange Juice Processing Waste by Illumina Sequencing

The Macherey-Nagel DNA extraction kit (Germany) was employed for crude DNA extraction from the orange juice processing waste, following the manufacturer’s protocol. The V4 region of the 16S rRNA gene was amplified by the universal primers 515F (5′-GTGYCAGCMGCCGCGGTA-3′) and reverse primer 909R (5′-CCCCGYCAATTCTTTRAGT-3′), while the ITS region was amplified by the primers ITS1Fgood (5′-TCCGTAGGGTGAACCTGCGG-3′) and ITS4R (5′-TCTCCGCTTAATTGATATGC-3′). The PCR mixture was prepared at “Mr DNA” (USA) by using the “HotStarTaq Plus Master Mix Kit” (QIAGEN, Germantown, MD, USA) and the amplified reads were obtained by applying an initial denaturation step of 3 min at 94 °C, and 28 thermocycles of 30 s at 94 °C, 40 s primers’ annealing at 53 °C and 1 min DNA extension at 72 °C. The amplification procedure was completed by applying a DNA elongation step of 5 min at 72 °C. Ampure beads of Agencourt Bioscience (USA) were used for the purification of the amplified products. Illumina sequencing was carried out at “Mr DNA” (USA) in a MiSeq platform.

The amplified sequencing data was demultiplexed and trimmed by using the “Pipeline Initial Process” platform at RDP pipeline [33]. Sequencing data containing N(s), amplicons of inappropriate length and reads of low quality score (<20) were excluded by employing PandaSeq [34]. Chimeric sequences were discarded by using the UCHIME algorithm [35]. The bioinformatic tool of Nawrocki et al. [36] was employed to align non-chimeric reads, which were further grouped by the “Complete Linkage Clustering” option of RDP pipeline. The Illumina sequences of the three individual orange juice processing waste samples were deposited in SRA database at NCBI under the “Bioproject” code PRJNA506784 (BioSamples’ accessions: SRX5058664-SRX5058669).

2.6. Estimation of Ecological Indicators and Statistical Analysis

The Chao’s richness, Shannon diversity and Pielou’s evenness indices were determined as previously described [37]. ANOVA (Analysis of variance) carried out by Duncan’s comparison tests (p < 0.05) were employed to identify statistically significant differences among microbial xylanolytic activities. Letters in common on the top of the chart bars denote non-statistically significant differences.

3. Results and Discussion

A combination of culture-dependent microbiological techniques and enzymatic assays, as well as next generation sequencing approaches, were employed to isolate the major xylan-degraders and to identify the entire microbial community structure in orange juice processing waste, which was mainly comprised of orange peels. Examination of physicochemical properties revealed that the orange processing waste had an acidic pH of 4.00 ± 0.01 and a high organic content, i.e., which exceeded 85% w/w of the dry weight. Moreover, an important part of the organic content, i.e., 28.16 ± 1.38% w/w, consisted of soluble carbohydrates, with anthrone-reactive C being equal to 9.63 ± 0.99% w/w (Table 1). Nitrogen content was estimated to be 1.21 ± 0.03% w/w, a fact that indicates a remarkable
high C/N ratio, whereas total phosphorus and soluble phenolics were determined to be equal as 2.38 ± 0.10 and 77.4 ± 4.0 mg/g d.w., respectively (Table 1).

Table 1. Physicochemical traits of orange juice processing waste.

| Parameter                          | Mean ± SE (n = 3) |
|------------------------------------|------------------|
| pH *                               | 4.00 ± 0.01      |
| EC (mS/cm) *                       | 1.60 ± 1.02      |
| Dry weight content (%)             | 14.18 ± 0.90     |
| Total organic matter (% w/w)       | 85.95 ± 1.78     |
| Carbon (% w/w)                     | 49.85 ± 1.03     |
| Ash content (% w/w)                | 14.05 ± 1.78     |
| Total nitrogen (% w/w)             | 1.21 ± 0.03      |
| Total soluble carbohydrates (% w/w)| 28.16 ± 1.38     |
| Anthrone-reactive C (% w/w)        | 9.63 ± 0.99      |
| Total phosphorus (mg/g d.w.)       | 2.38 ± 0.10      |
| Total soluble phenolics (mg/g d.w.)| 77.4 ± 4.0       |

* 1/10 w/v, orange juice processing waste-to-water.

By following an enrichment strategy, a total of 24 microbial strains were isolated using agar plates where xylan from corn core served as the sole carbon and energy source. All microbial isolates were further screened through estimation of their xylanolytic activities. To evaluate their xylanolytic nature, both endo-1,4-β-xylanase and 1,4-β-xylosidase activities were determined in their lysate and exhausted growth medium. Regarding endo-1,4-β-xylanase, none of the microbial strains isolated exhibited extracellular activities, whereas certain microbial isolates exerted surface/intracellular endo-1,4-β-xylanase activities (Figure 1). In contrast, low extracellular 1,4-β-xylosidase activities were exhibited by almost all microbial strains isolated, except for isolates XYLA-14 and XYLA-18 (Figure 2), while no intracellular 1,4-β-xylosidase activities were detected for any of the examined isolates. Moreover, a total of 7 and 2 microbial isolates that grew on xylan-containing plates lacked endo-1,4-β-xylanase and 1,4-β-xylosidase activity, respectively (Figures 1 and 2).

![Figure 1](image)

**Figure 1.** Endo-β-1,4-xylanase activities (surface/intracellular fraction) of the microbial strains isolated from orange juice processing waste.
Figure 2. 1,4-β-xylosidase activities (extracellular fraction) of the microbial strains isolated from orange juice processing waste.

Regarding endo-1,4-β-xylanase, a range of 11 microbial isolates, i.e., XYLA-1, -2, -4, -7, -9, -12, -13, -14, -21, -29 and -30, marginally exhibited endo-1,4-β-xylanase activities, with their values being lower than 10 U/mg protein (Figure 1). In contrast, the endo-1,4-β-xylanase activities of the microbial strains XYLA-22 and XYLA-3 exceeded 170 and 280 U/mg protein, respectively, whereas endo-1,4-β-xylanase activities of 20–50 U/mg protein were determined for the microbial isolates XYLA-8, XYLA-11, XYLA-15 and XYLA-18 (Figure 1). Based on 1,4-β-xylosidase activity data, all microbial isolates (apart from XYLA-14 and XYLA-18) exhibited 1,4-β-xylosidase activities lower than 5 U/mg protein (Figure 2). The microbial isolates exhibiting the greatest xylanolytic activity, i.e., XYLA-3 and XYLA-22, were further characterized through 16S rRNA gene sequencing, both showing high phylogenetic similarity (99.9% and 99.8% respectively) to the type strains of the species *Pseudomonas psychrotolerans* and *P. oryzihabitans* (Figure 3). Even though certain *Pseudomonas* strains have been found to possess the ability to degrade xylan [38,39], no *P. psychrotolerans* or *P. oryzihabitans* isolates capable of degrading xylan have been reported so far.

Figure 3. Phylogenetic position of the most effective xylanolytic micro-organisms isolated from orange juice processing waste, based on bootstrapping of 1000 trees. Scale bar represents substitutions per nucleotide site.

Reports on the induction of xylanases from allochthonous bacteria growing in orange processing (by)products are limited in number, while no work on indigenous xylanolytic microbiota isolated from orange juice processing waste exists in the international literature. *Streptomyces* [40–42] and *Bacillus/Geobacillus* [43,44] spp. isolated from soil are common bacterial strains that have found to exhibit endo-β-1,4-xylanase activities when tested on orange processing (by)products. In these studies, their endo-1,4-β-xylanase activities were in the same order with those determined in the microbial isolates XYLA-3 and XYLA-22. However, extracellular rather than intracellular endo-1,4-β-xylanase
activities were assessed in these studies. Even though the vast majority of xylanases are extracellularly secreted in order to breakdown this particular polysaccharide in the supernatant, the microbial strains in this work showed no detected extracellular activity. This means that either xylan is hydrolyzed to xylo-oligomers by an undetected negligible amount of extracellular endoxylanases, which can be then transported into the cells and broken down by intracellular endoxylanases [45], or more probably, peripheral cell-bound xylanases of the isolated microbial strains are involved in xylan hydrolysis [46,47], permitting the transport of oligomers and monomers into the cytoplasm. Interestingly, a \textit{Pseudomonas putida} strain displayed surface endo-1,4-β-xylanase and 1,4-β-xylosidase activities [39]. In addition, no studies regarding 1,4-β-xylosidase activity of bacterial isolates growing in orange processing residues have been performed. The lower 1,4-β-xylosidase as compared to endo-1,4-β-xylanase activities could be attributed to the terminal-edge specificity of β-xylosidases in contrast to the internal multi-cleavage caused by xylanases during xylan breakdown.

On the other hand, allochthonous fungi, mainly of the genera \textit{Penicillium} and \textit{Aspergillus} spp., have been reported to exhibit both endo-1,4-β-xylanase and 1,4-β-xylosidase activities [48–50]. However, their orange processing (by)product-induced xylanase activity appeared to be lower in comparison to the respective activities expressed by terrestrial streptomycetes and bacilli.

A total of 245,457 and 391,508 bacterial and fungal non-chimeric reads respectively were obtained during triplicate analysis of the orange juice processing waste examined. At phylum/class level, the major bacterial taxa identified in this waste analyzed were mainly placed in \textit{Firmicutes} and \textit{Alphaproteobacteria} taxa, representing 74.34% and 25.54% of the total bacterial relative abundance (Figure 4).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Bacterial diversity at phylum/class level in the orange juice processing waste examined by Illumina assay technology.}
\end{figure}

At the genus level, the main operational taxonomic units were related to members of the genera \textit{Lactobacillus} and \textit{Acetobacter}, corresponding to 74.19% and 25.52% of the relative bacterial abundance, i.e., 99.71% of the total reads (Figure 5). The remaining bacterial diversity consisted only of 31 taxa, as detected in the Illumina assay (Figure 5).
Figure 5. Bacterial diversity at genus level in the orange juice processing waste examined by Illumina assay technology.

Regarding fungal community structure, the majority of the taxa identified were classified into the classes Saccharomycetes and Tremellomycetes, representing 95.51% and 3.84% of the total fungal reads, respectively (Figure 6).

Figure 6. Fungal diversity at class level in the orange juice processing waste examined by Illumina assay technology.

Hanseniaspora Zygosaccharomyces, Saccharomyces, Pichia and Cystofilobasidium were the predominant taxa at genus level, corresponding to an overall proportion of 98.66%. In particular, Hanseniaspora and Zygosaccharomyces represented 59.66% and 21.97% of the total fungal reads, respectively (Figure 7). Ecological indicators of bacterial and fungal communities are presented in Table 2.
Table 2. Ecological indicator indices of bacterial and fungal communities in the orange juice processing waste examined.

| Ecological Indicator Index | Bacterial Community | Fungal Community |
|----------------------------|---------------------|------------------|
| Chao’s richness index      | 29.44 ± 0.89        | 43.67 ± 2.73     |
| Shannon diversity index    | 0.59 ± 0.02         | 1.15 ± 0.04      |
| Pielou’s Evenness index    | 0.18 ± 0.01         | 0.32 ± 0.01      |

Figure 7. Fungal diversity in the orange juice processing waste examined by Illumina assay technology. Only fungal taxa with relative abundance above 0.01% are shown in the bar chart.

The fermentation process proliferated various yeasts, since the latter covered 99.58% of the total fungal reads, whereas bacterial population was restricted only to lactic and acetic acid bacteria (Figure 5). However, the major yeast and bacterial taxa identified in the fermented orange juice processing waste, apart from Cystoflobasidium spp., appear to be common inhabitants of orange fruits [51–54].

Regarding the endo-1,4-β-xylanase activity in the fermented orange juice processing waste, non-statistically significant differences were detected between the lysed biomass and the unsonicated sample, indicating that endo-1,4-β-xylanase activity act exclusively extracellularly (Table 3). On the other hand, the statistical significant differences found in 1,4-β-xylosidase activity measurements with and without sonication ($p < 0.01$) can be attributed to the fact that part of such activity was exerted by intracellular or cell-bound enzymes. Despite the fact that Pseudomonas spp. expressed the highest endo-1,4-β-xylanase activities among the bacterial isolates obtained, they consisted only of a minor part of the bacterial diversity in the fermented orange juice processing waste examined. Thus, the proliferation of this particular taxon is due either to the enrichment procedure performed in order to isolate effective xylan-degraders and/or to the fact that the Pseudomonas species identified was among the taxa that its growth was self-sufficient and not reliant on interactions occurring in microbial food web. The fact that only a minor part of the microbiome is cultivated should not be excluded. Moreover, a range of microorganisms, such as the bacteria Paenibacillus, Clostridium, Anoxybacillus, Acinetobacter and Caulobacter as well as the fungi Pichia, Penicillium, Cladosporium and Aureobasidium, which constitute only a minor part of the microbial population, possess the genetic ability to degrade xylan, as was shown by MiSeq data comparisons against NCBI (Table 4). On the other hand, the fact that such microorganisms were not isolated indicates that either they are uncultured isolates, or they were not proliferated during the enrichment procedure.
Table 3. Xylanolytic activities of the naturally-fermented orange juice processing waste.

| Xylanolytic Activity | Lysate | Bulk/Surface | p-Value |
|----------------------|--------|--------------|---------|
| Endo-1,4-β-xylanase activity (U/g d.w.) | 5.21 ± 0.59 | 4.77 ± 0.11 | 0.509 |
| 1,4-β-xylosidase activity (U/g d.w.) | 3.41 ± 0.14 | 2.50 ± 0.13 | 0.009 |

Table 4. Potential xylan-degrading microbiota identified from MiSeq data.

| Taxon | 16S rRNA Gene Accession Number of the Closest Species | Closest Species | Identities | Reference |
|-------|-----------------------------------------------------|-----------------|------------|-----------|
|       |                                                     | P. xylanexedens | 370/370 (100%) | [55] |
| g__Paenibacillus | MF347934 | C. hiranonis | 350/396 (88.4%) | [56] |
| g__Clostridium | NR_028611 | A. genensis | 392/396 (99.0%) | [57] |
| g__Anoxybacillus | CP012152 | A. lwoffii | 394/395 (99.7%) | [58] |
| g__Acinetobacter | NR_113346 | C. segnis | 393/394 (99.7%) | [59] |

| Taxon | ITS-5.8S rRNA Gene Accession Number of the Closest Species | Closest Species | Identities | Reference |
|-------|----------------------------------------------------------|-----------------|------------|-----------|
| g__Pichia | NR_130688 | P. fermentans | 173/174 (99.4%) | [60] |
| g__Penicillium | MH856375 | P. italicum | 267/267 (100%) | [61] |
| g__Cladosporium | NR_152286 | C. angustiherbarum | 250/250 (100%) | [62] |
| g__Aureobasidium | NR_156246 | A. laevispermi | 275/275 (100%) | [63] |

It may be concluded that orange juice processing waste harbors a specialized xylanolytic community and should be considered as a source for the isolation of xylan-degraders and the recovery of xylanolytic enzymes with diverse environmental and industrial uses, such as in juice clarification, solid state fermentation and energy recovery from non-conventional organic resources.

Author Contributions: I.Z. performed the physicochemical analysis of orange juice processing waste and the isolation of xylan-degrading microbiota; I.Z. and N.R. performed the enzyme assays; I.Z. performed the identification of xylan-degrading microbiota; N.R. carried out the Illumina analysis; S.N. designed and supervised the work; S.N., N.R. and I.Z. drafted and edited the paper, read and approved the final manuscript.

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