JOURNAL OF AGROBIOTECHNOLOGY 2021, VOL 12(1):31-39
e-ISSN: 2180-1983
http://dx.doi.org/10.37231/jab.2021.12.1.243
https://journal.unisza.edu.my/agrobiotechnology/index.php/agrobiotechnology/index

Characterisation of *Stenotrophomonas maltophilia* Isolated from Marine Sponge Producing Salt Tolerant Proteases

Nurul Sherina Muzaini[a], Nurulhuda Mohd Jailani[a] and Tengku Haziyamin Tengku Abdul Hamid[a*]

[a]Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, Jalan Istana, 25200 Bandar Indera Mahkota, Kuantan, Malaysia.

***Corresponding author: haziyamin@iium.edu.my**

Received: 29/12/2020, Accepted: 05/02/2021, Available Online: 09/05/2021

ABSTRACT

Protease is an important industrial enzyme and salt tolerant protease in which has desirable properties that could enhance its uses not only in industries, but also in agriculture and environmental. Marine organisms usually harbour halophilic microorganisms which produce salt tolerant protease. In this study, salt tolerant protease producing bacteria from marine sponge were isolated and screened on skim milk marine agar supplemented with different NaCl concentrations (1.5 % w/v). Out of 11 isolates, 8 isolates (S1-1, S1-2, S1-3, S2-1, S2-3, S2-4, S2-5 and S2-6) showed clearing zones with ability to digest casein on the skim milk agar. Morphologically, these strains are gram negative bacilli which grow in yellow colonies and were found to be catalase-positive but oxidase-negative. They are also non-lactose fermenter that produce gelatinase but not α-amylase. The ribosomal 16S rRNA sequencing was used to identify each isolate (Acc. number of S1-1 for MT645770, S1-2 for MT645 771, S1-3 for MT645 772, S2-1 MT645 773, S2-3 MT645 774, S2-4 MT645 775, S2-5 MT645 776 and S2-6 MT645 7767). The 16S rRNA sequences showed that these isolates were highly similar to *Stenotrophomonas maltophilia* (S2-6, 99.87%) and to a related strain *Pseudomonas hibiscicola* (S1-1, S1-2, S1-3, S2-1, S2-3, S2-4, S2-5 (97.16-99.9 %). Further proteolytic studies were carried out using skim milk agar with 1.0 %, 2.0 %, 3.0 % and 4.o % (w/v) of NaCl concentrations. All isolates were able to hydrolyze casein which produced clear zones surrounding each colony at 1.0 % and 2.0 % (w/v) salt. However, only isolate S1-5 and S2-6 showed proteolytic activities at 3.0 % (w/v) salt but none of them at 4.0 % (w/v). The ability of these isolates to produce protease which active at higher salt may indicate their potential to be the sources for enzyme with useful properties.

Keywords: Halotolerant protease, slight halophilic organism, *Stenophomonas maltophilia;* skim milk marine agar, nematocidal

INTRODUCTION

Proteases are enzymes that break down peptide bond in proteins which producing smaller peptides and ultimately amino acids. Industrial proteases contributed to about two third of total global enzyme market

(Singh & Bajaj, 2017), in which their major applications are in many industries including textile detergents, leather or tanneries, food and beverages, pharmaceutical and in environmental bioremediation. Despite of their ubiquitous in nature, microorganisms are the preferred source of protease since they can rapidly multiply at low cost, cultivated in high density and their gene can easily be engineered to enhance their industrial properties. For instance, among microbial protease, *Bacillus* proteases have been widely studied and exploited commercially (Razzaq *et al.*, 2019).

Enzymes as in biological system, usually operate under milder or limited range of physiological condition. In order to maximise the industrial uses of protease, the enzymes must be able to withstand the harsh conditions during the manufacturing process. Enzymatic conversion may be subjected to inhibition under condition such as high salinity, extreme pH and high temperature (Samad *et al.*, 2017). Halophilic or halotolerant microorganisms are considered as good source for salt tolerant or halotolerant protease since they are more stable and active at a wide range of temperature, pH and ionic strength, the features desirable for industrial processes. The halophilic organism have two crucial characters, which are the ability to survive in high salt environment and capability of degrading contaminants or toxic chemicals. For these reasons they have huge potential to be employed in recovering saline waste water or contaminated soil (Zhuang *et al.*, 2010).

Exploration and research on marine microorganisms are highly beneficial in various applications in biotechnology. The ocean environment helps in nurturing bacteria endowed with unique characteristics such as psychrotolerant or halotolerant which are normally difficult to find in terrestrial microbes. Marine derived bacteria are potential source of a huge and diverse source of natural products (McCauley *et al.*, 2020), and this includes also many microbial enzymes of industrial or pharmaceutical interest. Sponges (phylum Porifera) are considered as ancient, multicellular and sessile organisms that can be found in many marine ecosystem such as coral reefs. Sponges have been explored since they are rich source of bioactive secondary metabolites of biotechnological interest (Santos *et al.*, 2010). It is hypothesized that sponge associated bacteria play huge role in building the richness of metabolites and their enzymes involve along the pathways in producing the metabolites.

In this work, sponge sample from Lang Tengah Island of Terengganu, Malaysia was collected and attempt was made to isolate microbes potentially producing halotolerant proteases. This report highlights on the isolation and characterization of several strains from *Stenotrophomonas maltophilia* or *Pseudomonas hibiscicola* which are able to produce salt tolerant protease. These strains have not only recognised as halophilic organisms (Zhuang *et al.*, 2010), they are so versatile that their enzymes were reported to have many biotechnological applications especially in agriculture and environment bioremediation.

MATERIALS AND METHODS

Sample collection and preparation

Two sponges samples (Gelliodes sp.) labelled with S1 and S2 were previously collected from Lang Tengah Island (GPS coordinate: 5.7960° N, 102.8961° E), an island situated off Terengganu coast, in peninsular Malaysia. The samples were aseptically cut into about 10 g each and homogenized in buffered saline. First, the homogenised mixtures were appropriately diluted and spread on ZorBell (Difco) marine agar which contains (at 5.5 % (w/v) in 1L) of Peptone (5.0 g); Yeast Extract 1.0 g; $C_6H_5FeO_7$ 0.1 g; NaCl 19.45 g; $MgCl_2$ 8.8 g Na_2SO_4 3.24 g $CaCl_2$ 1.8 g KCl 0.55 g $NaHCO_3$ 0.16 g KBr 0.08 g $SrCl_2$ 34.0 mg Boric Acid 22.0 mg $NaSiO_3$ 4.0 mg NaF 0.4 mg NH_4NO_3 1.6 mg $NaHPO_4$ 8.0 mg Agar 15.0. The plates were incubated at 37°C for 5 days and these were sub-cultured until well separated colonies formed. Whenever required, the strains were also propagated in liquid broth using Zorbell marine medium. Individual colonies were randomly selected for further tests and kept at -80°C under 25% v/v glycerol.

Screening of proteolytic activities from halophilic bacteria

Screening of proteolytic activity of the cultures was done using skim milk agar containing 10% (w/v) skim milk (Sunlac), 2% (w/v) Zobell marine agar (Difco), with no additional addition of NaCl. Single colony from each isolate was streaked on the agar surface and the plates were incubated for 48 hours at 37°C. Clear zones around the growth after 48 hours days were taken as evidence of proteolytic activity following milk casein digestion. These colonies were sub-cultured, kept in 30 % v/v glycerol and test again on skim milk agar with different percentage of added NaCl; 1.5 %, 3% and 5%. Similarly, clearing zones were observed after incubated at 37°C for 48 hours days. Production of clearing zones surrounding the colonies was taken as positive result.

Morphological and biochemical characterization

The morphologies of the isolated strains were characterized using standard techniques [Gram staining, cell shape, colony morphology on nutrient agar medium (colour, shape, elevation and margin)]. The biochemical tests such as catalase, oxidase, gelatin and amylase tests were carried out in a similar manner as done by Sivanandhini *et al.,* (2015) and these are based on Bergey's Manual of Determinative Bacteriology (Palleroni, 2015).

Genotypic characterization

To all selected 8 strains, the genomic DNA was extracted using GF1 DNA Extraction kit (Vivantis). The DNA sample were analysed using agarose gel (1.0%) and used as template for PCR amplification. For amplification, a pair of primer for 16S ribosomal RNA gene was used (forward, 27F): 5' TACGGYTACCTTGTTACGACTT 3' and reverse, 1492R: 5' AGAGTTTGATCMTGGCTCAG 3') (dos Santos *et al*., 2019). The PCR amplification was carried in a 50 µL reaction mixtures containing 1x of Master-premix buffer (Vivantis); 0.2 µM of each primer; 5.0 µL (~200ng) template. The PCR cycle began with an initial denaturation (95°C, 30s); followed by 30 cycles of denaturation (95°C, 30s); annealing (55°C, 1 min); amplification (72°C, 1 min, 30s); a final extension (72°C, 5 min) and a hold at 4°C. The amplified product were subjected to purification (Qiaquick PCR purification kit, Qiagen) and the purified DNA were sent to sequencing agency (Apical Scientific Sdn. Bhd., Malaysia). The generated sequence were curated and similarity searches were carried out using BLASTN, available NCBI website; *https://blast.ncbi.nlm.nih.gov)/*. The sequences were also deposited to NCBI database and given with accession numbers: S1-1 for MT645770, S1-2 for MT645 771, S1-3 for MT645 772, S2-1 MT645 773, S2-3 MT645 774, S2-4 MT645 775, S2-5 MT645 776 and S2-6 MT645 7767. To construct a phylogenetic tree, an online tool NGphylogeny.fr was used which is available at *https://ngphylogeny.fr/* (Lemoine *et al*., 2019). The 16S ribosomal DNA gene from all the major strains within Genus *Stenophomonas* were initially aligned using ClustalW, and constructed using Maximum likelihood (approximate likelihood ratio test of SH-like) using PhyML at 100 bootstrapping and 4 substitution rate. The generated tree was then viewed using interactive tree of life (iTOL v5).

RESULTS AND DISCUSSION

Morphological and biochemical characterisations

Based on morphological characterisation shown on **Table 1**, each isolate appeared yellowish that grew in well separated circular colonies in smooth texture, raised (convex) with entire margin. All strains appeared to be gram negative bacilli. Biochemical characterisation indicated that all of the 8 isolates were catalase positive and oxidase negative, consistent with other studies, for example by Hasan *et al.,* (2012). Despite of their abilities to grow on MacConkey agar, the lactose fermentation into lactate is however not observed as there is no change

in colouration. The colonies also were capable of hydrolysing gelatin, but not starch, consistent with other studies on *Stenotrophomonas maltophilia*. All of these observations are also described in Bergeys's manual; *S. maltophilia* is Gram negative rods, aerobic, catalase positive and oxidase negative (Palleroni, 2015).

Table 1. The result from biochemical tests carried out on all isolates from sponge.

Isolates	Catalase Test	Oxidase Test	MacConkey agar	Lactose Fermenting Bacteria Test	Starch Hydrolysis Test	Gelatin Hydrolysis Test
S1-1	+	-	+	-	-	-
S1-2	+	-	+	-	-	-
S1-5	+	-	+	-	-	-
S2-1	+	-	+	-	-	-
S2-3	+	-	+	-	-	-
S2-4	+	-	+	-	-	-
S2-5	+	-	+	-	-	-
S2-6	+	-	+	-	-	-

Note: '+' represents positive result and '-' is for negative result

Genotypic characterisation of isolates

For genotypic characterisation, 16S ribosomal DNA gene for each strain was amplified and sequenced. Similarity search BLASTN tool has shown that *S. maltophilia* strains have populated the blast hit list. Especially, strain S2-6 shows similarity with *S. maltophilia* at least at 99.87%. Other than *S. malthophilia*, strains *S. pavani* and *P. hibiscicola* also appeared among the top in the list. The majority of these strains, except for S2-6, showed high similarity with *P. hibiscicola* at least more than 99% (see **Table 2**). *P. hibiscicola* strain actually a misplaced strain which was regroup recently into *S. maltophilia* from Pseudomonas member. This strain despite of their plant origin has shown more similarity with *S. maltophilia* (Patil *et al.*, 2016). Thus, based on this recent nomenclature all other strains (S1-1, S1-2, S1-3, S2-1, S2-3, S2-4 and S2-5) should belong to *S. maltophilia*.

Table 2. BLASTN search similarity hit list for isolates from sponge

Strain	Strain with highest similarity	Total score	E value	% Identity
S1-1	*Pseudomonas hibiscicola*	1986	0.0	98.75
	Stenotrophomonas maltophilia	1975	0.0	98.49
S1-2	*Pseudomonas hibiscicola*	1895	0.0	99.9
	Stenotrophomonas maltophilia	1884	0.0	99.71
S1-5	*Pseudomonas hibiscicola*	1657	0.0	99.89
	Stenotrophomonas maltophilia	1652	0.0	99.78
S2-1	*Pseudomonas hibiscicola*	1624	0.0	99.22
	Stenotrophomonas maltophilia	1618	0.0	99.11
S2-3	*Pseudomonas hibiscicola*	1868	0.0	98.5
	Stenotrophomonas maltophilia	1857	0.0	98.31
S2-4	*Pseudomonas hibiscicola*	1293	0.0	97.16
	Stenotrophomonas maltophilia	1288	0.0	97.04
	Stenotrophomonas pavani	1288	0.0	97.04
S2-5	*Pseudomonas hibiscicola*	1766	0.0	99.09
	Stenotrophomonas maltophilia	1755	0.0	98.88
S2-6	*Stenotrophomonas maltophilia*	1456	0.0	99.87
	Stenotrophomonas pavani	1454	0.0	99.75

Phylogenetic tree

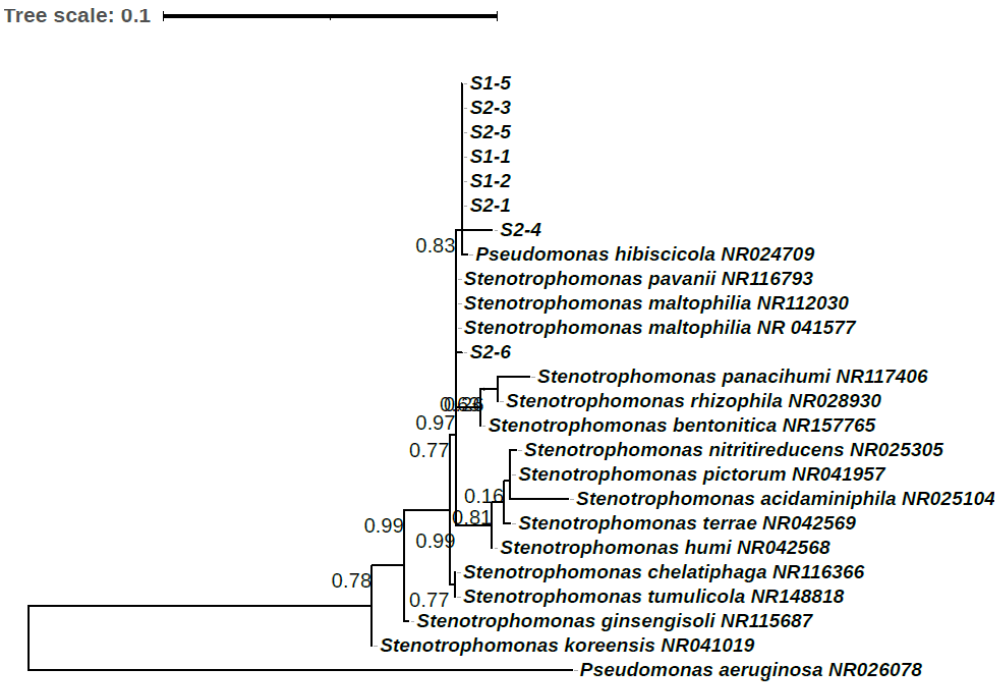

Fig. 1. Phylogenetic tree generated using 16 rRNA sequences from major species from genus Stenotrophomonas. The tree is constructed using Maximum likelihood at 100 bootstrapping and 4 substitution rate.

Based 16S ribosomal RNA sequences generated from each strain, phylogenetic tree was constructed as shown in **Fig. 1.** The 16S ribosomal DNA gene from all the major strains within Genus Stenotrophomonas were constructed using Maximum likelihood at 100 bootstrapping and 4 substitution rate. The sequence from *Pseudomonas aeruginosa* (NR 026078) was used as a distant outgroup. From the tree, strain *S. koreensis* becomes the earliest group to separate out, followed by those of *S. gingsengisoli* and a group containing both *S. tumulicola* and *S. chelatiphaga*. The remaining branch contains three major clusters comprising the rest of Stenophomonas species. Strain S2-6 and other strains (S1-1, S1-2, S1-3, S2-1, S2-3, S2-4 and S2-5) were clustered in a group that consists of *S. maltophilia, S. pavani* and *P. hibiscicola*. The presence of *S. pavani* in this cluster is consistent with NCBI similarity hit list, *S. pavani* closely follows *S. maltophilia* in for highest similarity. Meanwhile, the presents of *P. hibiscicola* in the cluster indicated that the strain is more similar to *S. maltophilia* rather than with Pseudomonas, justifying its reclassification (Patil *et al.*, 2016).

Proteolytic activities on salt added skim milk agar

Proteolytic activities from these strains were evaluated based on ability to grow on skim milk agar supplemented with extra NaCl at varying concentrations. Proteolytic activities on skim milk agar were recognised by the formation of clearing zones or halos. As shown on table 2, at 0% w/v salt, all strains were able to show active zone of proteolytic activities surrounding the growing colonies (**Fig. 2**). Similar proteolytic activities were observed at salt 1.0 % w/v, and at 2.0 % w/v. In general, slight decreases in proteolytic activities when salt in the growth medium was increased from 0 to 2.0 % w/v. At 3.0 % w/v NaCl, only strain S1-5 and S2-6 showed growth with zone of proteolysis, while other strains show growth with no clearing zone (see **Fig. 2**). Nevertheless, at 4.0 % w/v no clearing zone was observed for all strains. Ability of some strains to grow and degrade skim milk proteins at higher salt implies that the strains are potentially producing salt tolerant protease. Nevertheless, since all of these strains were able to tolerate at least 2.0 % w/v salt with proteolytic activities, they are considered to be slightly halophilic (Irshad *et al.*, 2014). Ability to tolerate high

salt could make this protease useful in certain industrial application; for instance, in high salt food fermentation, detergents formulation and leather processing (Gao *et al.*, 2019).

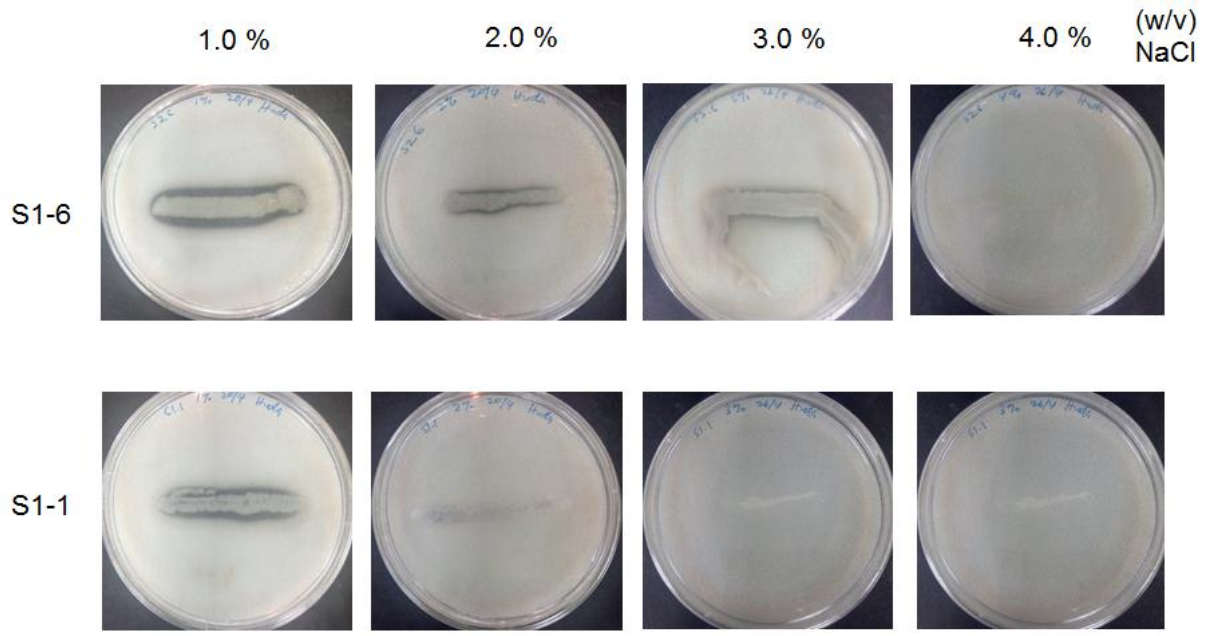

Fig. 2 Growth and proteolytic activities of isolates from sponge on skim milk agar supplemented with different level of salt. These examples shown are for isolate S1-6 and S1-1.

Table 3 Summary of proteolytic activities on skim milk agar with addition of 1.0, 2.0, 3.0 and 4.0 % (w/v) of NaCl for all isolates from sponge.

Isolates	Salt concentration, NaCl (w/v)				
	0%	1%	2%	3%	4%
S1-1	+++	++	+	-	-
S1-2	+++	++	++	-	-
S1-5	+++	++	++	+	-
S2-1	+++	++	++	-	-
S2-3	+++	++	++	-	-
S2-4	+++	++	++	-	-
S2-5	+++	++	+	-	-
S2-6	+++	++	++	++	-

Note: '+++' denotes width of the clearing zones (~ at or more than 2.0 mm), '++' denotes narrow clearing zones (~1.5 to 2.0 mm), '+' indicates small or a blot of clearing zones (less than 1.5 mm) and '-' implies no observable clearing zones.

The significance of *Stenotrophomonas maltophilia* strain

Results on morphological and biochemical tests were consistent with those from 16S rRNA sequencing. This indicated that strains S2-6 belongs to *Stenotrophomonas maltophilia* and other strains belong to *P. hibiscicola* which is still classified with *S. maltophilia*. *S. maltophilia* has undergone several taxonomic revisions. Previously, it was referred as *P. maltophilia* and later as *Xanthomonas maltophilia* (Palleroni, 2015). This bacterium can be found in wide variety of environments and it is often associated with plants rhizospheres (Ryan *et al.*, 2009). It has also been isolated from marine sponges (Manikandan *et al.*, 2014; Santos *et al.*, 2010). *S. maltophilia* has also become important nosocomial pathogen especially among those who are severely immunocompromised. In clinical setting, this bacterium has also emerged as multidrug-resistant bacteria (Zhao *et al.*, 2015). Nevertheless, the

member of the genus is so heterogeneous and the clinical derived strains are considered different from those isolated from the environment (Minkwitz & Berg, 2001). The versatility of non-clinical strains were reported to have great biotechnology applications especially in agriculture. Both the pathogenic virulence and biotechnological application of these strains are contributed by the suzbtilisin-like serine protease they produce. One of the application includes as a biological control agent against fungal plant pathogens (Messiha *et al*., 2007). In addition, ability to metabolize a wide range of organic compounds at a high metal tolerance makes it suitable to be employed in environmental bioremediation. *S. maltophilia* produces an extracellular protease targeting plant parasitic nematode (Ribitsch *et al*., 2012). Moreover, salt tolerant protease from *S. maltohilia* was shown to be stable in high salt or organic solvent, and therefore can be beneficial in many other industrial applications (Wang *et al*., 2016).

CONCLUSION

In this work, several strains from *Stenotrophomonas maltohilia* were isolated from marine sponge which was collected from Lang Tengah Island, Terengganu Malaysia. This is the first report on the isolation of this strain from the geographical region. This strains appeared to be slightly halophilic capable to produce salt tolerant proteases. These halophilic strains have potential to be used in many biotechnological applications, especially in agriculture and environmental bioremediation. The salt tolerant enzymes derived from these strains are also useful in various types of biotechnological applications.

ACKNOWLEDGMENTS

The authors wish to thank International Islamic University Malaysia for facilities provided in conducting this work.

REFFERENCES

dos Santos, H. R. M., Argolo, C. S., Argôlo-Filho, R. C., & Loguercio, L. L. (2019). A 16S rDNA PCR-based theoretical to actual delta approach on culturable mock communities revealed severe losses of diversity information. *BMC Microbiology, 19*(1), 74. doi: 10.1186/s12866-019-1446-2

Gao, X., Yin, Y., Yan, J., Zhang, J., Ma, H., & Zhou, C. (2019). Separation, biochemical characterization and salt-tolerant mechanisms of alkaline protease from *Aspergillus oryzae*. *Journal of the Science of Food and Agriculture, 99*(7), 3359-3366. doi: https://doi.org/10.1002/jsfa.9553

Hasan-Beikdashti, M., Forootanfar, H., Safiarian, M. S., Ameri, A., Ghahremani, M. H., Khoshayand, M. R., & Faramarzi, M. A. (2012). Optimization of culture conditions for production of lipase by a newly isolated bacterium Stenotrophomonas maltophilia. *Journal of the Taiwan Institute of Chemical Engineers, 43*(5), 670-677. doi: https://doi.org/10.1016/j.jtice.2012.03.005

Irshad, A., Ahmad, I., & Kim, S. B. (2014). Culturable diversity of halophilic bacteria in foreshore soils. *Brazilian Journal of Microbiology, 45*, 563-572.

Lemoine, F., Correia, D., Lefort, V., Doppelt-Azeroual, O., Mareuil, F., Cohen-Boulakia, S. , & Gascuel, O. (2019). NGPhylogeny.fr: new generation phylogenetic services for non-specialists. *Nucleic Acids Research, 47*(W1), W260-W265. doi: https://doi.org/10.1093/nar/gkz303

Manikandan, S., Ganesapandian, S., Sangeetha, N., & Kumaraguru, A. K. (2014). Antimicrobial activity of marine sponges associated some marine bacterial metabolites against multi drug resistance human pathogens. *Research Journal of Microbiology* 9, 25-33.

McCauley, E. P., Piña, I. C., Thompson, A. D., Bashir, K., Weinberg, M., Kurz, S. L., & Crews, P. (2020). Highlights of marine natural products having parallel scaffolds found from marine-derived bacteria, sponges, and tunicates. *The Journal of Antibiotics, 73*(8), 504-525. doi: 10.1038/s41429-020-0330-5

Messiha, N. A. S., van Diepeningen, A. D., Farag, N. S., Abdallah, S. A., Janse, J. D., & van Bruggen, A. H. C. (2007). *Stenotrophomonas maltophilia*: a new potential biocontrol agent of *Ralstonia solanacearum*, causal agent of potato brown rot. *European Journal of Plant Pathology, 118*(3), 211-225. doi: 10.1007/s10658-007-9136-6

Minkwitz, A., & Berg, G. (2001). Comparison of antifungal activities and 16S ribosomal DNA sequences of clinical and environmental isolates of *Stenotrophomonas maltophilia*. *Journal of Clinical Microbiology, 39*(1), 139-145. doi: 10.1128/jcm.39.1.139-145.2001

Palleroni, N. J. (2015). Stenotrophomonas. In M. E. Trujillo, S. Dedysh, P. DeVos, B. Hedlund, P. Kämpfer, R. F. A. & W. B. Whitman (Eds.), *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1-20): John Wiley & Sons-Bergey's Manual Trust.

Patil, P. P., Midha, S., Kumar, S., & Patil, P. B. (2016). Genome sequence of type strains of genus Stenotrophomonas. *Frontiers in Microbiology, 7*(309). doi: 10.3389/fmicb.2016.00309

Razzaq, A., Shamsi, S., Ali, A., Ali, Q., Sajjad, M., Malik, A., & Ashraf, M. (2019). Microbial Proteases Applications. *Frontiers in Bioengineering and Biotechnology, 7*(110). doi: 10.3389/fbioe.2019.00110

Ribitsch, D., Heumann, S., Karl, W., Gerlach, J., Leber, R., Birner-Gruenberger, R., . . . Schwab, H. (2012). Extracellular serine proteases from S*tenotrophomonas maltophilia*: Screening, isolation and heterologous expression in *E. coli*. *Journal of Biotechnology, 157*(1), 140-147. doi: https://doi.org/10.1016/j.jbiotec.2011.09.025

Ryan, R. P., Monchy, S., Cardinale, M., Taghavi, S., Crossman, L., Avison, M. B., . . . Dow, J. M. (2009). The versatility and adaptation of bacteria from the genus Stenotrophomonas. *Nature Reviews Microbiology, 7*(7), 514-525. doi: 10.1038/nrmicro2163

Samad, N. S. A., Amid, A., Jimat, D., & Shukor, N. A. A. (2017). Isolation and identification of halophilic bacteria producing halotolerant protease. *Science Heritage Journal, 1*(1), 7-9. doi: 10.26480/gws.01.2017.07.09

Santos, O. C. S., Pontes, P. V. M. L., Santos, J. F. M., Muricy, G., Giambiagi-deMarval, M., & Laport, M. S. (2010). Isolation, characterization and phylogeny of sponge-associated bacteria with antimicrobial activities from Brazil. *Research in Microbiology, 161*(7), 604-612. doi: https://doi.org/10.1016/j.resmic.2010.05.013

Singh, S, & Bajaj, B.K. (2017). Potential application spectrum of microbial proteases for clean and green industrial production. *Energy, Ecology and Environment, 2*(6), 370-386. doi: 10.1007/s40974-017-0076-5

Sivanandhini, T., Subbaiya, R., Gopinath, M., Mahavinod Angrasan, J. K. V., Kabilan, T., & Masilamani Selvam, M. (2015). An investigation on morphological characterization of actinomycetes isolated from marine sediments. *Research Journal of Pharmaceutical, Biological & Chemical Sciences, 6*(2), 1234-1243.

Vivantis. Nucleic acid extraction kit handbook. https://www.vivantechnologies.com/images/Resources/roomtempmanual/gf%201%20bacterial%20dna%20extraction%20kit.pdf

Wang, Q., Ji, F., Wang, J., Jiang, B., Li, L., An, L., . . . Bao, Y. (2016). Characterization of a salt-activated protease with temperature-dependent secretion in *Stenotrophomonas maltophilia* FF11 isolated from frozen Antarctic krill. *Journal of Indian Microbiolgy & Biotechnology, 43*(6), 829-840. doi: 10.1007/s10295-016-1749-3

Zhao, Y., Niu, W., Sun, Y., Hao, H., Yu, D., Xu, G., . . . Li, Y. (2015). Identification and characterization of a serious multidrug resistant *Stenotrophomonas maltophilia* strain in China. *BioMed Research International, 2015*, 580240. doi: 10.1155/2015/580240

Zhuang, X., Han, Z., Bai, Z., Zhuang, G., & Shim, H. (2010). Progress in decontamination by halophilic microorganisms in saline wastewater and soil. *Environmental Pollution, 158*(5), 1119-1126. doi: 10.1016/j.envpol.2010.01.007

How to cite this paper:
Muzaini, N.S., Jailani, N.M., and Tengku Abdul Hamid, T.H. (2021). Characterisation of *Stenotrophomonas maltophilia* Isolated from Marine Sponge Producing Salt Tolerant Proteases. Journal of Agrobiotechnology, *12*(1), 31-39