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Genetic Studies of Yeast Strains Isolated from Pineapple Pulp

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ABSTRACT

Seven (7) yeast strains were isolated from pineapple pulp and six (6) of them were tentatively identified as Saccharomyces species. Attempts to select prototrophs from these isolates considered eleven (11) different markers. Two (2) of the yeasts were prototrophic for tryptophan (trp-) and isoleucine/valine (iiv) marker respectively. Two (2) of the seven (7) yeasts isolated were self-sporulating. None of the isolates exhibited properties indicative of respiratory deficiency. This was also true for the hybrid produced. Two mating programmes were attempted involving the isolates and a standard Laboratory strain and between the isolates. Six (6) out of the twenty-one (21) attempts produced hybrids. All the hybrids sporulated.

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INTRODUCTION

The pineapple Ananas comosus belongs to the family Bromeliaceae, indigenous of the American tropics. Most of the Bromeliads are epiphytes. They live on trees. The pineapple and some relatives however live on the ground. There are many Ananas cultivars, but the predominant one is smooth caynnee (Alfredo and Stephanie, 2023).

The modem pineapple was domesticated in pre - columbian times in South Africa. It has since become a popular crop in most of the tropics. The centre of origin is probably in Parana - Paraqua iiver drainage area, where the related seedy species. A *bracteatus* (Lind. L) schultes A. anaassoides (B ak) L. B Smith, A. erectifolias L. B Smith and *Pseudomanas sagenarias Arudda* occur wild, It has been suggested that the Tupi - Guarani Indians first selected and cultivated A. comosus at its centre of origin and later took it with them on their subsequent migrations (Syafik *et al*., 2024)

Pineapples are propagated vegetatively with crowns, slips or suckers. The crown is the vegetative shoot on top of the fruit. Slips are side shoots arising from the fruiting stem just below the fruit. Suckers are side shoots developing from the main stem above ground level. New plants from crowns require about 24 months to fruit while those from suckers require 17 months (Amsal *et al*., 2021).

Each original plant set out produces one fruit at the top of the stem. This is called the plant crop. After these fruits are harvested, one or two suckers are allowed to develop from the mother plant. Each sucker, about a year later, produces one pineapple fruit ready to harvest. This is called ratoon crop. Sometimes a second ratoon crop is grown. However, highest yields and best quality fruits are obtained from the plant crop with solid block plantings of a single cultivar, pineapple fruits are seedless because each pineapple cultivar is self-incompatible i.e. its own pollen does not fertilize its egg (Sanewski *et al*., 2018)

Pineapples are rich in some chemical substances which serve as source of nutrients. The edible portion which constitutes about 60 percent of the fresh fruit; contains approximately 85 percent water, 0.4 percent protein, 14 percent of sugars (of which two - third is in the form of sucrose, the remaining are glucose and fructose), 0.1 percent fat, 0.5 percent fibre, 0.6 percent acid (of which 87 percent is citric acid, and several vitamins. Canned pineapple in syrup contains about 76 percent water, 0.4 percent protein, 22.6 percent sugar and acids. The milled juice from which sugar syrup and sometimes citric acids are obtained contains on a dry weight basis 75 - 85 percent sugar and 7 - 9 percent citric acid. Pineapple contains the protease, bromchia a protein digesting enzyme (Sanewski *et al.,* 2018).

The chemical composition of pineapple is suitable for the growth of some microorganisms. Generally, the microflora of fruits are different from that of leafy vegetables. This is because many fruits possess natural defence mechanisms. Pineapples as an example contain organic acids in quantities that are generally sufficient to produce pH values of about 43 or lower. The nature of the organic acid molecule itself and the low pH value determine the predominant microflora of pineapple, because of the low pH value of pineapple most of the soil and air borne bacteria are not encouraged (Almeida et *al., .*2024).

However, organisms like molds yeast and the laetics would do well at this pH level. Consequently, these organisms are often implicated as the predominant microflora of pineapple fruits. Yeasts occur earlier in exposed pineapples because of their capacity to tolerate wider temperature range (Lie *et al.,* 2020).

The Pineapples however are of immense economic importance. They are eaten as desert fruits throughout the tropics and sub tropics, for which they are best harvested fully ripe in order that the desired flavour may be developed. Ripe fruits soon deteriorate and must be eaten within 4 - 5 days. Most of the commercial crops of pineapple is canned in the producer countries. Mixed with other fruits pineapples, bananas and papayas are well known for this purpose. Sugar syrup is obtained from the mill juice which are formally a waste product. Alcohol and citric acid may also be manufactured from it. The fruit residues after the extraction of the mill juice may be made into bran white which is used as cattle feed. Wine was made from pineapple in tropical America (Sanewski, *et al.,* 2018).

The leaves yield 2 - 3 percent of a strong white silky fibre 38 - 90 cm in length which is used for making a fine fabric called pine cloth in the Philippines and Taiwan (purse glove, J. W 1972). In the Philippines vinegar is made from fruit juices, particularly pineapple, sugar cane, palm juice coconut toddy etc. vinegar offers a stable outlet for surplus fruit and fruit trimmings from large industries such as pineapple industry where at times surplus pineapple juice is discarded unless it is fermented. In addition to being used on the table as a condiment, vinegar is essential for manufacture of pickles and sauces for the canning of fish (Hikal *et al.,* 2024).

Also pineapples are used in making “Thai coconut vinegar”, which is made by fermentation of coconut toddy sugared coconut, pineapple etc. the alcoholic fermentation is carried out either by wild yeasts or by the use of Luk – paeng (Hikal *et al*., 2021).

The “Philippine Nata” is another product made from pineapple. Nata is the thick white or cream - coloured insoluble, gelatinous film of cells and polysaccharides that Acetobacter xvlinum forms on the surface of an acidified medium containing sugar, ethyl alcohol and other nutrients. It is a Philippine delicacy which when can dried resembles western gum drops in texture and flavour. Two types of Nata are well known: nata de pina produced on the juice from pineapple trimmings and nata de coco produced on coconut water or skin milk (Nurtjahtja, 2020).

It was first suggested that the organism actually was *Acetobacter aceti* and concluded that the causal organism was identical. In the most recent (8th) edition of Beergey’s manual of Determinative Bacteriology *A. xylinum* is classified as *A. aceti* sub species *xvlinum* (Yamada *et al*., 2013).

The problem was complicated by the fact that originally the essential organism in Nata was considered to be *J. mesenteroides*. The film therefore was expected

to be dextran. In fermented foods, acetic acid bacteria (AAB), kinds of bacteria with a long history of utilization, contribute to safety, nutritional, and sensory properties primarily through acetic acid fermentation. AAB are commonly found in various fermented foods such as vinegar, sour beer, fermented cocoa and coffee beans, kefir beverages, kombucha, and sourdough. They interact and cooperate with a variety of microorganisms, resulting in the formation of diverse metabolites and the production of fermented foods with distinct flavors and silky suspension (Han *et al*., 2024), The initial silky suspension turns to loose fluff and then flocculates to form larger bodies (Yamada *et al.,* 2013.)

The term "Yeast" has been used in a variety of ways and very often has no taxonomic significance. Thus one can talk of yeasts and Yeast-like fungi the latter being those that form true mycelium but reproduce by budding or arthro-spores. Yeasts occur in abundance in substrates that contain sugars, such as the nectar of flowers and the surface of fruits. They are also found in the soil, in animal droppings, in milk, on vegetative parts of plants and in association with insects. Yeasts are noted particularly for their ability to ferment carbohydrates (Kurtzman *et al.,* 2020))

Yotmg colonies of yeasts, when growing on solid media, have almost always a very characteristic appearance being moist and somewhat slimy. The colour of the colonies is usually whitish, cream or pink, but a few species are otherwise coloured. In some species colonies change with age but others gradually become wrinkled and dryer in appearance (Kurtzman *et al.,* 2020).

Yeasts offer many advantages as an organism for genetic studies. These include rapid growth, ability for cloning and ease of handling and storage. They are also adaptable to replica - plating, micromanipulation aid an array of biochemical procedure developed for bacteria. The genus Saccharomyceae is one of the thirty - nine genera of single - lled yeasts recognised in the treatise on yeasts (Lodder 1970). The genetic name was introduced by Meyen in 1838 and the genus was died by REES in 1870 (cited by Rose, 1977). The Saccharomycetaceae are unicellular organisms measuring about 6-12 in diameter, they possess a definite cell wall and a demonstrable nucleus. The nucleus of Saccharomyces cerevisiae contains eighteen chromosomes. Most of the chromosomes have been mapped (Mortimer and Schild, 1980) (Sa.raiva *et al.,* 2023)

The Saccharomyces are usually round cells, sometimes ovate or elongate only rarely producing pseudomycelium. Though, unicellular they are distinguished from bacteria by that size. All strains of Saccharomyces cerevisiae can use ammonia and urea as the sole nitrogen source but cannot utilise nitrate since they lack the ability to reduce it to ammonia ions (Lodder, 1970)(Parapouli *et al.,* 2020).

Saccharomyces contains both homothallic and heterothallic strains. Heterothallic strains of Saccharomvces cerevisiae have both haplo and diplo vegetative phases. In diploids, meiosis proceeds sporulation and normally four haploid spores are produced in ascus. The resulting spores in the ascus are products of a single meiotic process and are called tetrad. It was demonstrated that a single pair of alleles a and MAT (now MAT a and MAT oc) respectively, controlled mating response in these strains. Only cells of opposite mating types mate. These factors are secreted on the cell surface. The mating process are common several Saccharomyces species: *Saccharomvces bavanus, Saccharomyces chodati, Saccharomyces carlsbergensis, Saccharomvces chevalieirer. Saccharomvces diastaticus* and *Saharomyces oviformis* (old taxanomic names), most of which are not taxanomically termed *Saccharomyces cerevisiae* (Martin, 2022), but are mere strains.

Genetic analysis of such strains therefore becomes difficult and can only be tempted with a few strains. Good Sporulation depends upon the presence of nitrogenous compounds in the growth medium. The use of acetate, a non-fermentable carbon source is sporulation medium and the degree of sporulation and the proportion of four spored asci which demonstrate that respiratory - deficient diploids also do not sporulate even if they are heterozygous for the mating types locus (Neiman, 2024).

Respiratory deficiency, polyploidy and aneuploidy are amongst the major factors that inhibit sporulation and consequently disrupt the sexual cycle in *Saccharomyces* (Piekarska and Rytka, 2010).

Genetic work on yeasts isolated from local Nigerian fruits are beginning to appear. Preliminary genetic work had been done on yeasts isolated from pineapple fruit (Okwelle, 1987) where yeasts strains designated MAT a and MAT were isolated. Also yeasts strains were isolated from pawpaw fruits; and from banana fruit and from pineapple fruit respectively. Based on this further improvement on the preliminary genetic work on yeast was carried out (Nathaniel *et al*., 2025).

The purpose of the present study however, involves the isolation of yeast strains probably Saccharomyces cerevisiae or closely related to it from pineapple fruits. The mating and sporulation ability of these isolates were determined and hybrids were subsequently produced. The presence of respiratory sufficient mutant was also investigated (Nasir *et al*., 2017).

MATERIALS AND METHODS

Strains of Yeast

Genetically marked laboratory strains of Saccharomyces cerevisiae were used in this study. Strain S 732C is known to be MAT a. It also has requirement for Adenine. This strain as well as strain S 288C (Mat cc) were particularly useful in determining the mating type of yeast

strains isolated from pineapple pulp. Other laboratory strains were X 1069 — 2D (Mat cc) which had the following makers: leucine 2, aderine 1, histidine 4, threonine 4, methionine 2, uracil 1, tryptophan 5, as well as inability to ferment galactose and lysine strain (Mat cc) which carried a lysine maker. Table I presents the genotypes and sources of the laboratory strains of yeasts used in this project. All the strains were obtained from R.K. Mortimer in California.

Table 1: Laboratory Strains of Yeast

STRAIN	LOCUS	GENOTYPE	SOURCE
X1069-2D	leu2	aleu, adel, his4, thr4, met2, ural, trp5, gal2.	R.K.Mortimer
S732C	adel	a adel	R.K.Mortimer
S288C	gal2	ci suc2, mat, gal2, cupi.	R.K.Mortimer

The strains isolated had to be named for reference purposes. Names were assigned as follows: Isolated strains were given individual numbers prefixed with the letters Sp. This combination of strains of yeast used were followed by a sub fix, "Mp" for strain isolated from pineapple.

The prefix and subfix are specific for the substrate but the numbers are specific for the isolated strains. Strains produced from crosses (hybrids) were designed "XP" followed by a number and the usual subfix MP'

Spore cultures from such cultures had numbers specific for each tetrads and each Spore had a letter ABC or D. This Combination was separated from the parent hybrid number by a hyphen.

Pineapple

Healthy pineapple fruits were bought from the market. The apple were washed and cut into small pieces with a sterile scapel. The chunks were then transferred into a sterile blender. After blending the resultant pulp was collected in a sterile beaker.

Media

All media were autoclaved for 15 minutes at 121°C. The following were the media used in the project work: The media used includes: YPD agar, Malt extract agar, Minimal vitamin (MV) and Glycerol agar (YPGly)

Sterilization

All glass wares used autoclaved after washing with water at 121°c for 15 minutes. Loops were flamed to real hot and allowed to cool prior to use. Other materials including the blender, scapel for cutting the fruits and the inoculation hood were cleansed with alcohol.

ISOLATION AND MICROSCOPIC EXAMINATION

Plating from Exposed Pineapple Pulp

Blended pineapple pulp was exposed for 6 - 10 hours. With a sterile wire loop the pulp was aseptically streaked onto YPD agar plates in triplicate. Also a measured portion of the pineapple was cut into pieces (with the diameter of 2 cm) into lOml of distilled water (stock suspension). The stock suspension was serially diluted and 0.1 ml of the diluents from tubes containing the final dilution and the penultimate were plated and incubated at 28 Oc for 24 hours. The plates were then examined for growth, distinct colonies were picked using sterile wire loop and subcultured on malt extract agar plates. The Isolated organisms were then stored on YPD slants for further analysis. Before yeast strains were selected from among the isolates, they were examined microscopically and compared with the standard laboratory strains of yeast *(Saccharomyces cerevisiae).*

Gram stain

Organisms were gram - stained primarily to separate the bacterial flora from the other microorganisms in the mixture. The method involved flooding fixed films of the isolates or slides with crystal violet for about 10 seconds. This was followed by pouring excess of the dye and covering the film with iodine solutions for 10 seconds. The films were then decolorized, rinsed under tap water and the excess water allowed to drain off. This was followed by counter staining with safranin. Excess of safranin was poured out and excess water drained by placing the slide slanted on a filter which then absorbs the water.

The slide was then covered with oil - immersion and examined under the microscope (x 100).

Maintenance of Stock Culture

Isolated organisms i.e. yeast stains were stored in YPD agar slants contained in MacCartney bottles and refrigerated. The stock cultures were sub cultured at intervals of two weeks (or earlier) depending on the

prevailing circumstances. The sealed tube were wrapped with paraffin papers to prevent loss of moisture.

BIOCHEMICAL TESTS

The nutrient medium used for this test was peptone water. This formed the basal medium into which the test carbohydrate compounds were added.

Procedure

The component of the basal medium were prepared in the following proportion: peptone l0g, sodium chloride 5g and one litre of distilled water. The ingredients were dissolved in warm water and 25m I of 0.2 percent of Bromocresol purple was added to give a final concentration of 0.005 percent.

The pH was adjusted to 4.6. The solution was dispensed in a 90m1 amount into conical flasks. The flasks were plugged with cotton, covered with aluminum foil and autoclaved at 121°C for 15 minutes. One gram of the test sugar was dissolved in l0ml of distilled water to give 10% concentration and this was filter sterilized. Test sugars include sucrose, maltose mannitol, lactose, inositol and glucose in test tubes were sterilized by filtration and added aseptically to an autoclave Hugh and Leifson medium at 45°C to give a final concentration of 0. 5 to 1.0%. Durham tubes (sterile) were dropped invested, one into each tube.

Media was inoculated with test organism and incubated at 37°C for 48 hours. Positive Test: charge in colour of the indicator shows carbohydrate utilization. Gas given off during fermentation was collected in the invested Durham tube. The results are given in the table that follows.

Sporulation

The isolated strains on slants as well as cultures of mating mixtures obtained from mating hybridization were streaked using a flamed wire loop onto YPD plates (presporulation medium) and incubated at 30°C for 48 hours. Individual cultures were then transferred as a thin layer to potassium acetate (KAC) plates, incubated at 25°C and examined under the microscope (x40) for ascospore formation after 48-72 hours (Mclary et al, 1959).

Determination of Respiratory Sufficiency

Respiratory - deficient (petite) cells are unable to utilise glycerol as a carbon source, and were scored by their inability to grow on this medium (Ogur and St. John 1956). To determine if isolated strains were respiratory sufficient, the strains were plated on YPD agar plates containing glycerol a non-fermentable carbon source.

Mating and Hybridization

Standard Laboratory strains known to be Mat “a” as well as strain S 288c (Mat cc) were streaked on YPD plate and incubated for 18 hours, (overnight) at 300c. The isolated strains were also inoculated on YPD plates and incubated for 18 hours (overnight) at 3ODc also. The isolated yeasts were then mated individually with the laboratory strains. This was done by mixing the’ isolated standard strains on YPD plates. The mated mixtures was incubated at 3ODc for 4 - 6 hours. After which’ the mating mixture was examined microscopically (x 40) for the presence of zygote (Lindegre, 1949).

Mating was also carried out between the isolated strains. They were then incubated at 3ODc and examined microscopically (x 40) for the presence of zygote structures after 4- 6 hours.

RESULTS

Growth of the isolated yeast strains on YPD and malt extract agar medium revealed a wide range of colonies. Microscopic examination showed distinct morphological characteristics. Most of the colonies were creamy or whitish and ellipsoidal or spherical in shape.

Comparison of all the isolated strains with the standard laboratory strain of Saccharomvces cerevisiae indicated resemblance in cell size, shape and colonial fermentation reactions. These were therefore presumed to be yeasts probably Saccharomyces Sp.

Besides cultural characteristics and microscopic observation isolates obtained were further characterized by biochemical tests. Table 2a outlines the cultural, morphological, and biochemical traits of seven yeast strains (MP 001P to MP 007P).

Colony appearances vary: MP 001P is creamy and smooth, MP 002P is dull white with raised margins, MP 003P is dirty white with serrated edges, MP 004P is creamy and moist, MP 005P has a wrinkled edge, MP 006P is dirty white and raised, and MP 007P is moist and creamy with serrated edges.

Cell shapes differ, with MP 001P being spherical, MP 002P ellipsoidal, MP 003P special, MP 004P globose, and the rest spherical. All reproduce by budding and do not form pseudo-mycelium.

Biochemically, all strains ferment sucrose, glucose, and mannitose, but none ferment mannitol, lactose, or inositol. This diversity indicates their potential for various fermentation applications.

TABLE 2a: Characteristics of selected isolated

1	Characteristics	MP 001P	MP 002P	MP003P	MP004P	MP005P	MP006P	MP007P
	Cultural appearance	Creamy smooth and mucoid	Dull white raised colony with separated margin	Dirty white and seriated edge	Creamy moist and entire	Dull while and wrinkled edge	Dirty white and raised celony	Moisty creamy seriated edges
	Cell Morphology	Spherical single	Ellipsoidal budding	Special budding	Globose budding	Spherical budding	Spherical budding	Spherical budding
3.	Pseudo-mycelium formation	-	-	-	-	-	-	-
4.	Biochemical test							
	Sucrose	+	+	+	+	+	+	+
	Manitoal	-	-	-	-	-	-	-
	Lactose	-	-	-	-	-	-	-
	Inositol	-	-	-	-	-	-	-
	Glucose	+	+	+	+	+	+	+
	Manitose	+	+	+	+	+	+	+

KEY: (+) = Sugar Fermented; (-) = Sugar not Fermented

All the seven isolates fermented sucrose and glucose, but did not ferment lactose and inositol. While strains MP 001 P to MP 006 fermented maltose, strain MP 007P did not. Another difference is that MP 007P showed extensive pseudomycelial formation while others did not. Their possible identity (Lodder, 1970) is given below.

Table 2b: (Cont'd) Identification of strains

Strain	Genus
MP 001 P	*Saccharomyces Sp*
MP 002 P	*Saccharomyces Sp*
MP 003 P	*Saccharomyces Sp*
MP. 004 P	*Saccharomyces Sp*
MP 005 P	*Saccharomyces Sp*
MP 006 P	*Saccharomyces Sp*
MP 007 P	*Saccharomyces Sp*

The isolated strains presumed to be *Saccharomyces* species could be wild type for many genetic markers. However for the purpose of genetic work it is essential to determine the markers or the isolates. These isolates carry many genetic markers. With the use of complete minimal and selective media the markers on these strains were determined. The results of this determination is presented in Table 3.

Table 3: Ability of strains to Grow in Amino Acid Deficient medium

Selective	MP 001P	PMP 002P	MP003P	MP004P	MP005P	MP006P	MP007P
Omission Medium							
Adenine	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	+	+
Histidine	+	+	+	+	+	+	+
Isoleucine/valine	+	+	-	+	+	+	+
Lysine	+	+	+	+	+	+	+
Methronine	+	+	+	+	+	+	+
Phenylalanine	+	+	+	+	+	+	+
Threonine	+	+	+	+	+	+	+
Tryptophan	+	+	+	+	+	+	+
Uracil	+	+	+	+	+	+	+
Minimal medium without amino acid	-	+	-	+	+	+	+

Key: (+) = Means growth; (-) = No growth

Absence of growth of strain MP 001 P and MP 003 P on selective media lacking tryptophan and isoleucine/valine respectively suggests that these 2 strains require these amino acids for growth. That they are auxotrophic is further confirmed by their inability to grow on minimal medium.

Strain MP001P can therefore be designated tryptophan (-) i.e. requires trytophan in the medium for it to grow. In the same vain, strain MP003P carries a isoleucine/valine marker and can therefore be designated ILV. All other isolates seem to be wild type or prototrophic for the markers tested. Since the ploidy and the mating types of these strains t are not known it is probably that most apparent prototrophs will exhibit markers in their haploid states.

Table four presents the results of Sporulation experiments involving all the isolates. Two of the strains (MP 001 P and MP 003 P) Sporulated. Incidentally, these are the same strains that proved to be auxotrophic (tryptophan - and isoleucine/valine - respectively).

Ability to sporulate in yeasts is harnessed by heterozygosity for the mating type locus. This also implies that the strains cannot be haploid. Diploidy is the least requirement for sporulation at worse the strains would be aneuploid possessing a disomic condition for the mating type locus if this is the case. Spore viability could be a major problem.

The presumably heterozygous strains (MP001P and MP 003 P) might therefore not be useful for making crosses since they probably would not mate. This is a disadvantage because they carry genetic marker which should have been useful for selection of recombination in. the off spring. Since these strain sporulated. It is possible to analyse the spores produced by the strain for the marker loci. Patterns of segregation of the spores for the tryptophan and isoleucine/valine markers will indicate if the parent strain were homozygous or heterozygous for the loci. Information about the particular tryptophan or isoleucine/valine mutants will require the use of standard laboratory marker strains. Carrying different genetic markers for tryptophan and isoleucine valine. This is a more extensive genetic work which involves linkage studies.

Table 4: Sporulation of isolated strains and standard stains (S 732C MAT "a")

Strain	Sporulation
∀MP001P (trypt)	
MP 002 P	-
∀MP 003 P	+
MP. 004 P	–
MP 005 P	–
MP 006 P	_
S732C	-

KEY: (+) Means sporulation; (-) No Sporlation; V. The prototrophic Strains

This notwithstanding, it is worthwhile to carry out a mating programme between the isolated and standard laboratory strain. Because the mating type of the standard laboratory strain is known (MAT **"a")** all strains that mate with it are therefore or an opposite mating type that is Mat a. non maters are either Mat α or heterozygous for the mating type locus.

Table 5: crosses between isolated strains and standard strain (S 732 C MAT "α)

Strains	Mating	Hybrid	Product
MP00IP x S732C	-	-	
MP002 P x S732 C	+	XP 007P	
MP003P x S732C	-	-	
MP004P x S732C	+	XPOO8P	
MP005P x S732C	-	-	
MP006P x S732C	-	-	

Key: (+) Mating; (-) No mating

Strains MP 002 and MP 004 which mate with the standard laboratory strains S 732C are therefore probably MAT a while others are probably MAT a. The ploidies of these strains are not certain. The hybrids produced in crosses involving strains MP 002 P and 5732 C and MP 004 P and S 732 C have been designated XP 007 and XP 008 P respectively.

A second mating programme involved crosses between isolated strains of yeasts: The results of this mating programme are represented in Tables 6 it is expected that strains MP 001 P and MP 003 P which sporulated prior to mating (Table 4) will not mate with any other strain since they are presumably heterozygous diploids. In Table 6, it was also observed that strains **MP**002P and MP004P mated with S732C which is known to be (MAT a).

These two strains were therefore suggested to be of the opposite mating type (MAT a). Because the mating type of strains MP002P and MP004P were presumed to be (MAT a) all the strains that mate with then, can also be characterized with regard to mating type locus. These will be (MAT a) or higher ploidies of this mating type. Such strains would therefore resemble the standard laboratory strains in mating reaction and consequently, will not mate with (MAT a) cells.

Table 6: Cross between isolated strains

Strains	Mating	Hybrid	Product
MP00IP x MP 002P	-	-	
MP001P x MP003P	-	-	
MP001P x MP 004P	-	-	
MP001P x MP005P	-	-	
MP001P x MP 006P	-	-	
MP002P x MP003P	-	-	
MP002P x MP004P	-	-	
MP002P x MP005P	+	xp009p	
MP002P x MP006P	+	xp010p	
MP003P x MP004P	-	-	
MP003P x MP005P	-	-	
MP003P x MP006P	-	-	
MP004P x MP005P	-	-	
MP004P x MP006P	+	xP011P	
MP005P x MP006P	+	xP012P	

If these hybrid products are heterozygous diploid, they could also sporulate. They were then plated individually onto YPD agar (pre sporulation medium) plates and incubated overnight. Colonies were then transferred on to potassium acetate agar (KAC) plates (Sporulation medium) *to* determine if they could sporulate. After 2 to 4 days incubation cells from KAC plates were microscopically observed for the presence of asci. The results are represented in Table 8 below where all the hybrid products Sporulated.

Table 7: Sporulation of Hybrid products

Hybrid Products	Sporulation
xp007p	**+**
xp008p	+
xp009p	+
xpo1op	+
xp0110p	+
xp012p	+

Key: (+) Ascospores formed

Table 8: Respiratory-Sufficiency of isolated strains

Strain	Respiratory-Sufficiency
MP001P	+
MP002P	+
MP003P	+
MP004P	+
MP005P	+
MP006P	+
S 722C	+

The respiratory - sufficiency of all strains shown above excludes mitochondrial and non - mitochoridrial petites as part of the problems associated with the non - Sporulation of the strains of yeast.

DISCUSSION

Microscopic examination clearly showed that some isolated colonies were bacteria while others were yeasts. Of interest is the fact that all isolated and observed bacteria were gram negative and one yeast culture produced pseudomycelium. The later probably indicates that this yeast is not a Saccharomyces species (Sengupta *et al*., 2017).

The similarity evident between the selected strains and the standard laboratory yeast strain with respect to colony shape (spherical or ellipsoidal shape) and size allows for some degree of certainty in assuming that they were yeasts and presumably Saccharomyces species. Their sugar fermentation pattern seem to strengthen this presumptive identification (Chavez *et al.,* 2024).

Two of the yeast strains (MP001P and MP003P) were found to be prototrophic for the amino acid tryptophan and isoleucine/valine respectively. The mutant strains could be very useful for further genetic studies since they carry different genetic markers (Pronk, 2002).

More importantly, both strains seem to be heterozygous with respect to mating type locus, if this suggests diploidy, the strain could be a source for more marker strain on sporulation and isolation of spore cultures which would probably be haploids. Tetrad analysis on these strains would be most revealing and is therefore suggested (Lin *et al.,* 2008).

If they are triploid, spore viability might be a major problem, Strain MP 001 P and 003P are regarded as the marker strains produced in this project. Their importance is in determining the genotype of other isolated strains (Sampaio *et al.,* 2024).

The presence of mating between laboratory strain S732C (MAT “a’1) and two of the isolated colonies (MP 002P and MP 004P) of yeast suggests that the two isolated strains are of opposite mating type (MAT c) from the S732C strain. That, this is likely the case is evident in lack of mating when MP 002P and MP 004P were crossed as shown in Table 5. (Ejogbamu *et al.,* 2025).

Since these strain carry no auxotrophic makers and their mating type marker seem determined, they would be useful in future genetic studies as mating strain if their ploidies are determined. Determination of ploidy in yeast strains involves a combination of experiments including determination of DNA concentration in cells among others. This is an extensive process that can be done as further studies (Pronk, 2002).

The other isolated yeast colonies that did not mate with the strain S732C could be of the same mating type (MAT “a”) or higher ploides of this mating type. Cells of the same mating type however mate with each other occasionally (Howthome, 1963a), because all the isolated yeast Strains now seem to be of different mating types as revealed in Table 6, it is not surprising that mating was successful when attempted between the various strains (Haber, 2012).

Lack of mating ability could be due to heterozygousity for the mating locus, identical mating types between mating partners or aneuploidy, Heterozygous strains are expected to sporulate prior to mating. This statement is true with sporulation found in strains MP 001 P and MP 003P which did not mate with S732C and were self sporulating (Hanson, and Egel, 2022).

Furthermore, the failure of strains MP 001 P and MP 003P to mate when crossed further confirms their presumed heterozygous state for the mating type locus. Strains MP 001 P and MP 003P could be diploid, aneuploid or polyploids, they are however, heterozygous for their respective mating type loci ((Hanson, and Egel, 2022).

Diploid yeast strains that are MAT a MAT a frequently form ascospores. The sporulation of all the hybrids from crosses between the isolated strains (XMP 007P and XMP 008P) could mean that they are MAT a MAT a Since the ploidy of the hybrid strains were not determined, it might just be sufficient to describe them as heterozygous for the mating type locus (Hanson and Egel, 2022).

CONCLUSION

The results of the respiratory deficiency experiments indicates more strongly that lack of sporulation in any strain is not due to respiratory deficiency, Respiratory deficiency is any obvious disadvantage when genetic recombination is sort.

This phenomenon can become useful when strains have ‘been developed and stability is the desired property, Introduction of respiratory deficiency to such strain hinders sporulation an essential aspect of genetic recombination in *Saccharomyces* species.

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