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Exploring the biotechnological potential of *Acinetobacter soli* ANG344B: A novel bacterium for 2-phenylethanol production

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ABSTRACT

A bacterium, *Acinetobacter soli* ANG344B, isolated from river water, exhibited an exceptional capacity to produce 2-phenylethanol (2-PE) using L-phenylalanine (L-Phe) as a precursor—a capability typically observed in yeasts rather than bacteria. Bioreactor experiments were conducted to evaluate the production performance, using glucose as the carbon source for cellular growth and L-Phe as the precursor for 2-PE production. Remarkably, *A. soli* ANG344B achieved a 2-PE concentration of 2.35 ± 0.26 g/L in just 24.5 h of cultivation, exhibiting a global volumetric productivity of 0.10 ± 0.01 g/L.h and a production yield of 0.51 ± 0.01 $g_{2\text{-PE}}/g_{\text{L-Phe}}$, a result hitherto reported only for yeasts. These findings position *A. soli* ANG344B as a highly promising microorganism for 2-PE production.

Whole-genome sequencing of *A. soli* strain ANG344 revealed a genome size of 3.52 Mb with a GC content of 42.7 %. Utilizing the Rapid Annotation using Subsystem Technology (RAST) server, 3418 coding genes were predicted, including genes coding for enzymes previously associated with the metabolic pathway of 2-PE production in other microorganisms, yet unreported in *Acinetobacter* species. Through gene mapping, 299 subsystems were identified, exhibiting 30 % subsystem coverage. The whole genome sequence data was submitted to NCBI GeneBank with the BioProject ID PRJNA982713. These draft genome data offer significant potential for exploiting the biotechnological capabilities of *A. soli* strain ANG344 and for conducting further comparative genomic studies.

1. Introduction

The 2-phenylethanol (2-PE) is a highly significant flavoring compound renowned for its distinctive rose-like fragrance. Its versatile applications span various industries, including cosmetics, perfume, laundry, home care products, pharmaceuticals, as well as the food and beverage sector [1–3]. Moreover, 2-PE holds promise as a potential candidate for biofuel production [4].

The natural rose-like fragrance is traditionally obtained through extraction from essential oils derived from plants and flowers, notably roses. However, this method is associated with high production costs attributed to low recovery rates [5–8]. Consequently, natural 2-PE falls short of meeting market demand. As a result, the majority of 2-PE utilized in various industries is still obtained through chemical production, which offers significantly lower costs. The chemical synthesis process of 2-PE is accompanied by significant drawbacks, including the requirement for extreme operational and the use of toxic chemicals, having impact on product quality [7–11]. The utilization of chemically synthesized 2-PE in food and cosmetic products is subject to restrictions imposed by US and European legislations, reflecting growing concerns regarding its use and environmental impacts [12]. Consequently, microorganisms that possess the natural metabolic ability to produce 2-PE

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present a promising alternative. The aroma produced by this biotechnological approach can be considered as natural by the U. S. Food and Drug Administration and European legislation [2,7].

Ehrlich pathway is considered the most efficient metabolic approach for 2-PE production by microorganisms. This pathway starts with the transamination of l-Phe-to phenylpyruvate by aromatic amino acid aminotransferases, that is decarboxylated to phenylacetaldehyde and further reduced to 2-PE through the action of alcohol dehydrogenases [1,2,7]. The microbial production of 2-PE has primarily been reported in yeasts, being the most studied microorganism for 2-PE production. *Saccharomyces cerevisiae, Kluyveromyces marxianus, Pichia fermentans, Candida glycerinogenes*, and *Yarrowia lipolytica*, typically yield 2-PE production below 4 g/L [13–19]. In contrast, bacteria such as *Microbacterium* sp., *Brevibacterium linens, Proteus vulgaris, Proteus mirabilis, Psychrobacter* sp., and *Erwinia carotovora* have been reported to produce 2-PE, albeit at lower levels (below 152 mg/L), with limited subsequent advancements [20–22]. Remarkably, genetically engineered bacteria significantly enhanced the production of 2-PE, resulting in reported yields of 5.3 g/L for *E. coli* BW25113 and 4.7 g/L for *E. coli* BL21(DE3), both expressing yeast genes [23,24]. However, it is crucial to acknowledge that concerns regarding genetically modified organisms hinder their widespread adoption. Until now, only a few studies have explored the genomic information related to bacterial biosynthetic pathways for 2-PE production. *Enterobacter* sp. CGMCC 5087 stands out, as it has revealed a unique pathway involving the *de novo* synthesis of 2-PE from glucose via the phenylpyruvate pathway [25].

The genus *Acinetobacter* is widely distributed in nature and known for its versatile metabolism, allowing it to utilize various carbon sources depending on the ecological niche [26]. Consequently, *Acinetobacter* strains have been extensively studied for their biotechnological applications, including the degradation of diverse pollutants such as organic compounds, inorganic substances, and heavy metals [27]. Additionally, *Acinetobacter* strains have been explored for the production of valuable bio-products, including polysaccharides, polyesters, lipases, and commercially available bioemulsifiers such as emulsan, biodispersan, and alasan. These bioemulsifiers are currently utilized in microbial enhanced oil recovery and the biodegradation of toxic compounds [28–30]. *Acinetobacter soli* species was first identified in 2008 from forest soil in the Republic of Korea [31] and has demonstrated its potential in various biotechnological endeavors [32,33]. Interestingly, despite these various biotechnological applications, the production of 2-PE has never been reported among *Acinetobacter* isolates.

The aim of this study was to evaluate the capacity of the newly isolated bacterium *A. soli* ANG344B to produce 2-PE. Bioreactor experiments were performed using glucose as the carbon source for cellular growth and l-Phe-as the precursor for 2-PE biosynthesis. Furthermore, the study aimed to conduct a comprehensive genome analysis of *A. soli* ANG344B to identify key enzymes or metabolic pathways closely linked to 2-PE production, marking the first investigation of such genes within the *Acinetobacter* genus and advancing our understanding of *Acinetobacter* sp. metabolism.

2. Material and methods

2.1. Bacterial strain isolation

The strain ANG344B used in this study was obtained as part of a major project aiming the characterization of bacteria from non-clinical sources. During the laboratory screening performed with a water sample, obtained from the Catumbela river, in Benguela Province (Angola), it was perceived that this strain was able to emit a rose-flavor and was further identified and characterized.

2.2. Genome sequencing, assembly, annotation, and bioinformatic analysis

Total DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. DNA concentration was estimated using the Qubit dsDNA HS Assay Kit and the Qubit 3.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, USA). Extracted DNA was then sequenced using a standard protocol with 2 % 125 paired-end runs on an Illumina HiSeq 2000 (Illumina, San Diego, CA, USA). The quality of the high-throughput sequence data was assessed by FastQC (http://www.bioinformatics.babraham.ac.uk/projects/ fastqc/). Raw sequence reads were then de novo assembled using SPAdes 3.9.0 (http://cab.spbu.ru/software/spades/) and the quality was assessed by QUAST (http://quast.sourceforge.net/quast). Since average nucleotide identity (ANI) is a genomic parameter created to simulate DNA-DNA hybridization in silico, we performed whole-genome comparisons based on ANI with JSPecies Web Server (https://jspecies.ribohost.com/jspeciesws/#home) using *Acinetobacter* species type strains for analysis [34] using two methods, the BLAST algorithm (ANIb) and the MUMmer alignment tool (ANIm). While ANIb calculates ANI values using a BLAST (Basic Local Alignment Search Tool) algorithm, involving the alignment of short, overlapping DNA fragments (typically 1020 base pairs) from two genomes and calculating the percentage of nucleotide identity between these fragments, the ANIm (MUMmer-based ANI) uses the MUMmer (Maximal Unique Match) alignment tool to align the entire genomes of two organisms. It identifies unique regions (maximal unique matches) between the genomes and computes the ANI based on these alignments being more accurate than ANIm because it considers the entire genome, including regions of high conservation and variability [34].

The genome sequence data were uploaded to the Type (Strain) Genome Server (TYGS), a free bioinformatics platform available under https://tygs.dsmz.de, for a whole genome-based taxonomic analysis [35]. The TYGS analysis was subdivided into the following steps: (1) determination of closest type strain genomes by comparing against all type strain genomes available in the TYGS database via the MASH algorithm; and (2) against the 16S rRNA gene sequence of each of the currently 16,634 type strains available in the TYGS database. Secondary metabolites production was predicted using the Natural Product Domain Seeker.

SNP analysis was performed with the CSI Phylogeny tool from the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/CSIPhylogeny/) using all *A. soli* genomes available at NCBI on November 4th, 2022.

The RAST server (http://rast. nmpdr.org) was used for an automatic annotation.

The distribution and richness in antimicrobial resistance and virulence genes' content was compared between ANG344B using ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/), the Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca) and the Virulence Factors Database (VFDB) of pathogenic bacteria (http://www.mgc.ac.cn/cgi-bin/VFs/genus.cgi?Genus=Acinetobacter).

Genes coding for enzymes involved in phenylalanine metabolism according to KEGG information, were extracted from ANG344B whole-genome data and annotated with Geneious R10.

2.3. Experimental setup and conditions for 2-PE production

2.3.1. Culture preparation

A. soli ANG344B, preserved in cryovials containing glycerol (20 %, v/v) at -80°C was reactivated in Luria-Bertani (LB) medium plates (bacto-tryptone, 10 g/L; yeast extract, 5 g/L; sodium chloride, 10 g/L; agar, 18 g/L). For inoculum preparation, a colony was transferred to 100 mL of LB medium and incubated in an orbital shaker for 20 h (30°C, 200 rpm).

2.3.2. Batch bioreactor operation

Experiments were carried out in 2 L bioreactors (BioStat B-plus, Sartorius, Germany) using a 5 % (v/v) of inoculum (100 mL). The culture was grown in a medium containing Na_2HPO_4, 4 g/L; KH_2PO_4, 1 g/L; NaCl, 0.2 g/L, $MgSO_4{\cdot}7H_2O$, 0.2 g/L; $CaCl_2.H_2O$, 0.05 g/L; glucose anhydrous, 20 g/L; yeast extract, 5 g/L and l-Phe, 5 g/L. Temperature was controlled at 30.0 ± 0.1 °C and pH at 7.00 ± 0.02 by the automatically addition of 5 M NaOH and 5 M HCl. The aeration rate (1 vvm, volume of air per volume of reactor per minute) was kept constant during the experiments. The dissolved oxygen concentration was controlled at 30 % of the air saturation by the automatic variation of the stirrer rate between 300 and 1000 rpm. Foam formation was prevented by automatic addition of antifoam Y-30 emulsion (Sigma-Aldrich).

2.4. Analytical methods and quantification procedures

2.4.1. Cell separation and sample processing

Culture broth samples were taken periodically and centrifuged at 16 099 × *g*, for 12 min at 4 °C, for cell separation. The cell-free supernatant (stored at -20 °C) was used for determination of l-Phe, 2-PE, glucose and total nitrogen. The cell pellet was used for cell dry weigh (CDW) determination. After washing twice with deionized water (resuspension in water, centrifugation in the same conditions) the pellets were lyophilized and weighted.

2.4.2. l-Phenylalanine and 2-Phenylethanol quantification

L-Phe and 2-PE concentration was determined by high performance liquid chromatography (HPLC) (Alliance) equipped with a C18 3.9 mm × 150 mm column (particle diameter of 4 mm) (Nova-Pak®) and guard column, coupled to a PDA detector, set at 215 nm. Samples were diluted in deionized water and filtered through 0.2 μm centrifuge filters. The injection volume was 20 μL and compounds were separated at 25 °C. A gradient method comprising water/acetonitrile was applied as follows: 0–15 min 90/10 (flow 0.5 mL/min), 15–30 min 70/30 (flow 0.5 mL/min); 30–33 min 90/10 (flow 0.5 mL/min). l-Phe (pure, pharma grade, PanReac AppliChem) and 2-PE (≥99 %, Sigma-Aldrich) were used as standards in concentrations between 0.012 and 0.2 g/L.

2.4.3. Glucose quantification

For glucose quantification, the cell-free supernatant was filtered through 0.2 μm centrifuge filters and analyzed by HPLC (VWR Hitachi Organizer) with a detector Merck Differential Refractometer RI-71. The pre-column and the column were Metacarb 87H (VARIAN) and Aminex HPX −42A 125–0129 (Bio-Rad), respectively. The samples were separated during 15 min at 30 °C with 0.01 N H_2SO_4 as eluent at the flow rate of 0.5 $mL.min^{-1}$. D (+) glucose anhydrous (Scharlau) was used as standard, at concentrations between 0.03 and 1.0 g/L.

2.4.4. Nitrogen quantification

Total nitrogen was quantified using the Hach Lange Laton® total nitrogen (TN_b) kit protocol. The samples were diluted in deionized water to have a nitrogen concentration within the range measured by the kit (between 20 and 100 mg/L). After the procedure indicated in the protocol, the total nitrogen was measured in the spectrophotometer DR 2800™ by recognition of the bar code present in the sample cell.

2.4.5. Statistical analysis

All experiments were performed at least in duplicate and the results are expressed as mean ± standard deviation

3. Results and discussion

3.1. Isolation and characterization of strain ANG 344B

During laboratory experiments, strain ANG344B, isolated from river water, exhibited a distinctive rose-flavor profile, prompting further investigation. Partial sequencing of the *rpoB* gene revealed a high level of homology (98 %) with the *rpoB* gene from the *A. soli* type strain KCTC 22184(T). To preserve the isolate for patent purposes, it was deposited in the Microbial Strain Collection of Latvia (MSCL) under the accession number 1593.

3.2. A. soli ANG344B genome features

The main findings of the genomic analysis conducted in this study are summarized in Fig. 1. The genome of *A. soli* ANG344B was assembled into 123 contigs (N_{50} value of 98,983 bp, GC content of 42.7 %) with a total genome size of 3,524,841 bp and 3418 predicted coding sequences. To validate the identity of ANG344B as *A. soli*, a comparative analysis was performed with the reference strain *A. soli* KCTC 22184T (Genbank accession number GCA_000760595.1). Using the BLAST algorithm (ANIb) and the MUMmer alignment tool (ANIm) at JSpeciesWS, the average nucleotide identity (ANI) between ANG344B and the reference strain was found to be 98.29 % and 98.60 %, respectively (Table S1, Suppl. Material). These results provide strong evidence supporting the identification of ANG344B as *A. soli*. Additionally, the TYGS analysis (Fig. 1a) further confirmed the classification of ANG344B as *A. soli.*

Overall, *A. soli* isolates exhibited a range of 47–23,756 single nucleotide polymorphisms (SNPs) (Table S2, Suppl. Material). It is of note that, when compared to ANG344B, its closest relative exhibited a difference of 10,885 SNPs, highlighting the distinctive genomic characteristics of this particular isolate. However, given the diversity in years, sources, and geographic regions, the significance of the obtained SNP values is challenging to determine. It is noteworthy that *A. soli* isolates displayed a limited presence of virulence factors (VFs) and antimicrobial resistance genes (ARG) compared to *Acinetobacter* species commonly associated with infections (Table S3, Suppl. Material).

A minority of the identified gene clusters (33 %; 1107/3418) exhibited known functions based on the COG (Clusters of Orthologous Groups) database. These clusters were primarily associated with essential housekeeping functions, including amino acids and derivatives, proteins, and carbohydrates metabolism, but also associated with cofactors, biosynthesis (*e.g.*, biotin, thiamin) (Fig. 1b).

In addition, it is worth highlighting the presence of genes involved in the catabolism of aromatic compounds, including salicylate ester, quinate, biphenyl, benzoate and p-Hydroxybenzoate degradation, indicating the potential capability of the *A. soli* ANG344B strain to handle and process these types of compounds.

The search for enzymes involved in l-Phe-metabolism and potentially responsible for 2-PE production revealed the presence of the genes coding for the aromatic aminotransferase (AAAT) (EC 2.6.1.57) responsible for transamination of l-Phe-to α-ketoglutarate resulting in phenylpyruvate, the alpha-keto acid decarboxylase (KDC) responsible for decarboxylation of phenylpyruvate to phenylacetaldehyde (EC 4.1.1.1) and the ADH, alcohol dehydrogenase (EC 1.1.1.1) that catalyzes the oxidation of phenylethanol to aldehyde and ketone, respectively, and also can catalyze the reverse reaction. Analysis of their aminoacidic sequences revealed high identity (>99 %) with enzymes from other *Acinetobacter* isolates genomes deposited in NCBI, although it should be noted one aminoacidic substitution in the KDC at position 566 from a leucine to a serine, which may have impact in the protein polarity. For an overview of the l-Phenylalanine metabolic pathway and to showcase the predicted enzyme activities, Fig. 1c illustrates a visual representation, including the identified products in A. soli ANG344B. It is worth noting that the GC% content of the genes encoding the three enzymes exhibited variations. The GC% was observed to be 45.1 % for AAAT, 41.7 % for KDC, and 49.7 % for ADH. Notably, the GC% values for AAAT and ADH significantly differ from the overall GC% content of the ANG344B genome (42.7 %). This disparity in GC% content may suggest that these genes were acquired through horizontal gene transfer.

However, it is important to highlight that no plasmid-related

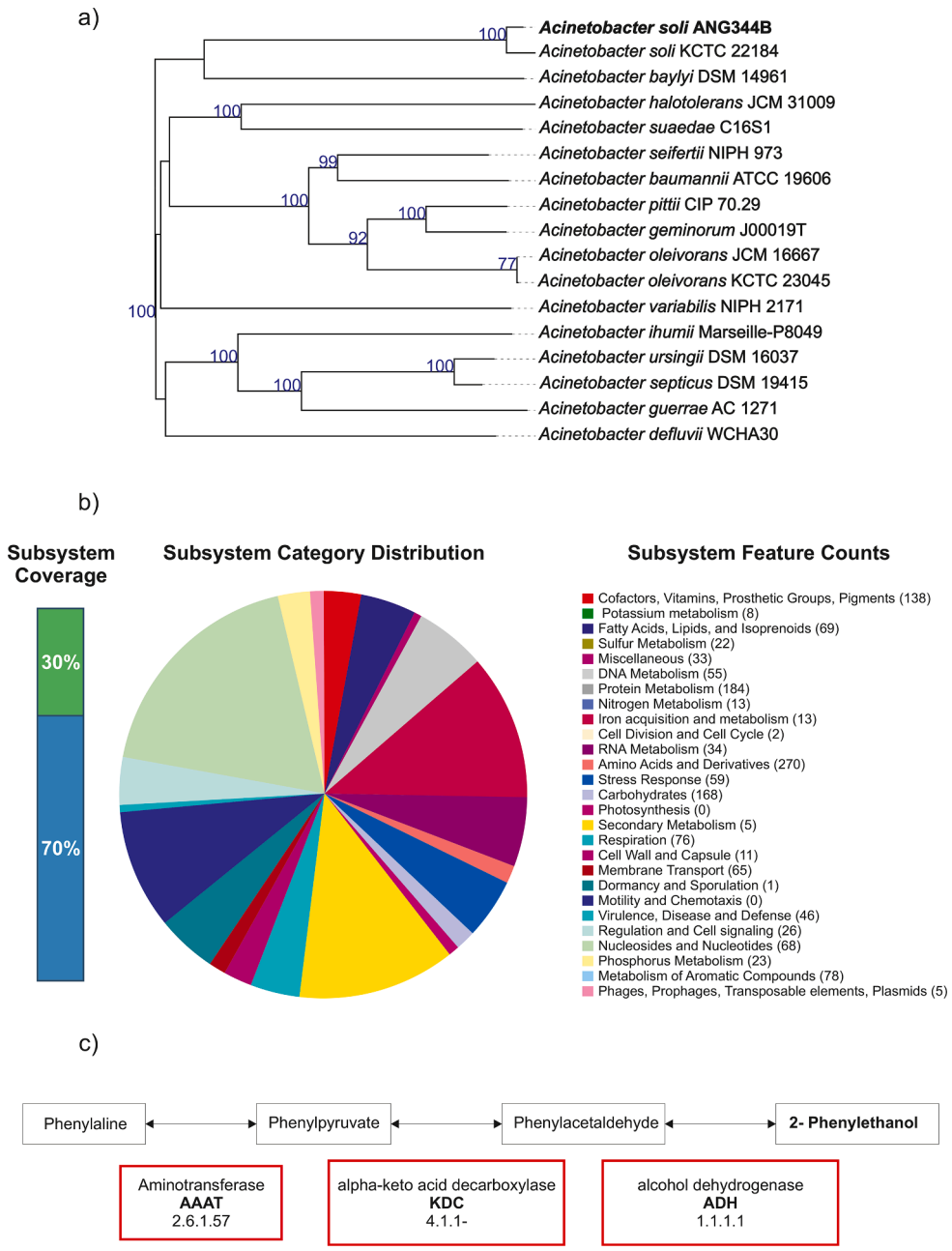

Fig. 1. Summary of the main findings from the genomic analysis conducted in this study. **a)** tree inferred with FastME 2.1.6.1 [36] from GBDP distances calculated from genome sequences. The numbers above the branches are GBDP pseudo-bootstrap support values > 60 % from 100 replications, with an average branch support of 86.1 %. The tree was rooted at the midpoint [37]; **b)** schematic overview of Subsystem Coverage, Subsystem Category Distribution and Subsystem feature counts predicted in *Acinetobacter soli* strain ANG344B using RAST server. Only 30 % of gene clusters identified had known COG function; **c)** schematic representation of the Ehrlich pathway involved in the synthesis of 2-phenylethanol. Enzymes predicted from the ANG344B genome are highlighted in red, including: AAAT (aromatic amino acid aminotransferase), responsible for the transamination of l-phenylalanine to α-ketoglutarate resulting in phenylpyruvate; KDC (alpha-keto acid decarboxylase), responsible for the decarboxylation of phenylpyruvate to phenylacetaldehyde; and ADH (alcohol dehydrogenase), which catalyzes the oxidation of phenylethanol to aldehyde and ketone, respectively, and can also catalyze the reverse reaction.

elements were identified during the RAST annotation process. Instead, elements associated with phage packaging machinery and phage capsid proteins were observed. This observation suggests the possible involvement of phages in the mobilization of these genes.

3.3. Bioproduction of 2-PE by A. soli ANG344B

To validate the 2-PE production capability of the newly isolated bacterium, a batch assay was conducted in a 2 L bioreactor. The bacterium was grown using 20 g/L glucose as the carbon source, 5 g/L yeast extract as nitrogen source, and 5 g/ l-Phe-as the precursor for 2-PE synthesis. Fig. 2 illustrates the dynamic profiles of cellular growth (cell dry weight - CDW), glucose, nitrogen and l-Phe-consumption and 2-PE production throughout the assay.

A. soli ANG344B exhibited exponential growth for approximately 13 h, with a specific growth rate of 0.21 ± 0.02 h^{-1}. During this phase, the biomass concentration reached a maximum of 5.25 ± 0.25 g_{CDW}/L, consuming 9.30 ± 0.28 g/L of glucose during that period, resulting in a cell growth yield on glucose ($Y_{X/S}$) of 0.56 ± 0.00 $g_{CDW}/g_{glucose}$. The cellular concentration remained constant throughout the remainder of

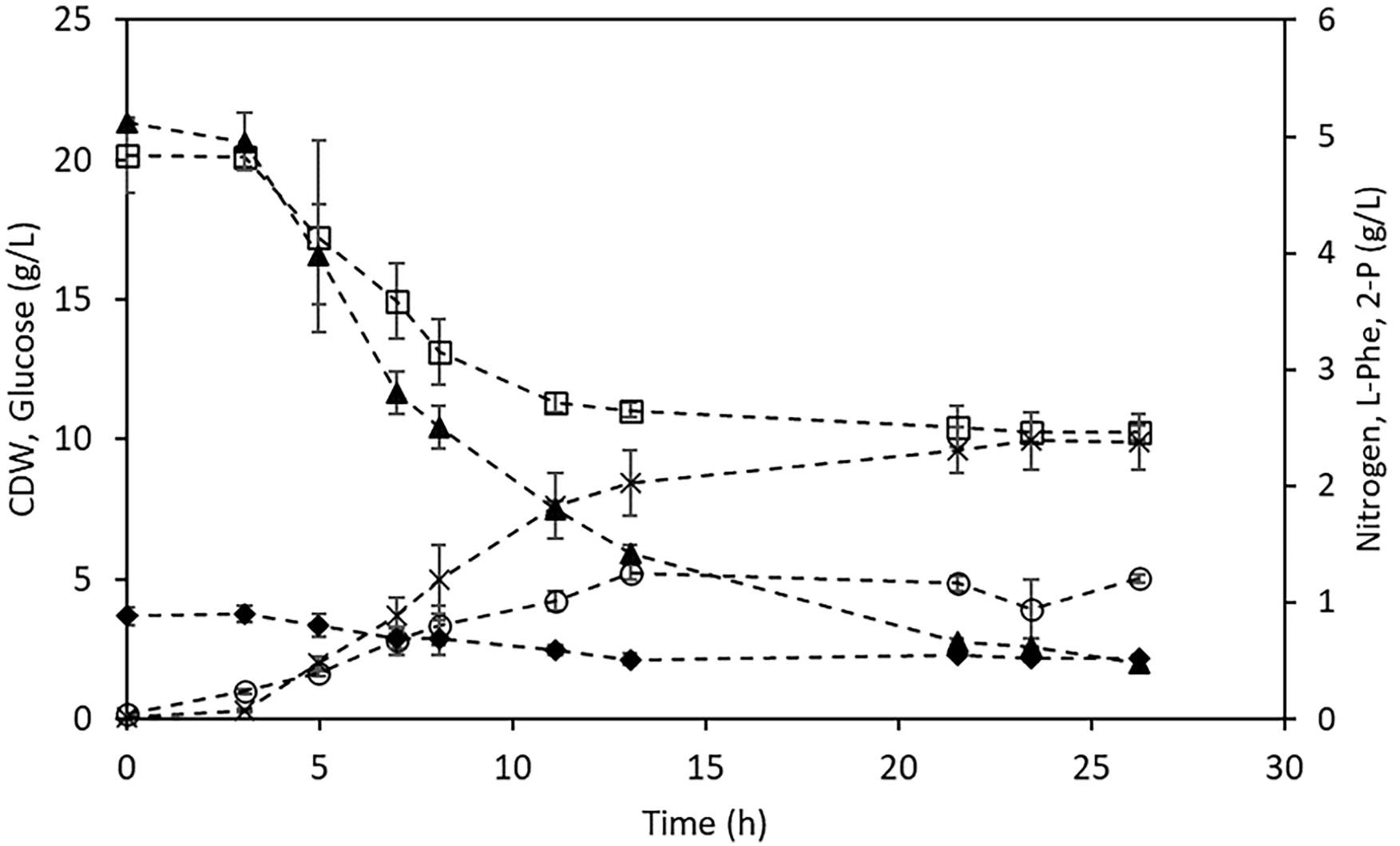

Fig. 2. *A.soli* ANG344B cultivation in batch operational mode showing the profiles of cell dry weight (CDW ○), glucose concentration (□), nitrogen concentration(◆), l-Phenylalanine concentration(▲), and 2-Phenylethanol concentration (x).

the experiment.

The production of 2-PE commenced 3 h into the process and reached its maximum concentration after 24.5 h. From the conversion of 4.60 ± 0.46 g/L of l-Phe, a significant 2-PE concentration of 2.35 ± 0.26 g/L was achieved. This corresponds to a production yield ($Y_{P/S}$) of 0.51 ± 0.01 $g_{2\text{-PE}}/g_{\text{L-Phe}}$.and a global volumetric productivity (r_p) of 0.10 ± 0.01 $g_{2\text{-PE}}$/L.h.

The production of 2-PE in this study was found to be growth-associated, as shown in Fig. 2, and as reported for yeasts [38,39]. Notably, during the exponential growth phase (up to the 13th hour), the production rate of 2-PE was significantly higher, reaching 0.15 ± 0.02 g/L.h. In contrast, during the stationary growth phase, the production rate decreased to 0.03 ± 0.00 g/L.h. Even though the nitrogen and carbon sources were not limiting the cultivation, the culture entered in stationary growth phase and decrease the 2-PE production. This might be caused by the decrease in other important nutrient concentrations or by the presence of high concentrations of 2-PE in the cultivation broth that might result in product toxicity towards the producing microorganism, avoiding further growth and consequently aroma production. In fact, the 2-PE production is reported to be limited due to its inhibitory effects towards microorganisms [2]. 2-PE concentrations between 2 and 4 g/L are reported to inhibit cellular growth of many yeasts capable of producing this aroma compound [2]. Strategies to improve 2-PE production by *A. soli* ANG344B might go through the evaluation of *in situ* product removal techniques to avoid product toxicity.

These findings demonstrate the competitiveness of our results compared to those reported in the literature for yeasts. The 2-PE production achieved by *A.* soli ANG344B (2.35 ± 0.26 g/L) is within the reported for yeasts. The most reported yeast strains producing 2-PE are *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* achieving 2-PE productions up to 4.5 and 1.45 g/L, respectively, but many other yeasts are being reported for 2-PE production, achieving similar 2-PE production levels to the aforementioned strains (as summarized in Table 1). The production yield achieved by *A. soli* ANG344B (0.51 ± 0.01 $g_{2\text{-PE}}/g_{\text{L-Phe}}$) also falls within the range of values reported for yeasts (0.31 to 0.89 g/g), further emphasizing the promising potential of our bacterium as a robust 2-PE producer [13,14,16–18,38,40–45]. Moreover, it is noteworthy that the global volumetric productivity achieved in this study is among the highest reported in the literature for a wild type microorganism in a batch operational mode, without the implementation of *in situ* product removal techniques. Specifically, the obtained value of 0.10 ± 0.01 g/L.h is comparable to the productivity reported for *Candida glycerinogenes* WL 2002–5 (0.11 g/L.h) [18]. This significant result underscores the tremendous promise of 2-PE production from *A. soli* ANG344B. The inherent advantages offered by bacteria in terms of their shorter growth time, simpler metabolic pathways with their streamlined metabolism, makes them ideal candidates for enhancing the natural production of 2-PE. The ability of *A. soli* ANG344B to achieve such a high yield of 2-PE production makes it particularly noteworthy in this regard.

Importantly, our study represents a novel contribution to the field as it highlights the ability of bacteria to produce 2-PE. Previous reports on bacterial 2-PE production were limited, with only a few bacteria, such as *Microbacterium* sp., *Brevibacterium linens, Proteus vulgaris, Psychrobacter* sp., and *Erwinia carotovora*, being documented as producers of 2-PE [20–22]. However, these bacteria exhibited very low production values, typically below 152 mg/L.

4. Conclusions

In this study, we have unveiled a strain from the *Acinetobacter soli* species, ANG344B, which exhibits an unprecedentedly high yield in 2-PE production. To the best of our knowledge, this is the first report of such a remarkable production level of 2-PE in a wild-type bacterium, also challenging the prevailing notion that only a limited number of bacteria possess the capability to produce 2-PE.

Furthermore, the investigation of the complete genome of *A. soli* ANG344B provided valuable insights into the genetic basis underlying its 2-PE production capability. Understanding the biosynthetic and metabolic potential of this strain is crucial for exploiting and harnessing its 2-PE production capabilities. The comprehensive dataset generated through this study serves as a valuable resource for further exploration and investigation of the biosynthetic pathways involved.

In addition to its role in 2-PE production, the study also shed light on the potential ecological significance of this capability in the natural environment. In the specific niche occupied by *A. soli* ANG344B, the production of 2-PE may confer certain advantages, as this compound has been shown to possess antimicrobial activity. The ability to produce 2-PE could serve as a competitive advantage for this strain in its ecological niche.

Table 1
Comparative analysis of production parameters in yeast without *in situ* product removal techniques and current study for 2-PE production.

Strain	Cultivation	L-Phe_i (g/L)	2-PE (g/L)	Y(P/S) (g/g)	r_p (g/L h)	Reference
Saccharomyces cerevisiae JM 2014	Batch, Bioreactor	5	3.60	0.79	0.05	[14]
Saccharomices cerevisiae Ye9-612	Fed-batch Bioreactor	10	4.5	0.81	0.065	[38]
Candida glycerinogenes WL 2002 - 5	Batch Bioreactor	7	5	0.71	0.11	[18]
Pichia fermentans L-5	Batch Shake flask	1	0.453	0.53	0.028	[17]
Kluyveromyces marxianus CBS 600	Batch Bioreactor	7	1.45	0.44	0.05	[16]
Yarrowia lipolytica	Shake flask	7	1.98	0.31	0.02	[13]
Wickerhamomyces anomalus	Fed-batch bioreactor	7 + 5	4.72	-	0.098	[44]
Zygosaccgaromyces rouxii M2013310	Batch Shake flask	-	3.58	-	0.05	[45]
Saccharomyces cerevisiae BCRC 21812	Batch Bioreactor	4	2.53	0.69	0.013	[43]
Pichia kudriavzevii YF1702	Batch Shake flask	10.7	5.09	-	-	[42]
Kluyveromyces marxianus and Debaryomyces hansenii (co-culture)	Batch Shake flask	4	2.55	0.89	0.03	[41]
Yarrowia lipolytica CH 1/5	Fed-batch Bioreactor	8 + 4	3.2	0.29	0.0143	[40]
Acinetobacter soli	Batch, Bioreactor	5.12 ± 0.01	2.35 ± 0.26	0.51 ± 0.01	0.10 ± 0.01	This study

Overall, the findings presented in this study have significant implications for both industrial and ecological perspectives. The exceptional 2-PE production capacity of *A. soli* ANG344B, coupled with its genetic insights, opens up new avenues for optimizing and leveraging bacterial metabolism for enhanced production of valuable compounds. The data generated in this study lays the foundation for future studies aimed at unraveling the biosynthetic and metabolic potential of this strain, thereby contributing to the broader understanding of bacterial physiology and ecological interactions.

Moving forward, it is imperative to explore optimal cultivation conditions and operational strategies for ANG344B, with the goal of enhancing cellular growth and 2-PE production. These future investigations will play a pivotal role in elevating the industrial relevance of this production process, ultimately enabling efficient and competitive production of this aromatic compound with a natural status.

CRediT authorship contribution statement

Ana R.S. Bernardino: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Filipa Grosso:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Cristiana A.V. Torres:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Maria A.M. Reis:** Writing – review & editing, Funding acquisition, Conceptualization. **Luísa Peixe:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Luisa Peixe, Cristiana A.V. Torres has patent #EP 4028535 A1 20220720 (EN) issued to UPorto and NOVA FCT. Maria A.M. Reis, Filipa Grosso has patent #EP 4028535 A1 20220720 (EN) issued to UPorto and NOVA FCT and declare that an European patent application (No. 20745298.8) protecting the 2-phenylethanol producing *Acinetobacter* soli ANG344B disclosed in this manuscript has been filed. The authors have nothing to declare.

Data availability

No data was used for the research described in the article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.btre.2024.e00839.

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