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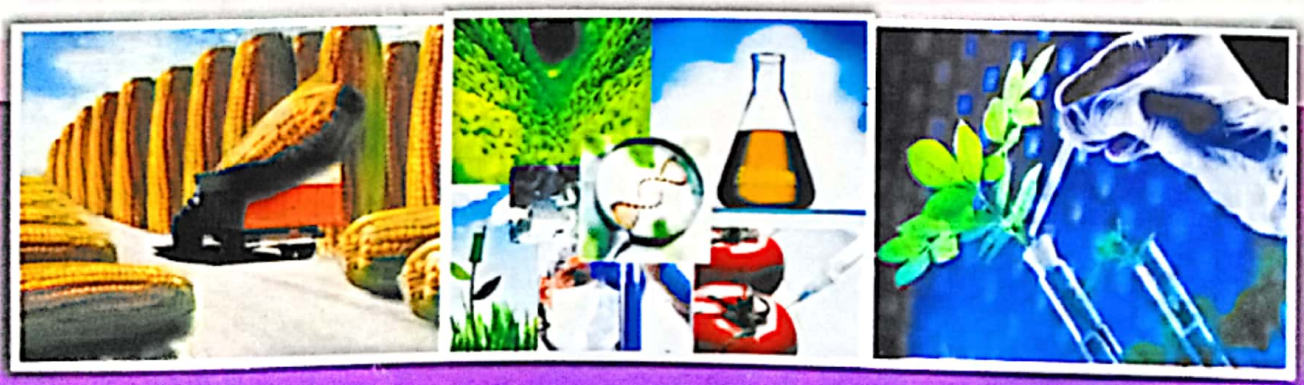
PROCEEDINGS

Theme:

BIOTECHNOLOGY AS A CHANGE AGENT FOR NATIONAL DEVELOPMENT



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POTENTIALS OF YEAST ISOLATES FOR BIOSURFACTANT PRODUCTION

[1]Oyeleke, S. B., [1]Goro, M. A., *[1]Oyewole, O. A., [2]Okeke, K. S., [3]Ayisa, T. T., [4]Mohammed, S. S. D.

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ABSTRACT

Twenty yeast isolates were screened for potential to produce biosurfactants using three different methods namely: collapse of oil drops, oil spreading or displacement and emulsification potential. Of the twenty yeast isolates screened, sixteen (80.0 %) showed collapse of oil drops. Eleven isolates (55 %) were able to displace the oil with *Saccharomyces cerevisiae* H02, *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15 having the highest diameter of displacement of 6.2 cm, 5.3 cm and 4.7 cm respectively. The isolates had emulsifying capacities that ranged from 6.67 % to 33.33 %. The results showed that *Saccharomyces cerevisiae* H02, *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15 were considered efficient potential candidates for biosurfactants production.

Keywords: yeast, biosurfactants, oil collapse, oil displacement, emulsification

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INTRODUCTION

Biosurfactants are amphiphilic compounds, which are secreted extracellularly by microorganisms (Luna *et al.,* 2013). They can be used in many industries as bio-emulsifiers in the food industries; as antimicrobial agent; in detergent formulation; in bioremediation processes as well as in enhanced crude oil recovery. Even though yeasts are known for producing biosurfactants in higher concentrations than bacteria, which is an advantage regarding these microorganisms, minimal studies have reported biosurfactants synthesized by yeasts (Hua *et al.,* 2003). Hence the need to explore yeasts in biosurfactant production, which was the basis for this study.

MATERIALS AND METHODS

Yeast strains

Twenty yeast isolates were used. The yeasts were obtained from Department of Microbiology, Federal University of Technology, Minna, Nigeria.

Drop collapse test: Oil drop collapse assay developed by Jain *et al.* (2011) was adopted.

Oil displacement test: The oil displacement test was done according to methods described by Rodrigues *et al.* (2006)

Emulsification activity: Emulsification ability of biosurfactants towards kerosene was studied using methods of Youssef *et al.* (2004).

Statistical analysis: Data was analyzed statistically using error mean square and

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Statistical analysis: Data was analyzed statistically using error mean square and

correlation analysis. Computer statistical package SPSS 9.0 was used.

RESULTS

Of the twenty yeast isolates screened, sixteen (80.0 %) showed positive result (that is, dispersed the oil around the liquid droplet). Of the sixteen positive isolates, only seven (43.75 %) were able to collapse the oil droplet (that is, caused it to spread out and appeared flat on the solid surface within one minute. The results of the oil drop collapse caused by yeast isolates are shown in Table 1.

Table 1: Extent of collapse of oil drops caused by yeast isolates

Coded yeast isolates	Reaction
Pichia strasburgensis H01*	+
Saccharomyces cerevisiae H02	++
Saccharomyces cerevisiae H03	++
Cryptococcus laurentii H04	+
Cryptococcus skinneri H05	+
Yarrowia lypolytica H06	++
Candida zelanoides H07	+
Candida apis H08	-
Candida boleticola H09	++
Candida spandovensis H10	+
Candida spandovensis H11	+
Candida tropicalis H12	+
Candida tropicalis H13	++
Rhodotorula glutinis H14	++
Rhodotorula bogoriensis H15	++
Rhodotorula bogoriensis H16	-
Candida acuta H17	-
Candida acuta H18	-
Candida acuta H19	+
Candida acuta H20	+
Control	-

Key: + = positive only; ++ = positive and collapsed; - : negative. Control: distilled water.
*Code assigned to yeasts in our laboratory.

Oil spreading or displacement potential of the yeast isolates

The twenty yeast isolates were also screened for their potential to displace and spread crude oil. Of this number, 11 isolates (55 %) were able to displace the oil. The diameter of oil spread ranged from 1.2 cm to 6.2 cm within 5 to 35 seconds (Table 2). It was observed that *Saccharomyces cerevisiae* H02, *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15 had the highest diameter of displacement of 6.2, 5.3 and 4.7 respectively within 10 seconds, meaning that they had strong ability to displace crude oil.

Emulsification potential of the yeast isolates

The emulsification capacities of the yeasts were tested and it was observed that the organisms had varying emulsifying capacities which ranged from 6.67 % to 33.33 % (Table 3). Twelve (60 %) of the

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Saccharomyces cerevisiae H02	++
Saccharomyces cerevisiae H03	++
Cryptococcus laurentii H04	+
Cryptococcus skinneri H05	+
Yarrowia lypolytica H06	++
Candida zelanoides H07	+
Candida apis H08	-
Candida boleticola H09	++
Candida spandovensis H10	+
Candida spandovensis H11	+
Candida tropicalis H12	+
Candida tropicalis H13	++
Rhodotorula glutinis H14	++
Rhodotorula bogoriensis H15	++
Rhodotorula bogoriensis H16	-
Candida acuta H17	-
Candida acuta H18	-
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Candida acuta H20	+
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isolates (*Pichia strasburgensis*, *Saccharomyces cerevisiae* H03, *Candida zelanoides* H07, *Candida apis* H08, *Candida spandovensis* H10, *Candida spandovensis* H11, *Candida tropicalis* H12, *Rhodotorula glutinis* H14, *Rhodotorula bogoriensis* H16, *Candida acuta* H17, *Candida acuta* H18, and *Candida acuta* H20) had no emulsifying capacity, 5 (25 %) of the isolates (*Yarrowia lipolytica* H06, *Candida acuta* H19, *Cryptococcus laurentii* H04, *Cryptococcus skinneri* H05, and *Candida tropicalis* H13) had very low emulsification indexes of 6.67 %, 8.11 %, 16.67 %, 16.67 % and 16.67 % respectively, 3 (15 %) isolates (*Saccharomyces cerevisiae* H02, *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15) had emulsification indexes of 33.33 %, 27.33 % and 24.20 % which is considered moderate. However none of the isolates showed percentage emulsification that was significantly high (50 % to 100 %).

Table 2: Diameter and time of displacement caused by yeast isolates

Coded yeast isolates	Diameter (cm)	Time (s)	Interpretation
Saccharomyces cerevisiae H02*	6.2	5	
Candida boleticola H09	5.3	7	
Rhodotorula bogoriensis H15	4.7	10	
Pichia strasburgensis H01	4.5	12	
Cryptococcus laurentii H04	3.8	35	
Saccharomyces cerevisiae H03	3.5	20	Positive
Candida acuta H19	3.2	34	
Yarrowia lypolytica H06	2.7	13	
Cryptococcus skinneri H05	2.3	9	
Candida zelanoides H07	1.2	7	
Candida tropicalis H13	1.2	16	
Candida apis H08	-	-	
Candida spandovensis H10	-	-	
Candida spandovensis H11	-	-	
Candida tropicalis H12	-	-	
Rhodotorula glutinis H14	-	-	Negative
Rhodotorula bogoriensis H16	-	-	
Candida acuta H17	-	-	
Candida acuta H18	-	-	
Candida acuta H20	-	-	
Control A	-	-	
Control B	-	-	

Key: Control A=Distilled water; Negative=diameter less than 4.5 and occurred after 30 seconds; Control B=Sterile broth; Positive=diameter from 4.5 and above and occurred within 30 seconds; cm=centimeter; s=seconds

Note: The results are arranged in ascending order of displacement with time.

*Code assigned to yeasts in our laboratory

Table 3: Emulsification capacity of yeast isolates

Coded yeast isolates	Emulsification index, E_{24} (%)
Pichia strasburgensis H01*	0 ± 0.00^{a}
Saccharomyces cerevisiae H02	33.33 ± 1.27^{c}
Saccharomyces cerevisiae H03	0 ± 0.00^{a}
Cryptococcus laurentii H04	16.67 ± 0.72^{b}
Cryptococcus skinneri H05	16.67 ± 0.93^{b}
Yarrowia lypolytica H06	6.67 ± 0.24^{a}
Candida zelanoides H07	0 ± 0.00^{a}
Candida apis H08	0 ± 0.00^{a}
Candida boleticola H09	27.33 ± 2.03^{bc}
Candida spandovensis H10	0 ± 0.00^{a}
Candida spandovensis H11	0 ± 0.00^{a}
Candida tropicalis H12	0 ± 0.00^{a}
Candida tropicalis H13	16.67 ± 1.14^{b}
Rhodotorula glutinis H14	0 ± 0.00^{a}
Rhodotorula bogoriensis H15	24.20 ± 0.87^{bc}
Rhodotorula bogoriensis H16	0 ± 0.00^{a}
Candida acuta H17	0 ± 0.00^{a}
Candida acuta H18	0 ± 0.00^{a}
Candida acuta H19	0 ± 0.00^{a}
Candida acuta H20	8.11 ± 0.45^{a}
Control	0 ± 0.00^{a}

Key: $\%E_{24}$=percentage emulsification index; control = distilled water; ± Se= standard error; the attached letters signifies significant difference ($p<0.05$). Numbers bearing same letters within rows are not significantly different.
*Code assigned to yeasts in our laboratory

DISCUSSION

Saravana and Vijayakumar (2012) screened 243 and 802 isolates from oil contaminated soil samples using oil drop collapse for biosurfactant production and only 10 (4.12 %) and 40 (4.99 %) respectively gave positive result. These differences in results may be attributed to the physiological characteristics of the organisms as well as their genetic and molecular composition. Several researchers (Satpute *et al.*, 2008; Thavasi, *et al.*, 2010; Jain *et al.*, 2011; Tarango *et al.*, 2012; Ibrahim *et al.*, 2013; Pereira *et al.*, 2013; Padmapriya *et al.*, 2013) have proved the oil drop collapse method to be highly sensitive, very effective, and reliable in identification of potent biosurfactant producers

In a study conducted by Chandran and Das (2011), biosurfactants produced by *Rhodotorula muciliginosa* and *Candida rugosa* could effectively emulsify (86 % and 78 %) diesel oil respectively. Other researchers have reported similar results (Chander *et al.*, 2012; Padmapriya *et al.*, 2013).

CONCLUSIONS

Saccharomyces cerevisiae H02, *Candida boleticola* H09 and *Rhodotorula bogoriensis* H15 were considered efficient potential candidates for biosurfactants production

REFERENCES

Chandran, P. and Das, N. (2011). Characterization of sophorolipid biosurfactants produced by yeast species grown on diesel oil. *International Journal of Science and Nature,* 2(1), 63-71.

Chander, C. R. S., Lohitnath, T., Kumar, D. J. M. and Kalaichelvan, P. T. (2012). Production and characterization of biosurfactant from *bacillus subtilis* MTCC441 and its evaluation to use as bioemulsifier for food biopreservative. *Advances in Applied Science Research,* 3(3), 1827-1831.

Hua, Z., Chen, J., Lun, S. and Wang, X. (2003). Influence of biosurfactants produced by
Candida antarctica on surface properties of microorganism and biodegradation of *n*-alkanes. *Water Resources,* 37, 4143-4150.

Ibrahim, M. L., Ijah, U. J. J., Manga, S. B., Bilbis, L. S. and Umar, S. (2013). Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. *Internnational Biodeterioration and Biodegradation,* 81, 28-34.

Jain, R. M., Mody, K., Mishra, A. and Jha , B. (2011). Physicochemical characterization of
biosurfactant and its potential to remove oil from soil and cotton cloth. *Carbohydrate Polymers,* 10, 10-16.

Luna, J. M., Rufinho, R. D., Sarubbo, L. A. and Campos-Takaki, G. M. (2013). Characterization, surface properties and biological activity of a biosurfactant produced from industrial waste by *Candida Sphearica* ucp0995 for application in the petroleum industry. *Colloids Surface Biointerfaces,* 102, 202-209.

Padmapriya, B., Suganthi, S. and Anishya, R.S. (2013). Screening, optimization and production of biosurfactants by *Candida* Species isolated from oil polluted soils. *American-Eurasian Journal of Agriculture and Environmental Science,* 13(2), 227-233.

Pereira, F. B., Gudina, E. J., Costa, R., Vitorino, R., Teixeira, J. A., Coutinho, A. P. and Rodrigues, L. R. (2013). Optimization and characterization of biosurfactant production by Bacillus subtilis isolates. *Fuel,* 111(3), 259-268.

Rodrigues, L. R., Teixeira, J. A., Van Der Mei, H. C. and Oliveira, R. (2006). Physiochemical and functional characterization of a biosurfactant produced by *Lactococcus lactis. Colloid Surface Bioinformatics,* 49, 79-86.

Saravanan, V. and Vijayakumar, S. (2012). Isolation and screening of biosurfactant producing microorganisms from oil contaminated soil. Journal of academic and industrial resources, 1(5), 264-268.

Satpute, S. K., Bhawsar, B. D., Dhakephalkar, P. K. and Chopade, B. A. (2008). Assessment of

different screening methods for selecting biosurfactant producing marine bacteria. *Indian Journal of Marine Science*, 37, 243-250.

Tarango, O. A. L., Moorillon, N. G. V., Casarrubias, B. M. L., Chavira, R. B. E., and Borunda, O. E. (2012). Isolation and characterization of biosurfactant producing bacteria. *Environmental Engineering and Management Journal*, 11(3), 1-12.

Thavasi, R., Jayalakshmi, S., Balasubramanian, T. and Ibrahim, M. B. (2009). Production and characterization of a glycolipid biosurfactant from *Bacillus megaterium* using economically cheaper sources. *World Journal of Microbiology and Biotechnology*, 24(7), 917- 925.

Youssef, H. N., Duncan, K. E., Nagle, D. P., Savage, K. N., Knapp, R. M. and McInerney, M. J. (2004). Comparison of method to detect biosurfactant production by diverse microorganisms. *Journal of Microbiological Methods*, 56, 339-347.