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Draft genomic DNA sequence of strain *Halomonas* sp. FS-N4 exhibiting high catalase activity

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ABSTRACT

Halomonas sp. FS-N4 is a bacterium, which can grow in the medium Marine Broth 2216 with 5 M initial hydrogen peroxide concentration, shows a strong oxidation resistance, and the crude enzyme activity can reach as high as 13.33 katal/mg. We reported the draft genome sequence of *H.* sp. FS-N4, showing that it contains 3434 protein-coding genes, including the genes putatively involved in the response to the oxidative stress, among which a phytochrome-like gene might be a key point to survive in the environment with high concentration of hydrogen peroxide and exhibit high catalase activity.

1. Introduction

Since genus *Halomonas* had been organized as a genus in 1980 (Vreeland et al., 1980), there was no report about any members whose catalase activity was above 1 katal/mg. Recently, we isolated a strain, FS-N4, which can grow in the medium Marine Broth 2216 (Difco; MB) with initial hydrogen peroxide concentration of 5 M, shows a strong oxidation resistance, and the cell-free extract enzyme catalase activity can reach 13.33 katal/mg. To attain deeper insight, a whole-genome sequence of the strain FS-N4 was established, the genome sequence may provide a basis to improve the growth of the strain FS-N4, and the probable industrial application.

The seawater was collected in the China East Sea, near the Zhoushan City, China. The seawater (5 mL) was added to 50 mL MB, and incubated at 28 °C for 2 days. The cultures were added to the fresh medium with 5 mM hydrogen peroxide by 10% volume. After cultured at 28 °C for 2 days, the cultures were added to the fresh medium with 10 mM hydrogen peroxide. By repeated selection with increasing amounts of hydrogen peroxide, the initial concentration reached 5 M. After spreading on plate without hydrogen peroxide for purifying, strain FS-N4 (=CGMCC 9352) was isolated.

The cells were collected and disrupted in the phosphate buffer (same volume of the culture broth) by ultrasonic wave, cell-free extracts were harvested by centrifugation. Catalase activity was measured spectrophotometrically by monitoring the decrease in absorbance at 240 nm caused by the disappearance of hydrogen peroxide (Beers and Sizer, 1952), using a spectrophotometer (DU 800; BECKMAN). The ε at 240 nm for hydrogen peroxide was assumed to be 43.6 $M^{-1} \cdot cm^{-1}$ (Hildebrandt and Roots, 1975). After cultured for 27 h, catalase activity of the strain FS-N4 reached the peak, 13.33 katal/mg (=79997.36 U/mg; the amount of enzyme that decomposed 1 μmol of hydrogen peroxide per minute was defined as 1 U of activity). Catalase activity in the cell-free extracts of the strain FS-N4 and other typical catalase producers were showed in Table 1. The specific activity of the catalase of the strain FS-N4 was more than 2.5-fold that of the catalase of *Rhizobium radiobacter* 2-1, which exhibits the highest activity shown in the references (Nakayama et al., 2008).

Genomic DNA sequencing of strain FS-N4 was performed using Solexa paired-end sequencing technology (HiSeq 2000 System, Illumina, Inc., USA) (Bentley et al., 2008) with a whole-genome shotgun (WGS) strategy, with a 500 bp-span paired-end library (546 Mb available

Table 1
Catalase activities in cell extracts of *Halomonas* sp. FS-N4 and other catalase producers.

Strain or species	Activity ($U \cdot mL^{-1}$)	Protein ($mg \cdot mL^{-1}$)	Specific activity ($U \cdot mg^{-1}$)
H. sp. FS-N4	75,523	0.9	79,997
Strain 2-1[a]	87,594	2.9	31,503
M. luteus[a]	36,986	3.4	11,365
P. fluorescens[a]	892	6.8	133
R. radiobacter (*A. tumefaciens*)[a]	129	2.6	50
E. coli[a]	117	3.5	36

[a] Data were taken from Nakayama et al., 2008. Cells were suspended in the buffer of 1/5 amount of the culture broth.

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t2.1 **Table 2**
t2.2 Genome features.

t2.3		*Halomonas* sp. FS-N4
t2.4	Size (bp)	3,797,897
t2.5	Contigs	20
t2.6	GC content (%)	51.87
t2.7	Coding (%)	88.93
t2.8	CDS	3434
t2.9	rRNA genes	6
t2.10	tRNA genes	60
t2.11	16S rDNA identity[a]	98.8

t2.12 [a] Compared to the type strain *Halomonas andesensis* LC6^T (Daniel et al., 2010).

reads). All these clean reads were assembled into 20 scaffolds with total 3,797,897 bp (coverage: 142.9×) using the Velvet 1.2.07 (Zerbino et al., 2009). The detail of FS-N4 genomic sequencing results was showed in Table 2. The results were extracted using Rapid Annotation using Subsystem Technology (RAST) (Aziz et al., 2008), and functions of the gene products were annotated by the same program.

This draft genome shotgun project has been deposited as a primary project at DDBJ BioProject (the accession number: PRJNA241396). The draft genome sequence of the strain FS-N4 was deposited in the GenBank database under the accession number JHQL00000000.

The GenBank accession number for the 16S rRNA gene sequence of strain FS-N4 is KM079655. Neighbor-joining phylogenetic tree based on the 16S rRNA gene of FS-N4 and related species was showed in Fig. 1. According to the tree, strain FS-N4 shared the highest sequence

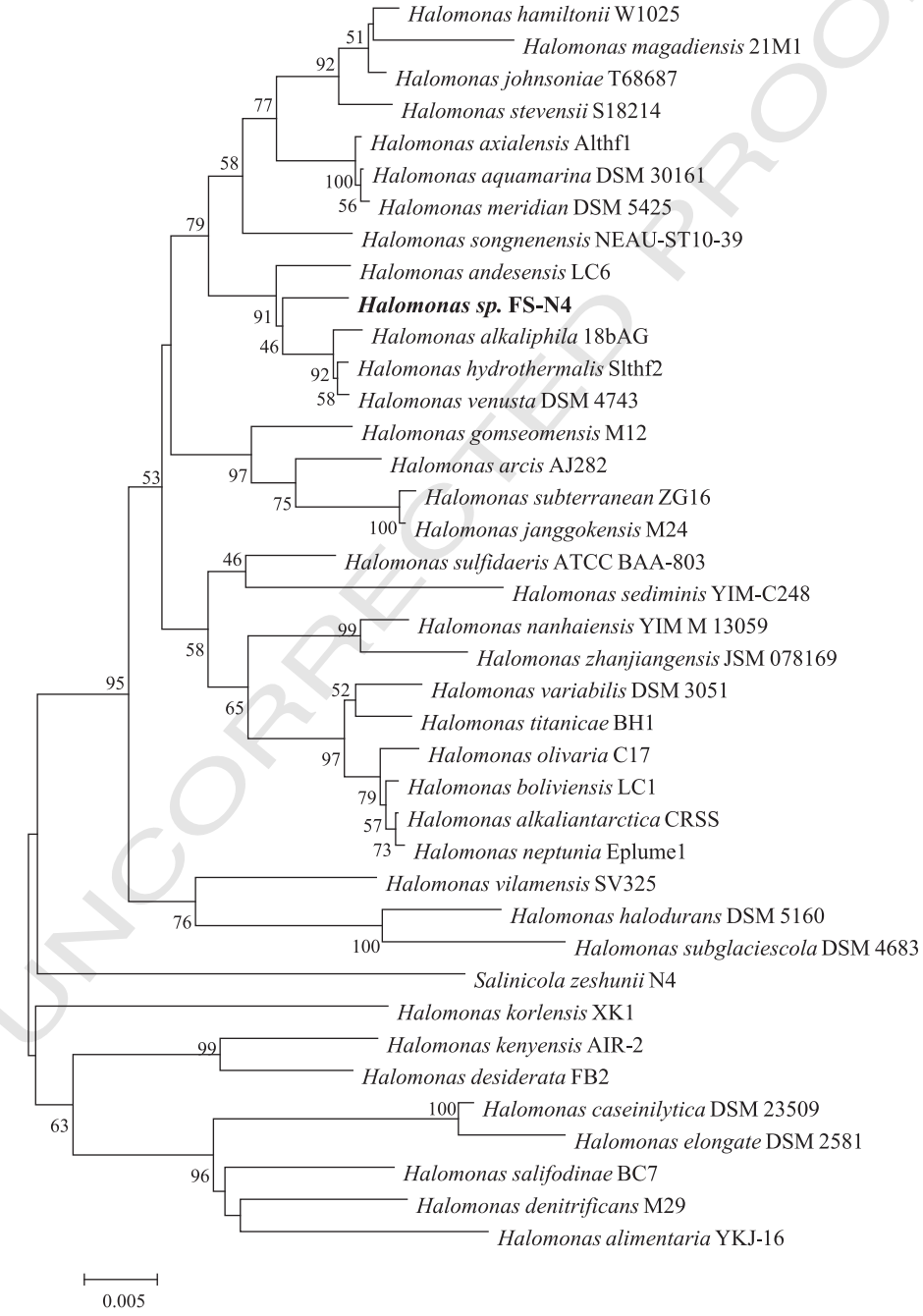

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain FS-N4 and other species of the genus *Halomonas*. Numbers at branch points are bootstrap values (percentages based on 1000 replications; only showed values above 45% are shown). Bar, 0.5% substitution per nucleotide position.

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similarity of 98.8% with *Halomonas andesensis* LC6^T, but did not cluster with it in the phylogenetic tree. It showed ambiguous taxonomic status of strain FS-N4, so we named it *H.* sp. FS-N4.

Bioinformatics analyses use Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1997) and RAST. The analyzed results were showed in Fig. S1 and also could be found on the web (http://rast.nmpdr.org/rast.cgi?page=JobDetails&job=140167), demonstrated that the *H.* sp. FS-N4 genome contained genes coding for 24 oxidative stress related proteins. Among them, the only superoxide dismutase shared the highest similarity with manganese superoxide dismutase (*sodA*), which was the enzyme that catalyzes the dismutation of superoxide (O_2^-) into oxygen and hydrogen peroxide (Keele et al., 1970), and two of catalases were most similar to hydroperoxidase I (catalase, *katG*), Manganese containing catalase, which was an important antioxidant enzyme that catalyzes decomposition and disproportionation of hydrogen peroxide, respectively (Chelikani et al., 2004), forming dioxygen and water. The other catalase was most similar to hydroperoxidase II (catalase, *katE*), which were haem-containing enzymes that use hydrogen peroxide as the electron acceptor to catalyze a number of oxidative reactions (Nelson et al., 1994).

Moreover, FS-N4 genome contained genes coding for proteins regulating the responses to hydrogen peroxide (H_2O_2) and superoxide (O_2^-), including alkyl hydroperoxide reductase subunits (*ahpC* and *ahpF*), glutaredoxin I (*grxA*), glutathione reductase (*gorA*), Fur repressor, Zinc uptake regulation protein ZUR, and peptide methionine sulfoxide reductase (*gsrA*).

Alkyl hydroperoxide reductase (Ahp), KatG and KatE were the most important proteins in the process of scavenging hydrogen peroxide in vivo (Seaver and Imlay, 2001). The thiol-based peroxidase Ahp consisted of two subunits, AhpC and AhpF, it transfered electrons from NADH to H_2O_2 and reduced H_2O_2 to water. Fur repressor and Zinc uptake regulation protein ZUR were both involved in the PerR regulon, which was known to be highly induced by oxidative stress caused by hydrogen peroxide or paraquat.

According to the annotation results of RAST, the genes related to the oxidative-stress-inducible activities were compared with those of *Hestiasula zhejiangensis*, the results showed that the related genes were almost the same, except a phytochrome-like gene. As the definite enhancement by phytochrome of the catalase level was demonstrated in mustard (Drumm and Schopfer, 1974) and the induction of the Cat3 expression is probably regulated by a very low fluence phytochrome response (Polidoros and Scandalios, 1997), the phytochrome-like gene might be an important gene for strain FS-N4 to survive in the high-hydrogen-peroxide environment and produce high-catalase-activity extract. It needed more works to reveal it.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.margen.2014.08.002.

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