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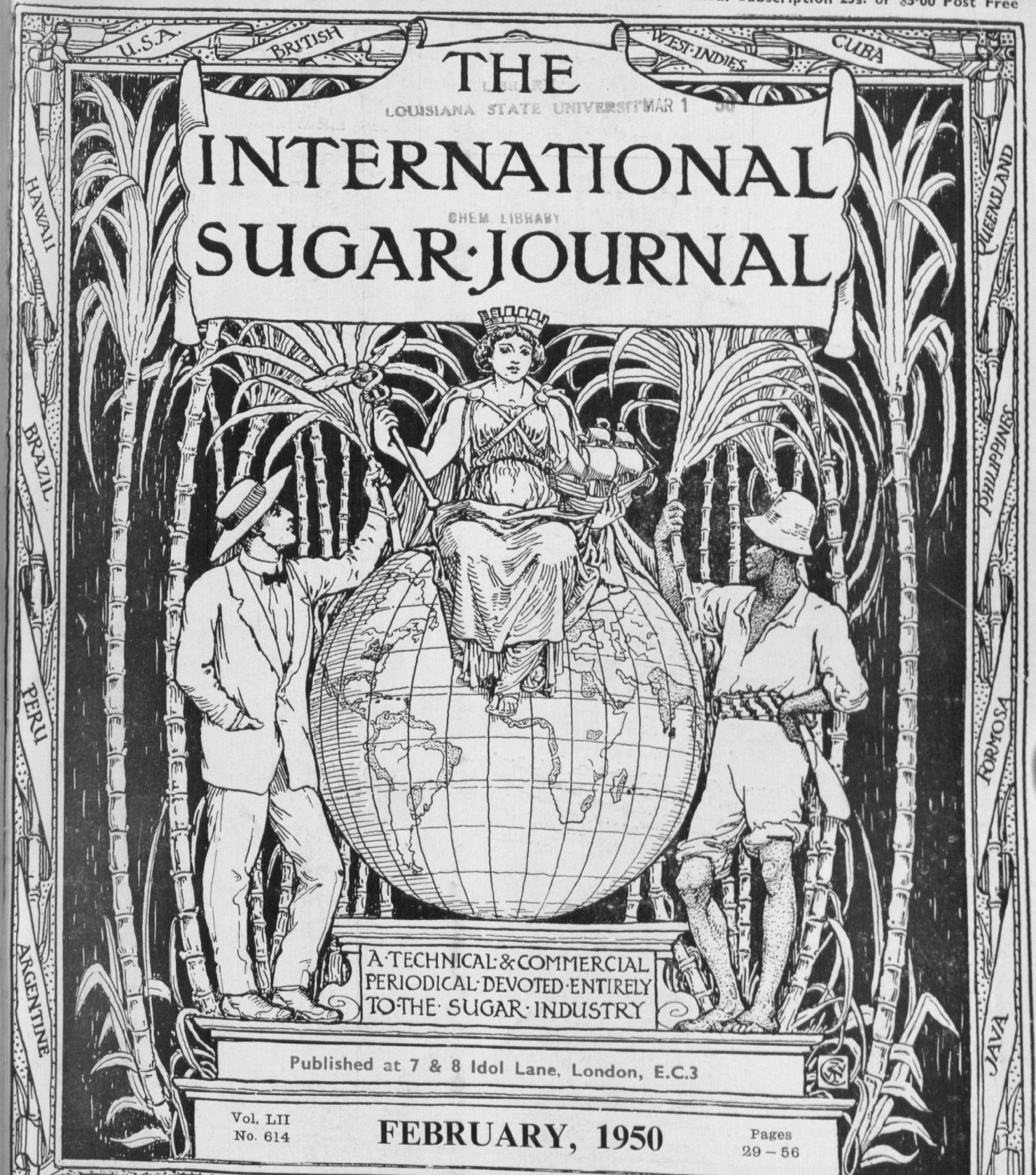

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ADVANCED FEATURES IN RUM FERMENTATION

(1) Multiple Culture Seed; (2) Yeast Hybridization

By RAFAEL ARROYO, Ch.E. & S.E., F.A.A.A.S.,
Consulting Chemical Engineer and Fermentologist, Rio Piedras, Puerto Rico.

WHILE rum distillers seem generally interested in new developments of a mechanical or a chemical nature that may improve either the efficiency of the process or the quality of end-products, they show little or no interest in ameliorations brought about through biological means. Scanty attention is given to the development of improved rum yeast strains, so that, in each particular case and for each particular rum, the most appropriate yeast is used for the development during fermentation of the right congeneric products of alcoholic fermentation, conjointly with profitable yields of alcohol in the optimum period of fermentation.

Our researches on rum[1] published in 1945 were designed to do their best to arouse the interest of rum producers to the paramount rôle of the yeast strain selected in rum fermentation, the most important and valuable chapter of the book being devoted to this subject. Subsequent studies since then, plus the knowledge gained from our consulting practice both at home and abroad, have convinced us that rum distillers would do well to invest some of their time and money in rum yeast research, in both extensive and intensive ways. However fully convinced the writer may be, convincing others is quite an arduous task. Nevertheless, the fact is that there is no single thing the rum industry needs more than the development of adequate rum yeast strains capable of meeting the exacting requirements that the near future may impose on all rums.

The ideal yeast strains for the successful production of specific rum types have not hitherto been provided by Nature, and, in our belief, the shortest route to that goal is for man, co-operating with Nature, to develop them artificially through the process of rum yeast hybridization under scientific control. Should this still fail to produce single hybrids capable of meeting all requirements, recourse may be had to the practice of multiple culture footings as a complementary feature.

The ideal yeast strain, we repeat, has not been found for the production of rum. The difficulty in obtaining the proper strain for a specific type of rum has led to the practice of utilizing auxiliary ferments in the form of bacteria and moulds for the sake of obtaining the desired aromatic gama in the end-products of fermentation. Good results have *sometimes* been obtained with this practice ; but always at the risk of decreased yields, costly infections, complicated additional equipment, extra labour, and costly and often irreparable mistakes in operation. Only with the help and assistance of the experienced bacteriologist or the expert fermentologist has this practice obtained partial success in specific cases, and it cannot be recommended as general practice for all distilleries nor for all types of rums.

Another factor that has retarded the advent of proper and adequate rum yeast strains is that rum producers have in the past often tried to draw too liberally from industrial alcohol distillery practices and methods ; failing to grasp the idea that, while the requirements of yeasts for the production of industrial alcohol are few and simple, the case varies considerably when the production of high quality rum is the objective. Industrial alcohol yeasts are mainly selected for their ability to produce high alcohol beers and good yields of alcohol on total sugars used in the shortest possible fermentative period. The amount and kind of congeneric products of the alcoholic fermentation have practically no influence or bearing on the selection of the yeast strain.

Contrary to this, in the judicious selection of the most suitable yeast strain for rum production, both the *quantity* and *quality* of the congeners produced form the point of greatest interest ; the *kind* and *organoleptic* qualities of these congeners is far more important to the rum distiller than the *quantity*. Moreover, it has been found in practice that often the same physiological, bio-chemical and other characteristics that prove a given strain well adapted for the production of a given type of rum, may militate adversely in its employment for the production of another rum type. Which brings to mind again the idea of having recourse to artificial rum yeast hybridization for securing specific hybrids for the performance of specific work.

We have, then, that in the proper selection of a veritable rum yeast, its products of metabolism, other than the ethyl alcohol, should receive very careful attention and study. That the separation, identification and quantitative determination of each individual constituent is not an easy task has been demonstrated by the hitherto mediocre knowledge existing on these substances. However, modern analytical chemistry should not consider as an insurmountable task the finding of better analytical procedures for the accurate isolation and determination of these congeneric substances.

With the exception of a few obstinate and backward cases, spontaneous fermentation is rapidly

becoming a thing of the past. The main objection to such a method of rum fermentation, aside from its poor economic results, rests in its failure to produce rums with consistency of flavour and aroma. The consuming public is beginning to demand rums of standard consistency in body, aroma and flavour—which means rums of a constant specific chemical composition. The use of cultured yeasts in an attempt to meet this demand is well established, but the selection of the best adaptable strains remains problematical. New methods seem necessary.

We shall bring to the attention of our readers two new developments that offer possible improvement of the situation, opening new horizons in the field of rum fermentation : (I) Multiple Yeast Culture for Seeding, and (II) Rum Yeast Hybridization.

(I) Multiple Yeast Culture for Seeding.

By this term is meant the practice of pitching the mash with a footing or seed composed of more than one cultured yeast strain. Having experienced the difficulties involved in obtaining a single strain possessing all the required characteristics for the production of high yields, coupled with the right amount and kind of aroma and taste, rum research workers and a few progressive distillers sought in this practice the remedy desired. It is supposed that each individual strain of the multiple culture used for seeding purposes would be complementary to the others in some respects, thus obtaining the desired characteristics from the well-balanced collaboration of the different strains used originally in the multiple culture. One desideratum would come from one member, another from a fellow member, and so on. In this manner it is thought logical and possible to obtain the result from the combination of the different strains that become unattainable through the use of a single yeast. This sounds very easy on paper, but actually it is not so ; the technique has its drawbacks and limitations, some of which we shall discuss later on. However, it represents an improvement and, under the guidance and supervision of one experienced in the art, it is capable of producing quite satisfactory results.

At first thought this multiple culture technique would appear flawless if a large enough number of collaborating different strains can be brought into play. But those who have experimented with it soon became aware of the drawbacks and limitations mentioned earlier. In the first place, it is soon found that the number of collaborating strains must be limited if a well-balanced effect of the whole is to be kept, to say nothing of the physical labour and manipulation necessary. In the second place, it is often found that in the building up of this compound footing to the size required for seeding the main fermenter, by the time the laboratory original footing reaches the fermenter a predominating strain has gained control of the medium and becomes practically the only one effective during the main fermentation ; the action upon the substrate of the others is so weak as to become insignificant. At other times it becomes apparent that the metabolic products of a strain are not the same when working in pure culture by itself, as when it becomes one of the members of a multiple culture. All of which tend either to nullify the end-thought, or at least to alter it appreciably. Also, strains characterized by producing highly desirable effects in aromatic gama and good taste often result in weak fermenters which cannot keep pace with others of less aromatic effect, but have stronger zymogenic action.

These, and many other problems, are met in the practice of this technique, even in an experimental way. Hence the need at the distillery of an experienced fermentologist using this procedure. In fact, in our opinion, in order to obtain frank success with this practice, the cultural characteristics and physiology of each strain composing the multiple culture must be well known. It seems of advantage to bring together in the formation of the multiple culture, strains having practically the same, or nearly the same, characteristics in regard to : (a) *Time of Generation* (the time interval for obtaining one cell from another cell) ; (b) *Energy of Multiplication* (the number of cells formed from another cell in a certain time ; and (c) *Power of Multiplication* (the total number of cells that can be produced from the same cell). This alone will explain the difficulties that must be faced and overcome in the practice of multiple culture seeding. However, we repeat that in the hands of the initiated this technique may attain, and has actually attained, considerable success.

A modification of the above procedure has been tried experimentally in order to obviate some of the difficulties outlined above. This modification tends to obtain the same results without the building up of the multiple culture. It consists in making several separate individual footings with each of the selected strains, and then pitching different fermenting vats each with one of the footings. After fermentation is over, then the different beers are blended in the required proportions before submitting them to distillation.

From a biological viewpoint this would be the easiest and safest way out ; but from economical and mechanical aspects this modification could hardly be recommended. Multiple yeasting equipment would be necessary, both in the laboratory and at the works ; extra time and labour would be required in the building up of the different seeds from laboratory to factory size ; the danger

of infection is multiplied ; more technical and skilled help would be necessary ; and extra equipment (tanks, pipe-lines, pumps, motors, etc.) would have to be installed. All these things would create undue expense and general complication of equipment and performance.

From these considerations we favour the employment of yeast hybridization ; and the use of multiple culture only as a complementary device in the case of failure to produce hybrids that would meet all requirements in single pure culture.

(II) Rum Yeast Hybridization.

Hybridization in general has done more than its share in fostering and improving the lot of mankind. No one can foretell what yeast hybridization may have in store for many agricultural industries; but, be that as it may, we feel that the rum industry has much to expect in this respect. The artificial and scientifically controlled hybridization of rum yeasts may provide the surest and simplest means to obtain the desired improvements in rum fermentation. When we published our book entitled "Studies on Rum"[1] we mentioned the work of Winge and Laustsen[2, 3, 4, 5, 6] who developed methods of yeast hybridization, some of which proved economically or industrially superior. Previous to the work of Winge and Laustsen, Kruis and Satava[7] had discovered that the large, vegetative, ellipsoidal cells of *Saccharomyces cerevisiae* are descended from cells produced from the fusion of two small, round, sexual (gametic) cells, arising from ascospores.

Due to the language in which it was published, this basic work passed unnoticed by other workers, and in 1935 Winge[8] independently rediscovered this fact ; opening the way for the subsequent work on yeast hybridization already mentioned, as performed by Winge and Laustsen. More recently, Lindegren, of Southern Illinois University[9, 10, 11], has made very valuable and extremely important contributions in this field, developing practical and simple methods of yeast hybridization. We shall treat these practical methods in more detail, since these are the methods we advocate and would recommend to industrial laboratories that may become interested in this type of work.

The outstanding work of these distinguished scientists is here mentioned as a demonstration of how fundamental research has beneficial results in practical industrial work. At the time we published our researches on rum, we expressed the hope that in the none too distant future the rum industry of the world would grasp the advantages of applying the work of yeast hybridization in the solution of some of its problems. Again we wish to state that it is high time for the consideration of such movement. These activities, well directed, will greatly benefit rum distillers in particular and all rum-producing countries in general.

After having studied the technique of Lindegren for practical methods of yeast hybridization, we agree with him in that the work should present no great difficulty, since it will not be necessary to adopt the complicated technique used in genetical analysis of yeast, where single ascospore cultures isolated from the asci with the micro-manipulator are exclusively relied upon. For the industrial laboratory worker such refinement is unnecessary. It is simply sufficient to follow the practical simplified method of Lindegren, which requires neither advanced knowledge in genetics nor specialized equipment. Standard bacteriological technique and equipment plus the willing worker is all that becomes necessary. This method is described succinctly below.

The worker may start with a sporulating yeast of known industrial value, or with any sporulating yeast. This is grown in a pre-sporulation medium, and is then transferred to the sporulating gypsum slant until an abundance of spores is obtained. The vegetative cells which may remain in the suspension are killed by heating to about 58°C. from 2–4 min., the spores alone remaining, since these are more heat-resistant than the vegetative cells. The culture is then quickly cooled and plated on solid medium. The colonies of haplophase will soon appear, which, after isolation, are tested for characteristics. In this way is secured a large stock of breeding haploids and, after a large collection of haploid variants has been obtained, mating of these haploids with each other will produce the ellipsoidal cells of the new hybrids.

With such a controlled hybridization programme in the case of our rum yeasts, it becomes possible to acquire artificially what Nature has not provided for the industry—the ideal rum yeast.

FOOTNOTES.

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10 C. C. Lindegren and G. Lindegren : "A New Method for hybridizing Yeast." *Proc. Natl. Acad. Sci. U.S.*, 1943, **29**, pp. 306–308.

11 C. C. Lindegren : "Practical Methods of Yeast Hybridization." *VII^e Cong. Internat. des Ind. Agri., Paris*, 1948.