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Research article

# *Dyadobacter* and *Sphingobacterium* isolated from herbivore manure in Thailand and their cellulolytic activity in various organic waste substrates

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**Abstract**

Nineteen isolates of carboxy methyl cellulose (CMC) degrading bacteria were screened from various herbivore manures. The strain V5 and W2, isolated from elephant and cow manures, were the top two isolates that produces high cellulase activity of 0.16±0.005 and 0.14±0.0043 U/mL, respectively. Study of nitrogen sources showed that 0.6% yeast extract and 0.8% peptone were favorable for V5 and W2, respectively, to maximize their cellulase activity. Different organic wastes for example rice straw, bagasse, grass and garland, were also applied as carbon sources for cellulase enzyme induction of the isolates. The two isolates (V5 and W2) possess 2-fold higher cellulase activity, when grown in the presence of garland waste (maximum at 0.60±0.0086 U/mL), than the other organic wastes. The molecular identification of the V5 and W2 isolates based on 16S rDNA sequencing analysis appeared in the genus *Dyadobacter* and *Sphingobacterium*, respectively.

## Introduction

Thailand is an agricultural country and has also become an agro-industrial country; therefore, organic waste accounts for most discarded biomass per annum (Chaiprasert, 2011). Over 100 million tonnes of agricultural wastes (mainly rice straw and sugarcane bagasse) are generated in a year (Jiménez et al., 2014). Furthermore, the volume of municipal solid waste (MSW), mainly (64%) comprising organic waste, has continued to grow as the result of economic development, urbanisation, and the rapid growth of the Thai population (Chiemchaisri et al., 2007; Cherdsatirkul, 2012). Discarded flower garlands constitute a type of waste that has been overlooked in Thailand. However, great quantities of flower garland waste are generated in the temples and other religious sites in the country. Although some organic waste has been used as an alternative energy source to produce biogas, only 36% of all agro-industrial waste and 4% of MSW is used for energy production (Chaiprasert, 2011). Thus, tremendous quantities of waste are being discarded in landfills, which can cause pollution when such waste decomposes under uncontrolled and unhygienic conditions. Damaging leachates are also being released into the water system. When such types of waste are moved around by rainwater run-offs or floods, waterways can be obstructed, resulting in further environmental hazards (Akpomie et al., 2013). If such waste is burnt, greenhouse gases and toxic fumes are emitted, which can cause various illnesses, including cancer.

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Major components of organic waste are plant materials containing cellulose, hemicelluloses and lignin, which are rather difficult to biodegrade (Liu et al., 2006). Cellulose is a linear polysaccharide of glucose residues with β-1, 4-glycosidic linkage, and it forms a crystalline structure (Sarker et al., 2012). It has long been known that microorganisms can degrade cellulosic compounds. The degradation proceeds through the action of different cellulases, which can be divided into three types: endoglucanase (endo-1, 4-β-D-glucanase); cellobiohydrolase or exoglucanase (exo-1, 4-β-D-glucanase) and β-glucosidase (1,4-β-D-glucosidase) (Bhat and Bhat, 1997). There are many microorganisms in the environment that degrade cellulose very efficiently, including many bacterial and fungal strains.

Cellulose-degrading bacteria have been isolated from a variety of sources, such as soil, decayed plant materials, organic matter, and composts (Pourramezan et al., 2012). Diverse microorganisms that have the potential to hydrolyze cellulose can also be found in the gut and faeces of various animals (Al Jassim et al., 2005; Oyeleke and Okusanmi, 2008; Anand et al., 2012; Huang et al., 2012; Padmavathi et al., 2012; Vilanova et al., 2012; Dar et al., 2015). However, not much relevant research has been conducted on the microorganisms present in herbivore manure (Salunke, 2012). Most commercial cellulases are produced by fungi (Percival Zhang et al., 2006), but they may not be cost-effective for waste management, owing to the time consumption involved in the cultivation of the fungi and degradation of waste using fungal cellulases. The authors could find no previous research conducted in Thailand documenting the screening of cellulose-degrading bacteria from local herbivore manure. In the present study, cellulose-degrading bacteria were isolated from herbivore manure. The isolates that had high hydrolysis capacity were further identified and their potential for cellulase production was examined using various types of organic waste as a substrate.

## Materials and Methods

### *Collection of samples*

Herbivore droppings were collected from upcountry farms in Thailand as followed: horse (*Equus caballus*) and cow (*Bos taurus* Linn) from Nakhon Ratchasima; elephant (*Elephas maximus*) from Ayutthaya; pig (*Sus domesticus*) and rabbit (*Oryctolagus cuniculus*) from Chachoengsao; bat (*Oreoglanis siamensis*), deer (*Cervus unicolor*) and sheep (*Ammotragus lervia*) from Ratchaburi. Samples were stored in sterile containers and sent to a laboratory, where they were stored at 4°C.

### *Isolation of cellulose-degrading bacteria*

The isolation of cellulose degrading bacteria was performed on mineral salts medium (MSM) supplemented with 1% carboxymethyl cellulose (CMC) as the sole carbon source. The MSM medium with 1% CMC contained (g/L) the following: CMC 10.0, $MgSO_4 \cdot 7H_2O$ 0.7, $K_2HPO_4$ 1.0, $KH_2PO_4$ 1.0, NaCl 1.0, $(NH_4)_2SO_4$ 4.0, $Na_3C_6H_5O_7 \cdot 2H_2O$ 0.5. One gram of each sample was added to 9 mL of sterile distilled water and thoroughly mixed and serially diluted. An aliquot of 0.1 mL diluted sample was spread on the surface of MSM plus 1% CMC agar and incubated at 37°C for 48 hr (Salunke, 2012). After incubation, the plates were stained by 0.1% (w/v) Congo red dye for 20 min (Congo red assay) and destained with 1 M NaCl (Padmavathi et al., 2012). The cellulose-degrading bacteria produced a zone of clearance around growing bacterial colonies. Bacterial colonies were purified and characterised by their morphology and Gram's staining. For assessing cellulose hydrolysis, the CMC hydrolysis capacity (HC value) was calculated from the ratio of the clear zone diameter divided by the colony diameter. The ratios considered to have high activity were in the range of 3.1–4.0, whereas moderate activity ratios were in the range of 2.1–3.0 (Kanokkanjana and Garivait, 2013).

### *Preparation of crude enzyme*

Cellulose-degrading bacterial isolates were pre-incubated in the MSM broth, supplemented with 1% CMC for 24 hr prior to their use as inocula. Then the MSM broth containing 1% CMC was inoculated with 1 mL of the bacterial inoculum ($OD_{600}$=1.0) and further incubated at 37°C for 48 hr while being shaken at 180 rpm. The cells were removed by centrifugation at 5,000 rpm for 30 min at 4°C. The supernatant was collected as an extracellular crude enzyme.

### *Assay of cellulolytic activity*

Cellulase activities were measured in terms of their CMCase activity and Filter paper hydrolytic activity (FPase) following a method adopted from Ghose (1987). CMCase activity was determined by incubating 0.5 mL of crude enzyme with 0.5 mL of 1% CMC in 0.05 M phosphate buffer at 50°C for 30 min and then cooled down in an ice bath. Thereafter 1 mL of DNS reagent was added and the mixture was well mixed. The tube was then heated at 100°C in boiling water for 10 min and immediately cooled down. The resulting colour was measured by a UV-Vis spectrophotometer (UV 1280 Shimadzu Corporation, Tokyo, Japan) at 540 nm in order to estimate the quantity of reducing sugar when compared to a glucose standard curve (Miller, 1959). One unit (U) of enzyme activity is expressed as the quantity of an enzyme required to release 1 μmol of glucose per minute under standard assay conditions. For FPase activity, a similar procedure was carried out with filter paper (Whatman® No.1) in place of CMC; the reaction mixture was incubated at 50°C for 1 hr before the reaction was terminated.

### *Utilisation of different nitrogen sources and organic wastes*

To evaluate the effect of nitrogen concentration on cellulase activities, the MSM with 1% CMC medium was supplemented with different concentrations (0.2%, 0.4%, 0.6% and 0.8%) of each nitrogen source for example yeast extract, peptone, ammonium sulphate, or ammonium chloride.

Various types of organic waste (rice straw collected from a paddy field; bagasse collected from a sugar refinery; flower garlands collected

from a temple, mainly consisting of jasmine and rose flowers; savanna grass collected from a public garden), were also determined. Each type of organic waste was oven dried at 65–70°C for 48 hr. The dried samples were reduced to 0.25 mm particle size with a 60-mesh sieve using an electrical blender. The powdered samples were then packed into plastic bags and kept at 62±1% relative humidity at room temperature until they were used. Then, different concentrations (0.5%, 1.0%, 2.0% and 4.0%) of organic wastes was added to the MSM medium.

The cellulose-degrading bacterial isolates at an initial concentration of absorbance 0.5 at $OD_{600}$ with 1 mL were then inoculated into the prepared media (50 mL volumes into 125 mL flasks) and incubated at 37°C for 48 hr while shaking at 180 rpm. Cellulolytic activity was measured as described above.

*16S rRNA gene sequencing analysis*

Sequencing of the 16S rDNA genes of bacterial isolates using universal bacterial primers, 27F (Lane, 1991) and 1492R (Turner et al., 1999), was carried out by the Bioscience Department at Thailand Institute of Scientific and Technological Research (TISTR). The phylogenetic trees were constructed using the neighbour-joining (Saitou and Nei, 1987) and pairwise deletion models in the MEGA5 (version 5.05) software. Megablast searches of the same 16S rDNA sequences were also comparatively conducted on the National Center for Biotechnology Information (NCBI) webpage.

*Statistical analysis*

The data are statistically analysed by a one-way analysis of variance (ANOVA), followed by Student's t test, using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The cut-off point for statistical significance was set at $p < 0.05$.

**Results**

*Cellulose-degrading bacteria isolated from herbivore manure*

Altogether 42 colonies were isolated from elephant, cow, snail, bat, goat, sheep, pig, deer, buffalo, monkey, rabbit, barking deer, langur and horse manure. Nineteen isolates of these samples showed clear zones around colonies after they had been flooded with Congo red. This indicated the presence of cellulose-degrading bacteria in the 19 isolates. The primary morphological characteristics of the isolates and their hydrolysis capacity (HC) values are shown in Table 1. Seven isolates were found in elephant manure, three isolates were found in horse manure and others were found in cow, pig, bat, rabbit, deer and sheep manure. All isolates were Gram-negative; 12 isolates were rods, while six were short rods. Merely one isolate was a coccus. Among these isolates, V5 provided the highest HC value (3.38±0.20), followed by W2 (3.24±0.21). Out of these, four bacterial isolates showed high cellulolytic activity (ratios ranging from 3.1 to 4.0), nine isolates showed moderate cellulolytic activity (ratios ranging from 2.1 to 3.0), and six isolates showed low cellulolytic activity (ratios ranging from 1.1 to 2.0).

**Table 1** Morphological characteristics and hydrolysis capacity of cellulose-degrading bacterial isolates

| Isolate | Manure | Colony characteristics | | | | Gram's | Shape | HC value |
|---|---|---|---|---|---|---|---|---|
| | | Color | Form | Margin | Elevation | | | |
| D1 | horse | white | circular | entire | convex | negative | coccus | 2.29 ± 0.13 |
| D6(1) | horse | white | circular | entire | convex | negative | rod | 3.17 ± 0.29 |
| D6(2) | horse | white | circular | entire | convex | negative | rod | 3.10 ± 0.17 |
| H3 | cow | white | circular | entire | convex | negative | rod | 2.56 ± 0.20 |
| S1 | elephant | yellow | circular | entire | convex | negative | rod | 2.33 ± 0.31 |
| S3 | elephant | yellow | circular | entire | convex | negative | rod | 2.38 ± 0.38 |
| V1 | elephant | yellow | irregular | undulate | raised | negative | rod | 2.53 ± 0.18 |
| V5 | elephant | yellow | circular | entire | convex | negative | rod | 3.38 ± 0.20 |
| U5 | elephant | yellow | circular | entire | convex | negative | short rod | 2.35 ± 0.04 |
| U7 | elephant | yellow | circular | entire | convex | negative | short rod | 2.53 ± 0.18 |
| U8 | elephant | yellow | circular | entire | convex | negative | short rod | 2.13 ± 0.13 |
| W2 | cow | yellow | circular | entire | convex | negative | rod | 3.24 ± 0.21 |
| T4 | pig | white | circular | entire | convex | negative | rod | 2.93 ± 0.81 |
| Y8 | bat | white | circular | entire | raised | negative | short rod | 1.61 ± 0.10 |
| L4 | rabbit | white | circular | entire | raised | negative | short rod | 1.47 ± 0.24 |
| L7 | rabbit | white | circular | entire | raised | negative | short rod | 1.39 ± 0.10 |
| G1 | deer | white | irregular | undulate | convex | negative | rod | 1.50 ± 0.00 |
| F2 | sheep | white | circular | entire | convex | negative | rod | 1.72 ± 0.26 |
| F8 | sheep | white | circular | entire | raised | negative | rod | 1.22 ± 0.19 |

*Determination of cellulase activity*

Cellulase activities of all bacterial isolates are presented in Fig. 1. All isolates showed higher cellulase activity in CMC than in filter paper. In particular, 11 isolates (D6(1), D6(2), H3, V1, V5, U5, U7, U8, W2, T4 and F8) had higher CMCase activity than FPase activity ($p < 0.05$). The strain V5 showed the highest level of cellulase activity at 0.16±0.0031 and 0.13±0.0069 U/mL in CMC and filter paper, respectively. The W2 isolate had the second highest cellulase activity in CMC and filter paper at 0.14±0.0043 and 0.12±0.0036 U/mL, respectively. In contrast, L4, L7, G1, F2 and F8 had a low level of activity in both substrates (0.02–0.03 U/mL). Further analyses were carried out with the V5 and W2 isolates.

*Utilisation of nitrogen sources*

The efficiency of the V5 and W2 isolates in producing cellulase with different nitrogen sources is shown in Fig. 2. The V5 isolate had the highest cellulase activity ($p < 0.05$) when yeast extract was used as a nitrogen source. The digestibility against CMC and filter paper was 0.22±0.0098 and 0.19±0.0027 U/mL, respectively. Peptone was favourable for the W2 isolate, producing the maximum levels of CMCase and FPase at 0.19±0.0047 and 0.15±0.001 U/mL, respectively ($p < 0.05$). Fig. 3 displays the effect of the nitrogen source concentration on the optimal cellulase activities of the V5 and W2 isolates. Yeast extract at 0.6% delivered the highest cellulase activity ($p < 0.05$) for V5. The digestibility in CMC and filter paper was 0.28±0.0045 and 0.23±0.0068 U/mL, respectively. For the W2 isolate, the highest cellulase activity ($p < 0.05$) was exhibited when 0.8% of peptone was used. The activities in CMC and filter paper were 0.24±0.0091 and 0.20±0.004 U/mL, respectively.

*Utilisation of carbon sources from different types of organic waste*

Cellulase activities of the V5 and W2 isolates in the presence of various types of organic waste shows in the Fig. 4. Flower garlands enhanced the cellulase activities of V5 and W2 up to 0.39±0.0048 and 0.30±0.0075 U/mL, respectively, when measured in terms of CMCase. With filter paper, the activity was 0.33±0.0123 U/mL for V5 and 0.24±0.0091 U/mL for W2. The photographs of each organic wastes before and after culturing with the isolate V5 are illustrated in Fig.5. The effect of the concentration of flower garlands on cellulase activity was further investigated, as shown in Fig. 6. Both V5 and W2 displayed the highest activity ($p < 0.05$), when cultivated with 4.0% of flower garlands for 48 hr: 0.60±0.0086 and 0.43±0.01 U/mL, respectively, for CMCase. In contrast, lower activities of 0.53±0.006 and 0.37±0.008 U/mL were found for FPase, respectively. All activities detected were solely due to the V5 and W2 cultures. The enzyme activity of merely organic wastes (4% W/V) showed very low level. The detected activities of rice straw, bagasse, flower garlands and savanna grass were 0.007±0.0001, 0.009±0.001, 0.010±0.001 and 0.007±0.001 U/mL, respectively, and those activities were equal with both CMCase and FPase.

*Molecular identification and phylogenetic tree of V5 and W2 isolates*

The 16S rDNA sequences of V5 and W2 were deposited into the NCBI database and the accession numbers provided were KT216624 and KT216625, respectively. After megablast searches were performed, V5 had the closest identities (99%) with five strains of *Dyadobacter fermentans* and *Dyadobacter soli* MJ20. Although the constructed phylogram in Fig. 7 placed V5 in the same branch as those two species, it appeared in the same node as *Dyadobacter beijingensis* (98% identity). For W2, the position shown in the tree (Fig. 8), the isolate was identified as *Sphingobacterium multivorum* (99% identity).

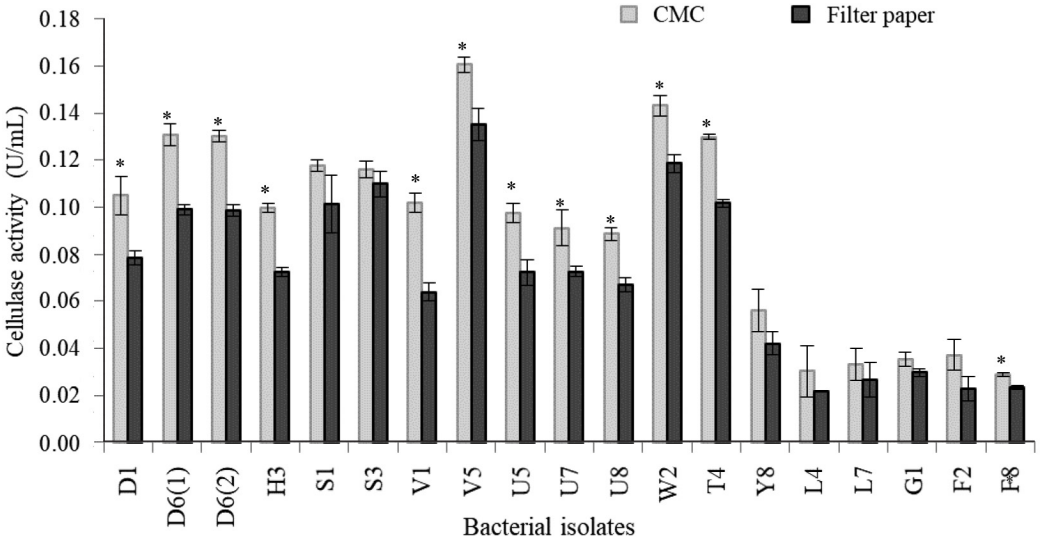


**Fig. 1** Cellulolytic activities measured in terms of CMCase and FPase from different bacterial isolates. Error bars represent the standard deviation of triplicate data. *Significant difference ($p < 0.05$) indicated by Student's t test between each pair of adjacent bars.

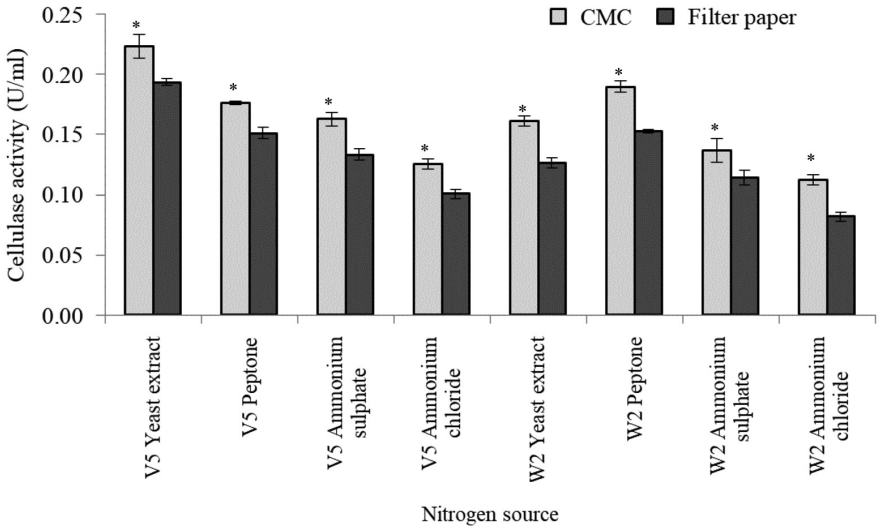


**Fig. 2** Effect of different nitrogen sources on the cellulase activity of the V5 and W2 isolates. Error bars represent the standard deviation of triplicate data. *Significant difference ($p < 0.05$) indicated by Student's t test between each pair of adjacent bars.

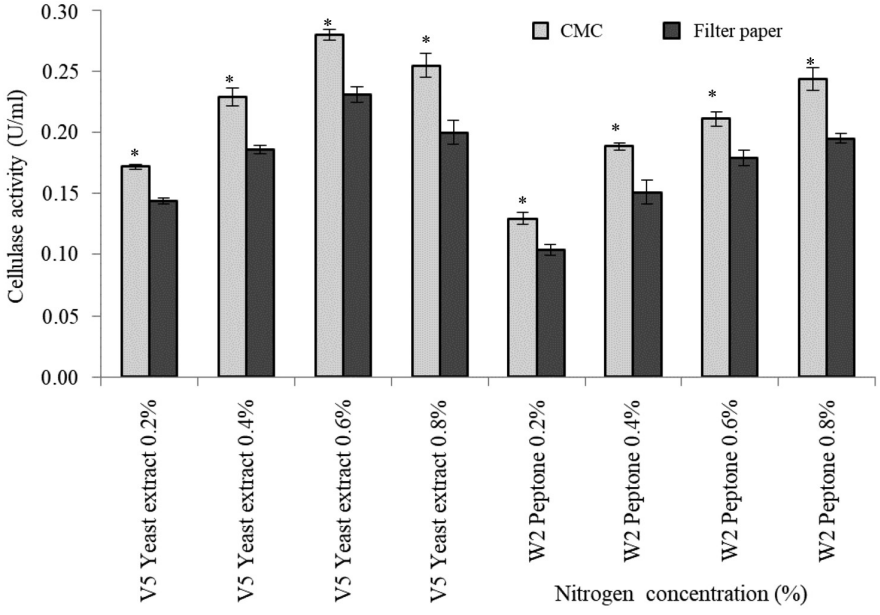


**Fig. 3** Effect of nitrogen source concentrations on cellulase activities of the V5 and W2 isolates. Error bars represent the standard deviation of triplicate data. *Significant difference ($p < 0.05$) indicated by Student's t test between each pair of adjacent bars.

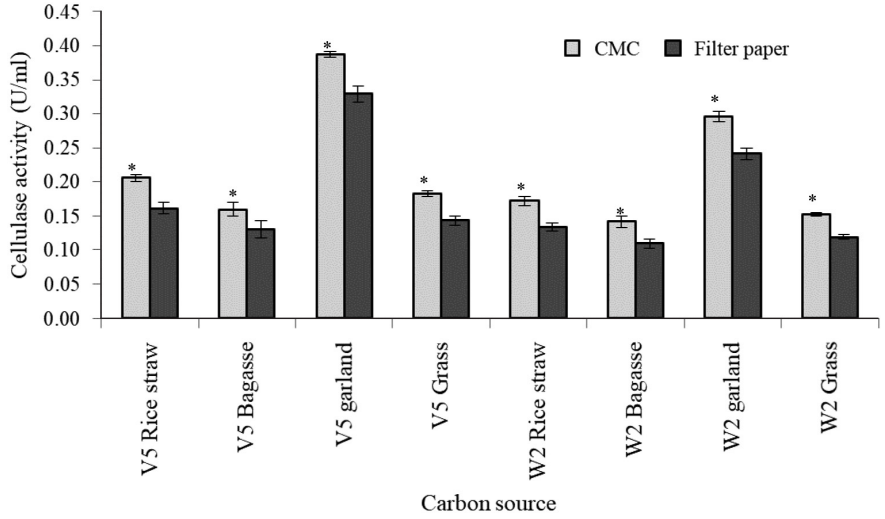


**Fig. 4** Effect of organic wastes on cellulase activities of V5 and W2 isolates. Error bars represent the standard deviation of triplicate data. *Significant difference ($p < 0.05$) indicated by Student's t test between each pair of adjacent bars.

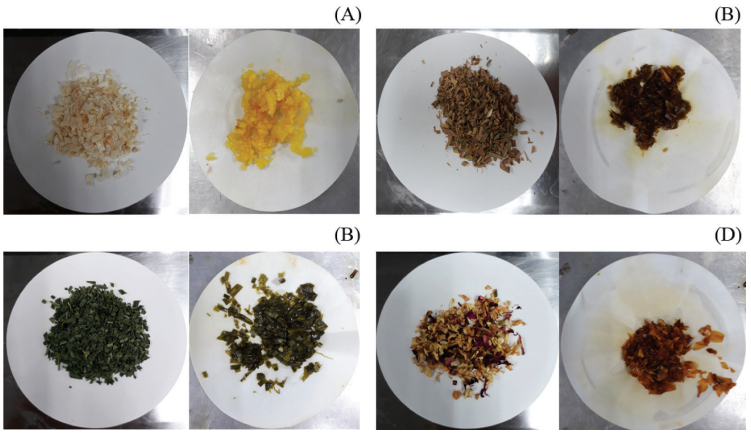


**Fig. 5** Appearance of organic wastes before (left) and after (right) incubation with isolate V5; bagasse (A), rice straw (B), savanna grass (C) and flower garland (D)

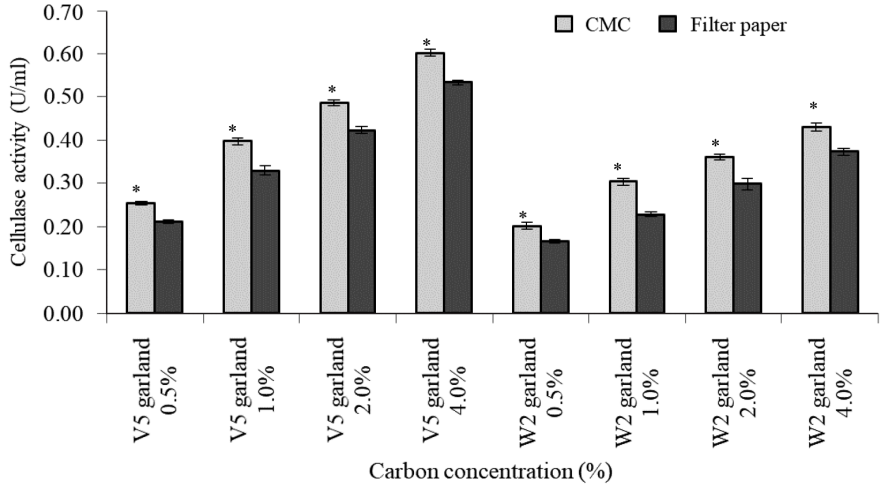


**Fig. 6** Effect of garland waste concentrations on cellulase activities of V5 and W2 isolates. Error bars represent the standard deviation of triplicate data. *Significant difference ($p < 0.05$) indicated by Student's t test between each pair of adjacent bars.

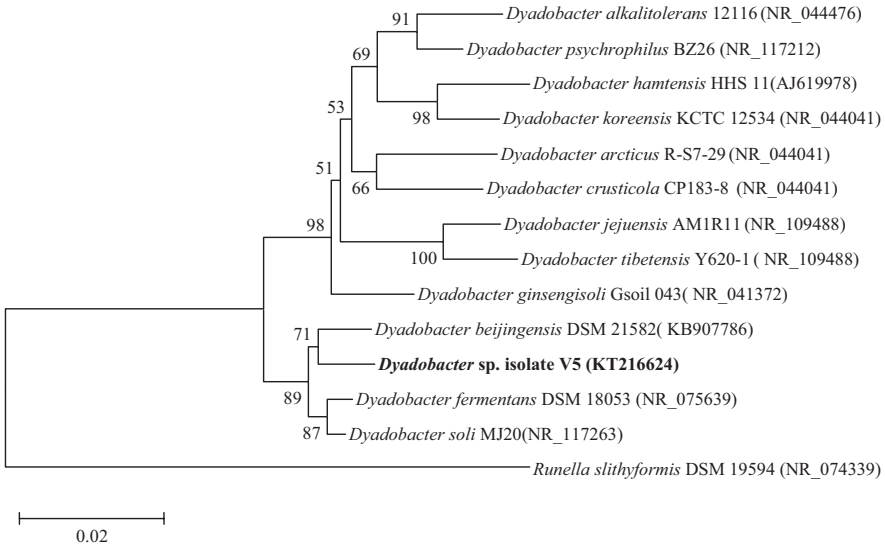


**Fig. 7** Neighbour-joining phylogenetic tree, based on 16S rDNA gene sequences, showing the position of the isolate V5, recognised members of the genus *Dyadobacter* and representatives of related taxa. Bootstrap values (expressed as percentages of 1000 resamplings) >50% are shown at branch points. Bar, 0.02 substitutions per nucleotide position.

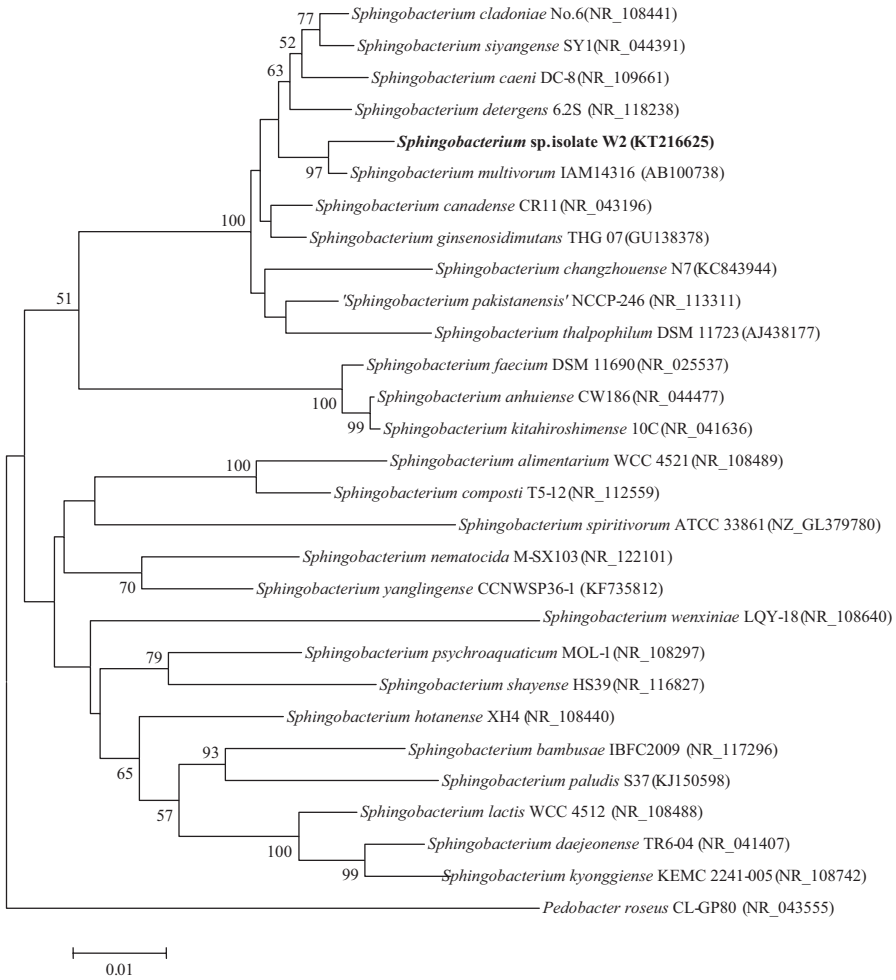


**Fig. 8** Neighbour-joining phylogenetic tree, based on 16S rDNA gene sequences, showing the position of the isolate W2, recognised members of the genus *Sphingobacterium* and representatives of related taxa. Bootstrap values (expressed as percentages of 1000 resamplings) >50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

**Discussion**

In this study, 19 isolates were obtained from the manure of various herbivores: horse (15%), cow (11%), elephant (37%), pig (5%), bat (5%), rabbit (11%), deer (5%) and sheep (11%) manure. Cellulose-degrading bacteria were obtained from eight out of 14 animal faeces samples, mostly from elephant manure. One similar study on herbivore manure in India found isolates only from elephant and deer excreta (Salunke, 2012). Many cellulose-degrading bacteria have been isolated from the droppings of herbivores, such as poultry (Akpomie et al., 2013), gut of the termite (Pourramezan et al., 2012), gut of *Holotrichia parallela* larvae (Huang et al., 2012), insect midgets (Vilanova et al., 2012), rumen of ruminants (cow, sheep, and goat) (Oyeleke and Okusanmi, 2008) and gastrointestinal tract of *Achatina fulica* (Giant African snail) (Dar et al., 2015).The predominant cellulose-degrading bacteria identified so far are *Acinetobacter*, *Bacillus*, *Cellulomonas*, *Clostridium*, *Comamonas*, *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Microbacterium*, *Micrococcus*, *Pseudomonas*, *Ruminococcus flavefaciens*, *Staphylococcus* and *Streptococcus* (Al Jassim et al., 2005; Anand et al., 2012; Huang et al., 2012; Pourramezan et al., 2012; Salunke, 2012; Vilanova et al., 2012; Akpomie et al., 2013; Dar et al., 2015). Although the temperature for screening and cultivation of cellulose degrading bacteria varied from 28–37°C (Premalatha et al. 2015), most of the literature used 37°C for this purpose (Oyeleke and Okusanmi, 2008; Salunke, 2012; Akpomie et al., 2013). The source of feces in this work was also from the warm-blooded animal, thus the body temperature would be the most suitable to isolate the actual inhabitant bacteria.

When comparing the decomposition of cellulose substrates (1% CMC vs. 1% filter paper), all 19 isolates showed higher activity in the CMC than in the filter paper (Fig. 1). CMC is a soluble form of amorphous cellulose which is easily digested, whereas filter paper is insoluble (Wirth and Ulrich, 2002). The types of enzyme are also specific to the substrate. Endoglucanase targets soluble substrates, whereas exoglucanase targets insoluble cellulose (Wood and Garcia-Campayo, 1990). The 19 isolates presented the ability to produce both endo- and exo-cellulolytic enzymes.

In the present study, the V5 isolate showed the highest hydrolyzing capacity (HC value) and enzymatic activity. Nucleotide blast performance using 16S rDNA sequence revealed 99% correspondence with *Dyadobacter fermentans* and *D. soli.* However, the phylogram generated by the neighbour-joining method showed a closer correspondence to *D. beijingensis.* Therefore, the identification could only ascertain that this isolate was in the genus *Dyadobacter.* Genus *Dyadobacter* has a Gram-negative rod shape; its cells occur in pairs in young cultures, form chains in older cultures and produce a flexirubin-like yellow pigment (Chelius and Triplett, 2000). According to previous studies, members of the genus *Dyadobacter* have been isolated from farm soil (Lee et al., 2010), the soil of a ginseng field (Liu et al., 2006), hydrocarbon contaminated soil (Zhang et al., 2010), desert sand in Xinjiang, China (Tang et al., 2009), rhizospheric soil of turf grasses irrigated with reclaimed water in Taoranting Park, Beijing, China (Dong et al., 2007) and the gut of *Holotrichia parallela* larvae (Huang et al., 2012). Nevertheless, those literatures did not examine the cellulolytic activity of *Dyadobacter* in term of enzyme unit. The current study has shown that *Dyadobacter* sp. can also be isolated from elephant manure and that it exhibits cellulase activity presented in CMCase and FPase unit.

The W2 isolated from cow dung had the second-best HC value and enzyme activity. The identification using 16S rRNA gene sequence analysis revealed a 99% correspondence with *Sphingobacterium multivorum*. This was agreeable to the phylogenetic tree that demonstrated 97% bootstrap value to the same node of this species. The member of this genus has been isolated from activated sludge of a wastewater treatment plant (Zhang et al., 2012), organic materials compost (Chiemchaisri et al., 2007), agricultural soil (Gautam et al., 2011) and forest soil (Irfan et al., 2012). The current study indicated the proficiency of *S. multivorum* from cow excreta to degrade cellulosic matter.

Nitrogen is one of the important constituents of proteins as well as enzymes. Irfan et al. (2012) experimented with various nitrogen sources and amino acids to determine the maximum cellulase activity of *Cellulomonas* sp. ASN2 isolated from soil. They found that peptone (0.25%) produced the highest activity (0.54±0.03 U) with CMC. In the present study, four nitrogen sources were compared. The yeast extract was preferable for *Dyadobacter* sp. and peptone for *S. multivorum.* The optimum concentrations of yeast extract and peptone for cellulase activity produced by *Dyadobacter* sp. and *S. multivorum* were 0.6% and 0.8%, respectively. This indicates that the cellulase enzymes produced by these two isolates operate at maximum capacity with a different organic nitrogen source. According to the literature, organic nitrogen sources such as beef extract, peptone and yeast extract have been reported to maximise the growth and enzyme production of fungi and result in higher activity than inorganic nitrogen sources (Gautam et al., 2011; Kumar et al., 2012; Pourramezan et al., 2012; El-Hadi et al., 2014). Yang et al. (2014) pointed out that the enzyme characteristic and production can be influent by the medium condition comprises the original environment. Therefore, the isolates from faces could prefer to organic nitrogen source than inorganic content. Furthermore, the yeast extract and peptone contain a mixture of various amino acids, vitamins and mineral to support the growth of bacteria.

The ability of *Dyadobacter* sp. and *S. multivorum* to utilise organic residue was further tested. This was aimed to find suitable cheap carbon sources as well as methods for decomposing common types of organic waste without generating environmental pollution. Interestingly, both isolates were capable of efficiently degrading garland waste collected from a temple, demonstrating high activity of endo- and exo-cellulase of up to 0.60±0.0086 and 0.43±0.01 U/mL, respectively. However, the activity of such isolates in other organic wastes such as rice straw, bagasse and savanna grass, seems to be low (in the range of 0.14–0.21 U/mL). Such activity seemed to be enhanced when the concentration of the garland was increased. However, Aguiar (2001) reported that the cellulase activity produced by *Aspergillus niger* IZ 9, cultivated with non-treated bagasse for 192 h, was less than 0.5 U/mL. Other studies on the utilisation of cellulose-degrading bacteria to degrade

complex organic waste, such as sawdust, have shown quite low activity, 0.19±0.034 U/mL for *Bacillus* sp., 0.25±0.041U/mL for *Pseudomonas* sp., and 0.26±0.042 U/mL for *Cellulomonas* sp. (Akpomie et al., 2013). Dantur et al. (2015) isolated bacteria from the intestine of the moth larvae, they found that the highest CMCase activity was 0.32±0.002 for the *B. pumilus* Kd 101 when using 0.5% sugar cane biomass as a sole carbon source. In the present study, the cellulase enzyme induction produced by garland waste seems promising. Garland waste contains polysaccharides with hexoses and pentoses (Nowak et al., 2014). In contrast to the other plant biomasses which contain cellulose (polymer of glucose) (36% in rice straw, 24% in grass), hemicellulose (21–24%) (5-, 6- carbon sugar and uronic acid) (Bakker et al. 2013; Ismail et al., 2015). These structures embedded in a matrix of lignin (polyphenylpropanoid unit) (15%) (Rezende, et al., 2011; Cheng and Wang, 2013). Meanwhile, the dried flower petals constituent in the garland contain soluble sugar (2.39–5.14 g/100 g dw) as well as protein (7 g/100 g dry weight) (Pires et al., 2017). These nutrients became more readily for bacteria to access than cellulose and hemicelluloses. Therefore, our work has indicated that the garland waste could be applied as an effective carbon source to induce the production of cellulase by the bacterial isolates. As these wastes are generated daily in various temples in the southeast Asian countries, these could be applied for high-value enzyme production in further study.

## Conflict of Interest

The authors declare that there are no conflicts of interest in this research.

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