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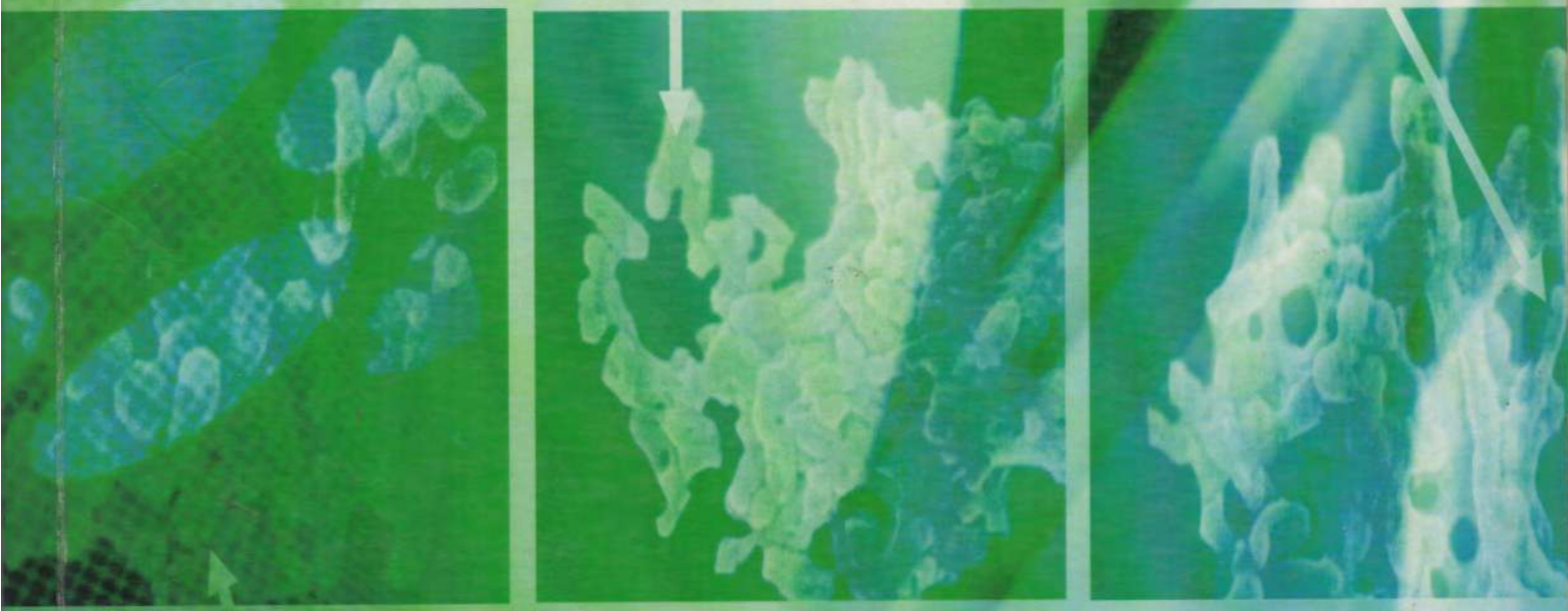
PROCEEDINGS

The 4th International Seminar of Indonesia Society for Microbiology and IUMS-ISM Outreach Program on Food Safety

INDONESIAN MICROBIAL RESOURCES: DIVERSITY AND GLOBAL IMPACT



22nd-24th June 2011, Udayana University Denpasar-Bali, Indonesia

EDITORS:

DR. YAN RAMONA • DR. YENNY CIAWI • DR. DR. DEWA MADE SUKRAMA, SPMK • DR. I NENGAH SUJAYA
DR. I NENGAH KENCANA PUTRA, MS. • PROF. IR I NYOMAN SEMADI ANTARA, MP., PHD.
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Dr. dr. Dewa Made Sukrama, Sp.MK.
Dr. I Nengah Sujaya
Dr. I Nengah Kencana Putra, MS.
Prof. Ir I Nyoman Semadi Antara, MP., Ph.D.
Nuki B Nugroho, MSi.
Prof. Ir I Wayan Redi Aryanta, M.AppSc., Ph.D.
Prof. Dr. dr. I Ketut Sukardika, Sp.MK.
Dr. I Putu Suparthana, SP., M.Agr.
Ir Ida Bagus Wayan Gunam, MP., Ph.D.
Dr. Ir. Koesnandar , MEng.
Diana Nurani, MSi.
Prof. Dr. Endang S Rahayu

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OPENING REMARK OF THE PRESIDENT OF THE INDONESIAN SOCIETY FOR MICROBIOLOGY

The Indonesian Society for Microbiology is proud to present the 4th International Seminar of Indonesian Society for Microbiology, in June 22-23, 2011 with the theme "Indonesian Microbial Resources: Diversity and Global Impact". I am also very proud to inform you that the International Union of Microbiological Societies (IUMS) in collaboration with the Indonesian Society of Microbiology(ISM), the International Commission on Food Mycology (ICFM) and the International Committee on Food Microbiology and Hygiene (ICFHM) is also organizing an IUMS-Outreach Program in Food Safety on 22-24 June 2011. I have the great pleasure in welcoming all of you to these events.

Over the past decades, there has been impressive progress in the area of microbiology. Microbiology and biotechnology have been playing important roles in the area of medical and veterinary, food and industry, agriculture and environment. This seminar will be an excellent event for exchange and sharing information, progress and experiences among the participants and more importantly to encourage collaboration and business interaction among participants. The workshop on food safety is also very important especially for countries like Asia because transfer of knowledge I believe will be very useful

The Indonesian Society for Microbiology, a member of the International Union of Microbiological Societies (IUMS), is a scientific organization in microbiology, with 1067 members spreads out in 20 regions in Indonesia. ISM has also published an English peer-reviewed journal namely *Microbiology Indonesia*, that has been given the accreditation at level "A" by Department of National Education, since 2001. The journal was published in *Bahasa* Indonesia from 1999-2006 but later on become English journal. I do expect that the journal will become a real international journal. In this regards, I would like to invite all of you to submit your manuscript and give contribution as editor or reviewer of the *Microbiology Indonesia* starting from 2012.

The society also conducts Annual Meeting which comprises of scientific meeting both national and international, and take place in the society branch all over Indonesia. Papers presented in the annual meeting are usually selected and published in the journal Microbiology Indonesia. In this regards, the society would like to thank the Department of Higher Education for granting a financial support in collecting appropriate and acceptable papers to be publish in the journal.

We still have to intensify and extend on the networking with international communities. Indonesia being privilege with a large diversity in microbes, we therefore invite our colleague from abroad to explore of the Indonesian microbes based on benefit sharing.

On behalf of the Indonesian Society for Microbiology, I would like to express our sincere appreciation and gratitude to the International Union of Microbiological Societies (IUMS), the International Commission on Food Mycology (ICFM) and the International Committee on Food Microbiology and Hygiene (ICFMH). We would also like to extend our appreciation to Udayana University, research institution, private sector and all supporting parties for the success of the seminar and workshop on food safety.

I would like to extend our appreciation to invited speaker and guest from foreign country. I believed your great contribution will be very fruitful and provide significant role in developing microbiology and its application in these region.

Special thanks are addressed to the organizing committee chaired by Prof Dewa Sukrama his excellent effort to conduct this successful seminar.

I do hope that this international seminar and workshop will strengthen our collaboration in exploring the role and application of microbiology.

With warm regards,

Dr. Koesnandar, M.Eng
President of the Indonesian Society for Microbiology

OPENING REMARK OF THE SECRETARY GENERAL OF THE IUMS

The INTERNATIONAL UNION OF MICROBIOLOGICAL SOCIETIES (IUMS) is one of the 26 Scientific Unions of the International Council of Science (ICSU). It was founded in 1927 as the International Society of Microbiology, and became the International Association of Microbiological Societies affiliated to the International Union of Biological Sciences (IUBS) as a Division in 1967. It acquired independence in 1980 and became a Union Member of ICSU in 1982. IUMS has 109 member societies and 16 associate members, altogether with more than 60.000 microbiologists.

The Divisions are responsible for the organization of their International Congresses (International Congress of Bacteriology and Applied Microbiology, International Congress of Mycology, and International Congress of Virology) and the committees, commissions and federations organize their own meetings. The next IUMS congresses will be in Sapporo Japan from 6 to 16 September 2011 (http://www.congre.co.jp/iums2011sapporo/index.html) .

The scientific activities of the Union are conducted by the three Divisions namely Bacteriology & Applied Microbiology (BAM), Mycology and Virology, by six specialist international committees, eight international commissions and two international federations (COMCOFs). Their major activities include the classification and nomenclature of bacteria, fungi and viruses, food microbiology, medical microbiology and diagnostics, culture collections, education, and biological standardization.

In support of its mission to enhance the scientific background and professional effectiveness of basic and applied microbiologists, the IUMS is embarking on a program of educational outreach to developing countries and their microbiologists. The Union envisions an IUMS series of courses that will be offered to groups of microbiologists that may include graduate students, postdoctoral fellows, and practicing professionals from developing countries within a given geographic region. These will be offered periodically in various regions and on different topics of interest and importance.

The first IUMS Regional Course was offered in Singapore during June 15-17, 2010 on Antimicrobial Resistance in Bacteria, Fungi and Viruses and was great success. The IUMS is very happy that the second IUMS outreach programme could be organized together with the Indonesian Society of Microbiology on the topic of Food safety. The International Commission on Food Mycology (Mycology Division) and International Committee on Food Microbiology and Hygiene (Bacteriology and Applied Microbiology Division) kindly sponsored the workshop by providing the experts on food microbiology.

On behalf of the IUMS Executive Board I like to thank the ISM and the local organizers to make this meeting possible and wish you all a very successful and productive workshop.

Robert A Samson - IUMS Secretary General.

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ORAL PRESENTATION: FOOD AND AGRICULTURE (OFA)

PRESENTATION: INDUSTRY AND ENVIRENMENT (PIE)

VINASSE BASED MEDIUM FOR EXOXYLANASE PRODUCTION

Kahar Muzakhar
Biology Dept., Faculty of Mathematics and Natural Sciences, the University of Jember
Jl. Kalimantan 37 Jember-Indonesia 68121, Email:kaharmzk@unej.ac.id

ABSTRACT

Vinasse is a liquid waste, produced during alcohol production was utilized for an isolate AA1 bacterium to produce exoxylanase. Without any supplement as nutrients added, this isolate capable utilized remaining sugar and nitrogen from vinasse. An extracellular enzyme was released which qualitatively detected as xylanase when spot-platted on agar oat spelt xylan medium. The hydrolyzation of xylan was indicated by clearance zone in medium, and further analysis by using TLC of hydrolyzates showed that only xylose was produced. Suggested, this enzyme is exoxylanase which attacked xylan exowise from reducing end. The enzyme stable at a range pH 4.5-8.5 and temperature below 55°C with optimum activity at pH 5.5 and temperature 50°C. The exoxylanase with 72.7 KDa of molecular weight estimated by SDS-PAGE was achieved after ammonium sulfate precipitation followed by 2 steps purification using a size exclusive chromatography Sephadex G100 and a weak anion exchanger DEAE Sepharose CL-6B. No significant results when AA1 grown at vinasse or using formulated medium containing 1% xylan, 0.5% pepton and 0.25% malt extract. Using the same purification procedure, the yield of exoxylanase recovery were 46% and 48% when AA1 cultivated at vinasse and formulated medium.

Keywords: vinasse, exoxylanase

BACKGROUND

A high pollution load with BOD values ranging from 30 to 60 g O2/l called vinasses were released from a alcohol distillery [1]. It was reported that about 13-15 times vinasse were produced much bigger than alcohol production itself so that these wastes to be potential as pollutant. It means, from 1 liter alcohol produced, up to 13-15 liters of vinasse were released [2]. Base on these problems, the management strategy of distillery wastewater is one of the most significant and challenging issues. Some of the existing methods for the disposal of vinasse are direct application such as can be spread on agricultural fields or used as organic fertilizer [3, 4], and methane production [5]. In the other side, vinasse caused the alkalinity of the soil is reduced so that crops may be destroyed [6], a manganese deficiency in the soil occurs [7] and can retarded of seed germination [8]. Another option, the concentration-incineration of vinasse, is the only system that can provide a satisfactory solution to the pollution problem but consumed much energy and expensive [9]. Vinasse also contains nitrogenous compounds as betain [4], cellulose and hemicellulose [10] which may possible for the microorganism to utilize it as carbon and nitrogen source. In this work, we investigated the utilization of organic-rich vinasse as substrate for microorganism to produce exoxylanase. The properties of the enzyme had also been characterized.

MATERIALS AND METHOD

Sampling and screening of isolates

Ten grams of soil from sugarcane farm Jatiroto Indonesia which treated by vinasse were sampled and directly suspended to 0.9% sterilized NaCl 100 ml in Erlenmeyer flask. For screening, sample was diluted 10^{-2} to 10^{-8} and direct plated to NA medium followed by incubation for 48 hours at 30°C. This step was repeated until getting the single colony. The screened microorganisms were stored at 4°C and further tested for xylanase activity.

Detection of xylanase activity

The xylanase activity every single colony was analyzed qualitatively on solid medium. For this purpose, the method Chasana *et. al.* [11] with few modifications was employed. Screening of xylanase producers was done by inoculation 1µl of pre-culture on xylan agar (0.05% K2HPO4, 0.025% MgSO4, 0.25% oat spelt xylan, 0.5% pepton, 0.25% malt extract and 1.5% agar) and incubated at 30°C for 48 hours. For the detection, plates were flooded with Gram's iodine (2.0 g KI and 1.0 g iodine in 300 ml distilled water) for 3 to 5 minutes. The clearance zone of around colony indicated xylanase activity. Activity index

was calculated by percentage of clearance zone diameter divided by colony diameter. To quantify the hydrolysis product, the same medium above containing 1% xylan in 100 ml volume and without agar was used. The incubation was done aerobically at 30°C, 120 rpm and hydrolysis activity was determined by measuring of reducing sugar released using the method of Nelson [12] as modified by Somogy [13] after 48 hours incubation.

Analysis of adaptability and capability of isolates to grow and produce xylanase on vinasse medium

The reason of these analyses are try to select among isolates which can grow and produce xylanase in vinasse based medium without any dilution or nutrients added. The experiments were done in 100 ml of vinasse medium and analyzed after 48 hours incubation at 30°C and 120 rpm. The growth or the density of the cell was estimated by spectrophotometer at 600nm. Xylanase production was analyzed based of crude xylanase activity by measuring reducing sugar released as described above after 48 hours hydrolization in 1% xylan substrate on 20mM phosphate buffer pH 6. The best isolate was used for a source xylanase production and purification. For this investigation, we utilized vinasse as medium, no other carbon and nitrogen sources added.

Xylanase purification

For purification, 900 ml of crude xylanase obtained from both cultivation using formulated and vinasse medium were used. The purifications were done separately by using same procedure and also using same material purification. Detail of this experiment was explained under result and discussion.

Molecular weight analysis

The molecular weight of purified xylanase was estimated by comparison of its migration rates with protein standards (in KDa); Phosphorylase (97.4), Albumin (66.3), Aldolase (42.4), Carbonic anhydrase (30) and Trypsin inhibitor (20.1) on Sodium Deodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Effects of pH and temperature on the enzyme activity and stability

The stability of purified xylanase on pH and temperature was examined after incubation for 30 minutes at a range pH 3-9 and temperature values 25-70°C. The buffers used were Na-acetate for pH 3-5; Na-citrate pH 6-6.5; and Tris/HCl, pH 7-9. All concentrations of buffer used were 50 mM. The effects of pH and temperature on the optimum activity of enzyme were also measured in series pH and temperature range as mentioned above. All assayed in 1% xylan substrate.

Xylanase hydrolysis product analysis

The enzymatic hydrolysis product every 30 minutes during 1% xylan hydrolyzation by purified xylanase was sampled and analyzed by Thin Layer Chromatography (TLC) in silica gel plate (Merck, silica gel 60 F254). Butanolethanol-chloroform-amonia 25% (4:5:2:8) was employed as solvent system. Sulphuric acid with 1% vanilin was used for detection.

RESULTS AND DISCUSSION

From soil sample, sixteen isolates were successfully screened on nutrient agar and maintained on the same medium at 4°C for further analyses of xylanase activity. Among of them we found that only 3 isolates (AA1, AC2 and AD1) produced xylanase when 1µl of pre-culture grown on solid medium containing 0.25% oat spelt xylan, 0.05% K2HPO4, 0.025% MgSO4, 0.5% pepton, 0.25% malt extract and 1.5% agar and incubated at 30°C for 48 hours. They identified as bacteria and capable to hydrolyze xylan which were indicated by clearance zones after they grown on xylan medium followed by staining with Gram's iodine. The activity index of each isolate was summarized at the Table 1 as follow.

Table 1. Activity index and reducing sugar released during cultivated at solid and liquid medium containing oat-spelt xylan.

Isolate	Activity index	Reducing Sugar Released (mg/L)
AA1	6.8	598
AC2	3.6	323
AD1	4	282

Table 1 showed that among 3 selected isolates, AA1 gave highest activity index comparing with others. AA1 also readily hydrolyzed the xylan when this isolate grown in liquid medium containing 1% xylan. AA1 produced 598 mg/L reducing sugar higher than AC2 or AD1. By these results proved that xylanase activity secreted by AA1 higher than AC2 or AD1 when cultivated on both solid and liquid medium containing 1% xylan. Base on this evidence, we further selected and optimized the growth of AA1 on vinasse for xylanase production. Optimization was done in order to obtained the period of incubation time (hours) of xylanase produced in maximum which was reflected on xylanase activity. One liter of vinasse was inoculated by AA1 pre-culture, incubated aerobically at 120 rpm 30°C, followed by measuring the xylanase activity on 1% oat spelt xylan substrate every 12 hours by using the method as described above. For the xylanase production, this step was repeated and the crude xylanase was harvested when the optimum time of incubation obtained. To harvest crude xylanase, the filtration using 0.2 µm cellulose acetate filter paper was employed. Further, the filtrate was centrifugated at 8000 rpm for 5 minutes to remove the remaining cells. The supernatant containing crude xylanase was stored at 4°C and used for the source xylanase purification.

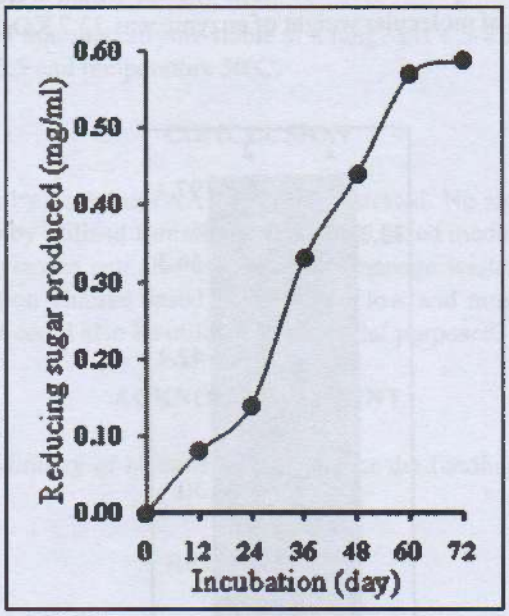


Figure 1. Xylanase activity of AA1 cultivated on Vinasse. Enzyme activity was measured on 1% oat spelt xylan substrate.

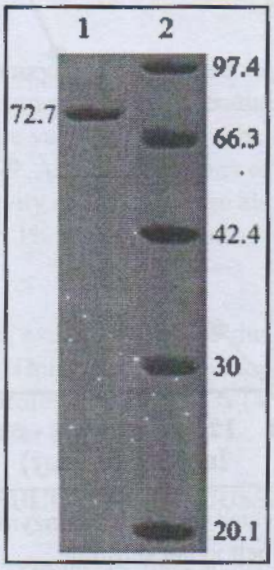
As shown at Figure 1, xylanase activity increased gradually within 0 to 24 hours incubation and activity increased exponentially when incubation between 24 up to 60 hours. However, no significant amount of reducing sugar produced between 60-72 hours incubation. We found that the optimum for producing xylanase after 60 hours incubation with the reducing sugar produced was about 55 mg/ml. For the comparison, the xylanase was also produced using formulated medium containing 1% xylan, 0.5% pepton and 0.25% malt extract. The cultivation incubated at 30°C for 48 hours. The crude xylanase from the both cultivations were harvested and then purified using steps as summarized at the Table 2.

Table 2. Comparison of xylanase production of AA1 from different medium.

Purification step	Total ABS-280		Total Activity (unit)		Specific Activity		Yield (%)		Fold	
	*	**	*	**	*	**	*	**	*	**
Ammonium sulfate Precipitation	234200	123,400	6,500	4,300	0.0	0.0	100	100	1	1
Sephadex G100	9820	7,240	4,300	3,657	0.4	0.5	66	85	16	14
DEAE Sepharose CL-6B.	124	84	3,150	1,980	25.4	23.6	48	46	915	676

* : Medium containing 1% xylan, 0.5% peptone and 0.25% malt extract
** : Vinasse based medium

From the two samples, firstly, the crude xylanases were concentrated by precipitation with 60% saturated of ammonium sulfate using centrifugation at 12000 rpm for 20 minutes. The pellet was then collected, dissolved with 5 ml buffer, and followed by overnight dialysis using Microkros Hollow Fiber Modules polysulfone 10KDa against 20 mM acetate buffer pH 5. The dialyzates was then loaded onto open column using Sephadex G100 and a weak anion exchanger DEAE Sepharose CL-6B. All steps of purification were carried out at 25°C and 20 mM acetate buffer pH 5. As shown from Table 2, the yields obtained of purified xylanases were not significant different when the cultures were cultivated in formulated medium (containing 1% xylan, 0.5% peptone and 0.25% malt extract) comparing with vinasse medium. Formulated and vinasse medium gave the yield 48 and 46 %. However, xylanase obtained from formulated medium had high puritiy comparing with xylanase from vinasse which were indicated by the results of their purification folds. Analysis of molecular weight of enzyme was 72.7 KDa as estimated by SDS-PAGE (Figure 2).



SDS-PAGE analysis of xylanase. Purified xylanase 72.7 KDa (lane 1) and Standards (lane 2)

To indentify the mode of action of this enzyme, the hydrolyzates which produced during xylan hydrolyzation was also examined by using TLC. We found that this enzyme could hydrolyze xylan from reducing end so that only monomer xylose will be produced (Figure 3).

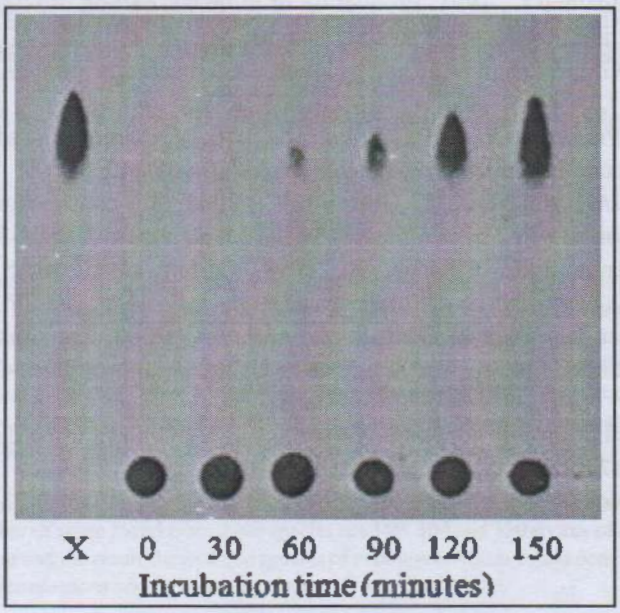


Figure 3. TLC analysis of hydrolyzates after xylan hydrolysis by purified xylanase in time series of incubation and xylose (X) was used as standard.

Further analysis showed that this enzyme stable at a range pH 4.5-8.5 and temperature below 55°C with optimum activity at pH 5.5 and temperature 50°C.

CONCLUSION

Production of xylanase by bacterium AA1 was demonstrated. No significant differences on yields when the enzyme productions by utilized formulated or vinasse based medium. This result can be adopted as a cheap way to produce xylanase and also as strategy to manage wastes. However, on this result the purity of xylanase by utilization vinasse based medium still low and must be improved. Further, it is expected that selected isolates could also be utilized in industrial purposed.

ACKNOWLEDGMENT

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