

Strain Improvement of Yoghurt Starter Cultures for the Use in Food & Dairy Industry

Birajadar Suryakant Ramchandrappa¹, M.Rajashekhar², R. Kelamani Chandrakanth³, K Vijaykumar⁴

¹Department of Botany, S.S.Margol Degree College, Shahabad, Kalaburagi Dist, Karnataka, India

^{2,4}Department of Zoology, Gulbarga University, Kalaburagi, Karnataka, India

³Department of Biotechnology, Gulbarga University Kalaburagi, Karnataka, India

Abstract—The dairy and food industry is continually endeavoring to grow new items to satisfy the perpetually changing requests of buyers and the strict necessities of administrative offices. For food based on microbial aging, this pushes the limits of microbial execution and requires the consistent improvement of new starter societies with novel properties. Since the utilization of fixings in the nourishment business is firmly managed and under close examination by shoppers, the utilization of recombinant DNA innovation to enhance microbial execution is at present impossible. Therefore, the center for enhancing strains for microbial aging is on traditional strain change strategies. Here we audit the utilization of these strategies to enhance the usefulness of lactic corrosive microscopic organisms starter societies for application in modern scale nourishment creation. Techniques will be portrayed for enhancing the bacteriophage resistance of particular strains, enhancing their composition shaping capacity, expanding their resilience to push and tweaking both the sum and character of acids created amid maturation. What's more, ways to deal with killing undesirable properties will be portrayed. Methods incorporate arbitrary mutagenesis, coordinated development and prevailing determination plans.

Keywords—Yoghurt, Lactic acid Bactria, Food industry

I. INTRODUCTION

Lactic acid bacteria (LAB) are gram –positive bacteria that include *Lactococci*, *Streptococci* and *Lactobacilli*, all of which have long been used as starters for food fermentations Caplice et, al (1999). As such, live LAB are regularly and widely consumed by human's and animals, Although numerous in VIVO beneficial effects of LAB have been claimed only a few have been unambiguously demonstrated experimentally Martean , P. et, al (1993) and Salminen et, al (1998).

By definition yoghurt is fermented milk with custard like consistency flavoured or non flavoured product fermented by *Streptococcus thermophilus* & *Lactobacillus bulgaricus*.

1.1. Historical Development of Yoghurt

The origin of yoghurt is not very clear it was known to ancient turks who lived as nomads, Balkans and slavs, yoghurt has higher nutritive value compared to other fermented milks because of its higher milk solid contents, The essential bacteria used in mild fermentation for yoghurts are *S.thermophilus* and *L.bulgancus* which are grown in approximately equal number to obtain most desirable consistency and flavour. The yoghurt & cultured, milk are virtually identical with the first stage of manufacture of curd and soft cheeses, when no rennet is used. The traditional yoghurt was made by heating milk in open pans; concentrating it in this way to 2/3 volume. The higher solids content milk, if used, would give a thicker or more viscous yoghurt, sheep milk, if used, would also give a thicker yoghurt because it is about 50% richer in solids than ordinary cow milk.

Yoghurt is generally made from a mix of standardized whole, partially defatted milk, condensed skim milk, cream and nonfat dry milk, Alternatively, milk may be partly concentrated by removal of 15-20% water in a vacuum pan, supplementation of milk solids not fat with non fat dry milk is the preferred industrial procedure, (All dairy raw materials should be selected for high) bacteriological quality.

Ingredients containing mastitis milk and rancid milk should be avoided. All workers agree that yoghurt is basically a culture of *L.bulgaricus* (Metchnikoff 1907, Orla-Jenson 1919) and at least in recent years, growing in association with *S.thermophilus* (Vanenberg, 1993). The two organisms are mutually beneficial . *S.thermophilus* by removing oxygen and producing the weak acid conditions favouring *L.bulgaricus*, and the casein, Although the lactobacilli be considered more proteolytic, & thermophilus

produces four peptidases in addition to a general caseolytic activity (Langsrud and Reinbold, 1974).

Both organisms convert nearly all the sugar to lactic acid, producing only trace amounts of by products. These are however, very important for yoghurt and are responsible for the 'roundness' of the characteristic yoghurt flavour, *S.thermophilus* by producing diacetyl and *L.bulagaricus* by producing acetaldehyde (Molimard and Spinner, 1996). Acetaldehyde production is decreased if 8% or more sucrose is present (Bills et al 1972).

Commercial production of yoghurt in India dates back to early seventies. However, it is not so popular fermented product being marketed in our country. The present demand for yoghurt in india is about 23 thousands tonnes. The demand for quality yoghurt & other fermented product from the urban population is increasing day by day (Sukumaran, 1982). But the preparation of quality yoghurt depends on the starter culture because the preparation of desired type of products in yoghurt depends on the ability of selected starter cultures to produce acid from lactose and to degrade milk proteins.

1.2. Strain Development

Most organisms used in industry for producing antibiotics, enzymes, amino acids, vitamins, and other chemicals are **mutants** derived from wild-type organisms that initially produced small amounts of the substances desired, and have been genetically altered to produce the product of interest in an unregulated manner. **Wild-type** organisms are used primarily in the food industry for making dairy products like cheese and yogurt or for alcoholic fermentations.

The traditional sequence of events that lead to the creation of a production strain that makes, for example, an antibiotic has been discovery through screening of wild type strains, followed by continual improvement through **mutation programs** and the constant search for optimized "fermentation" conditions. It is important to note that, from historical usage, an industrial "fermentation" can mean both aerobic and anaerobic processes. Discovery and strain improvement programs have traditionally been labor intensive, brute force, occupations. Typically, thousands of organisms are screened for novel compounds. Strains that show some promise are randomly mutated until **production strains** are created that yield the desired product at high levels and that behave well during scale-up to large volume fermentation vessels.

Recent decades have seen the introduction of **genetic engineering** technologies to the armamentarium of applied microbiologists. These technologies have allowed scientists to manipulate rationally organisms to produce more of a

given product. In addition, entire metabolic pathways or portions of pathways have been transferred from one organism to another as a way to optimize production or create novel compounds. In cases where donor organisms have not been isolated, **DNA** has been harvested from environmental samples and cloned into another organism where genes of interest are screened and expressed.

Today, developing a wild-type microorganism to create a production strain generally involves parallel approaches of optimizing the fermentation environment, and by altering the organism through mutagenesis or recombinant **DNA** technology. The actual mix and timing of approaches used in environmental optimization and genetic manipulation depends to a great extent on the desired product, on the cost of production, and increasingly on rules governing the use, release or consumption of genetically modified organisms.

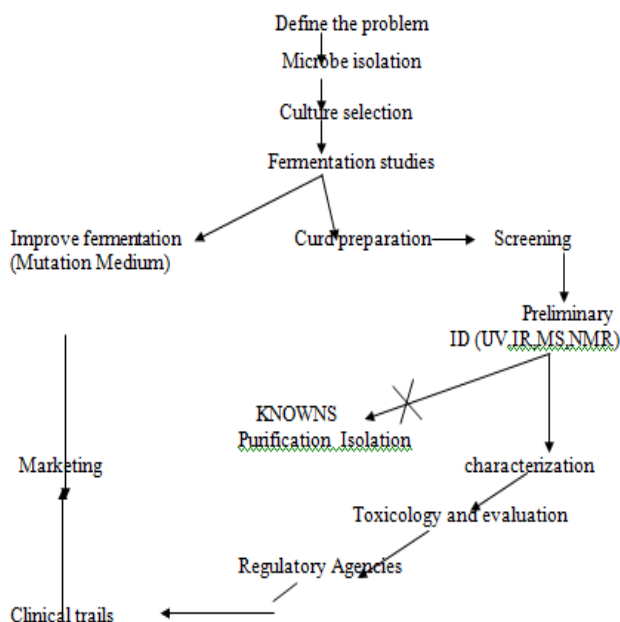
Why manipulate the microorganism? The simplest reason is to make more products at less cost. That is, the goal is to increase the **volumetric productivity**, or **Qp**, of a fermentation reaction. The **Qp** of the fermentation is defined in terms of grams product/liter/hour, simply the rate of product formation. As can be seen in the figure above, as the concentration of cells in a fermentor increases to a maximum (**Xmax**), the volumetric productivity will increase to a limit defined as the **Qpmax**. The limit of cell growth depends on several factors including delivery of nutrients (carbon, nitrogen, oxygen etc.) to the microorganism, and removal of wastes from the vicinity of the microorganism. As a practical matter, the maximum cell yields of optimized fermentations run in the neighborhood of 200 g/l and usually much less.

The limit of volumetric productivity (**Qpmax**) depends on the number of cells present and also on the efficiency of product formation by the cells. Thus, mutants that produce more product at the same cell density as the wild type will provide a higher volumetric productivity and thus yield more at less cost in a shorter period of time. The **specific activity** (**Sd**) of the reaction therefore changes to a more favorable dimension, where **Sd** is defined as $\frac{Qp}{X}$ or (g product/l/hr)/(g cells/l) –g product/h/g cells. This is the description of the slope in the figure above.

The bottom line for an industrial microbiologist is to create organisms that have an optimized specific activity to yield the most product per unit cost per unit time resulting in lower raw material costs, less down-stream processing and higher yields. This contribution defines the niche many microbiologists have in industrial processes.

II. SCREENING PROGRAMS

The general process of new product development in industry occurs as follows:



Perhaps the most critical first step in any industrial process is to carefully define the problem to be addressed. Sometimes the process of definition is relatively easy, for example in the early days of antibiotic discovery scientists were looking for a “magic bullet” that would kill pathogenic bacteria while having no effect on humans. In later days, questions have become more complex. For example, a search for antibiotics will likely yield many “known” compounds as well as a few new ones that can be further studied for their toxicity, effectiveness and spectrum of activities that may include antimicrobial, antitumor, antiparasitic, immunoactive, herbicidal, insecticidal or other activities. With a greater understanding of biology has come the potential to focus question and approaches ever more narrowly in the quest for new compounds. For example, the rise of antibiotic resistance has led to a search for compounds that either resist or circumvent bacteria defenses. The age of genomics has added a new and perhaps immense dimension by providing thousands of new biochemical targets for interaction with secondary metabolites of microbial origin.

2.1. Screening for Novel Metabolites

Obtaining a new microorganism from environmental samples requires a certain amount of ingenuity and persistence. Traditionally, plating techniques were used to isolate organisms from soil samples collected from

throughout the world. The joke in graduate schools of the 60s and 70s was that students often paid for tips to exotic parts of the world by volunteering to collect samples from mountain tops, dry valleys, beaches, and other environments while enjoying a break from the laboratory. Indeed, diversity of environmental sampling frequently leads to the discovery of novel organisms, and this search for diversity is the first step in the process of screening for novel metabolites.

Microorganisms inhabit and have adapted to virtually all areas of the earth. Their diversity reflects the sheer number of ecological niches to which they have adapted, so it makes sense that the microorganisms “out there” have yet to be fully sampled. It is almost impossible to estimate the number of “useful” compounds remaining to be discovered. In the case of antibiotics from *streptomyces*, statistical methods have been used to estimate the approximate number remaining to be discovered. Based on the rate of discovery of new compounds through the years. Somewhere around 150,000 to 300,000 compounds were estimated to be produced by *streptomyces sp.* Alone with only about 4500 compounds already described. Thus, the continued screening of *streptomyces* seems to be a relatively efficient way to come up with more compounds. However, since “old” compounds are rediscovered at an increasing rate as more are characterized, novel approaches for screening need to be used for discovering “new” compounds.

Screening protocols that are used for isolating new organisms basically are a variation on the theme of diluting, plating, fermenting in small shake flasks and screening culture supernatants. The variations include different sources of soil, water or sediments, different growth media, different pre selection treatments and different screening methods.

In addition to traditional culture-based screening methods, newer approaches circumvent the necessity to cultivate a novel organism. It is possible to isolate **DNA** directly from environmental samples and express a gene or genes in a test organism. Such an approach is being used by companies or industries.

2.2. Screening Mutants

Once an organism has been selected from its peers for its unique abilities, the process of strain improvement can proceed. Traditionally, the procedure used for this process entails several iterations of the following three steps:

- 1) Mutation to introduce genetic variability
- 2) Fermentation of several individual mutants

- 3) Selection of the most effective mutants by analysis of fermentation broths

The process is repeated several times over until an acceptable strain has been obtained. This procedure is essentially that followed during the first “strain improvement program” instituted by a famous microbiologist Charles Thom. He worked with **penicillium** species to improve the production of penicillin in the 1940s.

2.3. Mutagenesis

In discussing mutagenesis it is important to remember that a **mutation** is a change in genotype at the level of the **DNA** sequence. Not all mutations lead to an identifiable change in phenotype. Indeed most mutations are simply not observed either because their effect is neutral, or because their effect is lethal and the cells do not grow. Unless introduced by an error in **DNA** copying which is extremely rare, mutations are generally preceded by a **premutational structural change** in the **DNA**. This change is a chemical change that is caused by chemical or physical agents. The premutational structural change can include **DNA** alkylation, deamination, inter or intrastrand cross-linking or other changes that, if uncorrected, will lead to a mutation. Such lesions are not mutations. They lead to mutations if they remain uncorrected and cause the incorporation of incorrect bases during replication.

Thus mutations only arise if a premutational structural change is not corrected by one of many **repair systems** in the cell, and if **DNA** duplication leads to the insertion of an incorrect base. A mutation is referred to as a **transition** if a purine is replaced by another purine ($T \leftrightarrow C$), or a pyrimidine is replaced by another pyrimidine ($A \leftrightarrow G$). a mutation is referred to as a **transversion** if a purine is replaced by a pyrimidine or vice versa ($T \text{ or } C \leftrightarrow A \text{ or } G$).

Mutagens increase the frequency of mutations. Some mutagens are more potent than others since they are more chemically reactive, or cause changes more likely to lead to mutant formation. All mutagens must be handled with care since they do not discriminate between **DNA** from microorganisms or, say, humans. Various types of chemical and physical mutagens are available. Some examples of both are given below.

2.3.1. Base Analogs

Base analogs look like normal nucleotide bases and so may be incorporated into replicating DNA. Once incorporated, they may base-pair with different bases than the normal ones, leading to base substitutions. Common examples include 5-bromouracil that is incorporated as 5-bromo-deoxyuridine(5-BU). The keto tautomer pairs with A, but the enol tautomer pairs with G. thus, in the round of

replication after 5-BU is incorporated there is a chance that $AT \rightarrow GC$ or $GC \rightarrow AT$ transitions will become fixed in the DNA. In practice, analog mutation is infrequently used in industry since setting up the conditions for mutation can be complicated. For example, incorporation of 5-BU is generally done using thymine auxotrophs and such organisms may have undesirable qualities for growth in large scale fermentations.

2.3.2. Alkylation

Several chemicals are used as reagents to directly modify DNA with a methyl or ethyl group to alter base pairing characteristics. Such “alkylating chemicals” include ethyl methane sulfonate (EMS), methyl methane sulfonate (MMS), diethylsulfate (DES), and nitrosoguanidine(NTG). When added to cultures, they tend to cause lesions at G-rich regions leading to depurination or methylation. Because of their drastic effect on DNA, many of these substances induce the **SOS** or **Error-prone** repair systems. The most common effect of these mutagens is to cause base substitutions but they also cause a variety of other mutations by virtue of their ability to induce the error-prone repair system.

2.3.3. Nitrous Acid and deaminating agents

Nitrous acid (HNO_2) treatment leads to oxidative deamination of adenine to produce hypoxanthine, of cytosine to produce uracil and of guanine to produce xanthine(while still continues to pair with C). Since hypoxanthine pairs with cytosine, and uracil pairs with adenine, $AT \rightarrow GC$ and $GC \rightarrow AT$ transition mutations are generated. Other deaminating agents are hydroxylamine(NH_2OH) and bisulfite (HSO_3). Only nitrous acid can enter cells and is therefore used for **in vivo** mutagenesis. Bisulfite reacts with ssDNA. Hydroxylamine can be used to mutate DNA **in vitro** where it specifically induces $GC \rightarrow AT$ transitions.

2.3.4. Intercalation Mutagens

Some dyes such as acriflavine and ethidium bromide exert a mutagenic effect by intercalating between the stacked bases on DNA leading to formation of “frameshift” mutations where a base is inserted where none existed before. These agents require DNA synthesis to occur before become fixed.

2.3.5. UV Mutagenesis

Instead of using chemical agents that are somewhat dangerous to handle and discard, physical agents such as UV irradiation can be used for mutagenesis. Typically, cells are spread on the surface of an agar plate and then exposed to UV radiation for a calibrated exposure at wavelengths between 200-320nm (254 is optimal).

Exposure of DNA to UV light generates primarily cyclobutane dimers of adjacent thymine residues linking carbons 5 and 6 to one another. Adjacent thymine and cytidine bases can also form a 6-4 link. Usually such lesions are repaired in an error-free manner (see below) but if the damage is extensive, the error-prone DNA repair systems are induced and mutations arise through errors introduced during repair.

Realising that the starter cultures influence the body consistence and flavour of yoghurt during incubation period as well as refrigeration, many workers in recent years have engaged themselves in the heritable determinants of these culture bacteria i.e. by strain improvement by mutation and genetic manipulation, in strain improvement programme they used certain curing agents (Chassy et,al 1978, Khul et,al 1979 and Georghieu et,al 1981) and also protofusion techniques (Kondo and Mckay 1982, Hofer, 1985 and Davey et,al 1986).

REFERENCE

- [1] Bills D.D. Morgan, M.E, Libbey, L.M and Day, E.A.(1965) :- Identification of compounds responsible for fruity flavor defect of experimental cheddar cheeses. J. Dairy Sci., 48:1168.
- [2] Caplice, E. and G.E., Fitzgerald 1999, Food fermentations; role of microorganisms in Food production and preservation [nt.J. Food microbial. 50: 131-149/ Cross Ref/Medline].
- [3] Langsrud T, Reinbold G. W.(1974):- Flavor development and microbiology of Swiss cheese –a review, J. Milk Food Technol 37:26.
- [4] Martean, P. and J.C. Rambaud.1993. potential of using lactic acid bacteria for therapy and immunomodulation in man.FEMS microbiol Rev.12:207-220/Medline/
- [5] Molimard.P, Spinner H.E. (1996), Review :- Compounds involved in the flavor of surface mold ripened cheeses: Origins and Properties. J.Dairy Sci 79:169.
- [6] Salminen, S.C. Bouley, M.C. Boutron-Ruault, J.H. Cummings, A.Frank, G.R. Gibson, E. Isolauri, M.C. morean, M.Roberfroid and I. Rowland. 1998. Functional Food Science and gastrointestinal physiology and function. Br. J. Nutr. 80(Suppl.I):147-171.
- [7] Sukumaran, M.V. (1982):- The effect of some factors on acceleration of ripening of cheddar cheese, Ph, d. Thesis submitted to the Kurukshetra University.
- [8] Vanenbergh P.A.(1993) :- Lactic acid bacteria their metabolic products and interference with microbial growth. FEMS microbial Rev 12:221.