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A META-REVIEW ON PRINCIPLES OF RECOMBINANT DNA TECHNOLOGY

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ABSTRACTS

This article is an examination of the principles of recombinant DNA technology. The scientific development and subsequent analysis of rDNA and its applications in various areas such as the production of vaccines, antibiotics, hormones and in distant hybridisation continues to influence the researchers all over the globe today. This article examines the research done and published by researchers and scientists. Consideration of current trends and data in scientific queries and demonstrates further aspects of principles of recombinant DNA technology. Additionally, this article explores options for uses of rDNA in various different areas such as vaccines for cancer immunotherapy and lassa and filo virus, production of antibiotics using Acremonium chrysogenum strains for rDNA, treatment of diabetes using insulin analogs, production of various hormones like human erythropoietin, gonadotropins, etc using synthetic bacteria, amongst others and use of this technology in distant hybridisation.

KEYWORDS: Recombinant DNA, Vaccines, Hormones, Antibiotics, Distant Hybridisation.

1. INTRODUCTION

Human and animal life heavily depends on good health, and safe and efficient methods to remain healthy and combat various diseases. Since olden times, various methods have been introduced to produce medicines, drugs, vaccines, antibiotics, etc to aid in a healthy life. A modern, widely used technique that is gaining more popularity amongst scientists all over the world today is recombinant DNA technology. Unlike traditional approaches to overcoming health problems through medicines of the older generations and conventional techniques,

genetic engineering makes use of modern methods and approaches, such as molecular cloning and transformation, which are faster and produce more accurate results. It is a technology in which enzymes are used to cut and paste DNA sequences of interest to form a "recombinant DNA". The recombined DNA sequences can then be inserted into vectors, which transport the DNA to a suitable host cell where it can be copied or expressed. This is revolutionary as the recombinant DNA can be manipulated depending on the need and use, and can be made to meet many problems with optimum solutions. This technology has a wide range of applications and has the ability to enhance important aspects of life, such as health, food protection, and resistance to a variety of adverse environmental effects. In this literature review, we will be focusing on thoroughly researched aspects of rDNA technology including the use of rDNA in the production of vaccines, antibiotics, hormones and in distant hybridisation. The review highlights use of escherichia coli for production of DNA vaccines and how recombinant DNA vaccines are used to aid in cancer immunotherapy and the vaccine's effectiveness against infections caused by lassa and filo virus, as well as the use of yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) i.e. artificially constructed vector systems that are useful in creating the recombinant DNA which is further used for the production of vaccines, amongst other things. In order to produce various antibiotics, rDNA technologies use a number of different strains that aid in this technology. These include recombinant acremonium chrysogenum strains, for example: for production of cephalosporin, lactobacillus, streptomyces, bacillus, pseudomonas etc. Research has also been done in the use of rDNA in production applications of distant hybridisation. This includes the manipulation of chromosomes, nucleolar chromatin restructuring, introgression breeding and the applications of this technique, along with its advantages and disadvantages when employed in plants and animals. Finally, this review analyses the production of various hormones using rDNA technology such as human erythropoietin, gonadotropins, etc using synthetic bacteria. This technology also shows a great promise for the production of human insulin by forming insulin analogs in order to treat diabetes mellitus. This review article was written after having gathered information from a wide range of databases, over a wide time period, including the most recent advances. Comparing, analysing and studying these principles of rDNA technology in depth will help us gain a broader view on this technique and will give us insights into the scope of devising better therapies and making the treatment robust for future medicinal healthcare.

2. METHODS

The study was conducted using four databases Google Scholars SAGE, DOAJ and PubMed. Selection of papers were done based on keywords and themes relevant to this review. Further the published papers from these databases were arranged in systematic order with respect to the year of publication.

3. RESULTS AND DISCUSSION

rDNA in Production of Vaccines[1-4]

Sr. No	Title of research paper	Year of publication
1	Recombinant dna vaccines	1984
2	Progress in recombinant dna derived vaccines in lassa virus and filo virus	2011
3	Recombinant dna vaccine technology and applications in cancer immunotherapy	2016
4	Role of recombinant dna technology in vaccine development	2016

1. RECOMBINANT DNA VACCINES (1984)

Escherichia coli is the bacterium most widely used for the production of recombinant DNA technology. There are many reasons for its widespread use like ease of growth, its well studied Genomic structure, and an in depth knowledge of the basic mechanisms for its replication, E. coli-derived plasmids, extrachromosomal DNA elements and selectable markers, is extensive. It is these extrachromosomal apparatus that serve as vectors for the expression of foreign genes. For the production of vaccines, the RNA genome of the virus is first transcribed into DNA with reverse transcriptase enzyme and the resultant DNA was cloned and its nucleic acid sequence was determined using traditional methods. The translated protein sequence is now utilized to determine an efficient method for expressing the requisite antigen in E. coli. The resulting expressed proteins in E. coli may be a polypeptide fused to a naturally occurring bacterial protein or a directly expressed protein that contains only the amino acid sequences encoded by the gene of interest. In E.coli the expression of DNA product is dependent on inducible promoter from tryptophan operon. Due to this the bacteria can be grown into high concentrations before expression of protein takes place by regulating the trpAmino acid concentration. Although the E. coli system can synthesize several potential R-DNA vaccine antigens, it has the intrinsic problem that it is gram negative and cannot secrete antigens into the extracellular medium. Thus, any antigens synthesized in E. coli must be purified from killed cells which is a costly process and is difficult. As a result, attempts have been made to produce R-DNA vaccines in two different

microorganisms which can secrete proteins normally. Although the potential for vaccines manufactured E.coli is large, there are inherent problems with the use of these bacteria as an R-DNA vaccine source. Of major concern is the potency of the immunogen and the secondary structure of the antigen. It is, of course, of paramount importance that the potency and structure of the bacterially-expressed antigen be similar, if not identical, to the native molecule. It becomes necessary, therefore, to examine, sometimes extensively, the isolation and purification conditions that give rise to a properly folded immunogenic protein. This can be, in many cases, difficult if not impossible. rDNA vaccines from tissue culture: There are now several examples where foreign gene products have been expressed in mammalian tissue culture cells. Generally, by placing the desired gene under the control of a eukaryotic transcriptional promoter, usually of viral origin, one may express the protein product after the transfection of the DNA construction into cells. Several different viral and cellular promoters have been isolated and characterized, the transcriptional signals thus far most commonly used in tissue culture expression systems are derived from the small DNA papovavirus, SV-40. The entire nucleotide sequence of this virus has aided in characterizing the genome regions that are involved in viral DNA replication and control of early and late viral gene transcription. The techniques of R-DNA technology should allow the precise manipulation of viral genes for the production of attenuated live virus vaccines. For example, a normally virulent virus may be attenuated by cotransfection of the wild type viral genome with a cloned, mutated viral region that is necessary for virulence but not replication. In vivo recombination with this cloned region should result in incorporation of the mutated region into the wild type viral genome. Although this technique is limited to DNA viruses, it has apparently been successful with Herpes Simplex Virus.

2. Use of R Dna Vaccines in Treatment of Lassa Virus and Filo Virus (2011)

The first recombinant DNA-based vaccine tested for LASV was a recombinant vaccinia virus (VACV) expressing various combinations of the NP, GP1 and GP2 genes of LASV, strain Josiah. Results from a large nonhuman primate study demonstrated that it was possible to protect the animals from death, but not viremia, by vaccinating with viruses expressing all three proteins, or with a combination of GP1 and GP2, but not with NP alone There was no correlation of protection with the antibody response elicited by the vaccines. While these studies with VACV-vectored vaccines clearly showed the potential for recombinant DNA-based vaccines against LASV, the live VACV-vector cannot be used in immune – suppressed humans, thus is not a suitable vaccine platform for LASV. In addition, in other studies with

recombinant VACV-vectored vaccines, pre-existing immunity to the VACV-vectored (i.e., smallpox vaccination) was found to interfere with the ability of the recombinant vaccine to elicit immune responses to the foreign-expressed gene product. Two other replication-competent viral vector approaches were tested more recently, one of which was derived from vesicular stomatitis virus (VSV) and the other from yellow fever virus (YFV). To generate the recombinant VSV vaccine, the gene encoding the normal VSV surface glycoprotein (G) was replaced with the LASV GPC gene; consequently, the recombinant viruses have GP1 and GP2 on the surface instead of VSV G. The recombinant YFV replication-competent LASV vaccine candidate was derived from the live-attenuated flavivirus YFV-17D human vaccine, which has been used to vaccinate millions of humans over more than 70 years. This platform has been used for construction of chimeric vaccines in which the envelope protein genes of similar flaviviruses (i.e., dengue, Japanese encephalitis and West Nile viruses) replace those of YFV-17D. Commercial versions of these vaccines (ChimeriVaxTM) have already been tested in Phase 2 and Phase 3 clinical studies. In a plasmid DNA vector, the gene of interest is cloned into a plasmid downstream of a promoter region which is recognized by mammalian cell polymerase, early promoter/enhancer which is transcribed by Pol II. A transcription termination and polyadenylation signal sequence is placed downstream of the gene. When the plasmid is introduced into a host cell, it is transcribed by host cell machinery to yield the gene products in a manner that is similar to authentic viral protein production. DNA vaccines can be introduced into animals in a variety of methods. The methods that facilitate the transport of the DNA across cell membranes, e.g., gene gun or electroporation, are the best options for eliciting strong immune responses. Through AdenoVirus based approach it was shown that rAd vectors could be easily manipulated to insert foreign genes of interest, rapidly grown to high titers, are thermostable for clinical use, and can transduce mammalian cells both in vitro and in vivo. Interestingly, there are approximately 51 serotypes for humans and 27 serotypes for simians (including seven from chimpanzees), but the human serotypes have been the most extensively studied and based on their sequence homology are further broken down into six subgroups 2.DNA Vaccines: Plasmid DNA vaccines are expressed within host cells and can elicit immune responses that correspond to those induced by infection. Unlike rAd vaccines, DNA vaccines have the additional advantages of circumventing issues relating to pre-existing immunity and safety in immunocompromised individuals. Further, multi-agent DNA vaccines can be developed and rapidly altered as pathogens evolve or new species emerge, are non infectious, and are easy to produce in large quantities. In Spite of the various aforementioned advantages of DNA vaccines there is a large disadvantage which the

researchers will have to counter if the Dna vaccines are to truly revolutionise the field of medicine that is the potential integration of the viral DNA with the cellular DNA resulting in unknown and potentially dangerous mutationsThe main advantage of DNA virus is the specificity of these vaccines and their long term effects as opposed to other conventional treatment methods.

3. rDNA Vaccines and Use in Cancer Immunotherapy (2016)

Immune system activation against cancer is a useful method in taking a fight against this deadly disease. rDNA vaccine platforms have a lot of applications in this field and are considered to be a novel way to fight against cancer. A DNA vaccine can be manipulated to express multiple antigens in a single plasmid vector and can generate a broad range of immune response. Immunization with DNA is done by introducing it into intramuscular, epidermal, mucosal, and intravenous routes. The insertion of the gene of interest into muscle fibers is done with the help of reporter genes which is β-galactosidase. The delivery method of DNA is very important to determine the amount of DNA required to induce immune response in animals. The gene-gun introduction of DNA is the most efficient mode of delivery of foreign DNA. The development of a DNA based vaccine to target cancer revolves around the introduction of tumour antigens into the host cells to produce an immune response that should target the tumour cells. The immune response induced against tumour cells is useful and effective because of its specificity. These vaccines can also elicit antigen specific humoral and cell mediated immune responses. The plasmids used in DNA vaccines are of bacterial or viral origin which signals the innate immune responses to form antibodies. The bacterial plasmid also serves as a ligand and stimulates Toll-like receptors (TLRs) which are membrane spanning proteins essential for recognizing pathogen-associated molecular patterns. Activation of TLRs signals a cellular pathway to begin a cascade of pro-inflammatory responses that leads to the production of various cytokines and attract other immune cells and induces specific immune responses. For the selective targeting of malignant carcinoma cells, DNA based vaccine encoding tumor associated antigens (TAAs) are developed and tested in clinical trials. A DNA based vaccine is a novel approach to counter many diseases. It can be effective against cancer if it can be made to express antigens exclusively limited to the tumor cells. The ability of DNA vaccine to induce long term immune response makes it a valuable asset as cancer as a disease is notorious for its relapse which can now be countered by immunological memory created against antigens by DNA vaccine. Genes coding for multiple antigens are inserted into a single plasmid from which a

wide range of immune response can be induced. Considering the advantages of DNA vaccine over other conventional vaccines, it can be the decent immunotherapeutic approach against cancer.

4. The Role of Recombinant DNA Technology in Vaccine Development (2016)

Recombinant DNA technology is one of the techniques used in genetic modification of organisms to help the organism code for a specific gene product. It is done by combining artificially obtained DNA from various organisms in one single organism. The term vaccine is derived from Latin word 'vacca' meaning cow after the first known vaccine for cowpox discovered by Edward Jenner. 'Techniques in genetic engineering include; the isolation, cutting and transfer of specific DNA pieces, corresponding to specific genes'. 1.Vectors Vectors are DNA molecules used to transfer a gene into a host (microbial, plant, animal) cell; and to provide control elements for replication, selection and expression. Artificial vectors are constructed by cutting and joining DNA molecules from different sources using various restriction endonucleases and DNA ligase. The minimal features of a vector consist of origin of replication, a selection gene (usually an antibiotic resistant gene), and a cloning site to introduce foreign DNA: 1.1 Yeast artificial chromosome (YAC) It is a vector used to clone DNA fragments larger than 100 kb and up to 3000kb. A YAC is an artificially constructed chromosome that contains a centromere, telomeres and an autonomous replicating sequence (ARS) element required for replication and preservation in yeast cells. ARS elements are thought to act as replication origin. A YAC is built using an initial circular plasmid, which is typically broken into two linear molecules using restriction enzymes. DNA ligase is used to ligate a sequence or gene of interest between two linear molecules, forming a single large linear piece of DNA. YACs can replicate and be selected in common bacterial and yeast hosts like Saccharomyces cerevisiae and Escherichia coli. 1.2. Bacterial artificial chromosomes (BACs) are based on fertility (F) factors, the naturally occurring sex factor plasmid of E.coli. They were developed as insert cloning systems to facilitate the development of DNA libraries. 1.3 Plasmids- Plasmids are small circular DNA molecules that can replicate independently from chromosomal DNA within a cell. It is found as dsDNA in bacteria, Archaea and some other life forms. They carry genes required for the survival of organisms like antibody resistance and can be transmitted amongst bacteria by horizontal gene transfer. 1.4 Bacteriophages -they are viruses which infect bacteria .they have a protein coated(Capsid) head made up of Icosamers within which DNA is housed.e.g Lambda phage. Recombination is Exchange of genetic information between chromosomes or other molecules of DNA. In all

cases of recombination, two DNA molecules are broken and rejoined to each other forming a crossover. Foreign DNA is inserted into host cells by combining the foreign DNA with DNA of a vector. If the recombinant DNA gets inside a host cell, it can replicate along with the DNA of the host cell. This means that every time the host bacterium undergoes cell division, each new daughter cell receives a copy of the recombinant DNA, thus amplifying the recombinant DNA with each cell division.

rDNA in Production of Antibiotics[5-10]

Sr.No	Title of Research Paper	Year of Publication
1	Production of 'hybrid' antibiotics by genetic engineering	1985
2	Application of Recombinant DNA Technology in Antibiotic-Producing Microorganisms.	1987
3	Recombinant Acremonium chrysogenum strains for the industrial production of cephalosporin	1996
4	Recombinant microorganisms for industrial production of antibiotics	1997
5	Improvement of Cephalosporin C Production by Recombinant DNA Integration in Acremonium chrysogenum	2010
6	Recombinant organisms for production of industrial products	2010

1. Production of 'hybrid' antibiotics by genetic engineering - 1985

The development of recombinant DNA technology in Streptomyces has culminated in the production of novel hybrid antibiotics by introducing antibiotic biosynthetic genes, subcloned on plasmids, into different antibiotic-producing Streptomyces strains. The isolation of biosynthetic genes for some of the many antibiotic producing genus of bacteria is possible through recent development of molecular cloning systems in Streptomyces. Novel antibiotics arise through the transfer of biosynthetic genes between Streptomycetes producing different antibiotics. The factors with which the hybrid compounds being produced depends upon the substrate specificities of the biosynthetic enzymes. In order to maximize the chance of success, strains producing different members of the same chemical class of compounds are used to demonstrate hybrid antibiotic production. Here, the production of novel antibiotic products obtained by gene transfer between strains producing the isochroman quinone antibiotics actinorhodin, granaticin and medermycin. The above-mentioned conclusions are made possible by the recent cloning of the entire set of genes for the biosynthetic pathway of actinorhodin from Streptomyces coelicolor. The demonstration of novel compounds in hybrid strains prepared by transferring actinorhodin genes into both granaticin and medermycin producer for the widespread usefulness of genetic engineering as a new tool in antibiotic discovery. The efficiency of transformed cultures, using standard conditions developed for

Streptomyces lividans and S. coelicolor 16, by DNA of the clones (isolated from S.coelicolor A3(2) derivatives) was only 1-10 per μg of plasmid DNA, compared with 10^6-10^7 in S.coelicolor. These decreased frequencies may consider the operation of restriction systems, as well as the fact that no attempt was made to improve transformation conditions for these strains. The two base products like dihydrogranatirhodin and the medderhodins are indeed products of hybrid biosynthetic pathways arising through close relatable interaction between enzymes encoded by structural genes originated from two different streptomycetes, rather than results of the activation of latent genetic information in the recipient strains. Antibiotic hybrids indeed have a promising future as a therapeutic strategy to overcome drug resistance or to expand the usefulness of our currently available hybrid antibiotics.

2. Application of Recombinant DNA Technology in Antibiotic-Producing Microorganisms - 1987

A large part of biotechnology cares with the invention and subsequent optimization of useful biological processes or products. Recent years have witnessed the transfer of academically established genetic techniques into the sensible, applied area of antibiotic production. Genetic improvement of antibiotic producing species was carried out through development of suitable cloning vectors. Those vectors were available within the major antibiotic producing groups and account for the progress to date in the cloning of genes involved in antibiotic biosynthesis. The plasmids available, together with antibiotic resistance genes found in antibiotic-producing strains were used to construct a range of plasmid-derived vectors with a number of different characteristics. From the aim of view of antibiotic production, such vectors have already been constructed from an honest range of species of Streptomyces, Bacillus and Pseudomonas. It appears that at this time little work involving the appliance of those vectors to antibiotic production by species of Bacillus or Pseudomonas is ongoing. Cloning of genes in primary metabolic pathways provide the precursors for antibiotic structure. Manipulating primary metabolic pathways by genetic engineering made changes in the enzymes actually, concerned with assembling the subunits of the antibiotic. The most commonly used technique to detect recombinant plasmids carrying cloned copies of antibiotic pathway genes was the complementation of antibiotic nonproducing derivatives with restoration of antibiotic production and bioactivity as the criteria for success. Numerous genes for antibiotic resistance identified in Streptomyces and such antibiotic resistance genes had been inserted into those plasmids yielding vectors which can be positively selected for and used to generate recombinants via insertional inactivation. Several new types of vectors

have also been developed, including promoter probe vectors as well as expression vectors such as pARC1 and pBGH007 respectively, thereby permitting the production of antibiotics. A very good cloning system was available for the minor antibiotic producing genera Bacillus and Pseudomonas. Species of bacilli produce mainly peptide antibiotics, B.subtilis being the main producer. The study of antibiotic biosynthesis applying recombinant DNA techniques would be greatly facilitated by parallel biochemical investigations of the pathway of interest. In this connection the utilization of "blocked mutants " has proved very effective within the study of secondary metabolic pathways.

3. Recombinant Acremonium chrysogenum strains for the industrial production of cephalosporin - 1996

Conventional strain improvement programs assist random mutagenesis and rational screening have meant beneficial results to the antibiotic producing companies. The development of recombinant deoxyribonucleic acid techniques and their applications to the industrially-used cephalosporin-producing fungus Acremonium chrysogenum has provided a replacement tool, supportive to classical mutation, advancing the planning of alternative biosynthetic pathways making it possible to get new antibiotics and to intensify cephalosporin production. Yield increases have been achieved by elevating the dosage of the biosynthetic genes cefEF and cefG or increasing the oxygen uptake by expressing a bacterial oxygen-binding heme protein (Vitreoscilla hemoglobin). New biosynthetic capacities like the assembly of 7-aminocephalosporanic acid (7-ACA) or penicillin G are achieved through the expression of the foreign genes dao (D-amino acid oxidase) association with cephalosporin acylase or penDE(acyl-CoA:6-APA acyltransferase) respectively. Narrow manipulation of the above-mentioned recombinant strains must be performed consistent with standing rules. A study on Penicillium chrysogenum disclose that 950 proteins involved in precursor biosynthesis, stress response and pentose phosphate pathway were found to be associated with the fermentation yield in three penicillin-producing strains. Several differentially expressed proteins that are associated with several metabolic pathways are of importance especially those involved in primary metabolism of A. chrysogenum. Comparative transcriptomics study revealed more information. A total of 4329 genes were identified that have significantly differential transcription levels in which 1737 were upregulated and 2592 were downregulated in HY strain. These differentially expressed genes belong to the pathways of carbohydrate metabolism and energy, defense and virulence mechanisms, CPC biosynthetic precursor amino acids, oxidative stress defenses, and other secondary metabolites. By identifying

differentially expressed proteins during CPC fermentation in wide-type strain and high-yield strain, a comprehensive regulation system of A. chrysogenum fermentation proposed based on the popular theory of metabolic engineering and systems biology because omics study has shown to be a powerful tool to study the metabolic pathway from a systemic scope. Because of the limitation of traditional techniques on strain improvement for A. chrysogenum, along with the ubiquitous applications of molecular biology, genetic engineering has become a powerful tool to manipulate the antibiotic producing strain and to obtain high-yield mutant strain. CPC production was surprisingly enhanced in the Acatg1 disruption mutant and PcbC expression was kept at high level all through the fermentation process. Results revealed that regulatory genes cefM and cefR were upregulated in the CPC high-producing strain.

4. Recombinant microorganisms for industrial production of antibiotics - 1997

The enhancement of commercial antibiotic yield has been achieved through technological innovations and traditional strain improvement programs supported random mutation and screening. The event of recombinant deoxyribonucleic acid techniques and therefore their implementation to antibiotic producing microorganisms has authorized yield increments and the design of biosynthetic pathways giving rise to new antibiotics. Genetic manipulations of the cephalosporin producing fungus Cephalosporium acremonium have incorporate yield improvements, accomplished increasing biosynthetic gene dosage or increasing oxygen uptake, and new biosynthetic capacities as 7-aminocephalosporanic acid (7-ACA) or penicillin G production. In Penicillium chrysogenum, the economic penicillin producing fungus, heterologous expression of cephalosporin biosynthetic genes has led to the biosynthesis of adipoyl-7-amino deacetoxy cephalosporanic acid and adipoyl-7-ACA, compounds which will be transformed into the economically relevant 7-ADCA and 7-ACA intermediates. Escherichia coli expression of the genes encoding D-amino acid oxidase and cephalosporin acylase activities has simplified the bioconversion of cephalosporin C into 7-ACA, eliminating the utilization of organic solvents. The genetic manipulation of antibiotic producing actinomycetes has allowed productivity increments and therefore the development of the latest hybrid antibiotics. A legal framework has been developed for the limited manipulation of genetically modified organisms. Much more promising results have been generated setting up novel fermentative processes for the development of economically relevant intermediates by biosynthetic pathway engineering in recombinant industrial strains. Studies on biochemical and genetic properties of different high producing strains inspected by independent laboratories have shown that the transcription level of the biosynthetic genes

and their corresponding enzymatic activities are closely related with the penicillin production capability. 7-ACA, the starting material for the production of many clinically important cephem antibiotics, is obtained from cephalosporin C by an enzymatic procedure involving DAO and cephalosporin acylase activities. Southern analysis of the DNA region covering the whole biosynthetic gene cluster have shown that it was amplified a number of times and in general correlated with the strain productivity.

5. Improvement of Cephalosporin C Production by Recombinant DNA Integration in Acremonium chrysogenum - 2010

Acremonium chrysogenum is used for industrial production of the B.lactam antibiotic cephalosporin C (CPC). Chrysogenum showed that most transformants had a higher CPC production level than the parental strain. Multiple transformants containing an extra copy of cefG showed a remarkable increase in CPC production. Chrysogenum transformed with pYG858 which contained a vgb gene. Fermentation results demonstrated that the CPC production of these recombinant strains had no obvious changes. Since molecular biology and genetic engineering techniques were applied to strain improvement, known as molecular breeding, production of a large variety of antibiotics has been further enhanced by introduction of biosynthesis gene encoding key enzyme or disruption of by-path metabolism. By introducing cefG and a more powerful promoter, cephalosporins titer was increased by 2–3-folds, in addition, more copy numbers of cefG resulted in more production of CPC. Gene cefT encoding a putative multidrug efflux pump protein was found to be capable of doubling CPC production in recombinant strain. CPC production of independent transformants suggested that the introducing genes were integrated into the chromosome for one extra copy, different integration sites may influence the final yields. cefT had a clearly positive effect on CPC production since cefT was not a limiting factor in high CPC producer because strain 84-3-87 has a much higher original CPC yield than C10. Compared to transformants bearing pYG858 and pYG877, there shows no significant improvement in CPC production when cafEF gene was introduced. Introduction of an extra copy of cafEF in Chrysogenum, could reduce the accumulation and increase CPC production. All the recombinant plasmids were verified by restriction enzyme digestion before transformed into A. chrysogenum 84-3-87 via PEG-CaCl2-mediated protoplast transformation. For instance, pYG877 was digested by several restriction enzymes including NotI, HindIII, EcoRI, BglII, SphI, and ClaI. CPC biosynthesis pathway necessitates multiple steps of high oxygen consumption. Previous research study demonstrated that conveying Vitreoscilla hemoglobin in CPC producing strain

led to 4–5-folds increment in CPC production. Phleomycin- and hygromycin-resistant genes are the most frequently used markers. Albeit, geneticin has shown to be a suitable selection marker, the vector used has the advantages in heterologous genes introduction of A. chrysogenum and also in genetic engineering of filamentous fungi.

6. Recombinant organisms for production of industrial products - 2010

Traditional industrial microbiology was merged with molecular biology to getting improved recombinant processes for the industrial production of primary and secondary metabolites, protein biopharmaceuticals and industrial enzymes. Functional genomics, proteomics and metabolomics are being exploited for the invention of novel valuable small molecules for medicine also as enzymes for catalysis. Genetic recombination was virtually ignored in industry, mainly due to the low frequency of recombination. When an industrial production strain of A. chrysogenum 394-4 was transformed with a plasmid containing the pcbC and the cefEF gene from an early strain of the mutant line, a transformant producing 50% more cephalosporin C than the assembly strain, also as less penicillin N, was obtained. A transgenic wine yeast genetically engineered to contain six copies of the bovine L-lactate dehydrogenase gene produced L-(+)-lactate at 122 g l-1.71 Whole genome shuffling was used to improve the acid-tolerance of a commercial lactic acid producing Lactobacillus sp. Further approaches using the recursive protoplast fusion technique yielded strains of Lactobacillus rhamnosus ATCC 11443 with improved glucose tolerance, while simultaneously enhancing L-lactic acid production by 71% as compared to the wild type. Ethanol constituted over 95% of the fermentation products in the genetically-engineered strain. Its cycle of fermentation was not only abridged, but an increment in ethanol yields up to 11% in contrast with the control in very-high-gravity (VHG) fermentations. The increments were 95% in production of acetone, 37% for butanol, 90% for ethanol, 50% for solvent yield from glucose and a 22-fold lower production of undesirable acids. On top of 60% of the enzymes used in the detergent, food and starch refining industry are recombinant proteins. Recombinant DNA technology has been used effectively in the enzyme industry in the following ways: to put together in industrial organisms' enzymes obtained from microbes which are hard to grow or alter genetically, to increase enzyme yield by use of multiple gene copies, strong promoters, and well organised signal sequences, to produce in a safe host useful enzyme procured from a pathogenic or toxin-producing microorganism and, to improve the stability, activity or specificity of an enzyme by protein engineering.

rDNA in Distant Hybridization[11-18]

Sr no.	Title of research paper	Date
1	Evolutionary Role of Interspecies Hybridization and Genetic Exchanges in Yeasts	2012
2	Distant Hybridization: A tool for interspecific Manipulation of Chromosomes	2013
3	Research advances in animal distant hybridization	2014
4	rDNA Genetic Imbalance and Nucleolar Chromatin Restructuring	2015
5	A new type of homodiploid fish derived from the interspecific hybridization of female common carp × male blunt snout bream	2017
6	Comparative study of distant hybridization in plants and animals	2017
7	Current Perspectives on Introgression Breeding in Food Legumes	2021
8	Distant Hybridization: Types and Applications | Crop Improvement | Botany	NA

1. Evolutionary Role of Interspecies Hybridization and Genetic Exchanges in Yeasts

Rephrased Title: Combating Meiotic fertility in Yeast using recombinant DNA**.** One of the first indications of the existence of natural interspecies hybrids in yeasts came from an early genetic characterization of a commonly used brewer's yeast strain, Saccharomyces carlsbergensis. Use of cerevisiae for molecular genetic studies, stimulated numerous investigations on the Saccharomyces sense stricto complex of species and their hybrids during the last decade. Contrary to classical expectations, the main cause of reproductive isolation between Saccharomyces species is not the genetic incompatibility between the two parental genomes, but the combination of other molecular mechanisms. The chromosomal translocations observed between the different Saccharomyces sensu stricto genomes do not play a major role in the meiotic infertility of hybrids. In general, a smooth and monotonous relationship between meiotic fertility and the degree of sequence divergence between the parents of a crossing is observed. In general, a mild monotonous relationship is observed between meiotic fertility and the variance in sequence deviation between the parents of a cross. In this case, a deep sequencing study of 11 diploid strains from wild, viticulture, or clinical origins revealed the presence of multiple large homozygous regions within the otherwise heterozygous genomes. In this case, possession of one or another member of a pair of genes (called "OHNOLOGS" to distinguish them from other classic paralogues) leaves the remaining active copy of the gene on different chromosomes in each of the two species. By analogy to the recombination-dependent chromosome loss mechanism (RDCL) proposed to explain infertile meiosis of diploid Saccharomyces hybrids, formation of chimeric chromosomes has been suggested. Whether the differences between these parental pools are adaptive or not has been discussed elsewhere, but the very limited number of DM incompatibility genes presently identified in yeasts in Yeast Interspecific Hybrids and

Genome Evolution suggests that this reshuffling may be extensive and that, at least in yeasts, hybridization may play a greater role in the formation of novel species than generally considered.

2. Distant Hybridization: A tool for interspecific Manipulation of Chromosomes

Rephrased Title: rDNA in controlling inheritance of chromosomes**.** The main goal of hybridization is to provide genetic variation when two multiple plants are genotypically assembled in F1. The degree of variation generated in the separate generations depends on the number of heterozygous genes in F1, which in turn depends on the number of genes for which the two parents differ. Most efforts to transfer a useful feature of wild plants to crops have so far closed the species gap by exotic chromosome translocation lines. The duplicated chromosome in somatic cells or gametes of F1-Hybrids, followed by the installation of all aliens of chromosomes, was used in the production of amphidiploids. Amphidiploid can be used as a bridge to move the individual chromosome from a species to another or develop new plants. Chromosome deletion of an uniparian genome during the development of F1-hybrid embryos was used in the production of haploids. Hybrids that produce clonal gametes are common in plants and invertebrates; Invertebrates often reported on amphibians, fish and reptiles, but not about birds or mammals. It can also be helpful to understand how different polyploid forms of hybrid origin can persist over long periods of time. Interspecific hybridization could also be used for developing a replacement variety, e.g., Clinton oat variety was developed from a cross between cereal oat x A. byzantina (both haploid oat species), and CO 31 rice variety was developed from the cross cultivated rice var. indica x O. perennis. Segregation and recombination produce an outsized number of genotypes in For the amount of various genotypes possible in F2 increases geometrically with a rise within the number of segregating genes. Homozygosity increases rapidly with continued selfing. The frequency of absolutely homozygous vegetation additionally will increase rapidly. By F7, approximately seventy three percent of the vegetation grow to be absolutely homozygous even if 20 genes are segregating. The increasing frequency of interspecific hybridization due to anthropogenic causes and global climate change is a growing problem in nature conservation biology, where efficient instruments are particularly welcome on the projection of consequences for demographic species.

3. Research advances in animal distant hybridization

Rephrased Title: rDNA in Fish Distant hybridisation. A genetic cross between two unrelated species or higher ranking taxas forming recombinants is known as Distant Hybridisation and results in rDNA which have interspecies genome and phenotype, genotypes according to two different unrelated species these repetitive rounds of genome alterations have enabled researchers to track the etymology of some organisms. Recombinant DNA using distant hybridisation can be formed inter-class, inter-families, inter-phylum, inter-genera and inter-species. Several factors can lead to a dearth of fertile progenies such as we are going against the standard definition of species by performing interspecies hybridisation, some early-stage recombinants such as in F1 or F2 generation have poorly developed sexual glands but the gametes are fully viable and we can store them for later use or even in some cases the investigation for the case is not done properly.

One major observation by the author is that whenever the chromosome number of a female parent is higher, the progeny formed is stable and fertile. Polyploidisation after recombination is a matter of great concern as the next set of a genetic pool depends on it. Recombinants with triploid polyploid offer an advantage that cannot contaminate the natural genetic pool as they are sterile, as well as they are more tolerant towards diseases. It is muddled whether physical cell DNA recombination is detectable from atomic record levels in more intently related fish mixtures**.** The genotypes of parental DNA for ideal distant hybridization should be identical. This helps in a stable inheritance of genetic material. Several other factors such as metabolism rate, reproductive cycles, phylogenetic relationships, diet, size etc. must also be noted. Here was a model describing this frequent but appallingly studied distant hybridization to assess its consequences on parental species and to anticipate the conditions under which they can reach fertility. This general model is useful to better comprehend the evolution of such hybrid systems and demonstrated its importance in the field of conservation biology to set up management recommendations when this increasingly frequent type of hybridization is in action.

4. rDNA Genetic Imbalance and Nucleolar Chromatin Restructuring Is Induced by Distant Hybridization between Raphanus sativus and Brassica alboglabra

Rephrased Title: Chromatin Restructuring between Raphanus sativus and Brassica alboglabra using rDNA**.** Hindrance of methyltransferases prompts changes in nucleolar design, ensnaring a vital job of methylation in charge of nucleolar predominance and fundamental

nucleolar arrangement progress. These genetic stocks or lines are useful in plant breeding Epigenetic changes occur in allopolyploids that result from distant hybridization events. In contrast, the silenced rRNA genes appear as heterochromatin, with characteristics including H3K9 methylation, histone hypoacetylation and DNA methylation. Hereditary lopsidedness actuated by far off hybridization connects with development of rDNA qualities (NORs) in the half and halves between Raphanus sativus L. furthermore, Brassica alboglabra Bailey. Also, expanded CCGG methylation of rDNA in F1 cross breeds is attending with Raphanus-inferred rDNA quality hushing and rDNA transcriptional dormancy uncovered by nucleolar design limitation. Recently shaped rDNA quality locus happened through chromosomal in F1 crossovers through chromosomal lopsidedness. NORs are acquired anew, lost, and additionally rendered in the new genome. Genomic DNA isolation and Southern blot analyses Seeds of Raphanus sativus and Brassica alboglabra were surface sterilized with 75% ethanol for 30 seconds, rinsed with sterile water, and then planted in a growth cabinet with 16h light at 22 °C. Spatial statistical analysis of rDNA in nucleolus Spatial statistical analysis of rDNA was carried out on transmission electron micrographs using an Image-Pro Plus software. RT-PCR was performed with a template of cDNA from reverse transcription of total RNA in leaves of Raphanus sativus, Brassica alboglabra and F1. Comparison of RT-PCR products from F1, Raphanus sativus and Brassica alboglabra was performed with Clustal x to determine nucleolar dominance. These results indicate that the methylated states of DNA in RNA genes may play a role in silencing the DNAR gene in this system of distant hybridization between the sexes. Considering that rRNA genes from Raphanus were expressed and those from Brassica were silenced, it is suggested that gene dosage was not the key factor in controlling rRNA gene expression, and that chromosomal remodeling and rearrangement during distant hybridization and polyploidy formation were predominant.

5. A new type of homodiploid fish derived from the interspecific hybridization of female common carp × male blunt snout bream

Rephrased Title: New progeny of homodiploid carp and snout bream using rDNA technology. A few investigations have given significant direct proof supporting the finding that removed hybridization can prompt the arrangement of allotetraploid and autotetraploid fish just as allodiploid fish. It is regularly accepted that hybridization may prompt the arrangement of new polyploidy species, yet it is indistinct whether hybridization can deliver another homodiploid species. FISH and 5S rDNA investigations uncovered that the genotype of the crucian carp-like homodiploid fish contrasts from those of its folks yet is firmly

identified with that of diploid crucian carp. Incompatibilities due to differences in chromosome number between the parent species can result in genomic shock in the F1 offspring of distant hybridization, which might lead to their mortality. Homoploid hybrid speciation (HHS) is an important mode of speciation derived from interspecific hybridization that does not alter the chromosome number 7, 8.The crossing procedure is as follows: 1]] The diploid natural gynogenesis common carp obtained from the 2nCOC (♀) × 2nBSB (♂) cross was denoted 2nGCOC, the diploid scattered mirror carp (a variety of common carp) derived from 2nCOC (♀) × 2nBSB (♂) was abbreviated 2nGMCC, and the tetraploid hybrid produced by 2nCOC (♀) × 2nBSB (♂) was denoted 4nCB. 2]] The 2nCOC (♀) × 2nBSB (♂) cross resulted in four offspring: the new crucian carp-like homodiploid fish, a diploid natural gynogenesis common carp, a diploid gynogenesis scattered mirror carp (a variety of common carp), and a tetraploid hybrid. 3]] The 2nCOC (♀) × 2nBSB (♂) cross showed high fertilization (85.8%) and hatching (72.1%) rates, but the offspring presented a relatively low survival rate (38.9%). The same-species mating of 2nCOC resulted in fertilization, hatching, and survival rates equal to 90.6, 86.3, and 78.9%, respectively, and that of 2nBSB showed rates of 92.9, 88.2, and 73.4%, respectively. Hereafter, the new crucian carp-like homodiploid fish produced by 2nCOC (♀) × 2nBSB (♂) is referred to as 2nNCRC, the self-cross offspring is denoted 2nNCRC-F2, and the subsequent self-cross offspring is called 2nNCRC-F3. The reverse cross of 2nBSB (♀) × 2nCOC (♂) did not produce any living progeny, and the 2nNCRC self-cross resulted in a single offspring. The unusual genomic DNA variations observed in 2nNCRC, which exhibited very low survival rates, suggest the effects of genomic incompatibilities in the process of homodiploid fish speciation

6. Comparative study of distant hybridization in plants and animals

Rephrased Title: Distant Hybridization in plants and animals using rDNA. Analysis and summaries of many interrelated documents in plants and animals, both nationally and internationally, including examples and a long-term study of remote hybridization in the fish of our laboratory, we grasp and compare the similarities and differences in the plant and long-range hybridization. Aspida to a large body analysis of the national and foreign literature on hybridization of distant animals and animals, and on the results of the author's laboratory on distant fish hybridization for a long time. The allopolyploidization resulting from the hybridization can underlie the dynamics of the new species formation, the agricultural hybridization is the main mediator of the evolution of the genome and the formation of new species**.** Genomic in situ hybridization (GISH) involves the use of genomic DNA from

heterologous species as a probe. Reproductive and selection revealed that the offspring of the various hybridized populations were all males (Panonychus Citri is a parthenogenetic and androgenic), suggesting that these two species have been subjected to reproductive isolation due to long-term selection pressures of host plants, which are Two species capable of producing healthy fertile offspring, but not different ecological areas or niches that can achieve remote hybridization. The reproductive isolation of remote hybridization between species generally involves a variety of mechanisms that work together and, according to the period of time, reproductive isolation in remote hybridization of animals and plants can be divided into precoral and postzygotic reproductive isolation, and isolation tests. The allotetraploid of the crucian carp red nucleus exhibits a dumbbell pattern. The somatic chromosome count assay is the simplest and most accurate method. Indirect assays primarily involve flow cytometry and determination of cell size by morphological analysis. The single repeat sequences of centering were used as red carp probes. Flow cytometry can be used to accurately determine nuclear DNA content by comparing reliable identifications of ploids.

7. Current Perspectives on Introgression Breeding in Food Legume

Rephrased Title: rDNA for genetically modified legume: Wild gene introgression as a reproducing methodology has been sent effectively in food vegetables for advancement of improved assortments, pre-reared lines, hereditary stocks, planning populaces, and extension species. The narrow genetic base of the elite genepool of food legumes and resultant vulnerability of the existing varieties to climate vagaries and changing insect-pest and disease scenario warrants introgression of novel genes or alleles through hybridization and deployment of more diverse germplasm including exotic lines and CWR(crop variety relatives) in crop improvement programs. Horizontal gene transfer from wild species and even across different genera has played a significant role in the evolution of eukaryotic genomes as wild species have evolved through different degrees of selection pressure exerted by environmental forces and biotic agents over a long period of time. In AB-QTL methodology, the revelation and further exchange of positive QTLs from unadapted foundation to first class pool are a solitary advance interaction where QTL investigation is acted in later ages to encourage sound measurable force for location of QTLs with little impact. Abdominal muscle populace was produced for improving the mineral status of the Andean assortment "Cerinza," a huge red cultivated shrubbery bean cultivar with wild genotype "G10022." The huge size of germplasm assortment, raisers' inclination for supreme × supreme crosses because of clear favourable circumstances of their flexibility to nearby

conditions, presence of enigmatic hereditary variety, and the linkage drag related with moving qualities from wild family members are different elements related with confined utilization of germplasm. This system revealed 13 QTLs for plant tallness, yield, and yield-crediting attributes alongside location of a QTL for seed size from the wild parent. The genetic base of the elite genepool of food legumes and resultant vulnerability of the present varieties to climate vagaries and ever-changing insect-pest and illness situation warrants introgression of novel genes or alleles through hybridisation and preparation of a lot of various germplasm as well as exotic lines and CWRs in crop improvement programs.

8. Distant Hybridization: Types and Applications | Crop Improvement | Botany

Rephrased Title: rDNA for Crop Improvement**:** Distant hybridization refers to the crossing between two different species of the same genus or two different genera of the same family, and such crosses are called distant crosses or wide crosses. Interspecific hybridization involves crossing or mating between two species of the same genus, it is also termed as intergeneric hybridization. Some of the various techniques used in acquiring zygote from distant crosses include choice of parents, reciprocal crosses, manipulation of ploidy, bridge crosses, use of pollen mixtures, manipulation of pistil, use of growth regulators, large number of crosses, protoplast fusion, embryo culture, grafting etc. Numerous modern cultivars of sugarcane have been developed from crosses of Saccharum officinarum with S. spontaneum or S. barberi. These crosses combine for high sugar content of S. officinarum with the disease resistance, cold tolerance and vigour of S. spontaneum and S. barberi. Cytoplasmic male sterility has been moved from wild species to developed ones in wheat, grain, cotton, tobacco, ryegrass and a few different yields. Resistance to boll weevil and jassids in cotton and leaf chewing insects in peanuts has been incorporated from wild species with interspecific hybrids developed for commercial cultivation in sugarcane. Distant crosses are often diagnosed with problems of cross incompatibility, hybrid inviability, hybrid sterility and hybrid breakdown which pose a severe threat to interspecific or intergeneric gene transfer. A few exceptional methods like ploidy control, pistil control, synthetic (development controller) treatment, connect crossing, joining, undeveloped organism culture and so forth must be utilized to make inaccessible half and halves effective now and again.

CONCLUSION

This research review’s purpose is to help the reader understand different aspects posed by the research on the principles of recombinant DNA technology . This is significant because it

gives insights about the applications of rDNA technology in the production of vaccines, antibiotics, hormones and distant hybridisation. There has been much research and discussion conducted on these opinions of recombinant DNA technology and its applications and principles**.** Most of the research found was on the recombinant DNA in vaccines for cancer immunotherapy and lassa and filo virus, production of antibiotics using Acremonium chrysogenum strains for rDNA, treatment of diabetes using insulin analogs, production of various hormones like human erythropoietin, gonadotropins, etc. and use of this technology in distant hybridisation for manipulation of chromosome and nucleolar chromatin restructuring in plants and animals. More research and testing is required to gain a better understanding of the principles of recombinant DNA technology.

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8. Ethics Approval and Consent To Participate

Not applicable.

9. Human and Animal Rights

No Animals/Humans were used for studies that are base of this research.

10. Consent For Publication

Not applicable.

11. Availability of Data And Materials

The author confirms that the data supporting the findings of this research are available within the article.

12. Funding Acknowledgement And Conflict Of Interest

The authors whose names are listed immediately above certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-

licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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