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Isolation of sodium benzoate-degrading bacteria from rice noodle manufacturing wastewater in Can Tho, Viet Nam

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ABSTRACT

Diverse starch-based foods have been widely consumed. To keep the products in good quality, preservatives are added. Sodium benzoate is one of the most commonly used food preservatives for starch-based products. As an antimicrobial compound, high concentrations of sodium benzoate in water can pose risks to aquatic indigenous microbial communities. From wastewater samples of three rice noodle facilities, 27 sodium benzoate-degrading bacteria were isolated on minimal mineral medium supplemented with sodium benzoate (50 mg/L). Isolates SB2.1, SB2.2, SB2.4, SB3.10, and SB3.13 demonstrated their best sodium benzoate degradation efficacy in liquid minimal mineral medium containing 1,250, 2,500, and 5,000 mg/L sodium benzoate, quantitatively measured by the spectrophotometric method at 225 nm. Optimal degradation efficacy (>93%) at 1,250 mg/L sodium benzoate was observed when isolates SB2.1, SB2.2, SB2.4, and SB3.10 were grown in a minimal mineral medium at pH 7-9. Notably, SB3.10 exhibited chemotaxis towards sodium benzoate at 24 hours of incubation. Based on 16S-rRNA gene sequencing and biochemical characterization, SB3.10 was identified as *Acinetobacter calcoaceticus* SB3.10.

1. INTRODUCTION

A food additive is one of the preservatives used to inhibit the growth of microorganisms, thereby extending the shelf life of products. Some commonly used preservatives for foods include sulfur dioxide, sodium benzoate, sorbic acid, propionic acid, sodium nitrate, and potassium nitrate (Lennerz et al., 2015). Sodium benzoate (SB) is the first chemical compound approved as a preservative and widely used in the food industry, such as jams, milk, carbonated beverages, and pickled vegetables. Additionally, SB is utilised in other industries, including cosmetics and pharmaceuticals (Davidson

et al., 2013). According to the World Health Organization, the acceptable daily intake of SB for humans is 5 mg/kg body weight (Wibbertmann et al., 2000). However, exposure to high concentration of SB can increase the risk of cancer by disrupting the oxygen absorption process and causing cell death at levels exceeding the permissible limit. Furthermore, SB can combine with ascorbic acid (vitamin C) or citric acid, forming benzene, a carcinogen linked to leukemia, DNA damage, mitochondrial dysfunction, cell death, and attention-deficit hyperactivity disorder (Noorafshan et al., 2014).

In addition to the direct health impacts of SB, a direct release of wastewater containing SB into the environment poses a significant pollution. If SB containing wastewater from factories and food production facilities is not treated, it can negatively affect native species due to its antimicrobial nature. In aquatic systems, SB reduces biological oxygen demand and affects aerobic microorganisms (Krishnani et al., 2022). Moreover, this compound can disrupt the natural balance of aquatic ecosystems by altering pH, thereby affecting the nutrient availability for aquatic organisms (Hun et al., 2024).

Bioremediation of SB-containing wastewater is a viable approach that can help reduce the environmental treatment cost and minimise the use of chemicals. Several bacteria capable of degrading SB have been isolated and environmental factors have been shown to significantly affect their biodegradation rate (Hun et al., 2024). Among the identified bacterial isolates were *Pseudomonas* sp. Ba-0511 isolated from soil (Wang et al., 2001), *Priestia aryabhatai* obtained from agricultural soil (Esikova et al., 2021), and *Bacillus subtilis* DSSK isolated from food processing effluent (Krishnani et al., 2022).

In the Mekong delta of Vietnam, particularly in Can Tho city, starch-based foods such as noodles, rice noodles, and vermicelli are daily consumed. As the demand for these foods continues to increase, the use of SB also rises. However, data on the biodegradation of SB in the Mekong Delta are very rare. Studies have primarily focused on developing technical methods for the detection and quantification of SB in foods (Ogunleye et al., 2017; Dinh et al., 2024). There is a lack of studies on the isolation of SB-degrading bacteria in this region. Therefore, this study aimed at isolating SB-degrading bacteria from wastewater, determining the influence of SB concentration and pH of the medium on the biodegradation of this compound, and determining the chemotactic response of the isolates towards SB. These data will lay the groundwork for applied research on the bioremediation of SB in wastewater, contributing to the reduction of environmental pollution and the protection of aquatic organisms and human health.

2. MATERIALS AND METHODS

2.1. Sample collection

Four fresh wastewater samples were collected including wastewater samples from the floor, output

surface wastewater, water from rice noodle rinsing and powder solution at each of three rice noodle production facilities in Can Tho, Viet Nam. The coordinates of the facilities are (1): 10°01'28.5"N 105°45'12.5"E (91B residential area), (2): 10°03'08.6"N 105°46'25.6"E (Nguyen Van Cu street), and (3): 9°59'53.1"N 105°44'20.9"E (Vong Cung road). Each sample was collected in triplicate along the water pathway, and the three subsamples were mixed thoroughly to obtain one representative sample. Sterile plastic bottles were used to store wastewater samples and these samples were transported to the laboratory for immediate measurements of pH, salinity, and SB concentration.

2.2. Enrichment and isolation of sodium benzoate-degrading bacteria

The collected wastewater samples were used for isolation of SB-degrading bacteria. A volume of 5 mL wastewater was inoculated into a 100 mL-Erlenmeyer flask containing 45 mL of minimal mineral (MM) medium supplemented with 50 mg/L SB. The cultures were incubated on a rotary shaker at 200 rpm for 1 week under laboratory conditions (30-32°C). After visualising the turbidity of the cultures, 5 mL of the enriched cultures was transferred into 45 mL of fresh MM medium containing 50 mg/L SB (Krishnani et al., 2022) under the same culture conditions and incubated for another week. The MM medium (pH 7.0) consists of 1.42 g Na₂HPO₄, 1.36 g KH₂PO₄, 0.3 g (NH₄)₂SO₄, 98.5 mg MgSO₄·7H₂O, 5.75 mg CaCl₂·2H₂O, 3.2 mg Na₂-EDTA, 2.75 mg FeSO₄·7H₂O, 1.7 mg MnSO₄·H₂O, 1.16 mg H₃BO₃, 1.15 mg ZnSO₄·7H₂O, 0.24 mg CuSO₄, 0.24 mg CoCl₂·6H₂O, 0.1 mg MoO₃, and 1 L water (Nguyen et al., 2014).

After two weeks of repeated enrichment, serial dilutions of the cultures ranging from 10⁻¹ to 10⁻⁵ were prepared using 0.9% NaCl solution. An aliquot of 50 µL bacterial suspension was spread onto solid MM agar plates supplemented with 50 mg/L SB and incubated at 32°C for seven days. Colonies that appeared morphologically distinct and well-isolated were selected by streaking on MM agar plates (1.5% agar, w/v) containing 50 mg/L SB. Purified colonies were further cultured on tryptone soya agar (TSA) medium (30 g tryptone soya broth, 15 g agar, and 1 L water) to obtain pure isolates. Colony morphology (colour, elevation, margin) was used to assess the purity of the isolates. After three days of incubation on TSA, the morphological characteristics of the

bacterial colony were recorded. Gram reaction was quickly determined using the string test method (Dash & Payyappilli, 2016) and the conventional Gram staining method (Gram, 1884) was used for the determination of cell shape and dimension of potential SB-degrading isolates.

2.3. Screening of sodium benzoate-degrading bacteria

Preparation of bacterial suspension: A single colony from each bacterial isolate was inoculated into tryptone soya broth (TSB) and incubated for 24 hours under laboratory conditions on the shaker at 200 rpm. Cells were then harvested by centrifuging and washed three times with 0.9% NaCl solution. The optical density at 600 nm (OD₆₀₀) of the bacterial suspension was adjusted to 0.7.

Degradation assay: 40 µL aliquot of each bacterial suspension was inoculated into a 15 mL test tube containing 4 mL of MM medium supplemented with 1 g/L SB. Two control treatments were included: (i) abiotic control - MM medium with 1 g/L SB addition but without bacterial inoculation; (ii) substrate control - MM medium with bacterial inoculation but without SB addition. Each treatment was performed in triplicate. All treatments were incubated in the laboratory on the shaker at 200 rpm for 24 hours. At 12 and 24 hours of incubation, the optical density and residual SB concentration (after the removal of bacterial cells by centrifugation at 13,000 rpm for 15 mins) of all treatments were recorded by spectrophotometric method at 600 nm and 225 nm, respectively (Wang et al., 2020).

2.4. Effects of sodium benzoate concentrations and pH of medium on the biodegradation efficacy

Bacterial isolates selected in Section 2.3 were used to investigate the effects of SB concentration and culture medium pH on SB degradation capacity. Bacterial suspensions were prepared as described in Section 2.3. A 40 µL aliquot of each bacterial suspension was inoculated into each 15 mL test tube containing 4 mL of MM medium. All cultures were incubated under laboratory conditions on the shaker at 200 rpm for 24 hours; each treatment was done in triplicate.

Effect of SB concentrations: MM medium was supplemented with SB at concentrations of 1,250, 2,500, and 5,000 mg/L before bacterial inoculation.

Effect of pH: MM medium supplemented with the appropriate SB concentration (1,250 mg/L as being

recorded in the previous experiment) was adjusted to pH levels of 5, 6, 7, 8, and 9 before bacterial inoculation.

At 12 and 24 hours of incubation, the turbidity (OD₆₀₀) of the cultures was monitored, the cell viability was determined by the drop plating method (Hoben & Somasegaram, 1982), and the residual SB in the medium was quantified (see Section 2.3) as described by Wang et al. (2020).

2.5. Chemotactic response of bacteria towards sodium benzoate

The chemotaxis of bacterial isolates towards SB was assessed using semi-solid MM medium 0.65% agar (w/v) (Partridge & Harshey, 2013). Sterile Whatman filter papers were cut into full and half circles with a radius of 1 cm and placed on the Petri dishes. SB crystals were sprinkled around the perimeter of the filter papers. Using a sterile forcep, the filter papers were carefully removed. Bacterial cultures (10 µL of the overnight cultures, OD₆₀₀ = 0.7) were then inoculated at the centre of the SB zone; a control treatment was done similarly, but without the presence of SB. The plates were incubated at 32°C. Bacterial biomass formation was visually monitored daily. Bacterial chemotaxis was confirmed by directional biomass growth towards SB crystals, and no biomass growth if there was no SB addition.

2.6. Identification of sodium benzoate-degrading bacteria

Bacterial isolates demonstrating SB degradation efficacy were inoculated on TSA medium and incubated at 32°C for 72 hours. Genomic DNA was extracted using Monarch Spin gDNA Extraction Kit (New England Biolabs) following the manufacturer's instructions. The 16S-rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATC-MTGGCTCAG-3') and 1492R (5'-TACGGYTA-CCTTGTTACGACTT-3') (Frank et al., 2008) and sequenced by Sanger method. The sequences were compared to NCBI database using BlastN.

Identification to the species level was based on 16S-rRNA gene sequence similarity and biochemical characteristics following Bergey's Manual of Systematic Bacteriology. The biochemical analysis included urease, oxidase, catalase activity and citrate assimilation. Additionally, the isolates' ability to ferment carbon sources such as glycerol, glucose, galactose, D-fructose, D-xylose, lactose, and maltose was tested (Chandra & Mani, 2011).

2.7. Data analysis

Raw data were processed in Microsoft Excel 2013 to calculate means and draw graphs. Minitab 18 was used to perform analysis of variance (ANOVA), and treatment means were compared using Tukey's test for multiple comparisons.

3. RESULTS AND DISCUSSION

3.1. Isolation of sodium benzoate-degrading bacteria

Wastewater samples collected from three different rice noodle production facilities exhibited variations in physicochemical properties such as pH, salinity,

Table 1. Physicochemical characteristics of wastewater samples collected at three different rice noodle processing facilities in Can Tho city

Facility	Source	pH	Salinity (‰)	SB (mg/L)
1 (91B)	Floor wastewater	5.79	4	23
	Output wastewater	6.42	10	35
	Powder solution	3.65	20	32
2 (Nguyen Van Cu)	Floor wastewater	4.36	5	24
	Output wastewater	6.12	12	19
	Rice noodle rinsing water	4.13	5.9	17
3 (Vong Cung)	Floor wastewater	3.77	5	28
	Powder solution	3.75	18	35

From the samples collected at three rice noodle production facilities, 35 bacterial isolates capable of degrading SB were isolated. Among these, 27 isolates were obtained from wastewater on the production floor, two from noodle-rinsing water, and six from output water. In terms of colony morphology, 25 out of 35 isolates formed circular colonies, while 10 isolates exhibited irregular colony shape. Thirty-three isolates showed an entire colony margin, and two isolates exhibited a lobate margin. Most colonies had a smooth surface. The majority of colonies were either opaque white (29 isolates) or milky white (six isolates). In terms of elevation, 31 colonies were convex, while four were low-raised. Colony diameter ranged from 1 to 5 mm. Gram staining revealed that 22 isolates were Gram-positive and 13 were Gram-negative. The colony morphology, cell morphology, and Gram staining of representative isolates are shown in Figure 1.

and SB concentration (Table 1). The pH of the wastewater samples ranged from 3.65 to 6.42, while the output water exhibited a pH from 6.12 to 6.42. The salinity of the powder solution samples was high, ranging from 18 to 20‰, whereas the output wastewater had lower salinity, and the floor wastewater showed the lowest salinity (<6‰). Although pH and salinity varied among facilities, the SB concentration in all samples remained relatively low (17-35 mg/L) with no significant difference observed between facilities.

Numerous studies on the biodegradation of SB were published. Elshahed et al. (2001) isolated *Syntrophus* that lived symbiotically with hydrogen-utilising bacteria; all were Gram-negative and capable of degrading SB under anaerobic conditions. Lindsay et al. (2008) isolated three Gram-negative bacteria capable of utilising SB as a carbon source. Krishnani et al. (2022) isolated a Gram-positive bacterium from industrial wastewater. This rod-shaped cell strain exhibited optimal growth at very high SB concentration (10,000 mg/L) and spore production. Lyakhovchenko et al. (2023) isolated a Gram-negative strain, BG28, capable of growing on a nutrient medium containing SB. The colony of BG28 was convex, round, and smooth. BG28 was a non-spore-forming and motile strain.

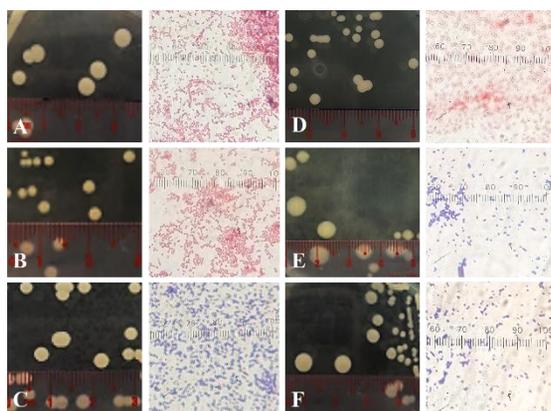


Figure 1. Colony, cell morphology and Gram staining of some representative SB-degrading bacteria

Note: A: SB2.9, B: SB3.8, C: SB3.4, D: SB3.13, E: SB2.10, F: SB1.7

3.2. Bacterial degradation of sodium benzoate

The data in Table 2 indicated that all bacterial isolates were capable of growing in MM medium supplemented with 1,000 mg/L SB, showing a statistically significant difference as compared to the control treatment. None of the isolates formed turbidity in MM medium without SB supplementation, meaning that our experiment was controlled well without cross-contamination with other organic chemicals. Bacterial density increased at 12 and 24 hours of incubation; the highest turbidity was observed at 24 hours of incubation for all isolates.

In addition, data obtained from the SB degradation assay showed that 35 bacterial isolates were capable

of degrading SB at 24 hours of incubation (Figure 2). SB concentration decreased from 12 to 24 hours. At 24 hours of incubation, SB degradation efficacy ranged from 97% to 98.3%. These data were inversely related to bacterial turbidity, indicating that the isolates utilised SB as a carbon source for growth. Among these, five isolates, including SB2.1, SB2.2, SB2.4, SB3.10, and SB3.13, exhibited low residual SB concentrations at 12 hours with the degradation efficacy of 66.9%, 45.7%, 35.5%, 44.2%, and 48%, respectively, suggesting that these five isolates are promising candidates for SB biodegradation.

According to Pyankova and Plotnikova (2022), 28 bacterial isolates of *Halomonas* obtained from potassium and magnesium salt mines in the Upper Kama were capable of growing in a medium supplemented with SB. In extreme environments such as hot spring samples, Hun et al. (2024) isolated *Benzoatithermus flavus* SYSU G07066^T from enriched cultures supplemented with 400 mg/L SB as the sole carbon source. Xie et al. (2009) reported that *Pseudomonas* sp. XP-M2 completely degraded 10 g/L of SB within 12 hours. Similarly, *Pseudomonas citronellolis* was shown to degrade over 95% of SB at concentrations of 1 to 5 mM (144 to 720 mg/L) at 24 hours of inoculation (Zaveri et al., 2021). In our study, all isolates significantly reduced SB 1,000 mg/L at 24 hours of incubation. Notably, SB2.1, SB2.2, SB2.4, SB3.10, and SB3.13 demonstrated rapid SB degradation at both 12 and 24 hours, ranging from 35.5% to 66.9% and 97.8% to 98.4%, respectively.

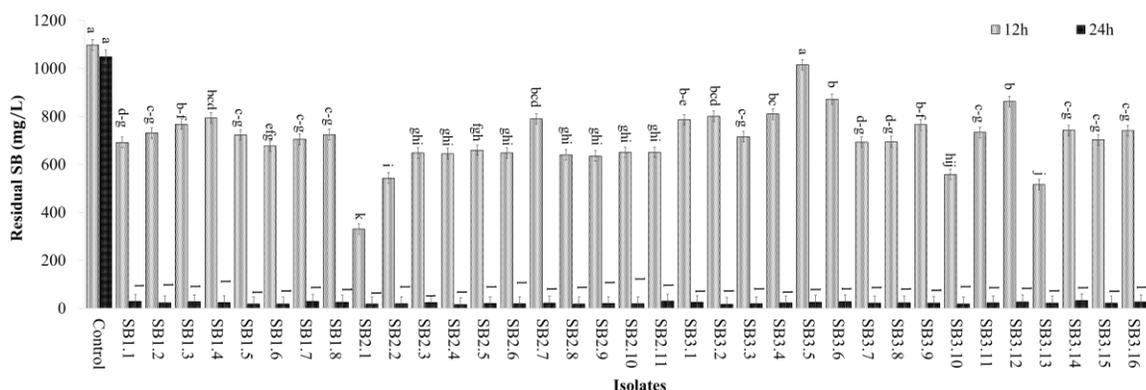


Figure 2. SB degradation efficacy by bacterial isolates at 12 and 24 hours of incubation under laboratory conditions

Note: At each time point, columns followed by the same letter(s) are not significantly different at the 5% level.

Table 2. Bacterial growth (OD₆₀₀) in MM medium supplemented with and without SB at 12 and 24 hours of incubation under laboratory conditions

No.	Isolates	MM medium with SB (1,000 mg/L) addition		MM medium without SB addition	
		12 hours	24 hours	12 hours	24 hours
1	Control	0.045±0.001 ^o	0.044±0.001 ⁿ	0.052±0.006 ^d	0.051±0.006 ^{de}
2	SB1.1	0.157±0.005 ^{b-g}	0.291±0.017 ^{g-k}	0.063±0.001 ^{a-d}	0.071±0.008 ^b
3	SB1.2	0.141±0.001 ^{f-k}	0.299±0.03 ^{f-j}	0.062±0.001 ^{a-d}	0.069±0.004 ^{bcd}
4	SB1.3	0.155±0.007 ^{b-h}	0.198±0.010 ^m	0.057±0.002 ^{cd}	0.07±0.003 ^{bcd}
5	SB1.4	0.145±0.011 ^{d-k}	0.247±0.016 ^{j-m}	0.055±0.002 ^d	0.061±0.008 ^{b-e}
6	SB1.5	0.143±0.007 ^{e-k}	0.230±0.034 ^{lm}	0.062±0.003 ^{a-d}	0.059±0.006 ^{b-e}
7	SB1.6	0.138±0.006 ^{g-k}	0.241±0.012 ^{klm}	0.060±0.003 ^{bcd}	0.06±0.001 ^{b-e}
8	SB1.7	0.132±0.007 ^{ijk}	0.256±0.013 ^{i-l}	0.063±0.002 ^{a-d}	0.063±0.003 ^{b-e}
9	SB1.8	0.134±0.002 ^{h-k}	0.287±0.028 ^{g-k}	0.075±0.007 ^a	0.062±0.005 ^{b-e}
10	SB2.1	0.186±0.002 ^a	0.397±0.007 ^{ab}	0.069±0.001 ^{abc}	0.070±0.005 ^{bc}
11	SB2.2	0.164±0.003 ^{b-e}	0.353±0.014 ^{b-f}	0.064±0.008 ^{a-d}	0.059±0.007 ^{b-e}
12	SB2.3	0.162±0.013 ^{b-f}	0.313±0.023 ^{e-h}	0.058±0.008 ^{cd}	0.047±0.001 ^e
13	SB2.4	0.162±0.003 ^{abc}	0.412±0.014 ^a	0.074±0.004 ^{ab}	0.068±0.001 ^{bcd}
14	SB2.5	0.173±0.006 ^{ab}	0.266±0.005 ^{h-l}	0.065±0.004 ^{a-d}	0.058±0.003 ^{b-e}
15	SB2.6	0.152±0.007 ^{b-i}	0.339±0.01 ^{c-g}	0.057±0.003 ^{cd}	0.05±0.003 ^e
16	SB2.7	0.131±0.003 ^{ijk}	0.328±0.007 ^{d-g}	0.060±0.003 ^{bcd}	0.046±0.002 ^e
17	SB2.8	0.172±0.005 ^{ab}	0.359±0.016 ^{a-e}	0.057±0.003 ^{cd}	0.056±0.003 ^{b-e}
18	SB2.9	0.155±0.003 ^{b-h}	0.318±0.013 ^{e-h}	0.065±0.004 ^{a-d}	0.053±0.004 ^{b-e}
19	SB2.10	0.150±0.007 ^{c-i}	0.330±0.014 ^{d-g}	0.052±0.003 ^d	0.053±0.003 ^{b-e}
20	SB2.11	0.145±0.010 ^{d-k}	0.306±0.005 ^{e-i}	0.058±0.005 ^{cd}	0.047±0.001 ^e
21	SB3.1	0.147±0.005 ^{c-j}	0.306±0.012 ^{e-i}	0.059±0.005 ^{cd}	0.058±0.006 ^{b-e}
22	SB3.2	0.108±0.009 ^{lm}	0.292±0.018 ^{g-k}	0.064±0.003 ^{a-d}	0.047±0.005 ^e
23	SB3.3	0.154±0.012 ^{b-h}	0.333±0.002 ^{c-g}	0.059±0.007 ^{cd}	0.054±0.005 ^{b-e}
24	SB3.4	0.125±0.006 ^{kl}	0.325±0.009 ^{d-g}	0.056±0.002 ^{cd}	0.051±0.004 ^{de}
25	SB3.5	0.083±0.007 ⁿ	0.316±0.002 ^{e-h}	0.061±0.005 ^{a-d}	0.052±0.007 ^{cde}
26	SB3.6	0.074±0.002 ⁿ	0.294±0.010 ^{g-k}	0.054±0.006 ^d	0.048±0.007 ^e
27	SB3.7	0.134±0.005 ^{h-k}	0.288±0.008 ^{g-k}	0.056±0.002 ^{cd}	0.054±0.002 ^{b-e}
28	SB3.8	0.126±0.001 ^{ijkl}	0.319±0.003 ^{d-h}	0.054±0.001 ^d	0.053±0.003 ^{b-e}
29	SB3.9	0.130±0.003 ^{i-l}	0.340±0.01 ^{c-g}	0.058±0.002 ^{cd}	0.057±0.003 ^{b-e}
30	SB3.10	0.158±0.007 ^{b-g}	0.387±0.033 ^{abc}	0.065±0.009 ^{a-d}	0.1±0.02 ^a
31	SB3.11	0.093±0.011 ^{mn}	0.352±0.014 ^{b-f}	0.053±0.003 ^d	0.053±0.006 ^{b-e}
32	SB3.12	0.166±0.004 ^{a-d}	0.322±0.035 ^{d-h}	0.059±0.006 ^{cd}	0.056±0.004 ^{b-e}
33	SB3.13	0.167±0.005 ^{abc}	0.375±0.014 ^{a-d}	0.058±0.004 ^{cd}	0.058±0.003 ^{b-e}
34	SB3.14	0.096±0.007 ^{mn}	0.334±0.006 ^{c-g}	0.054±0.006 ^d	0.047±0.001 ^e
35	SB3.15	0.083±0.005 ⁿ	0.335±0.003 ^{c-g}	0.057±0.003 ^{cd}	0.048±0.006 ^e
36	SB3.16	0.155±0.004 ^{b-h}	0.294±0.018 ^{g-k}	0.056±0.002 ^{cd}	0.056±0.002 ^{b-e}

Note: Within each column, numbers followed by the same letter(s) are not significantly different at the 5% level.

3.3. Effect of substrate concentrations on the growth of bacteria and sodium benzoate degradation in liquid minimal mineral medium

Our data indicated that five isolates, SB2.1, SB2.2, SB2.4, SB3.10, and SB3.13, were able to grow in MM medium containing different concentrations of SB (1,250, 2,500, and 5,000 mg/L), showing statistically significant differences as compared to the control treatment (Table 3). Among these, SB3.13 showed an optical density (OD₆₀₀) of 0.439,

but had the lowest viable cell count at 2,600 × 10⁷ CFU/mL in MM supplemented with 1,250 mg/L SB. In contrast, SB3.10 exhibited an OD₆₀₀ of 0.435 while achieving the highest cell number (348,889 × 10⁷ CFU/mL). When SB was added at concentrations of 2,500 mg/L and 5,000 mg/L, there was no correlation between optical density and viable cell number. The highest cell viability for all isolates was recorded at the 1,250 mg/L concentration, indicating that high concentrations of SB (2,500 and 5,000 mg/L) inhibited the growth of these isolates.

Table 3. Turbidity and bacterial cell number at 24 hours of incubation in liquid MM medium supplemented with different SB concentrations

SB conc. (mg/L)	Isolates	Turbidity (OD ₆₀₀)	Cell number (x10 ⁷ CFU/mL)
1,250	Control	0.052±0.002 ^g	0±0 ^m
	SB2.1	0.377±0.008 ^{ab}	24,667±273 ^c
	SB2.2	0.230±0.011 ^{de}	252,222±509 ^b
	SB2.4	0.428±0.025 ^a	3,000±120 ^d
	SB3.10	0.435±0.007 ^a	348,889±694 ^a
	SB3.13	0.439±0.065 ^a	2,600±153 ^{de}
2,500	Control	0.057±0.001 ^g	0±0 ^m
	SB2.1	0.225±0.008 ^{de}	1,933±100 ^f
	SB2.2	0.140±0.008 ^f	2,122±96 ^{ef}
	SB2.4	0.172±0.007 ^{ef}	224±19 ^g
	SB3.10	0.266±0.012 ^{cd}	2,511±135 ^{de}
	SB3.13	0.315±0.047 ^{bc}	160±9 ^h
5,000	Control	0.063±0.007 ^g	0±0 ^m
	SB2.1	0.045±0.002 ^g	14±1 ⁱ
	SB2.2	0.047±0.025 ^g	6±1 ^k
	SB2.4	0.064±0.010 ^g	13±0.6 ⁱ
	SB3.10	0.065±0.007 ^g	0.69±0.03 ^l
	SB3.13	0.151±0.001 ^f	8±1.5 ^j

Note: Within each column, numbers followed by the same letter(s) are not significantly different at the 5% level.

According to Krishnani et al. (2022), the Gram-positive *Bacillus subtilis* DSSK1 isolated from the effluent treatment plant of a food processing industry in Jaipur, Rajasthan, performed maximum growth at a very high concentration of SB (10,000 mg/L), revealing that bacterial degradation of SB is strain-specific, depending on the history and concentration of SB discharged at the sampling sites.

In addition, the SB degradation assay showed that at a SB concentration of 1,250 mg/L, all five bacterial isolates exhibited optimal SB degradation, with a significant reduction of SB concentration observed at 24 hours, ranging from 95.54% to 97.77% (Figure 3). At 2,500 mg/L of SB, the isolates showed degradation activity but lower efficacy (33.26% to 52.62%). Similarly, at 5,000 mg/L, the degradation efficacy decreased significantly (from 22.56% to 59.47%). These findings suggested that the five bacterial isolates showed the most effective SB degradation at 1,250 mg/L.

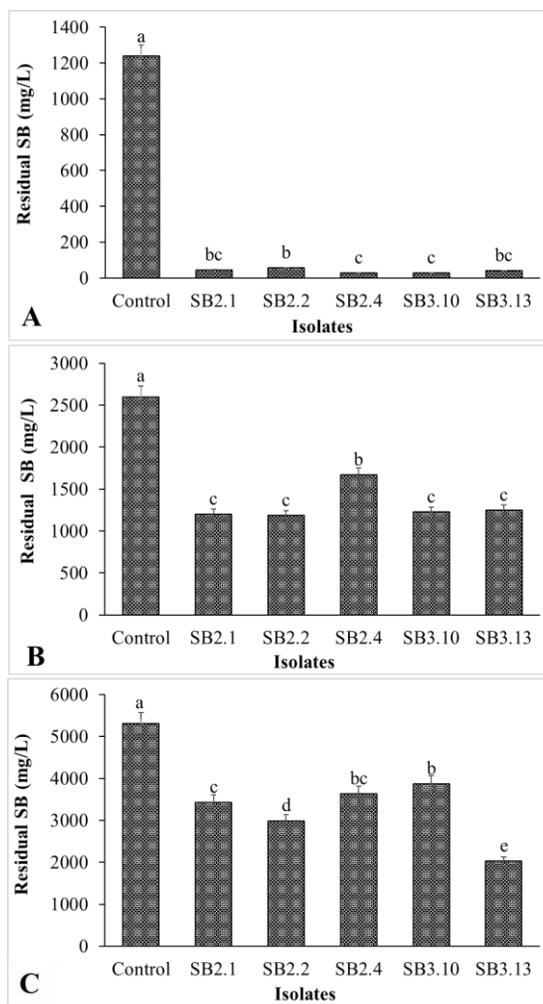


Figure 3. SB degradation by five bacterial isolates at different concentrations in liquid MM medium at 24 hours of incubation under laboratory conditions

Note: A: 1,250 mg SB/L; B: 2,500 mg SB/L; C: 5,000 mg SB/L. In each graph, columns with the same letter are not significantly different at the 5% level.

Previous studies have shown that bacterial isolates tend to degrade SB more efficiently at lower concentrations (typically <1,000 mg/L). For instance, Loh and Chua (2002) reported that *Pseudomonas putida* completely degraded 400 mg/L SB within 12 hours, achieving a degradation efficacy of 90%. At higher concentrations, degradation is often less efficient. Lyakhovchenko et al. (2023) indicated that isolate BG28 degraded SB at 5,000 mg/L and 15,000 mg/L, achieving 73%

to 75% after 11 days at 5,000 mg/L; however, degradation efficacy was significantly reduced at 15,000 mg/L. These results highlighted that the optimal degradation concentration varies among bacterial isolates, as each isolate may adapt and degrade SB effectively at a specific range. In this study, the five isolates exhibited optimal degradation at 1,250 mg/L SB, consistent with the findings of Loh and Chua (2002). Similarly, this study aligns with Lyakhovchenko et al. (2023) in that high SB concentrations reduced the efficacy of SB degradation.

3.4. Effect of medium pH on bacterial growth and sodium benzoate degradation efficacy

The results indicated that five bacterial isolates were capable of growing in SB-supplemented medium at different pH levels (pH 5, 6, 7, 8, and 9), with statistically significant differences as compared to the control treatment (Table 4). SB2.4 exhibited the highest optical density ($OD_{600} = 0.482$) at pH 9, but had low viable cell number ($2,111 \times 10^7$ CFU/mL). In contrast, SB2.1 had an OD_{600} of 0.478 but achieved the highest viable cell number of 321.111×10^7 CFU/mL at pH 7, indicating that pH affected the growth and development of the isolates.

Degradation assays also showed that the isolates exhibited the highest SB degradation efficacy at pH 7, approximately 98%, except for isolate SB3.13 (Figure 4). At pH 8, four isolates also showed high SB degradation activity, with efficacy ranging from 96.56% to 98.36%. Although the isolates remained capable of degrading SB at pH 9, the efficacy was lower (93.1% to 96.81%) compared to pH 7 and 8. At pH 5 and 6, degradation was notably low, ranging from 2.19% to 3.62% at pH 5, and from 0.54% to 14.8% at pH 6. In general, SB2.1, SB2.2, SB2.4, and SB3.10 demonstrated strong degradation activity at pH 7 and 8, while SB3.13 exhibited degradation ability at pH 6, 7, and 8 (approximately 46%).

These findings are consistent with previous studies indicating that optimal biodegradation of SB occurs in a neutral to slightly alkaline medium. Liu et al. (2019) isolated *Acinetobacter* sp. Tust-DM21 showing optimal SB degradation (97.6%) at pH 8. Similarly, Zaveri et al. (2021) reported that

Pseudomonas citronellolis achieved over 95% SB degradation within 24 hours at pH 7. These data are consistent with the current study, in which SB2.1, SB2.2, SB2.4, and SB3.10 exhibited over 98% SB degradation within 24 hours at pH 7. All five isolates demonstrated efficient SB degradation at pH 7 and 8. However, pH 7 was found to be the most favourable condition for SB degradation.

Table 4. Turbidity and viable cell number in liquid MM medium at 24 hours of incubation at different pH levels under laboratory conditions

Treat-ment	Isolates	Turbidity (OD_{600})	Cell number ($\times 10^7$ CFU/mL)
pH 5	Control	0.043±0.002 ^g	0±0 ^o
	SB2.1	0.061±0.002 ^g	9±1 ^j
	SB2.2	0.055±0.004 ^g	0.68±0.08 ^m
	SB2.4	0.049±0.002 ^g	7±0.8 ^j
	SB3.10	0.055±0.003 ^g	0.53±0.08 ^m
pH 6	Control	0.050±0.001 ^g	0±0 ^o
	SB2.1	0.064±0.002 ^g	12±1 ⁱ
	SB2.2	0.069±0.002 ^g	4±0.2 ^k
	SB2.4	0.076±0.004 ^g	8±1 ^j
	SB3.10	0.082±0.010 ^g	1.49±0.08 ^l
pH 7	Control	0.048±0.002 ^g	0±0 ^o
	SB2.1	0.478±0.002 ^{ab}	321,111±16,443 ^a
	SB2.2	0.292±0.052 ^c	33,444±1,836 ^b
	SB2.4	0.464±0.005 ^{ab}	33,444±1,953 ^b
	SB3.10	0.402±0.038 ^{bcd}	295,556±8,389 ^a
pH 8	Control	0.045±0.002 ^g	0±0 ^o
	SB2.1	0.462±0.007 ^{ab}	2,655±117 ^{cd}
	SB2.2	0.445±0.012 ^{ab}	2,878±77 ^c
	SB2.4	0.476±0.011 ^{ab}	2,878±204 ^c
	SB3.10	0.437±0.007 ^{abc}	2,533±88 ^{cd}
pH 9	Control	0.048±0.002 ^g	0±0 ^o
	SB2.1	0.452±0.008 ^{ab}	622±51 ^f
	SB2.2	0.364±0.070 ^{cde}	1,911±150 ^e
	SB2.4	0.482±0.010 ^a	2,111±107 ^{de}
	SB3.10	0.420±0.005 ^{a-d}	215±6 ^g
	SB3.13	0.090±0.006 ^g	0±0 ^o

Note: Within each column, numbers followed by the same letter(s) are not significantly different at the 5% level.

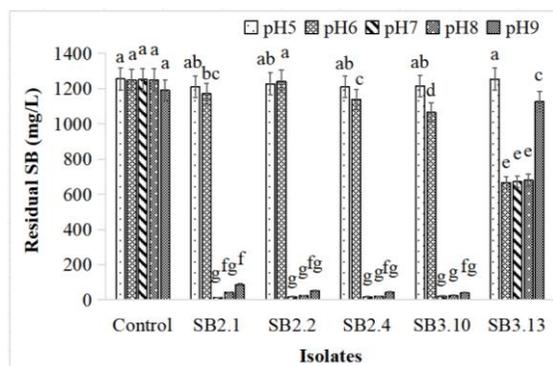


Figure 4. SB degradation efficacy by bacterial isolates grown in liquid MM medium with different pH levels under laboratory conditions

Note: Columns with the same letter(s) are not significantly different at the 5% level.

3.5. Bacterial chemotaxis towards sodium benzoate

Among SB-degrading isolates, only SB3.10 exhibited chemotactic response towards SB (Figure 5). At five days of incubation, SB3.10 did not produce biomass in MM medium without SB supplementation and only grew at the original inoculation point (Figure 5A). In contrast, when SB was added, SB3.10 produced biomass oriented towards SB crystals. When SB was sprinkled in a semicircular pattern, SB3.10 developed biomass towards SB crystals, and no growth in the opposite direction was visualised (Figure 5B). When SB crystals were sprinkled in a full circular pattern, biomass formation was directed from the centre towards the SB ring (Figure 5C).

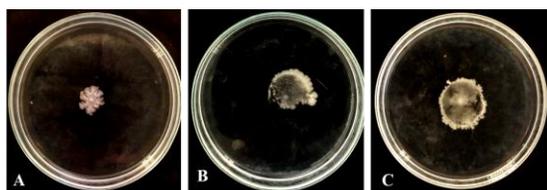


Figure 5. Chemotactic response of SB3.10 towards SB on semi-solid agar medium

Note: A: No SB added; B: SB crystals added in a semicircle; C: SB crystals added in a circle.

Bacteria have been shown to perform chemotaxis towards environmental pollutants that they can use as substrates for growth. Moriuchi et al. (2021) indicated that *Cupriavidus necator* NH9 showed chemotactic movement towards aromatic compounds on semi-solid agar plates. Zhou et al. (2022) revealed that *Sphingomonas* sp. contained a set of genes associated with bacterial chemotaxis

and flagellar assembly. These genes likely contributed to the organism's ability to adapt to harsh environmental conditions, enabling the metabolism of aromatic compounds and diverse substrates such as styrene and atrazine, thereby supporting its ecological competitiveness and niche specialisation. Bacterial chemotaxis demonstrates the adaptability of prokaryotic cells across diverse living environments (Xu et al., 2024). In this study, SB3.10 demonstrated efficient SB degradation and is the first bacterial isolate performing chemotaxis towards SB recorded, indicating its potential for SB bioremediation due to its ability to detect, move towards and metabolise this substrate in the environment.

3.6. Identification of sodium benzoate-degrading bacteria

SB3.10 was a promising candidate for SB degradation and was therefore selected for molecular identification. The amplified product of the 16S-rRNA gene was verified by electrophoresis on a 1.5% agarose gel, showing a band corresponding to the expected size. Sequence analysis revealed that the obtained fragment was 1372 bp in length. The 16S-rRNA gene sequence of SB3.10 showed high similarity (>99.85%) to *Acinetobacter* species.

In addition, biochemical characterisation of SB3.10 revealed that the strain performed citrate assimilation, exhibited catalase activity, but neither oxidase nor urease activities. SB3.10 was able to ferment carbon sources such as glycerol, glucose, galactose, D-fructose, D-xylose, lactose, and maltose (Table 5). These biochemical traits are consistent with those reported for *Acinetobacter calcoaceticus*. Based on the 16S-rRNA gene sequence analysis and biochemical characteristics, SB3.10 was identified as *Acinetobacter calcoaceticus* SB3.10.

Acinetobacter genus has been shown to degrade various aromatic compounds. Collier et al. (1998) isolated an *Acinetobacter* sp. capable of degrading SB via the β -keto adipate pathway, where benzoate is first converted to catechol (via *benABCDE* genes), followed by ring cleavage catalysed by cat-encoded enzymes. *A. calcoaceticus* PHEA-2 showed delayed phenol degradation when benzoate was present, phenol degradation was completely inhibited in a benzoate 1,2-dioxygenase knock out mutant when benzoate was supplemented (Zhan et al., 2009). Moreover, *A. calcoaceticus* CA16 grew in MM supplemented with diesel as the sole carbon

source and degraded 82% to 92% of aliphatic alkanes (C12-C18) within 28 days (Ho et al., 2020). In this study, *A. calcoaceticus* demonstrated efficient SB degradation and exhibited chemotactic

behaviour towards SB, revealing that this isolate is a promising candidate for further study on the treatment of SB in wastewater.

Table 5. Biochemical characterization of SB3.10 isolate and *Acinetobacter* species

Species	Biochemical tests										
	Urease	Oxidase	Glycerol	Glucose	Catalase	Galactose	D-fructose	D-xylose	Lactose	Citrate	Maltose
SB3.10	-	-	+	+	+	+	+	+	+	+	+
<i>A. calcoaceticus</i> ⁽¹⁾	-	-	+	+	+	+	+	+	±	+	±
<i>A. lactucae</i> ⁽²⁾	-	+	-	+	+	-	-	-	-	-	-
<i>A. baumannii</i> ⁽³⁾	-	-	+	+	+	+	+	-	-	+	-
<i>A. oleivorans</i> ⁽⁴⁾	-	+	+	nd	+	nd	nd	nd	nd	+	-
<i>A. pittii</i> ⁽⁵⁾	-	-	+	+	+	±	±	±	-	+	±

(+): positive; (-): negative; (±): difference depending on isolate; (nd): not determined

(1) Constantiniu et al., (2004); Glover et al., (2022); (2) Rooney et al., (2016); (3) Rossi et al., (2016); Ahuatzin-Flores et al., (2024); (4) Jung et al., (2011); (5) Nemeč et al., (2011); Vanechoutte et al., (2011)

4. CONCLUSION

From four wastewater samples collected at three rice noodle production facilities with SB concentrations ranging from 17 to 35 mg/L, 27 bacterial isolates capable of degrading SB were isolated. Among them, SB2.1, SB2.2, SB2.4, SB3.10, and SB3.13 showed effective SB degradation (1,250 mg/L) at 24 hours of incubation.

Notably, isolates SB2.1, SB2.2, SB2.4, and SB3.10 efficiently degraded more than 93% of SB (1,250 mg/L) in MM medium at pH 7-9, and only SB3.10 exhibited chemotactic behaviour towards SB. Based on 16S-rRNA gene sequencing and biochemical characterisation, SB3.10 was identified as *Acinetobacter calcoaceticus* SB3.10. This isolate represents a promising candidate for further study on SB biodegradation in wastewater.

REFERENCES

Ahuatzin-Flores, O. E., Torres, E., & Chávez-Bravo, E. (2024). *Acinetobacter baumannii*, a multidrug-resistant opportunistic pathogen in new habitats: A systematic review. *Microorganisms*, 12(4), 644. <https://doi.org/10.3390/microorganisms12040644>

Asejeje, F. O., Ajayi, B. O., Abiola, M. A., Samuel, O., Asejeje, G. I., Ajiboye, E. O., & Ajayi, A. M. (2022). Sodium benzoate induces neurobehavioral deficits and brain oxido-inflammatory stress in male Wistar rats: Ameliorative role of ascorbic acid. *Journal of Biochemical and Molecular Toxicology*, 36(5), e23010. <https://doi.org/10.1002/jbt.23010>

Collier, L. S., Gaines III, G. L., & Neidle, E. L. (1998). Regulation of benzoate degradation in *Acinetobacter* sp. isolate ADP1 by BenM, a LysR-type transcriptional activator. *Journal of Bacteriology*, 180(9), 2493-2501. <https://doi.org/10.1128/jb.180.9.2493-2501.1998>

Constantiniu, S., Romaniuc, A., Iancu, L. S., Filimon, R., & Tarași, I. (2004). Cultural and biochemical characteristics of *Acinetobacter* spp. isolates isolated from hospital units. *The Journal of Preventive Medicine*, 12(3-4), 35-42.

Davidson, P. M., Taylor, T. M., & Schmidt, S. E. (2013). Chemical preservatives and natural antimicrobial compounds. In Doyle, M. P. & Beuchat, L. R., *Food Microbiology* (pp. 713-745). ASM Press. <https://doi.org/10.1128/9781555818463.ch30>

Dinh, D. T., Van Anh, T. T., Van Khanh, N., Viet Hung, D., Thanh Hai, N., & Quoc Tuan, P. (2024). Development of a high-performance liquid chromatography method for simultaneous determination of hesperidin and sodium benzoate in Kien Ty syrup. *VNU Journal of Science: Medical and Pharmaceutical Sciences*, 40(2). <https://doi.org/10.25073/25881132/-vnumps.4585>

EIshahed, M. S., Bhupathiraju, V. K., Wofford, N. Q., Nanny, M. A., and Mcinerney, M. J. (2001). Metabolism of benzoate, cyclohex-1-ene carboxylate, and cyclohexane carboxylate by *Syntrophus aciditrophicus* isolate SB in syntrophic association with H₂-using microorganisms. *Applied and Environmental Microbiology*, 67(4), 1728-1738. <https://doi.org/10.1128/AEM.67.4.-1728-1738.2001>

- Esikova, T. Z., Anokhina, T. O., Abashina, T. N., Suzina, N. E., & Solyanikova, I. P. (2021). Characterization of soil bacteria with potential to degrade benzoate and antagonistic to fungal and bacterial phytopathogens. *Microorganisms*, 9(4), 755. <https://doi.org/10.3390/microorganisms-9040755>
- Gram, C. (1884). Ueber die isolirte Färbung der Schizomyeten in Schnitt- und Trockenpräparaten. *Fortschritte der Medicin*, 2, 185-189.
- Glover, J. S., Browning, B. D., Ticer, T. D., Engevik, A. C., & Engevik, M. A. (2022). *Acinetobacter calcoaceticus* is well adapted to withstand intestinal stressors and modulate the gut epithelium. *Frontiers in Physiology*, 13, 880024. <https://doi.org/10.3389/fphys.2022.880024>
- Ho, M. T., Li, M. S., McDowell, T., MacDonald, J., & Yuan, Z. C. (2020). Characterization and genomic analysis of a diesel-degrading bacterium, *Acinetobacter calcoaceticus* CA16, isolated from Canadian soil. *BMC Biotechnology*, 20(1), 39. <https://doi.org/10.1186/s12896-020-00632-z>
- Hun, C. J., Lv, Y. Q., Xian, W. D., Jiao, J. Y., Lian, Z. H., Tan, S., ... & Li, W. J. (2024). Multi-omics insights into the function and evolution of sodium benzoate biodegradation pathway in *Benzoatithermus flavus* gen. nov., sp. nov. from hot spring. *Journal of Hazardous Materials*, 476, 135017. <https://doi.org/10.1016/j.jhazmat.2024.135017>
- Jung, J., Madsen, E. L., Jeon, C. O., & Park, W. (2011). Comparative genomic analysis of *Acinetobacter oleivorans* DR1 to determine strain-specific genomic regions and gentisate biodegradation. *Applied and Environmental Microbiology*, 77(20), 7418-7424. <https://doi.org/10.1128/AEM.05231-11>
- Krishnani, S., Vineet, Tripathi, N. M., Kaur, R., Aseri, G. K., Khare, N., ... & Singh, D. (2022). Isolation and identification of benzoate degrading bacteria from food industry effluent. *Journal of Water Chemistry and Technology*, 44(3), 191-197. <https://doi.org/10.3103/S1063455X22030080>
- Lennerz, B. S., Vafai, S. B., Delaney, N. F., Clish, C. B., Deik, A. A., Pierce, K. A., ... & Mootha, V. K. (2015). Effects of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans. *Molecular genetics and metabolism*, 114(1), 73-79. <https://doi.org/10.1016/j.ymgme.2014.11.010>
- Lindsay, D., Ntoampe, M., & Gray, V. M. (2008). Biodegradation of sodium benzoate by a Gram-negative consortium in a laboratory-scale fluidized bed bioreactor. *Bioresource Technology*, 99(11), 5115-5119. <https://doi.org/10.1016/j.biortech.2007.09.020>
- Liu, N., Liu, Z., & Song, D. (2019). Degradation characteristics of catechol and sodium benzoate by a petroleum-degrading bacterium. *Biotechnology Bulletin*, 35(9), 156-164. <http://dx.doi.org/10.13560/j.cnki.biotech.bull.1985.2019-0578>
- Loh, K. C., & Chua, S. S. (2002). Ortho pathway of benzoate degradation in *Pseudomonas putida*: induction of meta pathway at high substrate concentrations. *Enzyme and Microbial Technology*, 30(5), 620-626. [https://doi.org/10.1016/S01410229\(02\)000169](https://doi.org/10.1016/S01410229(02)000169)
- Lyakhovchenko, N., Gubina, E., Senchenkov, V., Nikishin, I., & Solyanikova, I. (2023). Estimation of the ability to decompose sodium benzoate by a bacterium isolated from biohumans *Eicenia fetida*. *EDP Sciences*, 57, 09001. <https://doi.org/10.1051/bioconf/20235709001>
- Moriuchi, R., Dohra, H., Kanesaki, Y., & Ogawa, N. (2021). Transcriptome differences between *Cupriavidus necator* NH9 grown with 3-chlorobenzoate and that grown with benzoate. *Bioscience, Biotechnology, and Biochemistry*, 85(6), 1546-1561. <https://doi.org/10.1093/bbb/zbab044>
- Nemec, A., Krizova, L., Maixnerova, M., van der Reijden, T. J., Deschaght, P., Passet, V., ... & Dijkshoorn, L. (2011). Genotypic and phenotypic characterization of the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU). *Research in Microbiology*, 162(4), 393-404. <https://doi.org/10.1016/j.resmic.2011.02.006>
- Noorafshan, A., Erfanizadeh, M., & Karbalay-Doust, S. (2014). Stereological studies of the effects of sodium benzoate or ascorbic acid on rats' cerebellum. *Saudi Medical Journal*, 35(12), 1494-1500. PMID: 25491215; PMCID: PMC4362157.
- Nguyen, T. P. O., Helbling, D. E., Bers, K., Fida, T. T., Wattiez, R., Kohler, H. P. E., ... & De Mot, R. (2014). Genetic and metabolic analysis of the cobofuran catabolic pathway in *Novosphingobium* sp. KN65. *Applied Microbiology and Biotechnology*, 98(19), 8235-8252. <https://doi.org/10.1007/s00253-014-58585>
- Ogunleye, D. T., Oyeyiola, A. O., CT, O., Falana, T. G., & Abolade, O. M. (2017). Spectrophotometric and high performance liquid chromatographic determination of sodium benzoate and potassium sorbate in some soft drinks. *UNILAG Journal of Medicine, Science and Technology*, 5(1), 168-178. <https://ujmst.unilag.edu.ng/article/view/150>
- Partridge, J. D., & Harshey, R. M. (2013). Swarming: flexible roaming plans. *Journal of Bacteriology*, 195(5), 909-918. <https://doi.org/10.1128/JB.02063-12>
- Pyanikova, A. A., & Plotikova, E. G. (2022). Benzoate-degrading bacteria of the family *Halomonadaceae* isolated from a salt mining area: Species diversity and analysis of the benA genes. *Microbiology*, 91, 91-103. <https://doi.org/10.1134/S0026261722-010106>

- Rooney, A. P., Dunlap, C. A., & Flor-Weiler, L. B. (2016). *Acinetobacter lactucae* sp. nov., isolated from iceberg lettuce (Asteraceae: *Lactuca sativa*). *International Journal of Systematic and Evolutionary Microbiology*, 66(9), 3566-3572. <https://doi.org/10.1099/ijsem.0.001234>
- Rossi, E., Longo, F., Barbagallo, M., Peano, C., Consolandi, C., Pietrelli, A., ... & Landini, P. (2016). Glucose availability enhances lipopolysaccharide production and immunogenicity in the opportunistic pathogen *Acinetobacter baumannii*. *Future Microbiology*, 11(3), 335-349. <https://doi.org/10.2217/fmb.15.153>
- Vaneechoutte, M., Dijkshoorn, L., Nemeč, A., Kämpfer, P., & Wauters, G. (2011). *Acinetobacter*, *Chryseobacterium*, *Moraxella*, and other nonfermentative Gram-negative rods. In J. Versalovic, K. C. Carroll, G. Funke, J. H. Jorgensen, M. L. Landry, & D. W. Warnock (Eds.), *Manual of Clinical Microbiology* (10th ed., pp. 714–738). ASM Press. <https://doi.org/10.1128/9781555816728.ch42>
- Wang, M., Zhang, J., Zhang, Z., Liu, X., Fan, J., Liu, W., & Zhang, X. (2020). Simultaneous ultraviolet spectrophotometric determination of sodium benzoate and potassium sorbate by BP-neural network algorithm and partial least squares. *Optik*, 201, 163529. <https://doi.org/10.1016/j.ijleo.2019.163529>
- Wauters, G., & Vaneechoutte, M. (2015). Approaches to the identification of aerobic Gram-negative bacteria. *Manual of Clinical Microbiology*, 613-634. <https://doi.org/10.1128/9781555817381.ch33>
- Wibbertmann, A., Kielhorn, J., Koennecker, G., Mangelsdorf, L., & Melber, C. (2000). *Benzoic acid and sodium benzoate* (Concise International Chemical Assessment Document 26). World Health Organization.
- Xie, N., Tang, H., Feng, J., Tao, F., Ma, C., & Xu, P. (2009). Characterization of benzoate degradation by newly isolated bacterium *Pseudomonas* sp. XP-M2. *Biochemical Engineering Journal*, 46(1), 79-82. <https://doi.org/10.1016/j.bej.2009.04.019>
- Xu, Q., Ali, S., Afzal, M., Nizami, A. S., Han, S., Dar, M. A., & Zhu, D. (2024). Advancements in bacterial chemotaxis: Utilizing the navigational intelligence of bacteria and its practical applications. *Science of The Total Environment*, 931, 172967. <https://doi.org/10.1016/j.scitotenv.2024.172967>
- Zaveri, P., Iyer, A. R., Patel, R., & Munshi, N. S. (2021). Uncovering competitive and restorative effects of macro-and micronutrients on sodium benzoate biodegradation. *Frontiers in Microbiology*, 12, 634753. <https://doi.org/10.3389/fmicb.2021.634753>
- Zhan, Y., Yu, H., Yan, Y., Ping, S., Lu, W., Zhang, W., ... & Lin, M. (2009). Benzoate catabolite repression of the phenol degradation in *Acinetobacter calcoaceticus* PHEA-2. *Current Microbiology*, 59(4), 368-373. <https://doi.org/10.1007/s00284-009-9446-3>
- Zhou, M., Liu, Z., Wang, J., Zhao, Y., & Hu, B. (2022). *Sphingomonas* relies on chemotaxis to degrade polycyclic aromatic hydrocarbons and maintain dominance in coking sites. *Microorganisms*, 10(6), 1109. <https://doi.org/10.3390/microorganisms10061109>