Assessment of Hydrocarbon Utilization by Hybrids and Wild Type *Saccharomyces* spp. isolated from pawpaw fruit

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Abstract: Yeast, *Saccharomyces* sp., isolated from pawpaw fruit were mated to form hybrids and a comparative assessment of hydrocarbon utilization by the hybrids and the wild type *Saccharomyces* was carried out. A total of five isolates belonging to the genus S*accharomyces* were studied. Mating experiment was conducted using Yeast Peptone Dextrose (YPD) agar. Hybrids (xcpI, xcpII and xcpIII) resulted in three out of the ten crosses. A hydrocarbon biodegradation set-up was used to compare hydrocarbon utilization by the hybrids and wild types for a period of 15 days. The order of percentage changes in the viable counts was - hybrid xcpII (14.5 %) > hybrid xcpI (11.2 %) > hybrid xcpIII (10.8 %). The reduction in pH values was slightly greater in hybrid biodegradation systems (7.4 - 6.5) than the corresponding wild types biodegradation systems (7.4-6.6). Analysis of Variance (ANOVA), using the Statistical Package for Social Sciences (SPSS) shows that there was statistically significant difference ($p<0.05$) in the mean numbers of the viable cells growing in the various set-ups with the isolates, hybrids and the control. Differences in the pH values were however, not significant. Net hydrocarbon losses of 48.3%, 35.8% and 52.3% was recorded on day 15 for hybrids xcpI, xcpII and xcpIII respectively while corresponding wild type *Saccharomyces* recorded average net losses of 33.7%, 28.3% and 27.9%. On the whole, the hybrids proved to be more effective in hydrocarbon utilization.

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1.0 Introduction

Pollution of soils, underground and surface waters and air with toxic chemicals originating from oil spillages and via other industrial activities is a major problem facing developed and developing countries today. While regulatory steps have been implemented to reduce or eliminate the release of these toxicants to the environment, significant environmental contamination has occurred in the past and will probably continue to occur in the future. The need to remediate these sites of pollution has led to the development of new technologies that emphasize the detoxification and destruction of the contaminants rather than the conventional approach of disposal. Currently, physical and chemical methods are the most widely employed in the clean up of polluted sites but most often, these methods are grossly inadequate and ineffective, and may even result in further contamination of the environment (Steven, 1991). Bioremediation has been defined as a controlled treatment technology where environmental parameters are optimized to achieve the fastest and most complete biodegradation of petroleum hydrocarbon present in soils and waters.

Researches have indicated that biodegradation of hydrocarbon is not restricted to bacteria alone. Remediation of hydrocarbon polluted soil using spent mushroom compost has been reported by Ibiene *et al.* (2011). Okpokwasili and Amanchukwu (1988), Leahy and Colwell, (1990), have implicated *Candida* in hydrocarbon utilization. Okpokwasili and Okorie, (1988) isolated *Saccharomyces* from used and unused lubricating oil, while Leahy and Colwell, (1990) and Allsopp and Seal, (1986) mentioned *Aspergillus* as hydrocarbon degrader.

The yeast *Saccharomyces* is the most important organism in biotechnology. Besides its role in brewing and bread making industries, it has been used as a host organism for the synthesis of important pharmaceuticals from cloned genes. Yeast can grow in both haploid and diploid state.

The mating of yeast only occurs between haploids, which can either be "**a**" or "**α**" (alpha) mating type or thus display simple sexual differentiation. Through a form of genetic recombination, haploid yeast can switch mating type as often as every cell cycle. Haploid cells are capable of mating with other haploid cells of the opposite mating type (an "**a**" can only mate with an "**α**" cell, and vice versa) to produce a stable diploid cell. Diploid cells, usually upon facing stressful conditions such as nutrient depletion, can undergo meiosis to produce four haploid spores: two "**a**" spores and two "**α**" spores (Sherman, 2002).

Unlike most laboratory scale biodegradation test

process involving yeast, this experiment was aimed at isolating yeast *Saccharomyces* from pawpaw fruit, mating the wild type isolates to form hybrids and comparing the hydrocarbon utilization abilities of the hybrids and wild type parent isolates.

2.0 Materials and method

2.1 Source of materials

The crude oil type "Bonny Light" used in this research work was obtained from the Port Harcourt Refinery, Eleme Local Government Area of Rivers State. The yeasts were isolated from a pawpaw fruit, harvested in Gokana Local Government Area of Rivers state, Nigeria - an oil producing area.

2.2 Isolation of test organisms

Suspensions of pawpaw fruit in physiological saline were inoculated into mineral salt broth. The mineral salt broth had filter-sterilized antibiotics – tetracycline and streptomycin – to inhibit bacterial growth (Walker and Colwell, 1975). The concentration was 50µg/l each for the two antibiotics (Amanchukwu *et al*., 1988). A 50:50 mixture of kerosene and diesel was introduced into the media as the sole source of carbon at a concentration of 1.5% (v/v). Incubation was carried out for 21 days at 30^{o}C. Viable cultures from the above medium were inoculated onto mineral salt agar plates, made selective for yeast by addition of 50µg/ml each of filter sterilized antibiotics (streptomycin and tetracycline).The media contained 2.0% purified agar (Amanchukwu *et al*. 1998). The antibiotics were added just before the media solidified. Incubation was carried out at 30^{o}C for 5days. Sterile filter papers (Whatman no.1) saturated with filter-sterilized hydrocarbons (kerosene and diesel) were placed on the inside of the Petri dish and incubation done on the inverted position. The filter papers transferred the hydrocarbons to the organisms by vapour phase transfer method. Yeast isolates obtained were purified and prefixed with the letters Cp (*Carica papaya*), e.g. Cp01, Cp02, Cp03 etc for 1st, 2nd, 3rd etc. isolates respectively.

2.3 Characterization and identification of yeast

Identification of yeast is based upon a combination of morphological and biochemical criteria (Haley, 1971and Kreger-van Rij, 1984).

2.4 Mating and hybridization

The various isolates were studied for mating ability on PDA plates. Strains of each organism were picked with a pick stick and thoroughly mixed with each other on a surface of fresh PDA plates. The plates were incubated at 30^{o}C for 4 - 6 hours. Smears of the colonies were microscopically examined for dump-bell shape typical of zygote formation.

2.5 Monitoring hydrocarbon utilization

The pH, changes in total hydrocarbon utilizing yeast population and hydrocarbon content were parameters used to monitor hydrocarbon utilization (Ibiene *et al.,* 2011; Osuji and Ezebuiro. 2006; UNEP, 2004.).

Table 1. Hydrocarbon biodegradation set-up

Flasks	Inoculation options
I	medium + 1mg/L of crude oil + Cp01
II	medium + 1mg/L of crude oil + Cp02
III	medium + 1mg/L of crude oil + Cp03
IV	medium + 1mg/L of crude oil + Cp04
V	medium + 1mg/L of crude oil + Cp05
VI	medium + 1mg/L of crude oil + Hybrid xcpI
VII	medium + 1mg/L of crude oil + Hybrid xcpII
VIII	medium + 1mg/L of crude oil + HybridxcpIII
IX	medium + 1mg/L of crude oil (control)

3.0 Result Analysis

3.1 Yeast isolates and characterization

Five yeast isolates from pawpaw fruit designated - Cp01, Cp02, Cp03, Cp04 and Cp05 (Table 1) were identified as *Saccharomyces* spp.

3.2 Mating and hybridization of yeasts isolates

Out of the ten crosses, three crosses resulted n zygote/hybrid formation. The zygote positive crosses included the Cp01:Cp03; Cp02:Cp04, and Cp02:Cp05 and resulted in formation of hybrids xcpI, xcpII and xcpIII respectively. The zygote formation shows that these parent isolates were haploid cells of the opposite mating types, while those that did not result in hybrids either involved cells of similar mating types or diploid strains with heterozygous mating type locus. In industrial application, mating to some extent determines ploidy and stability of strains. The ability of yeast to mate increases the number of strains and hybrids. In strain development, crosses (mating) are carried out between two species, one with desired characteristics and the other without it, thereby transferring the desired characteristics to the later.

3.3 Changes in pH during hydrocarbon biodegradation

Figures 1 to 3 present data on pH changes during the 15-day study period. pH was observed to gradually decrease in the test systems except in the control, ranging between 7.4 – 6.5. The change in pH

from alkalinity to acidity is an indication of increased metabolic activities and biodegradation products, which eventually caused the lowering of pH. Jones and Greenfield, (1991) reported a pH range of 6.4 – 8.0 as ideal for optimum hydrocarbon mineralization. The widest margin in pH values between isolates and hybrids was recorded on day 15 in hybrid xcpI, where a difference of 0.3 was observed in pH of hybrid xcpI and parent isolate Cp03. The slight drop in the pH of the control, which may be termed insignificant, may be attributed to photo oxidation. The pH changes recorded for hybrids and the corresponding isolates were not statistically significant ($p<0.05$) using the Statistical Package for Social Sciences (SSPS) ANOVA test.

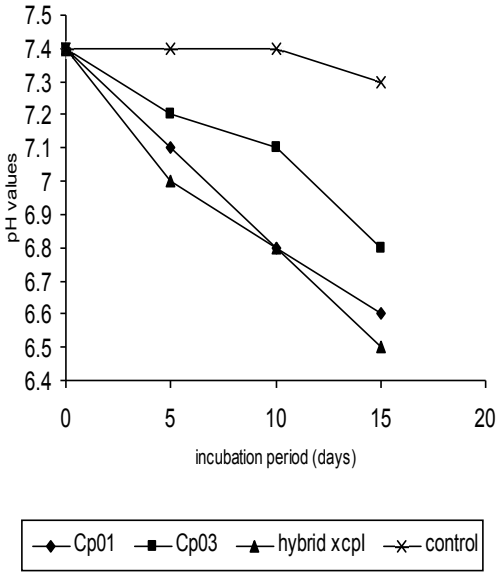

Fig. 1: pH profile of hybrid xcpI and wild type parents on hydrovcarbon medium

3.4 Changes in total hydrocarbon utilizing yeast count

Figures 4 to 6 present data on the percentage changes in population of the hydrocarbon utilizing yeasts during hydrocarbon biodegradation. The order of percentage changes in the viable counts was - hybrid xcpII (14.5%) > hybrid xcpI (11.2%) > hybrid xcpIII (10.8%). In general, the hybrids recorded higher percentage changes in the viable counts than the corresponding parent wild types.

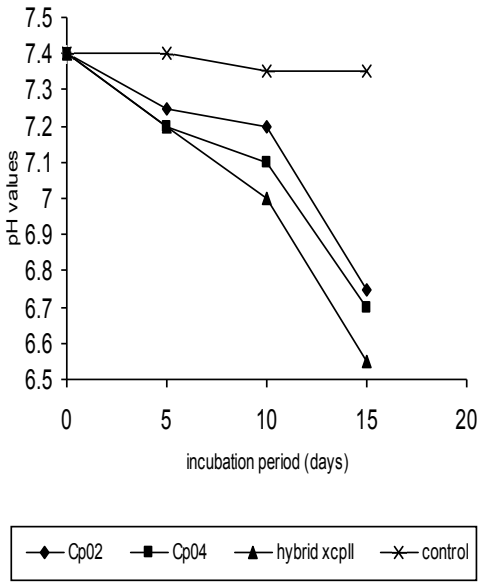

Fig. 2: pH profile of hybrid xcpII and wild type parents on hydrocarbon medium

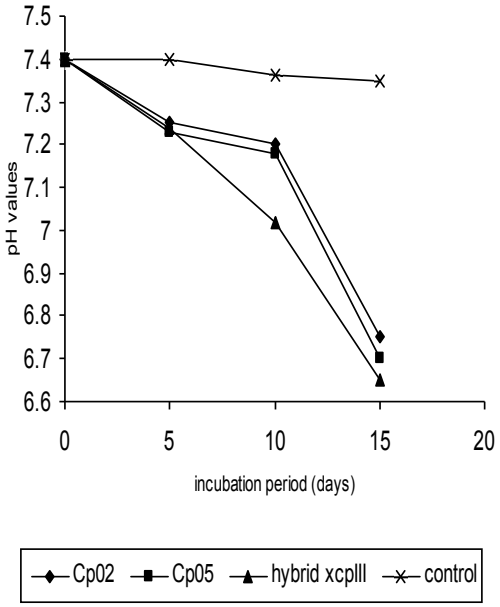

Fig. 3: pH profile of hybrid xcpIII and wild type parents on hydrocarbon medium

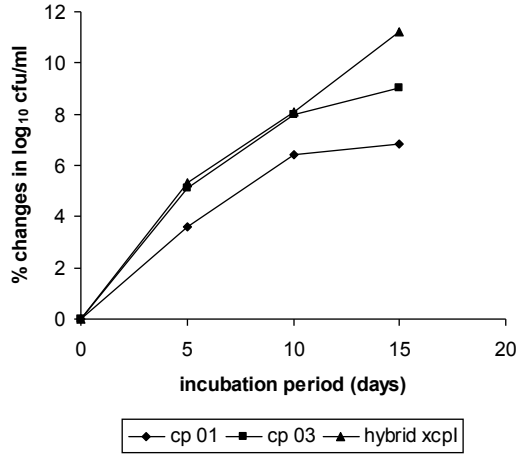

Fig.4: Growth profile of hybrid xcpI and wild type parents on hydrocarbon medium

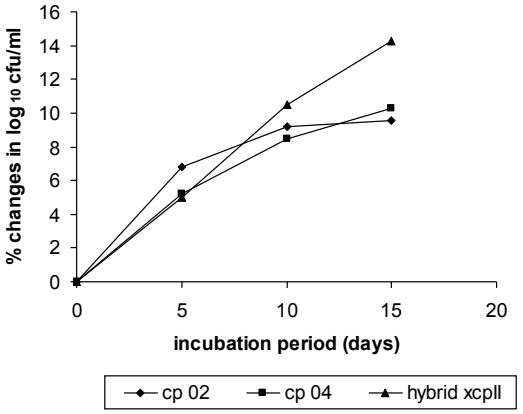

Fig. 5: Growth profile of hybrid xcpII and wild type parents on hydrocarbon medium

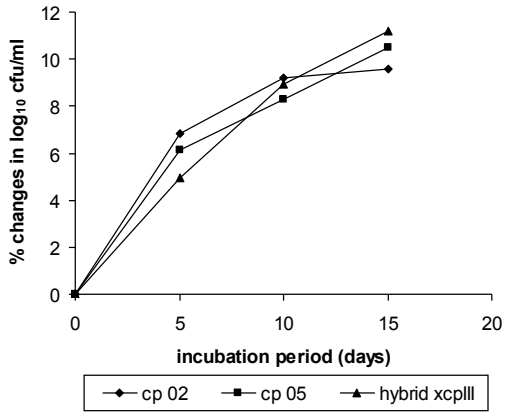

Fig. 6: Growth profile of hybrid xcpIII and wild type parents on hydrocarbon medium

3.5 Hydrocarbon utilization by hybrids and wild type parent isolates.

Hydrocarbon utilization by wild type and resultant hybrids during the study period are presented in Figures 7 to 9. The highest net hydrocarbon loss (52.3%) was recorded in hybrid xcpIII on day 15. Hybrids xcpI and xcpII recorded net losses of 48.3% and 35.8% respectively. Corresponding isolates that formed hybrids xcpI, xcpII and xcpIII recorded average net losses of 33.7%, 28.3% and 27.9% respectively. In general, net loss of crude oil was more in the hybrids than in the individual isolates.

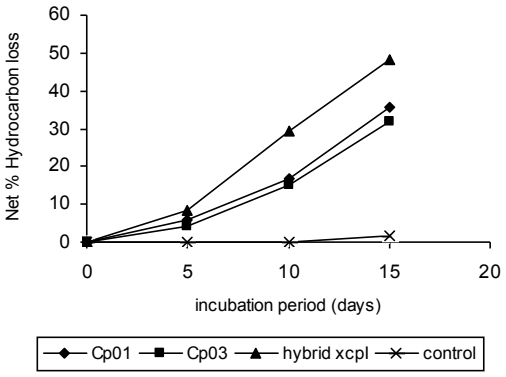

Fig. 7. Comparison of hydrocarbon utilization by hybrid xcpI and parent wild types

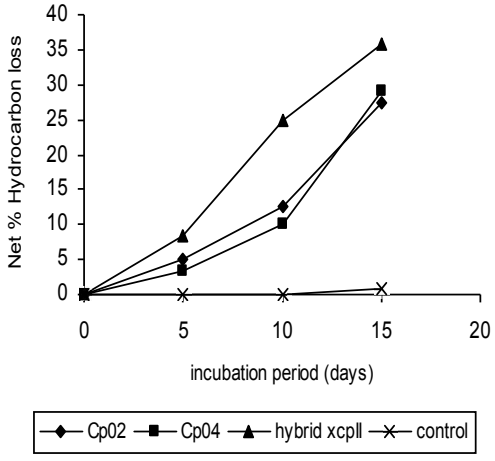

Fig. 8. Comparison of hydrocarbon utilization by hybrid xcpII and parent wild types

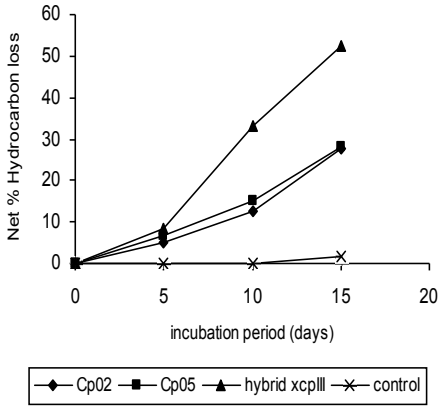

Fig. 9. Comparison of hydrocarbon utilization by hybrid xcpIII and parent wild types

4.0 Conclusion

Bioremediation has often been viewed as a veritable tool in the clean up of oil-polluted environment. It is economical, environmentally friendly and disrupts site minimally (Abu and Ogiji, 1996). Unlike most laboratory scale bioremediation, this study was undertaken to see if hybridization of yeast isolates could improve hydrocarbon utilization. Pawpaw fruit, a sugar-rich substrate, serves as a good medium for yeast growth and was a good source of *Saccharomyces* species.

The study showed that *Saccharomyces* isolated from pawpaw fruit was effective in utilizing the hydrocarbon. Furthermore, the ability to utilize the hydrocarbon was enhanced by mating the isolates to form hybrids. Thus hybridization of *Saccharomyces* can be a useful tool in increasing the rate and/or extent of clean-up of oil pollutants in soils and waters. However, it should be noted that this work is only a preliminary genetic study into the ability/potential of gene manipulation to facilitate remediation activities. This research coupled with other advances in molecular biological techniques – like isolation of plasmid DNA, construction of probes and use of genetically engineered microorganisms – can be used to increase rates of pollution clean-up.

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