UJMR, Special Conference Edition, Vol. 9 No. 3. June, 2024, pp.13 - 21

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.2493.003

Received: 17th March, 2024 **Accepted:** 1st June, 2024

Bio-Prospecting Xylose-Utilizing, Exopolysaccharide (EPS)-Producing Bacteria and EPS Quantification through Submerged Fermentation using Xylose as the Major Carbon Source

[1]*Ukponobong Antia, [1]Nsikak Stephen, [1]Victoria Umoh, [1]Maria Bassey, [2]Itorobong Udo, and [3]Adebare J. Adeleke.

[1]Department of Microbiology, Akwa Ibom State University, Mkpat Enin, 524106, Nigeria
[2]Department of Microbiology, University of Uyo, Uyo, 520101, Nigeria
[3]Department of Microbiology, Modibbo Adama University, Yola, 640230, Nigeria
*Correspondence author: ukponobongantia@aksu.edu.ng.

Abstract

Many microorganisms are capable of producing Exo-polysaccharides (EPS) while utilizing simple sugars and hexoses. These EPS found applications in various fields, such as agricultural biotechnology, pharmaceuticals, textiles, and food industries. However, there is a lack of studies on EPS-elaborating bacteria that can utilize pentoses like xylose. Therefore, the utilization of alternative carbon sources for EPS production has become a focus of recent research. This study aimed to prospect bacteria that can utilize xylose for EPS production. Samples from agricultural soil, dump sites, saline soil, cement-contaminated soil, fresh cow milk, cow dung, and yogurt were serially diluted and cultured in a salt-based medium with xylose as the primary carbon source. Slimy and mucoid colonies were selected as potential EPS-producing isolates and identified morphologically and biochemically using the VITEK 2 Automated identification system. The quantification of EPS production by these isolates was conducted through submerged fermentation with xylose as the sole carbon source. The mean heterotrophic bacterial count of xylose-utilizing bacteria ranged from $2.1x10^6$ CFU to $3.5x10^8$ CFU per gram of the samples analyzed. The slimy and mucoid colonies were identified as members of the genera Staphylococcus, Enterobacter, Kocuria, Klebsiella, Enterococcus, Serratia, and Burkholderia. The quantities of EPS produced by the isolates ranged from 0.04 g/L to 2.0 g/L, with E. cloacae D1, E. cloacae D2, K. oxytoca D2, and K. oxytoca G1 elaborating the highest amount of EPS. Bacterial isolates capable of utilizing xylose for EPS production were obtained from various sources, showing potential for further optimization.

Key words: Bioprospecting, exopolysaccharides, lignocellulosic biomass, submerged fermentation, xylose.

INTRODUCTION

Exopolysaccharides (EPS) are long-chain polysaccharides secreted by microorganisms to survive in harsh environments (Netrusov, 2023). They are the major components of microbial biofilms, known to protect the producing microorganisms against adverse effects of the host immune system, pH, temperature, and antibiotics (Nguyen *et al.*, 2020). EPS may be soluble or insoluble polymers and are commonly observed to accumulate around the cells (Moscovici, 2015). Exopolysaccharides are high molecular weight polymers of dextran, gellan, xanthan, alginate, levan, cellulose, pullulan, curdlan, hyaluronic acid, and succinoglycan (Mishra and Jha, 2013). The monomers making up these polymers include D-glucose, D-mannose, L-rhamnose, L-fucose, D-galacturonic acid, D-glucuronic acid, D-mannuronic acid, N-Acetyl-D-glucosamine, N-Acetyl-D-galactosamine, and some non-carbohydrate constituents such as carboxyl, sulfate, phosphate, and pyruvate substituents (Fretias *et al.*, 2011). Many bacteria have been reported to produce EPS, including *Acetobacter, Rhizobium, Alcaligenes, Pseudomonas, Enterobacter, Xanthomonas, Klebsiella, Bacillus*, and a host of Lactic Acid Bacteria (Netrusov *et al.*, 2023).

Lactic acid bacteria have been described as very versatile producers of exopolysaccharides applicable in the production of milk and milk products such as cheese, yogurt, cultured cream, and milk-based desserts (Mende *et al.*, 2016). Due to their unique characteristics, microbial exopolysaccharides have found

applications in various fields such as agricultural biotechnology, pharmaceuticals, food industries, detergents, beverages, textiles, cancer treatments, culture media formulation, paint, paper, and petroleum industries (Suryawanshi *et al.*, 2022).

The production of EPS by microbes is believed by many researchers to be affected by the medium composition; likewise, carbon and nitrogen sources are important parameters in their biosynthesis (Netrusov *et al.*, 2023). Several sugars have been used in the production of EPS including sucrose, glucose, lactose, and maltose, as well as sugar alcohols such as mannitol, sorbitol, and other substrates such as whey, starch, sugar concentrates, methanol, and alkenes (Kumar *et al.*, 2007).

Among these substrates, glucose and sucrose have been extensively used for the production of exopolysaccharides (Wu *et al.*, 2021). The importance of effectively utilizing carbon sources is underscored by the fact that the carbon source accounts for 30% of the running cost of EPS production (Onilude *et al.*, 2012; Oner, 2013). In an attempt to reduce overhead costs due to the high cost of carbon sources, researchers are currently experimenting on EPS production using lignocellulosic wastes such as sugarcane straw (Abdeshahian *et al.*, 2020), corn cob (Jesus *et al.*,2014), rice hull (Wang *et al.*, 2014), and rice straw (Jazini *et al.*, 2017) for EPS production. The results obtained so far indicate the selective utilization of glucose over xylose in these biomasses, potentially leading to the wastage of the hemicellulose fraction of these biomasses. Bioprospecting for microorganisms that are better producers of biomolecules is an essential aspect of research that should be constantly considered by researchers (Antia *et al.*, 2018; George *et al.*, 2023); hence, this research was carried out to bio-prospect bacterial isolates that can utilize xylose as the main carbon source for EPS production.

METHODS AND MATERIALS

Isolates and EPS production on solid media

Various samples from different sources including samples from agricultural, saline, cement-contaminated, and dump site soils, as well as fresh cow milk, cow dung, and yogurt were aseptically obtained and transported to the microbiology laboratory in Akwa Ibom State University (AKSU).

Serial dilutions of the samples were carried out sequentially up to 10-7, and bacterial isolates were obtained using the pour plate technique on a salt-based medium with the following composition (g/l): 0.15 KH2PO4, 0.5g/L K2HP04, 0.1g/L NaCl, 0.2g/L MgSO4, yeast extract 0.8%, 18 agar-agar, and 5% xylose (Ventorino et al., 2019). After 96 hours of incubation at 30 ℃, bacterial isolates displaying ropy or slimy colonies were chosen for further analysis to determine EPS production through submerged fermentation using a method adapted from Ventorino *et al.* (2019).

Identification of bacterial isolates using VITEX 2 Compact system

The bacterial isolates underwent cultural and morphological examination before being analyzed using the VITEK 2 compact identification system (bioMerieux). Fresh cultures of the isolates, aged 18–24 hours, were prepared and inoculated into the VITEK system. The results obtained were collected and automatically compared against an extensive microbial profile database to generate a tentative identity for the organisms.

Synthesis of EPS by the selected bacterial isolates

The ability to form slimy colonies in a salt-based medium supplemented with 5% w/v xylose as a carbon source was used as the criterion for selecting isolates for further evaluation of their ability to produce EPS in submerged fermentation (Ventorino *et al.*, 2019). The bacterial cells were pre-cultured in a 10 mL nutrient broth supplemented with 2% w/v xylose and incubated for 18 – 24 hours at 30 ℃. The concentration of cells in the broth was standardized using a haemocytometer. One (1) mL of the nutrient culture containing about 1×0^6 cells/mL in the nutrient broth was transferred into a broth of the same composition as the salt-based medium described above, without the agar.

After 48 hours of incubation at 30 ℃, the EPS produced was quantified following the method outlined by Palomba *et al.* (2012). In summary,

the culture underwent centrifugation at 5000 rpm for 10 minutes, and the EPS-containing supernatant was treated with 2 volumes of chilled 98% v/v ethanol before being left to incubate overnight at 4 ℃. Subsequent centrifugation at 5000 rpm for 10 minutes allowed for the recovery of pellets, which were then reconstituted in 1 mL of distilled water, dried in an oven, and weighed to determine the amount of EPS. The EPS quantity was expressed as polymer dry mass per liter (PDM, mg/L) of the initial wet medium.

The weight of EPS produced was calculated as the weight of the filter paper with the precipitate minus the weight of the filter paper. The resulting value was then converted to mg/L following standard conversion procedures.

RESULTS AND DISCUSSION

Bacterial isolation, enumeration and screening for EPS producers in xylose containing medium

The mean heterotrophic plate count of bacterial isolates capable of utilizing xylose for EPS production, obtained from the analyzed samples, ranged from 2.1x10^6 CFU/g in the agricultural soil sample to 3.5x10^8 CFU/mL in the yogurt sample (Table 1). This count was measured after 96 hours of incubation, reflecting the organisms' requirement to synthesize enzymes for xylose utilization as the sole carbon source in the medium.

The colonies of the isolates were mostly smooth, medium, slimy, and mucoid, in accordance with Mu'Minah *et al.* (2015) who reported that EPS-producing bacteria are characterized by mucoid colonies. The isolates obtained from cow dung, agricultural soil, fresh cow milk, dump site, and yogurt were found to have slimier colonies compared to isolates from other samples, and hence they were purified, identified, and used for further analysis.

Table 1: The Mean Heterotrophic Count of Xylose Utilizing Bacterial Isolates Obtained from various samples

Sample	Sample ID	Mean CFU
Normal soil	A	2.1 x 10^6CFU/g
Cow milk	B	4.5 x 10^6 CFU/mL
Saline soil	C	2.6× 10^6 CFU/g
Cow dung	D	2.3 × 10^7 CFU/g
Dumpsite soil	E	1.4 x 10^7 CFU/g
Cement contaminated soil	F	1.8 x 10^7 CFU/g
Yoghurt	G	3.5 x 10^8 CFU/mL

Identification of the screened bacterial isolates using VITEK 2.0 Compact System.

Twelve (12) of the isolates showing thick slimy colonies were characterized morphologically, and selected for identification based on VITEK 2.0 Compact identification system. Table 2 shows the percentage probability of similarity of the obtained isolates with those in the VITEX database. The organisms belonged to the genera: *Staphylococcus, Enterobacter, Kocuria, Klebsiella, Enterococcus, Serratia* and *Burkholderia*. Seven (7) out of the twelve isolates were members of the gram positive group while the remaining 5 were gram negative isolates. Torres et al. (2012) and other researchers have reported on EPS production by *Enterobacter* and *Klebsiella*, respectively. However, only few reports on EPS production using a pentose sugar as the carbon source have been made. The abundance of lignocellulosic hemicelluloses with their enormous amount of pentoses makes it very important to begin to pay attention to this biomass fraction to avoid its continuous wastage (Antia *et al.*, 2023). The VITEX 2 system by Bioérieux is relatively a new system for rapid bacteria identification and previous studies confirmed that this system gives rapid and reliable identification results with pure bacterial cultures within a short period (Ling *et al.*, 2003). Details of the Biochemical reactions as obtained from the VITEX analyses are presented in Table 3 and 4.

Table 2: Microscopic, Cultural Characteristics and Identification of the Isolates Based on VITEK 2.0 Similarity Index/Probability of Accurate Identification

Isolates	Gram reaction	Colonial morphology	Similarity Index/ Probability of Accurate Identification	Organism
Isolate A1	Gram positive cocci	Slimy, even, mucoid, transparent, Circular, opaque	86%	*Staphylococcus heamolyticus*
Isolate A2	Gram positive cocci	Slimy, even, mucoid, transparent, creamy, circular,	94%	*Staphylococcus warneri*
Isolate B1	Gram negative cocci	Mucoid, circular, even, creamy, slimy,	94%	*Enterobacter clocae complex*
Isolate C1	Gram positive	Mucoid, creamy white, shiny, even, circular	96%	*Kocuria kristinae*
Isolate C2	Gram negative	Mucoid, creamy white, shiny, even, circular	94%	*Enterobacter clocae* complex
Isolate D1	Gram negative	Mucoid, creamy, circular, opaque, entire,	94%	*Enterobacter clocae complex*
Isolate D2	Gram positive	Slimy, even, mucoid, transparent, creamy, circular	86%	*Staphylococcus heamolyticus*
Isolate D3	Gram negative	Mucoid, creamy white, circular, medium, entire	99%	*Klebsiella oxytoca*
Isolate E1	Gram positive	Mucoid, creamy white, circular, medium, entire	90%	*Enterococcus casseliflavus*
Isolate F1	Gram negative	Mucoid, creamy white, shiny, even, circular	95%	*Serratia marcescens*
Isolate G1	Gram negative	Mucoid, creamy white, circular, medium, entire	99%	*Klebsiella oxytoca*
Isolate G2	Gram negative	Mucoid, creamy, slimy, medium, circular	95%	*Burkholderia cepacia*

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

Table 3: VITEK 2 Testing for Gram positive organisms (*Staphylococcus heamolyticus,Staphylococcus warneri,Kocuria kristinae,Enterococcus casseliflavus)*

ID	A1	A2	C1	E1
AMY	-	-	-	+
APPA	-	-	-	-
LeuA	+	-	-	+
AlaA	-	-	-	-
dRIB	+	+	+	+
NOVO	-	-	-	-
dRAF	-	-	-	+
OPTO	+	+	-	+
PIPLC	-	-	-	-
CDEX	-	-	-	+
ProA	-	-	+	-
TyrA	-	-	-	+
ILATK	+	+	-	-
NC6.5	+	+	-	-
O129R	+	+	-	-
dXYL	-	-	-	-
AspA	-	-	-	-
BGURr	-	-	-	-
dSOR	+	-	-	+
LAC	-	-	-	+
dMAN	-	+	-	+
SAL	-	+	-	+
ADH1	+	-	+	+
BGAR	-	-	-	+
AGAL	-	+	-	+
URE	-	-	-	-
NAG	+	+	-	+
dMNE	-	+	-	+
SAC	+	+	-	+
BGAL	-	-	-	+
AMAN	-	-	-	-
PyrA	+	+	+	+
POLYB	+	-	-	-
dMAL	+	+	-	+
MBdG	-	+	-	+
dTRE	+	+	+	+
AGLU	+	+	-	+
PHOS	-	-	-	-
BGUR	-	-	-	-
dGAL	-	-	-	+
BAC	+	+	-	-
PUL	-	-	-	+
ADH2s	-	-	-	-
Probable Isolate	*Staphylococcus heamolyticus*	*Staphylococcus warneri*	*Kocuria kristinae*	*Enterococcus casseliflavus*

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

Table 4: VITEK 2 Testing for gram negative organisms (*Enterobacter cloacae, Burkholderia cepacia, Serratia marcescens, Klebsiella oxytoca)*

ID	B1	D3	F1	G2
APPA	-	-	+	-
ADO	-	-	-	+
PyrA	-	-	-	-
IarI	+	+	+	+
DceI	+	+	-	-
BGAL	+	+	-	-
H2S	-	-	-	-
BNAG	+	-	+	-
AGLTP	-	-	-	-
Dglu	+	+	-	+
GGT	+	-	+	-
OFF	+	+	-	-
BGLU	-	+	+	-
AGLU	+	+	-	-
dMAL	+	+	-	-
dMAN	+	+	-	+
dMNE	+	+	-	-
BXYL	-	-	-	-
BAlap	+	-	+	+
ProA	-	-	-	-
LIP	+	+	-	-
PLE	+	-	+	+
TyrA	-	+	-	-
PRE	+	-	-	-
dSOR	+	+	-	-
SAC	-	+	-	-
dTAG	+	+	-	-
dTRE	+	-	+	+
MMT	+	+	-	-
5KG	-	+	-	-
ILATk	+	-	+	+
AGLU	-	-	+	-
SUCT	+	-	+	+
NAGA	+	-	-	-
AGAL	+	+	-	-
PHOS	-	+	-	-
GlyA	+	-	-	-
ODC	+	-	-	-
LDC	-	+	-	-
IHISa	-	-	+	+
CMT	-	-	-	-
BGUR	-	-	-	-
0129R	+	-	-	-
Probable Isolate	*Enterobacter cloacae*	*Klebsiella oxytoca*	*Serratia marcescens*	*Burkholderia cepacia*

Quantification of EPS Production by the Identified Bacterial Isolates.

Previous studies showed that the quantities and composition of microbial EPS depends on species of organism and the cultural conditions used (Donot *et al.*, 2012). The utilization of alternative carbon sources for microbial production of exopolysaccharide (EPS) has gained significant attention in recent years. Though several studies had shown that many microorganisms are good producers of EPS, not all EPS producers are capable of utilizing xylose for EPS production (Zhao *et al.*, 2020).

In our study, the amount of EPS produced varied among the isolates obtained. When utilizing xylose as the sole carbon source, the isolates were capable of producing EPS in a range from 0.04 g/L to 2.0 g/L (Table 5). These quantities align with those reported for bacterial isolates cultured in the presence of glucose

(Vijayabaskar *et al.*, 2011; AMAO *et al.*, 2019). *Enterobacter cloacae* isolated from cow milk and cow dung, along with *klebsiella oxytoca* from cow dung and yogurt, exhibited the highest EPS production at 2.0 g/L, followed by *Burkholderia cepacia* from yogurt and *Staphylococcus heamolyticus* from cow dung producing 1.8 g/L of EPS.

However, some authors have reported higher EPS values under different culture conditions; the variations in EPS production could be attributed to the specific bacterial isolates and growth environments. In our research, we focused on EPS production by utilizing xylose as the primary carbon source among the isolates. Cultivating xylose-utilizing bacteria in the presence of other readily accessible sugars might enhance the EPS yield of these isolates (Antia *et al.*, 2019).

Table 5: Weight of Extracted EPS produced by the obtained isolates in a submerged fermentation using xylose as the sole carbon source.

Isolates ID	Organism	Mean Weight of filter paper with EPS	Weight of EPS (g/50mL)	EPS production (g/L)
A1	*Staphylococcus heamolyticus*	0.75g	0.08g	1.6g/L
A2	*Staphylococcus warneri*	0.71g	0.04g	0.8g/L
B1	*Enterobacter cloacae*	0.70g	0.03g	0.6g/L
B2	*Enterobacter cloacae*	0.77g	0.10g	2.0g/L
C1	*Kocuria kristinae*	0.75g	0.08g	1.6g/L
C2	*Enterobacter cloacae*	0.69g	0.02g	0.04g/L
D1	*Enterobacter cloacae*	0.77g	0.10g	2.0g/L
D2	*Staphylococcus heamolyticus*	0.73g	0.09g	1.8g/L
D3	*Klebsiella oxytoca*	0.77g	0.1g	2.0g/L
E1	*Enterococcus casseliflavus*	0.69g	0.02g	0.4g/L
F1	*Serratia marcescens*	0.73g	0.06g	1.2g/L
G1	*Klebsiella oxytoca*	0.77g	0.10g	2.0g/L
G2	*Burkholderia cepacia*	0.73g	0.09g	1.8g/L

CONCLUSION

This study presented compelling evidence of substantial EPS production by a variety of bacteria when exclusively utilizing xylose as the carbon source. *Enterobacter cloacae* and *Klebsiella oxytoca* stood out as top producers, yielding 2.0 g/L of EPS. The vitek 2.0 identification method proved to be more efficient in identifying the isolates compared to traditional biochemical techniques. By employing optimization techniques and exploring pentose sugars, there is potential to enhance EPS production when utilizing plant lignocellulosic biomass as the carbon source.

ACKNOWLEDGEMENT

The authors wish to thank the management of Akwa Ibom State University and the Laboratory staff of the Department of Microbiology of the same institution for their support towards the successful completion of this research.

REFERENCES

Abdeshahian, P., Ascencio, J. J., Philippini, R. R., Antunes, F. A. F., Dos Santos, J. C., and da Silva, S. S. (2020). Utilization of sugarcane straw for production of ß-glucan biopolymer by Lasiodiplodia theobromae CCT 3966 in batch fermentation process. Bioresource Technology, 314: 123716. **[Crossref]**

AMAO, J. A., Omojasola, P. F. and Barooah, M. (2019). Isolation and characterization of some exopolysaccharide producing bacteria from cassava peel heaps. Scientific African, 4: e00093. **[Crossref]**

Antia, U. E., Akan, O. D., Stephen, N. U., Eno-Ibanga, C. K., and Akpan, N. G. (2018). Isolation and Screening of Yeast Isolates Indigenous Palm Wine for Ethanol Production. Philippine Journal of Science, 147(3).

Anita, U. E., Stephen, N. U., Onilude, A. A., and Ibanga, I. A. (2019). Studies of the Nutritional, Environmental Effects and

Repressive Nature of Simple Sugars on the Production of endo-ß-mannanase by Aspergillus flavus PT7 on Solid State Fermentation. Journal of Advanced Biology, 21(4): 1-12. **[Crossref]**

Antia, U. E., Stephen, N. U., Onilude, A. A., Udo, I. O. M., & Amande, T. J. (2023).Bioconvertibility of mannan-containing polysaccharides to bioethanol: A comparative study of palm kernel cake and copra meal feedstocks. Biomass Conversion and Biorefinery, 13(6): 5175-5186. **[Crossref]**

Chowdhury, S. (2012). Heterotrophic bacteria in drinking water distribution system: are view. Environmental monitoring and assessment, 184: 6087-6137. **[Crossref]**

Donot, F., Fontana, A., Baccou, J. C., and Schorr-Galindo, S. (2012). Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. Carbohydrate Polymers, 87(2): 951-962. **[Crossref]**

Fretias, F., Alves, V. D., and Reis, M. A. (2011). Advances in bacterial exopolysaccharides:from production to biotechnological applications. Trends in biotechnology, 29(8): 388-398. **[Crossref]**

George, A. E., Antia, U. E., Adeleke, A. J., and Fatunla, O. K. (2023). Optimisation of Polyhydroxy Butyrate Production by Lysinibacillus fusiformis and Metabacillus indicus isolated from Spent Engine-oil Contaminated Soil. UMYU Journal of Microbiology Research (UJMR), 8(2): 30-39. **[Crossref]**

Jazini, M. H., Fereydouni, E., and Karimi, K. (2017). Microbial xanthan gum production from alkali pretreated rice straw. RSC advances, 7(6): 3507-3514. **[Crossref]**

Jesus, M., Araújo, P., Silva, T., Reis, E., Ruzene, D., Silva, D., and Padilha, F. (2014). Evaluation of production of xanthan gum utilizing the corn cob liquor as a carbon source in different strains of Xanthomonas campestres. In BMC Proceedings, 8(4): 1-2. **[Crossref]**

Kumar, A. S., Mody, K., and Jha, B. (2007). Bacterial exopolysaccharidesa perception. Journal of Basic Microbiology, 47: 103. **[Crossref]**

Ling, T.K, Liu, Z. K. and Cheng, A.F. (2003). Evaluation of the VITEK 2 system for rapid direct identification and susceptibility testing of gram negative bacilli from positive blood cultures. Journal of Clinical Microbiology, 41: 4705-4707. **[Crossref]**

Mende, S., Rohm, H., and Jaros, D. (2016). Influence of exopolysaccharides on the structure, texture, stability and sensory properties of yoghurt and related products. International Dairy Journal, 52: 57-71. **[Crossref]**

Mishra, A. and Jha, B. (2013). Microbial Exopolysacchrides. In: Rosenberg, E, DeLong, E. F., Thompson, F., Lory, S., Stackebrandt, E. (Eds.), The Prokaryotes: Applied Bacteriology and Biotechnology, 4th ed. Springer Berlin Heidelberg, pp. 179-192

Moscovici, M. (2015). Present and future medical applications of microbial exopolysaccharides. Frontiers in Microbiology, 6, 137616. **[Crossref]**

Mu'Minah, Baharuddin, Subair, H., and Fahruddin. (2015). Isolation and Screening Bacterial Exopolysaccharide (EPS) from Potato Rhizosphere in Highland and the Potential as a Producer Indole Acetic Acid (IAA). Procedia Food Science, 3: 74-81. **[Crossref]**

Nadzir, M. M., Nurhayati, R. W., Idris, F. N., and Nguyen, M. H. (2021). Biomedical Applications of Bacterial Exopolysaccharides: A Review. Polymers, 13(4). **[Crossref]**

Nguyen, T., Nguyen, T., Bui, C., Hong, T., Hoang, K., and Nguyen, T. (2020). Exopolysaccharide production by lactic acid bacteria: The manipulation of environmental stresses for industrial applications. AIMS Microbiology, 6(4): 451-469. **[Crossref]**

Netrusov, A.I., Liyaskina, E. V., Kurgaeva, I. V., Liyaskina, A. U., Yang, G., Revin, V. V. (2023) Exopolysaccharides Producing Bacteria: A Review. Microorganisms, 11(6): 1541. PMID: 37375041; PMCID: PMC10304161. **[Crossref]**

Oner, E. T. (2013). Microbial production of extracellular polysaccharides from biomass. In: Z. Fang, (Ed) Pretreatment techniques for biofuels and biorefineries. Springer, Berlin, pp 35-56. **[Crossref]**

Onilude, A. A., Fadahunsi, I. F., Antia, U. E., Garuba, E. O., & Ja'afaru, M. I. (2012). Characterization of crude alkaline ß-mannosidase produced by Bacillus sp. 3A isolated from degraded palm kernel cake. AU Journal of Technology, 15(3).

Palomba, S., Cavella, S., Torrieri, E., Piccolo, A., Mazzei, P., Blaiotta, G., Ventorino,

V., Pepe, O. (2012). Polyphasic screening, homopolysaccharide composition, and viscoelastic behavior of wheat sourdough from a Leuconostoc lactis and Lactobacillus curvatus exopolysaccharide-producing starter culture. Applied Environmental Microbiology, 78: 2737-47. **[Crossref]**

Ramírez-Castillo, M.L. and Uribelarrea, Jean-Louis. (2004). Improved process for exopolysaccharide production by Klebsiella pneumoniae sp. pneumoniae by a fed-batch strategy. Biotechnology letters, 26: 1301-6. **[Crossref]**

Suryawanshi, N., Naik, S. and Jujjawarapu, S. (2022). Exopolysaccharides and their Applications in Food Processing Industries. Food Science and Applied Biotechnology, 5: 22.**[Crossref]**

Torres, C. A., Antunes, S., Ricardo, A. R., Grandfils, C., Alves, V. D., Freitas, F., and Reis, M. A. (2012). Study of the interactive effect of temperature and pH on exopolysaccharide production by Enterobacter A47 using multivariate statistical analysis. Bioresource Technology, 119: 148-156. **[Crossref]**

Ventorino, V., Nicolaus, B., Di Donato P., Pagliano, G., Poli, A., Robertiello, A., Iavarone V. and Pepe. O. (2019). Bioprospecting of exopolysaccharide-producing bacteria from different natural ecosystems for biopolymer synthesis from vinasse. Chemical and Biological Technologies in Agriculture, 6: 1-9. **[Crossref]**

Vijayabaskar, P., Babinastarlin, S., Shankar, T., Sivakumar, T., and Anandapandian, K. T. K. (2011). Quantification and characterization of exopolysaccharides from Bacillus subtilis (MTCC 121). Advance Biological Resource, 5(2): 71-76.

Wang, D., Ju, X., Zhou, D., and Wei, G. (2014). Efficient production of pullulan using rice hull hydrolysate by adaptive laboratory evolution of Aureobasidium pullulans. Bioresource Technology, 164: 12-19. **[Crossref]**

Wu, J., Zhang, Y., Ye, L., and Wang, C. (2021). The anti-cancer effects and mechanisms of lactic acid bacteria exopolysaccharides in vitro: A review. Carbohydrate polymers, 253: 117308. **[Crossref]**

Zhao, Z., Xian, M., Liu, M., and Zhao, G. (2020). Biochemical routes for uptake and Conversion of xylose by microorganisms. Biotechnology for biofuels, 13: 1-12. **[Crossref]**