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Extracellular Metabolites of Clove-Endophytic Bacteria, *Niallia nealsonii* DCL1, Exhibit Potential Cellular Antioxidant and Antiaging Activities

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Abstract

The clove plant, *Syzygium aromaticum* L., is a highly valued spice in Indonesia, recognized for its extensive applications in food preservation and medicinal uses. In a prior study, six endophytic bacteria were successfully isolated from clove leaves. One of these isolates, designated as DCL1, is a Gram-positive bacterium identified as *Niallia nealsonii.* This study aimed to determine the antioxidant and antiaging properties of the extracellular metabolite of DCL1. Extracellular metabolites were extracted with ethyl acetate and evaluated for antioxidant activity via the DPPH assay. Total phenolic and flavonoid compounds were also quantified with further metabolilte profiling using Liquid Chromatography Tandem-Mass Spectrometry (LC-MS/MS) analysis. Further study of antiaging activity was assayed using the yeast model *Schizosacchromyces pombe*. The extract from *N. nealsonii* DCL1 cultures harvested at 28 and 40 hours yielded 0.01% and 0.009%, respectively. Our findings indicate that the 40-hour extract contains higher levels of phenolics (93.54 mg gallic acid equivalents/g extract) and flavonoids (12.09 mg quercetin equivalents/g extract) compared to the 28-hour extract. Additionally, the 40-hour extract exhibited greater antioxidant activity than the 28-hour extract, as demonstrated by DPPH (IC_{50} 222 µg/mL) and ABTS (IC_{50} 511.43 µg/mL) assays. Moreover, the 40-hour extract significantly prolonged the viability of *S. pombe*, suggesting its potential as an anti-aging agent. LC-MS/MS analysis identified several potential bioactive compounds, including gallic acid and N-carbamoyl-2,3-dihydroxybenzamide, which may contribute to its bioactivity. Ultimately, our study indicates that DCL1 40-h extract showed antiaging potential, which is promising as source and formulation of biopharmaceutical or cosmeceutical products.

Keywords: antiaging; antioxidant; LC-MS/MS; *Niallia nealsonii* DCL1; *Schizosaccharomyces pombe*.

INTRODUCTION

Cellular aging is one of the causes of functional decline in tissues and organs in the body, which can increase the risk of developing degenerative diseases such as cancer and diabetes. In 2019, Indonesia was ranked of 7th in the world with the highest number of diabetics and in 2045 it is predicted that Indonesia will remain in the top 10 countries with the most diabetes globally (Saeedi et al., 2019). Degenerative diseases are closely related to cell aging which can be caused by various factors such as genetics, lifestyle, exposure, and accumulation of toxic molecules reactive oxygen species (ROS) or advanced glycation end's products (AGEs) which can be originated from UV light, cigarette smoke, pesticide, and pollution (Lobo et al., 2010; Martemucci et al., 2022).

Antioxidants are important defence compounds to help slow down the cellular aging process because these compounds can inhibit cell damage reactions caused by free radicals (Li et al., 2021; Robert et al., 2020; Vaiserman et al., 2020). Of note, antioxidant constituents could be produced naturally from within cells in the form of enzymes or from outside cells, such as food and plant bioactive compounds (Shahidi et al., 2015; Stobiecka et al., 2022; Xu et al., 2017). Previous studies indicate the potential antioxidant activities from metabolites of clove (*Syzygium aromaticum*), *Asteraceae* plants, *Adenostemma lavenia* and plant-derived essential oils of nutmeg and patchouli (Astuti et al., 2021; Batubara et al., 2020; Rahmi et al., 2021).

Clove plants have many benefits in various fields of life, such as food industries, perfume, and medicinal plants. Most of the utilization of clove plants is carried out on the flower organs while the leaf organs are less utilized. Clove leaf extract has been shown to have potential as an antiaging agent (Amini et al., 2022; Ariybah et al., 2021; Cortés-Rojas et al., 2014; Fauzya et al., 2019; Lesmana et al., 2021). On the other hand, the

increasing needs of the community and international trade have also increased in clove plantations, of which 98% of the area is cultivated by smallholders (Riptanti et al., 2019). Therefore, an alternative approach is needed to overcome the high demand from the community for the efficacy of clove plants, one of which is by investigating the secondary metabolites of endophytic bacteria that live in clove leaf tissue.

Secondary metabolites can be produced not only by plants, but also by endophytic bacteria that live symbiotically in plant tissues. The ability of endophytic bacteria to produce the same active compounds as the host is thought to be the result of a genetic exchange over a long evolutionary period (Singh et al., 2017). In our previous research, six isolates of endophytic bacteria were isolated from clove leaf, one of which was identified as *N. nealsonii* DCL1 (Utami et al., 2023). Based on the 16S rRNA gene sequence analysis, the DCL1 isolate is 99.18% similar to *N. nealsonii* DSM 15077. DCL1 has good potential for antioxidant activity as this isolate was physiologically tolerant against H_2O_2-induced oxidative stress treatment and found as non-pathogenic strain (Utami et al., 2023). Based on this data, thus, in this study, we evaluate the potential activity of the extracellular metabolite from isolate DCL1 as an antioxidant in vitro and further determine the antiaging activity at the cellular level using the yeast *Schizosaccharomyces pombe* as model organism.

MATERIALS AND METHODS

Microbial strains and Medium

The endophytic bacteria *N. nealsonii* DCL1 were routinely grown on Luria Agar (LA). The bacterial culture was incubated for 24 hours at room temperature (± 27°C). Yeast *S. pombe* ARC039 was routinely cultured in Yeast Extract with Supplement Medium (YES medium) at 30 °C for 1-3 days.

Determination of the stationary phase of selected endophytic bacteria

Determination of the stationary phase was referred to previous study (Utami et al., 2023). *N. nealsonii* was precultured in Luria Bertani (LB) liquid medium for 24 hours at room temperature (± 27°C). Preculture was transferred to a new sterile LB liquid medium as the main culture at initial OD_{600} of 0.1. The main culture of 3 replicates was incubated in a shaker incubator (120 rpm) at room temperature. The OD600 value was then measured every 4 hours for 48 hours.

Extraction of endophytic bacterial extracellular metabolites

Endophytic bacteria were precultured as previously mentioned in the determination of the stationary phase procedure. The main culture was incubated for 28 and 40-hours with shaking (120 rpm) at room temperature (± 27°C). Subsequently, bacterial cultures were mixed with ethyl acetate solvent (96%) with a ratio of main culture volume and ethyl acetate of 1:1 and shaken as the modification technique was performed at 180 rpm for 2 hours at room temperature. The ethyl acetate layer formed was then separated and evaporated using a rotary evaporator at 50°C to obtain crude extracts of the bacterial extracellular metabolites (M.E. Prastya et al., 2018)

Antioxidant activities based on DPPH and ABTS assay

The antioxidant activity of the crude extract was assessed using the DPPH (2,2′-diphenyl-1-picrylhydrazyl) (Astuti et al., 2021)and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assays (Abubakar et al., 2022), following the methodology of a previous study. The crude extract was diluted with 96% methanol, mixed with a DPPH solution (125 µM in methanol), and incubated for 30 minutes. The absorbance was then measured at 514 nm using an ELISA microplate reader. For the ABTS assay, a 7 mM ABTS solution was oxidized with potassium persulfate for 12-16 hours to generate the ABTS radical, which was then reacted with the crude extract solution and incubated for 30 minutes. The absorbance was measured at 734 nm using an ELISA microplate reader. The extract's ability to scavenge DPPH and ABTS radicals was calculated as a percentage of inhibition, using the formula: % inhibition = [1 – ((A–C)/(B–C))] × 100%, where A is the absorbance of the radical with the crude extract or positive control, B is the absorbance of the radical with methanol, and C is the absorbance of methanol. The results were expressed as the inhibitory concentration of 50% (IC50). Ascorbic acid and trolox were used as positive controls.

Quantitation of total phenolic and flavonoid content

The total phenolic content of the extract was measured using the Folin-Ciocalteu reagent method, with gallic acid as the standard. The crude extract was diluted in 96% methanol to a concentration of 1000 µg/mL. This solution was then combined with 250 µL of Folin-Ciocalteu reagent and 3.5 mL of distilled water, shaken, and incubated for 8 minutes. Afterward, 750 µL of 20% sodium carbonate (Na2CO3) was added to the mixture, which was again shaken and incubated for 2 hours at room temperature. The absorbance of the solution was measured at 765 nm. Standard curves were created using gallic acid concentrations of 1000 µg/mL, prepared at various concentrations (5, 10, 15, 20, and 25 µg/mL). The total phenolic content in the extract was expressed as gallic acid equivalents (mg/g extract) based on the regression equation from the gallic acid standard curve (Afrendi et al., 2023).

The total flavonoid content was determined using the colorimetric aluminum chloride (AlCl3) method. A crude

extract solution (1000 µg/mL) was mixed with 2 mL of distilled water and 150 µL of 5% NaNO2, shaken, and incubated for 2 minutes. Then, 150 µL of 10% aluminum chloride was added, and the mixture was shaken again and incubated for 8 minutes. Following this, 2 mL of 1M NaOH was added, and the absorbance was measured at 510 nm using an ELISA microplate reader. Quercetin concentrations of 1000 µg/mL were prepared at various concentrations (10, 20, 30, 40, and 50 µg/mL) to generate standard curves. The total flavonoid content in the extract was expressed as quercetin equivalents (mg/g extract) based on the regression equation of the quercetin standard curve (Kefayati et al., 2017).

Antiaging activity based on yeast viability assay

The yeast viability assay was conducted using a spot assay. The wild-type strain *S. pombe* ARC039 (h-leu1-32 ura4-294) was initially cultured in yeast extract supplement (YES) liquid medium with 3% glucose and incubated for 24 hours at 30°C. The preculture was then transferred to 3 mL of YES liquid medium containing 3% glucose with an initial OD600 of 0.05, serving as the treatment culture. The test extract was diluted with 99% DMSO and added to each main culture at various concentrations, which were determined based on the IC50 value from a DPPH assay, including 1/2x, 1x, and 2x IC50. A negative control consisted of *S. pombe* cultured in YES medium with 3% glucose and DMSO, without the extract, while a positive control involved *S. pombe* cultured in YES medium with 0.3% glucose (calorie restriction) and DMSO, without the extract. All cultures were incubated for 11 days at 30°C with shaking at 120 rpm. Spot assays were performed on days 1, 7, and 11, starting from cultures adjusted to an OD600 of 1. Serial dilutions were then prepared from OD600 = 1 to 10^4 on a sterile 96-well plate, and 3 µL of each dilution was spotted onto YES solid medium, followed by incubation for 3 days at 30°C. The cell density from each spot was used to assess the viability of *S. pombe* cells (Lesmana et al., 2021).

Liquid Chromatography Tandem-Mass Spectrometry (LC-MS/MS) Analysis

LC-MS/MS analysis was used to determine the compounds in the extract of extracellular metabolites of *N. nealsonii* DCL1. The LCMS data were obtained by using ACQUITY UPLC I-Class Binary Solvent Manager. The columns used were the FTN Sample Manager with a temperature of 40°C. stepwise gradients achieved separation from 95% A (0.1% formic acid + distilled water) and 5% B (acetonitrile + 0.1% formic acid) to 5% A and 95% B for 17 minutes. The flow rate of the desolvation gas was set to 1000L/h, for cone gas it was set to 50L/h, and the source temperature was fixed to 120°C. The capillary voltage and cone voltage were set to 2.0 and 30kV, respectively. The mass spectrometry was determined using the type of electrospray ionization (ES) Xevo G2-S QTof (Waters) mass spectrometry in positive ion mode. Moreover, the raw MS fragmentation data were identified using NPAtlas, PubChem, and literature (Astuti et al., 2021).

Statistical Data Analysis

Data were presented as means with standard deviation. The total phenolic content, flavonoid contents and antioxidant activity of the extracellular metabolites were analysed statistically using the One way ANOVA test at $p<0.05$.

RESULTS AND DISCUSSION

Morphology and growth curve of endophytic bacteria *Niallia nealsonii* DCL1

The morphological characteristics of the endophytic bacteria *N. nealsonii* DCL1 are round in shape, convex elevation, smooth surface, slimy texture, and white colour (Figure 1A). The microscopic character of the bacterial cell is rod-shaped with a cell size of about ± 4 µm. The results of Gram staining showed purple bacterial cells which confirmed that the isolate belonged to the group of Gram-positive bacteria (Figure 1B). Based on the growth curve, isolate DCL1 began the stationary phase after 20 h of incubation and remained stable up to 48 hours of incubation (Figure 1C). Based on this data the growth curves obtained, the harvesting of secondary metabolites was determined at 28 and 40-hours.

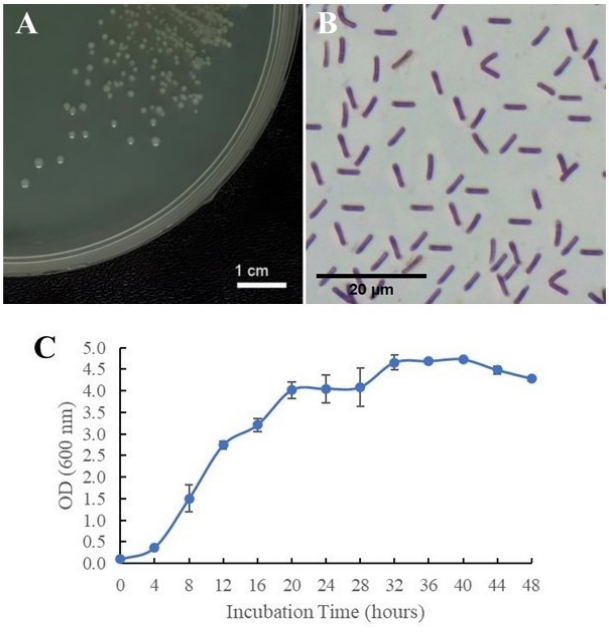

Figure 1. The morphological characteristics of the colony (A) and cell following Gram staining procedure (B) as well as the growth curve (C) of isolate *Niallia nealsonii* DCL1 on Luria Bertani (LB) medium at a temperature of 28-29 °C.

Extraction of extracellular metabolites

Extraction of bacterial extracellular metabolites was conducted by the maceration method using ethyl acetate as a solvent. The crude extract produced at 28 and 40-h had a brownish paste form (Figure 2). The amount of extract obtained is expressed in percentage yield (gram/mL). The yield percentage of the 28 h extract was higher than at 40-h extract (Table 1).

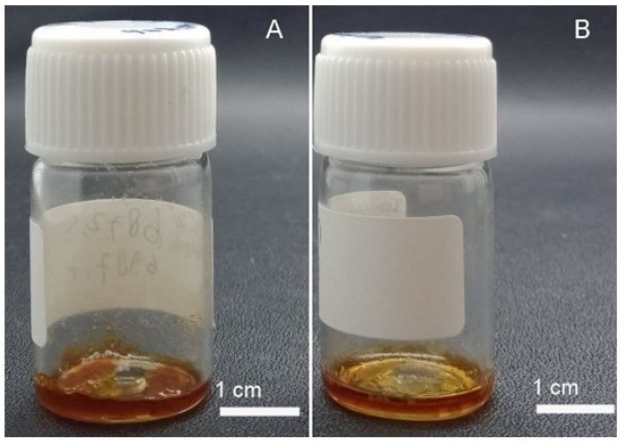

Figure 2. The resulted extract of extracellular metabolites from *N. nealsonii* DCL1 using ethyl acetate solvent. (A) 28-h extract and (B) 40-h extract.

Table 1. The yield percentage of the of extracellular metabolite extract from *Niallia nealsonii* DCL1.

Sample	Culture volume (mL)	Extract weight (gram)	Yield (%)
28-h extract	2000	0.2085	0.010
40-h extract	3000	0.2672	0.009

Quantification of total phenolic and flavonoid

From our data it is confirmed that the total phenol was higher than the total flavonoid concentration. The highest concentration of total phenols and flavonoids were found in the 40-h extract. The value of total phenol content in the 40-h extract was 93.54 EAG/g extract. As for, the total level of flavonoids in the 40-h extract was 12.09 QE/g extract (Table 2). Based on the results of the total phenol and flavonoid measurements and the antioxidant activity, the 40-h extract has the potential to be analyzed further.

Table 2. Total phenolic and flavonoid contents of the extracellular metabolites extract of *Niallia nealsonii* DCL1.

Sample	Total phenolic* (mg GAE/g extract)	Total flavonoid** (mg QE/g extract)
28 h extract	27.44 ± 0.33^{a}	14.78 ± 3.69^{a}
40-h extract	93.54 ± 0.48^{b}	12.09 ± 0.11^{ab}

*The total phenolic content is expressed in mg gallic acid equivalent/gram of extract (GAE/g extract). **The total flavonoids are expressed in mg quercetin equivalent /gram of extract (QE/g extract). Different superscript letters in the same column showed significant differences ($p<0.05$).

Antioxidant activities based on DPPH and ABTS assay

Antioxidant activities were determined using the radicals of DPPH and ABTS. The strongest antioxidant activity of DPPH and ABTS was shown by the 40-h extract with an IC_{50} value of 222 µg/mL and 511.43 µg/mL, respectively. This value indicates the activity of each extract in degrading free radicals by 50% at each IC_{50} value (Table 3).

Table 3. Antioxidant activities of the extracellular metabolites extract of *Niallia nealsonii* DCL1.

Sample	Antioxidant activities	
	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)
Ascorbic acid	4.00 ± 2.10^{ab}	11.52 ± 3.89^{a}
Trolox	2.97 ± 0.35^{a}	3.82 ± 0.64^{b}
28 h extract	838.11 ± 57.51^{c}	586.80 ± 41.72^{c}
40-h extract	222.00 ± 20.33^{d}	511.43 ± 32.76^{cd}

Note: The ascrobic acid and Trolox were used as positive control. Different superscript letters in the same column showed significant differences ($p<0.05$).

Antiaging activity based on yeast viability assay.

The antiaging activity was determined using the spot method to observe the effect of bacterial extracts on the viability of *S. pombe* yeast cells under glucose-rich conditions. The results indicated that the 40-h extract treatment supports yeast cells viability up to 11 days (considered as the stationary phase) better than that of the control negative treatment (Figure 3). It is worth noting that all control positive treatment of calorie restriction resulted relatively better growth than all treatment of extract at 11 days. As for the treatment of 444 µg/mL, yeast cells viability was found higher than lower extract treatment and the control negative (non-calorie restriction treatment without extract). Our data indicate the potential of extracellular metabolite of DCL1 in prolonging and maintaining yeast cells viability, during stationary phase.

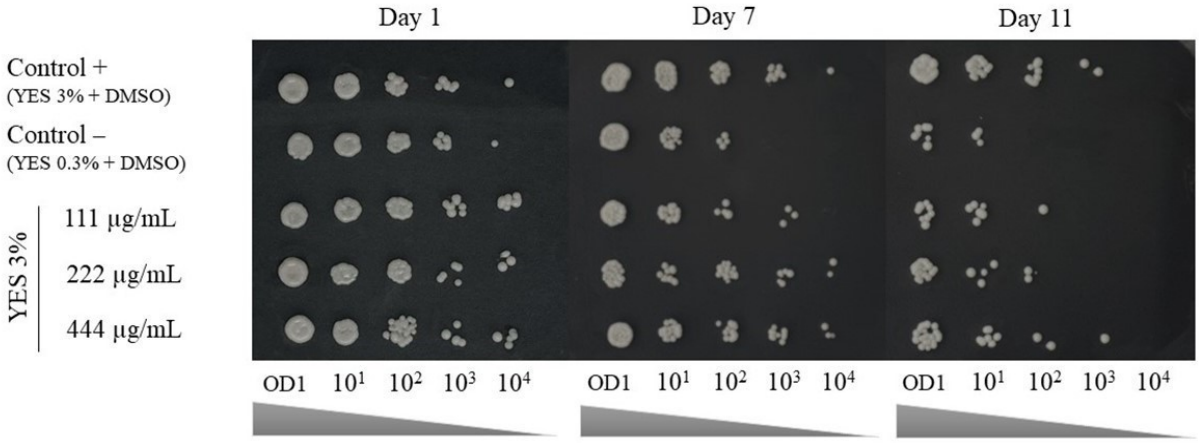

Figure 3. The antiaging effect of 40-h extract at various concentrations on the life span analysis of *S. pombe* was observed using the spot method. Positive control was yeast grown on 0.3% glucose medium. Negative control was yeast grown on 3% glucose medium without extract treatment.

Liquid Chromatography Tandem-Mass Spectrometry (LC-MS/MS) Analysis

The content of active compounds from bacterial extracts was identified using the LC-MS/MS method. The results on the chromatogram pattern show several peaks of dominant compounds which are thought to have a role in antioxidant and antiaging activities. Based on the literature review, the predominant compounds are Gallic acid, N-carbamoyl-2,3-dihydroxybenzamide, Methyl 6-carbamoylphenazine-1-carboxylate, N-carbamoyl-2-hydroxy-3-methoxybenzamide, Prenostodione, 12E-bromoisorumbrin, and two unknown dominant compounds are unknown1 and unknown2 (Figure 4).

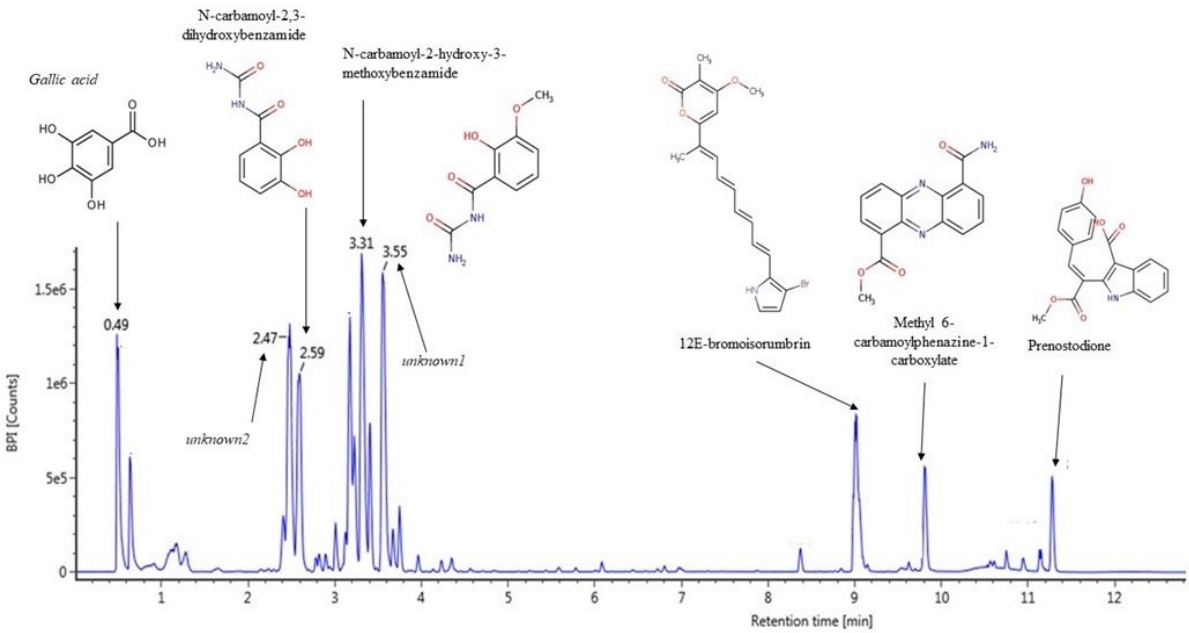

Figure 4. Metabolite profile of 40-h extract of extracellular metabolites from *N. nealsonii* DCL1. The metabolites were analysed using LC-MS/MS analysis. The dominant peaks were identified based on the specific mass identity.

Based on previous literatures, the three compounds namely N-carbamoyl-2-hydroxy-3-methoxybenzamide, Gallic acid, and N-carbamoyl-2,3-dihydroxybenzamide are known to have antioxidant activity. In addition, gallic acid has been reported to exhibit antiaging activity. As for, Methyl 6-carbamoylphenazine-1-carboxylate and Prenostodione showed antibacterial activity, while prenostodione compounds were also reported to have antifungal bioactivity. All those reports are summarized in Table 4.

Table 4. The peak identity of highly abundance compounds in the extracellular extract of 40h-culture of *Niallia nealsonii* DCL1and its corresponding potential bioactivity based on the previous reports.

No	Putative compound (Molecular formula)	Retention time (minutes)	Molecular weight	Bioactivity	References
1.	*N-carbamoyl-2-hydroxy-3-methoxybenzamide* ($C_9H_{10}N_2O_4$)	3.31	210.1323	Antioxidant	(Liang et al., 2020)
2.	*Unknown1*	3.55	244.1174	Unknown	-
3.	*Gallic acid* ($C_7H_6O_5$)	0.49	169.0671	Antioxidant, Antiaging	(Lesmana et al., 2021; Utami et al., 2023)
4.	*Unknown2*	2.47	260.1113	Unknown	-
5.	*N-carbamoyl-2,3-dihydroxybenzamide* ($C_8H_8N_2O_4$)	2.59	196.1146	Antioxidant	(Sugiyama et al., 2009)
6.	*12E-bromoisorumbrin* ($C_{20}H_{20}BrNO_3$)	9.04	402.2258	Unknown	-
7.	*Methyl 6-carbamoylphenazine-1-carboxylate* ($C_{15}H_{11}N_3O_3$)	9.80	281.2697	Antibacterial, Antifungal	(Hu et al., 2019)
8.	*Prenostodione* ($C_{19}H_{15}NO_5$)	11.28	337.3293	Antibacterial	(Utami et al., 2023)

Discussion

Niallia nealsonii was previously known as *Bacillus nealsonii*, but there was a change in nomenclature based on evidence of collecting phylogenetic, molecular, and protein data into a new genus *Niallia* which belongs to the Bacillacea family (Gupta et al., 2020; Patel et al., 2019). The isolate DCL1 has been identified as *N. nealsonii* based on the 16SrRNA gene which was deposited in the GenBank database with accession number OP363654 (Utami et al., 2023). Physiological properties of DCL1 in terms of growth phase have shown that the stationary phase was started after 20 h of incubation in the liquid Luria Bertani (LB) medium under aerobic conditions. Thus, harvesting the extracellular metabolites at 28 and 40-h of incubation would give insight towards the secondary metabolites secreted by DCL1.

In this study, the extraction of metabolites was conducted using maceration method and employing ethyl acetate as solvent. Ethyl acetate has been reported to be an effective solvent to obtain an extract rich in various groups of bioactive compounds such as polyphenols (Pintać et al., 2018), flavonoids (Nurcholis et al., 2021; Ren et al., 2020; Thavamoney et al., 2018), and terpenoids (Jiang et al., 2016; Truong et al., 2021). Those groups of bioactive compounds have also been reported to be contained in clove leaves (Haro-González et al., 2021; Lesmana et al., 2021; Plata-Rueda et al., 2018). However, limited information is available in the potential endophytic bacteria of clove in producing the similar bioactive compounds to its plant host.

As revealed by our experiments, the endophytic bacteria DCL1 were confirmed to produce phenol and flavonoids as part of the secreted extracellular metabolites. The total phenol levels were higher than the total flavonoid levels both in 28 and 40-h culture. However, the levels of those compounds were higher in metabolites produced by 40 than 28-h bacterial culture. In accordance with that, the antioxidant activity of metabolites in 40-h culture was found stronger than the 28- h bacterial culture. However, the IC_{50} DPPH value of the crude extract of *N. nealsonii* 40-h was still lower by about 55x than ascorbic acid and about 74x lower than trolox. It is expected that the *N. nealsonii* extract is still a crude extract, so it contains various types of active compounds that have an impact on their non-specific activity compared to the positive control, which is already a pure compound. It is worth noting that the IC_{50} using DPPH and ABTS radicals has a significant difference (Table 2), yet both activities are considered to have low antioxidant activity. Similar results occurred in the extracts of endophytic sponge *Pseudoalteromonas flavipulchra* STILL-33 (Prastya et al., 2020), which had a higher IC_{50} ABTS value than IC_{50} value of DPPH radicals. This was influenced by the different types of radicals used, so the degradation mechanism of each radical is different [22, 23]. Several previous studies reported that the bacterial extract associated with clove leaves had an IC_{50} DPPH antioxidant activity of 262.3 µg/mL (Utami et al., 2023). When compared with this study, the 40-h *N. nealsonii* extract had a DPPH antioxidant activity value of about 0.85x stronger. Notably, antioxidant activity is essential to promote cellular longevity. It is mostly due to the free radicals-causing oxidative stress are common causes of cellular aging (Pizzino et al., 2017; Tan et al., 2018). Thus, we further elucidated the antioxidant and antiaging properties at the cellular level.

The yeast model organism *S. pombe* was used for the antiaging assay at cellular level because it has a relatively short life span, easy to reproduce, is not pathogenic, quickly obtains test results, and provides comprehensive results because it has similarities with mammalian cells. The similarities between mammalian cells and the yeast *S. pombe* are observed in the inheritance of mitochondria through the microtubule network (Ruetenik et al., 2015). Another similarity was in the stress response which activates TORC1 and mitogen-activated protein (MAP) kinases (Pan et al., 2011). In this assay, the 40-h extract of *N. nealsonii* potentially influence aging mechanisms

so that it could prolong yeast life span in YES medium compared to without extract treatment (Figure 4). It is worth noting that treatment of 2 x IC_{50} DPPH markedly maintained yeast viability comparable to that of calorie restriction treatment (YES with 0.3% glucose) up to 11 days of incubation. The calorie restriction treatment has been known to promote life span to various organisms including yeast *Saccharomyces cerevisiae* (Ariybah et al., 2021), *S. pombe* (Astuti et al., 2021), worm *Caenorhabditis elegans* (Yang et al., 2010), mice *Mus musculus* as well as mammalian cells (Chung et al., 2019; Khan et al., 2019; Pitt et al., 2015). Such antiaging activity of calorie restriction has been proposed through various mechanisms including induction of Sirtuin activity (Wierman et al., 2014), autophagy (Chung et al., 2019), and adaptive-mitochondrial signalling (Lesmana et al., 2021; Wierman et al., 2014; Yusuf et al., 2021). It is worth noting that the metabolite extract of DCL1 shows potential antiaging activity even in non-calorie restricted conditions. However, further study is required to elucidate the potential mechanism elicited by the metabolites of DCL1.

Compound profiles in potential extracts were analysed using the LC-MS/MS method to identify dominant compounds that are potentially related to antioxidant and antiaging activities. The peak of the most dominant compound is suspected to be N-carbamoyl-2-hydroxy-3-methoxybenzamide (m/z 210.1), followed by unknown1 (m/z 244.1), gallic acid (m/z 169.0), unknown2 (m/z 260.1), and N-carbamoyl-2,3-dihydroxybenzamide (m/z 196.1), respectively. Based on literature studies, gallic acid and N-carbamoyl-2,3-dihydroxybenzamide compounds were reported to have pharmaceutical activities related to antioxidant and antiaging activities (Riptanti et al., 2019; Singh et al., 2017). The N-carbamoyl-2-hydroxy-3-methoxybenzamide compound has nuclear magnetic resonance (NMR) data which is very similar to N-carbamoyl-2,3-dihydroxybenzamide and were reported to have antioxidant activity (Lesmana et al., 2021). A previous study reported that the ethanol extract of clove leaves and ethyl acetate-derived extract from clove endophytic bacteria *Bacillus cereus* DCN1 produced metabolites rich in gallic acid. Those extracts were found to have both antioxidant and antiaging properties (Lesmana et al., 2021; Utami et al., 2023). Thus, our study enriched the potential source of important bioactive compounds that are profoundly used for the development of drugs and cosmeceuticals.

CONCLUSIONS

The ethyl acetate extract of clove endophytic bacteria *N. nealsonii* DCL1 has extracellular metabolites with low antioxidant activity in vitro. However, a concentration of 2x IC_{50} of antioxidant activity could prolong yeast *S. pombe* cells. Thus, showing the potential of the extract as antiaging agent at cellular levels. The extract was found to be rich in gallic acid and N-carbamoyl-2,3-dihydroxybenzamide which potentially be developed further for pharmacological purposes.

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Competing Interests: The authors declare that there are no competing interests.

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