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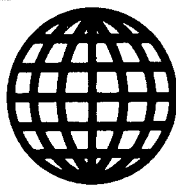

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China

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SCIENCE & TECHNOLOGY

CHINA

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Importance of Technical-Base Activities to Defense S&T Outlined

40080163a Beijing ZHONGGUO KEJI LUNTAN [FORUM ON SCIENCE AND TECHNOLOGY IN CHINA] in Chinese No 3, 1988 pp 10-12, 48

[Article by Ding Henggao [0002 5899 7559]: "Explore New Paths in Defense Technical Base Activities"]

[Text] I. Comrade Zhao Ziyang proposed in his report at the 13th plenum that the development of science and technology be accorded top priority. China's economic development experience demonstrates that both economic invigoration and modernization must rely on science and technology. Modernizing science and technology is the key to the four modernizations. Defense science and technology shoulder heavy assignments in the service of defense modernization. Defense science and technology form a part of science and technology in general, so that their development is closely connected with both defense modernization and scientific and technical modernization.

The immediate objective of developing defense science and technology is to produce continuous scientific and technical results, to develop new and advanced arms and equipment, and to accelerate the pace of defense modernization. Without development of new prototypes, we will be unable to produce arms and equipment, and only the strengthening of basic and applied research will make it possible to raise the level of science and technology and to provide technical reserves and increased staying power for the development of arms and equipment. But the development of arms and equipment, long-range research, and even the operation and maintenance of arms and equipment, are all inseparable from technical base activities, which include standardization, metrology, information management, and management of scientific and technical results. Technical-base activities provide essential technical services and technical safeguards for prototype development and long-range research and prototype development and long-range research continually stimulate progress in technical-base activities. To summarize, prototype development and long-range research form with technical-base activities a closely connected, mutually stimulating unity, and the three are inseparably related. We should therefore understand the importance of technical-base activities in terms of the totality of defense scientific and technical work. Technical-base activities are the foundation on which science and technology rely for their progress: it permeates the entire process of research, testing, production and operation and has extensive, deep-seated influences on every aspect of defense modernization and development.

As early as the 1950's, Comrade Nie Rongzhen pointed out that defense metrology is one of the seven keys to the development of advanced technology. In 1983, he stated: "Science and technology must be developed, and metrology must lead the way." Launching geostationary satellites is a major systems engineering project, and a major problem involved in it is to assure that the dozens of monitoring and control units distributed throughout the country, the launch vehicle and the satellite have highly accurate time and frequency synchronization. For this purpose, the launch vehicle, the satellite and the ground-based units must be equipped with more than a thousand instruments and devices for unified measurement. This is just a single example of the importance of metrology in experimentation. Standardization is an important yardstick of the country's modernization. If the development of advanced technology and of arms and equipment is not based on standardization, there will be grave consequences in production and operation. In addition, the economic benefits resulting from standardization are obvious. For example, the adoption of three types of standardization in relation to cerium-doped quartz glass tubes and their testing is likely to save more than 1.2 million yuan and 1.2 million kilowatt-hours of electricity a year.

The importance of scientific information work becomes increasingly obvious as science and technology, the economy, and society develop. First, policymaking is inseparable from information. Scientific policymaking can in a certain sense be said to be information-dependent policymaking. Adequate, correct information and correct analysis and understanding of it form the basis for correct policymaking. Today, not only science and technology themselves, but policymaking in military affairs, economics and even government, require scientific and technical information. Second, information is a concrete component of science and technology; and for our developing nation, scientific and technical shortcuts and the saving of funds and time in research and production will require information. For example, information and document collection work was very important in the development of the Yinhe 100-MIPS [million instructions per second] computer. Our current high-technology exploratory research requires information and document collection to keep up to date. Management of scientific and technical results, which includes results evaluation, record keeping, decisions on awards, dissemination, development, patents and the like, has an effect on whether results can be accepted and properly appreciated by society, whether they are used and whether proprietary rights are protected; they also affect the vital interests of scientific and technical personnel and the national interest. We must appreciate that results management simulates the commercialization of technological results, develops the commodity economy, and promotes technological progress.

To summarize, in order to raise China's scientific and technical level and decrease the gap between China and the most advanced countries, we must appreciate the importance of technical-base activities in strategic terms as affecting the quality of the foundations; we must focus in our work on strengthening scientific research and adapt to the objective need to emphasize key technical breakthroughs and pioneering projects and strengthen emerging fields of technology.

II. Defense science and technical base activities must adapt to the requirements of the new situation, which involves primarily a series of new problems and requirements arising from the challenge of the imminent world revolution in new technology, restructuring of the domestic economic and political system, and the strategic shift in the guiding concepts of defense development. As regards the technical base, the new situation is expressed primarily in discrepancies between existing concepts, management and methods and the objective requirements. We must thus begin the reform with these aspects.

A. Reform of Concepts

1. Develop Awareness of the Commodity Economy. The commodity economy concept has two primary aspects: the need to recognize and respect price laws, and the need to be competition-minded and to rely on our competitive capabilities in order to survive and develop. Because the technical base by its nature serves the public interest and safeguards research and production, it has long been supported by the state. But we must also be aware of another aspect, namely that technical-base activities must adapt to the commodity economy environment if they are to survive and develop. Otherwise, if we rely entirely on guaranteed state funding, we will be in a state of "neither feast nor famine." We must therefore explore the possibility of gradually implementing the system of inviting contract bids for certain research topics in the technical base. We must develop paid information search, data-base and technical consulting services related to standardization, metrology, information management, and scientific results management. If we do this effectively, excellent benefits are sure to result. In the developed commodity-economy nations, and in the information society, scientific and technical information is a key aspect of the information business; it has a variety of operating techniques, such as computer search services, data-base services, topic-research, commodity-sample and market-information consulting, exhibitions, exchange activities and the like. But we still subsist almost entirely on state funding to survive, and many comrades believe that this is as it should be: they lack the spirit of winning markets and engaging in competition.

2. Develop Legal Awareness. Standardization, metrology management and patent activities all have a very pronounced legal aspect, and they all use legal forms to prescribe and restrict scientific and technical work. We must correct our previous exclusive reliance on administrative commands and administrative coordination, act in terms of the law, and replace government of men by government of laws. For technical-base personnel engaged in compiling laws and regulations and monitoring their observance, developing legal awareness is of particularly great importance.

3. Develop Awareness of Scientific and Democratic Policymaking. Management is policymaking. Technical-base activities include both research and management. In order to raise technical-base activities to a new level, we must develop an awareness of scientific, democratic policymaking and adopt methods such as forecasting, substantiation and consultation in all stages of basic technical work. Future standards publications, and the specification

of metrological practices and information research topics must all be based on scientific supporting documentation; investigation of standards, evaluation of results, and evaluation of personnel must be done strictly in terms of prescribed procedure. Objective laws must be fully respected, democracy must be emphasized, and experts must be used, so that our work will be placed on an increasingly scientific basis.

4. Develop an Awareness of Benefits and Efficiency. A striving for benefits and efficiency is an increasingly pressing requirement that science and technology, the economy, and society impose on management and on scientific and technical personnel. We must consider the economic and social benefits of every technical base activity. For example, when considering the establishment of metrological practices, we should not purely pursue higher and higher standards, but must relate them more effectively to the development of future new technologies and prototypes. We must also emphasize efficiency: the drafting of standards now requires a very long time period, and when they have been drafted, if disagreements arise in the review process, it may drag on for a year or two. If everyone emphasizes benefits and efficiency, costs and time, far more will be accomplished.

B. Reform of Management

Management reform is really a problem of establishing a high-efficiency transmission mechanism. In the area of technical-base activities too there is a need to correct direct administrative intervention in and control of the economy and the scientific and technical process. The cognizant administrative departments should focus on indirect control; their main function should be to formulate laws and policies and carry out long-term planning, making of broad policy, and coordination and oversight. Units involved in technical-base activities must actively search for ways to improve economic self-sufficiency such as establishing technical consulting organizations and the like, and must socialize their activities. The state should primarily approve and hand down research topics, subsidize key projects and provide support. We are the scientific and technical base of national defense, and consequently serving national defense is our basic function. Subject to this principle, we should vigorously promote compensated services and development organizations.

Unity of the army and people is the basic policy for development of the defense science and technology industry and of all technical-base activities, whether standardization, metrology, information management, or results management, must implement this policy. For several years we have had considerable success in implementing the policy of unity of the army and people. For example, in the past few years there have been considerable efforts in the transfer of military technology to the civilian sector; and it appears that both social efficiency and economic benefits have been good, and all parties involved have expressed satisfaction. But the results are uneven in different fields and geographic areas broadly speaking, we have just taken a few steps, the road ahead is still very long, and there is still much unused potential. We cannot deny that there are problems in this area. For example, the less-than-ideal progress in declassification of technological

results is a case in point. In the 30-odd years since the state was founded, we have accumulated a great many technical results related to the development, production and operation of arms and equipment, but more than 80 percent of these are still classified and have always been kept under lock and key. Last year we began a declassification effort. After a year, only about 3 percent of the total has been declassified. It must be realized that the value of technical results decreases over time, and eventually is entirely lost. If these results are not declassified in timely fashion, it will not be possible to exploit them, to say nothing of turning them over to the civilian sector, and this will be a great waste. It is evident that although the policy of transfer of military technology to the civilian sector has been in effect for several years, in several respects it is still not being treated seriously enough. The current approach to declassification of technical results is a drop-by-drop method. This will not do: we must take the opposite approach of determining what things should still be kept secret. Things that should not be kept classified are in the majority, and we should open them all up. If we do this effectively, a great deal of technology can be released to the civilian sector.

In terms of the technical base, union of the army and the people involves the turning over of military industrial technology to the people, an orientation toward society, and use of superior methods and technology to provide services; it also requires that we absorb advanced civilian technologies and that, while promoting the transfer of military technology to the civilian sector, we also continuously raise our own technical and management standards and consistently strengthen and improve ourselves. These two aspects must be emphasized equally.

C. Modernization of Personnel and Resources

We must make full use of modernization of resources to create a reliable, highly efficient information collection, analysis, transmission and feedback system, establish a variety of data bases and systems for servicing them, and make effective use of them in our work so as to increase working efficiency. Modernizing the resources of the technical base includes both hardware and software. Hardware include various types of measuring and testing equipment, equipment for information and document storage, processing, transmission and exchange equipment and the like; it constitutes the material base of technical-base activities. The slogan "Develop science and technology and give priority to the base" means first of all that we must assure that the hardware of the technical base is appropriately advanced. But merely having advanced equipment is not enough: we must have the corresponding software support if we are to make full use of the equipment. In addition, we must make an effort to develop personnel. Personnel engaged in technical-base activities must first of all develop a sense of the gloriousness and importance of their jobs. Next, job training must be organized at every level and in every specialty. Third, a vigorous effort must be made to train a new generation of specialists, to bring in new blood, to solve the problem of "aging" of personnel. We must draft a set of procedures in technical services, academic research, foreign contacts and

proficiency testing appropriate to the special characteristics of technical-base activities.

Technical-base activities are an extensive field, each aspect of which is a science. As the world revolution in new technology proceeds, these fields face a series of new problems; they must therefore be reinforced, modernized, or even reconstructed. Intensified academic research is the key to Chinese-style development of technical-base activities. We already have so large a contingent of personnel and have done so much work in defense development that although our technical level cannot compare with those of developed countries like the United States and the Soviet Union, and we should not underrate ourselves in terms of our management and practical experience. We must have a bold spirit and China must establish itself in respected schools of thought, publications and organizations related to standardization, metrology, technical information, and technical results. Establishing our prestige cannot be done overnight: we currently are far behind in all respects, but we must have a bold spirit.

8480/6091

Strengthened Basic, Applied Research Called Basic to S&T Progress

40080163b Beijing ZHONGGUO KEJI LUNTAN [FORUM ON SCIENCE AND TECHNOLOGY IN CHINA] in Chinese No 3, 1988 pp 13-15

[Article by Shi Changxu [1597 2490 4872], vice chairman of State Natural Science Fund: "Continue To Strengthen China's Basic and Applied Research"]

[Text] I. China must achieve a comfortable standard of living by the end of the present century and become a moderately developed nation that has essentially completed its modernization by the middle of the next century. The key is accelerated scientific and technical progress; and intensified basic and applied research is the basis for scientific and technical progress.

Creative scientific and technical activity can generally be subdivided into the scientific research stage and the development stage; research itself is subdivided into basic and applied research.

Basic research is intellectual labor that makes use of scientific experiments and theoretical investigations to reveal previously unknown natural laws, to discover new phenomena, and to establish new theories. Basic research both directs and serves as a reserve for scientific and technical development. The formulation of electromagnetic theory and quantum dynamics, the discovery of semiconductors and superconductivity, and the discovery of the gene and of lasers, indicate clearly that major breakthroughs in basic research often will fundamentally alter a country's technical and economic character and give rise to major alterations in human society. Applied research is a creative research activity dealing with a specific subject which solves scientific problems arising in the course of industrial and agricultural production and in engineering and technical applications, and which has an even more direct and closer relationship to economic development and to defense development.

As a result, in the initial stage of socialism, continuing to intensify basic and applied research is of extremely great strategic significance in energizing China's economy. How great a contribution a people makes to mankind, and whether a country can establish itself among the great nations, depends not only on its economic capabilities, but also on its scientific and cultural level, on how many creative scientific results it achieves, and

on how many top-flight world-class scientific and technical personnel it produces. Continuing to intensify basic and applied research will provide a historic impetus and serve a vanguard function in raising the cultural level of the Chinese people, producing top personnel catching up with the world revolution in new technology and making an even greater contribution to humanity, and functioning as a historic motive force and vanguard.

Since the state was founded, China has had great achievements in scientific research. In some fields China is close to or at the world state of the art; a contingent capable of keeping up with advanced work in science and technology and independently carrying on creative research has been created, and several research organizations with advanced equipment and a fairly full complement of personnel has been created. But overall, China is still far behind the developed countires: teaching, research and production are out of touch with each other, and the development of various fields is not coordinated with economic development; investments in research are too low, and research personnel cannot make a full contribution; the research contingent is insufficiently adaptable, and research organizations are not flexible enough; there is not a consistent recognition of the importance of basic research, and this has had adverse effects on the consistency of policy.

II. To narrow the scientific and technical gap between China and the advanced countries, in the 70-odd years between now and the middle of the 21st century, i.e., the initial stage of socialism as a whole, we must emphasize the use of science and technology to make the country develop vigorously. The focus of China's basic and applied research should be on economic development, but with adequate emphasis on developing the leading edge of science. We must strive to establish China among the scientifically and technically strong nations as quickly as possible. This is China's medium and long-term strategic goal for basic and applied research.

This objective can be realized in two stages. From 1988 to 2000, the objective of persistently strengthening scientific research is to quadruple China's output value, emphasize technical modernization of traditional industries, and promote the development of the agricultural and defense industries, while at the same time pursuing scientific research in leading-edge fields in which China is well situated and high technology industries and focusing on breakthroughs to limited far-advanced objectives. In the first half of the next century, the objective of continuing to intensify scientific research will involve an emphasis on achieving essentially complete modernization of China's science and technology in order to promote the modernization of agriculture, industry and defense, further accelerating the high-level development of science and technology markets and high-level conversion of scientific and technical results to commodities, greatly decreasing the time between basic research and the building up of production, and accelerating the development of the high-technology industries, so as to bring China into the world forefront in more fields.

In the process of implementing the above strategic objectives, there are two guiding strategic concepts for basic and applied research:

1. In connection with a focus on modernization of economic development, scientific research must lead the way. When dealing with the relationship between research and the economy, we must persistently adhere to the principles of "Scientific research as the base and the economy as the objective" and "Science and technology for prosperity, research for applications."

2. We must proceed in terms of China's characteristics and capabilities, persistently rely on our strengths and avoid our weak points, and make full use of China's distinctive characteristics. We must work vigorously to strengthen the country, dare to make breakthroughs, and in addition emphasize the main focus, proceed under a realistic evaluation of our capabilities, and consistently adhere to the principle of obtaining benefits from research and to the competitive system. When dealing with the relationship between acting in terms of China's specific situation and keeping aware of the world situation, we must adhere to the principle of "Learning from others when they are ahead, being creative when we are ahead, competing when we are equal to others, and biding our time where we have no capabilities."

Based on the above strategic objectives and guiding concepts, in the next 20 or 30 years the following should be the key areas of research:

1. The life sciences and biotechnology. This is basic research that bears on agriculture, medicine, public health and eugenics in China. We should also focus research on the basic theory of Chinese medicine and Chinese materia medica.

2. Information science and communications, and particularly computer science.

3, Materials science and engineering science. Our traditional industries are still very backward, which is retarding to some extent the development of new industries. As a consequence, the appropriate importance should be attached to materials science and engineering science during the present century, so as to increase labor productivity, improve quality, decrease costs, and make China's products competitive internationally.

4. Develop a distinctively Chinese earth science, make thorough use of China's advantages in natural resources, create new industries, and decrease natural disasters.

5. Emphasize leading-edge fields and new areas of growth in basic science, and in particular make thorough use of China's intellectual advantages in sciences in which we can achieve a leading position in not too long a time, such as mathematics, theoretical physics, and software.

6. Create and develop distinctively Chinese software sciences.

III. China's basic and applied research is still far behind the advanced countries and behind the world revolution in new technology, and task of catching up will be extremely arduous. We must persist in reforming, opening up and invigorating basic and applied research in order to assure their vitality. The following basic strategic measures are proposed for this purpose.

1. We must resolve to greatly expand operating-expense allocations for science and technology and for education. Operating-expense allocations for science and technology must reach 1.5 percent of GNP during the Seventh 5-Year Plan and 2-3 percent by the end of the century. Basic research should account for 10-15 percent of operating-expense allocations for science and technology, and applied research should account for 20-25 percent.

2. Stringent reforms are needed in the funds allocation system; we must break down rigid stereotypes and persist in introducing the competitive system and the principle of democratic criticism and evaluation by personnel in the same field. Operating expenses for basic and applied research should be obtained primarily by application. In order to assure that the country's key research units can continue to function normally, the state should allocate operating funds, equipment procurement funds, maintenance and operating funds, and necessary special-topic funds. Special funds allocations should be made for projects of strategic significance.

The Central Committee Decision on Reform of the Science and Technology System states, "The scientific fund system should be gradually implemented on a trial basis for basic research and some applied research," and "The organizations mainly involved in this type of research should gradually change over to obtaining their research funding by application." As a consequence, the State Natural Science Fund Committee was set up in February 1986. Experience in the years following demonstrates that the fund system is feasible and effective in China. The essence of the fund system is that it ends the "big cook pot" system of lump-sum allocations, introduces the competitive system into research, and allows a nationwide search for the best based on democratic review by personnel in the same field. We should make increasing use of the fund system and steadily improve it.

3. Effective steps should be taken to assure that basic and applied research results are converted into productive capabilities and commodities as quickly as possible and to promote ties between research and production.

First, when the state drafts guidelines for various types of projects in basic and applied research, in addition to topics in the leading-edge areas of each field, it should also include directive topics related to key scientific problems that stem from production and should bring together the best capabilities nationwide. Second, it should quickly draft policies to encourage equal or proportional enterprise-state joint investment and encourage scientific and technical personnel to visit

industry in order to find research topics. Third, we should vigorously support unity between research, education, and the enterprises, and vigorously establish and promote university-enterprise or institute-enterprise joint research centers. Fourth, research institutes and universities should constantly absorb enterprise scientific personnel and involve them in joint research, thus both refreshing their knowledge and establishing strong ties with the enterprises so as to promote direct use of research results to advance production.

4. We should improve personnel management techniques in basic and applied science, make use of the initiative and creativity of research personnel, use flexibility in the training and selection of personnel, promote continuous renewal of the ranks of research personnel, and increase the vitality of research institutes.

First, we should institute the institute director and project group leader responsibility system by giving the directors and group leaders the necessary powers and autonomy for independent research. We should decrease and regulate the fixed staff of specialized research institutes and expand temporary staff, and large numbers of young people should be drawn into research work. Second, certain strong research institutes with a high ratio of basic to applied research should recruit increasing numbers of graduate students in order to make use of latent capabilities and increase their vitality, and should train advanced scientific and technical personnel capable of doing independent research. Third, we should make thorough use of advanced institutes and schools in basic and applied research, we should promote contacts between the personnel of research institutes, universities and enterprises, and simultaneous membership in different organizations. Fourth, we should encourage scientific and technical personnel to have broad interests and to expand their range of knowledge. We should enliven the academic atmosphere, promote scholarly interchange, and oppose any refusal to exchange scientific information. Scholarly exchange centers should be opened in certain areas with large concentrations of research personnel in order to promote and facilitate timely exchange. Research personnel in basic and applied fields who have genuine ability and knowledge and have made major contributions in basic and applied research should receive preferential treatment. Effective measures should be adopted to solve the problem of the illogically higher compensation for physical than for mental work, and we should eliminate egalitarianism in the distribution of profits among scientific research personnel. Fifth, we should develop the personnel market, promote personnel mobility, and allow the use of funds to recruit supernumerary research personnel or support personnel.

5. Key state laboratories, open laboratories and key topic areas should engage in overall planning so as to make full use of their equipment and personnel.

Research funds for the research projects of state key laboratories and advanced open laboratories should be given priority. They should be allowed to organize their outstanding personnel to develop research topics, submit funding proposals, and take part in evaluation, so that the laboratories can

realize their full potential. We should organize experts to evaluate these laboratories' operations and results and to determine rewards and penalties. The utilization rate of major equipment in the country is low, and fees for its use are incorrectly set, which is hindering the improvement of China's research level. This state of affairs should be given serious attention and be quickly and appropriately resolved.

Advancing state key topics in basic and applied research should be accorded full seriousness and full support should be given to topics of major academic importance or creative topics in connection with progress in key state topics.

6. Basic and applied research should compete on an international scale. Today, when exchange of scientific information is becoming increasingly rapid and frequent, international cooperation becomes increasingly important. As a result, while making thorough use of domestic potential, full importance should be accorded to making use of the international environment, maintaining and encouraging cooperative research, and bringing in foreign funds for Chinese research that benefits China. We should encourage scientists in China to make use of advanced major scientific research facilities abroad through international cooperative research.

7. We should promote scientific morality, establish a good research style, and encourage exchange and cooperation.

The rapid advance of scientific research requires not only excellent personnel and material conditions but also an emphasis on a good research style, creation of a spirit of openness, and an atmosphere of free exchange. There are still a few problems in this area which must be quickly rectified, and when necessary legal safeguards should be provided. This is the only way to assure mutual support and thorough utilization of collective capabilities.

8480/6091

Approaches for Establishing Distinctly Chinese High-Tech Centers

40080168 Beijing BEIJING KEJI BAO in Chinese 11 Jun 88 p 3

[Article by Zhou Anshi [6650 1344 4258]]

[Excerpts] High-Tech promotes the Development of Manufacturing Technology. A technology and its industry rely on manufacturing technology to develop. Clearly, the emergence and development of high-tech cannot be separated from manufacturing technology. Originally a traditional, basic technology, manufacturing technology has followed progress in scientific techniques and industrialization, modernization, and development of a new technological revolution. It not only serves as the foundation of high-tech development but is also elevated through rapid transformation. Currently, manufacturing technology has found applications in some high-tech fields. Meanwhile, certain aspects have begun to become the mark of its high-tech field. For example, the traditional metal cutting and screw-mating, and riveting and ordinary welding in parts processing have been partially replaced by high-tech breakthroughs in plasma and particle beams. Traditional machining and mechanical assembly have been extensively applied to micro-electronics, computer and robot technology, and other high-tech artificial intelligence technologies.

In the manufacturing technology field, aerospace has unique characteristics. Its manufacturing technology has more opportunity for elevation, due to the fact that it is an important component of aerospace technology. Meanwhile, due to the progressiveness, leadership and driving force of the aerospace high-tech, it will bring about a more vigorous impact in the development of the driven manufacturing technology.

It should be pointed out that although manufacturing technology has gradually achieved high quality, in many situations, its important effect of manufacturing is not recognized as being valuable nor has it been given sufficient importance. In the past, Western public opinion considered it extremely difficult for the research work of manufacturing technology to achieve comparably great results. A specialist in theoretical research work can produce in a year or two, or less, a quality paper good enough for a degree. A design engineer can similarly come up in a shorter time with a design or product which will garnish him technical acclaim. However, a manufacturing engineer needs more expensive conditions and equipment and to repeatedly conduct experiments (these conditions are hard to obtain). After several years of practical experience, even if some technical difficulties are overcome, it is still not easy for him to write a sensational dissertation.

Even if a manufacturing technology specialist can, with more effort and time and better equipment, obtain results, he will have a hard time gaining the recognition of the evaluating experts. Under many situations, only when the results of the design of a product or a system appear can the fruit of manufacturing technology show up in the form of a by-product. The salary of a manufacturing specialist is usually lower than that in theoretical or design work and this is still true in China today. However, many changes have taken place in the West. In the U.S. the importance of manufacturing technology was recognized in the early fifties. Although it "cooled off" for a while, it has been revived with more investment in research, and, with the new priority technologies to promote manufacturing technology, it is being driven into the realm of high technology. Japan emphasized manufacturing technology so that it could quickly develop high tech to surpass Europe to become the world's second economic power.

One can see the importance placed by the West on manufacturing technology by the secrecy surrounding it. One can find many reference on systems, theory, and configuration of high technology from foreign treatises, journals, and theses, but not the contents of manufacturing technology in high-tech areas. In surveys abroad, one can find various new products and new configurations, but not material on manufacturing technology, indicating the high level of secrecy the West has placed on this subject. We have emphasized security regarding theory and configuration, but have neglected secrecy on manufacturing technology, so that secrets are stolen by foreigners. These "leaks" are due to the low value given manufacturing technology. We still have a crisis in scientific development, especially in this area. A large number of people still want to use low technology, traditional manual, imitation methods that are labor intensive, not knowledge intentive, to achieve modernization and to develop high technology; they do not know that this high plateau cannot be reached through such methods. First, we must heavily stress research and investment and the nurturing of senior-level human resources in manufacturing technology. This is vital to developing high-tech. Even if the policy-makers have subjectively decided not to emphasize manufacturing technology, the objective needs for high-tech development will still impact on it, forcing its development, and enabling it to satisfy the developmental needs of high-tech and its industries. Otherwise, any talk of high-tech and its products is just an illusion.

Three Approaches for Establishing a High-Tech Center

In describing these characteristics, we point out that high-tech development must rely on concentration and integration. In order to develop scientific research, there must be close cooperation between academia and industry. This means that scientific research organizations, institutions of higher learning, and industry must be tightly integrated in order to develop high-tech research and development, concentrating knowledge, technology, and talent to create high-tech research centers or a "science parks". Abroad these are usually centered about one or two technical universities, augmented by scientific research organizations, surrounded by medium and small enterprises, to form an integrated high-tech development system. Some examples of these science parks are "Silicon Valley" in the U.S., Cambridge

in the U.K., Tsukuba in Japan, and Munich in West Germany. In recent years, China has successively formed technological knowledge-concentrated centers such as Zhongguan Village in Beijing, Jiading in Shanghai, Nankai in Tianjing, Donghu in Wuhan, Yuelixuan in Changsha, and Xinju in Taiwan. These are embryonic compared to (foreign) science parks and technological cities in both level and scale. In terms of technological results, economic and social benefits, risk investment, competitiveness, environmental conditions, and integrated management there is a large gap between our centers and those abroad. In some aspects, ours do not even reach basic levels. In order to satisfy the strategic requirements of our high-tech development, we should emphasize the establishment of high-tech centers. This writer considers it necessary to combine the conditions of our country with the adoptation of the following three approaches to establish distinctly Chinese high-tech research centers or science parks.

The first is centered on institutions of higher learning. One can select one or two, with superior conditions, from the aforementioned areas to build experimental, typical science parks, on a priority basis. The selection of this priority experimental area can go through the overall report, and be settled by the evaluating committee consisting of national-level high-tech experts. Then, it should be given priority investments by national, provincial, and municipal organizations. The leadership cadre will be selected through advertised applications.

The second is centered on scientific research organizations. There are several research institutes in our Ministry of Astronautics, and a few high-tech institutes now in the embryonic stage, which can be used. Some of these already possess strong foundations and have shown outstanding results with good national and international standing. The state should give them careful attention and assistance. Currently, there are two key points for building such institutes: management and investment. As in building the typical science park mentioned above, the state can only select one or two of these institutes, and give them priority assistance. However, one needs to reform management systems and solve policy problems: to adjust for economic cooperation between business and industry, and reduce friction and internal waste. In doing so, one can promote strengths and avoid weaknesses within each unit, and use other's strength to compensate for one's weakness, thus making it into a viable technical, economic entity bringing many benefits. Simultaneously, if the state provides it with a large amount of investment, especially investment in astronautical high-tech and in manufacturing technology, to strengthen scientific research and its technological installations and to stimulate science/technology researchers and technicians, it is feasible to establish a Chinese-style high-tech center.

The third is to set up a "spanning" high-tech entity. This entity is not necessarily situated in one area, but may span region, city, and the integrated multi-municipal economic body.

Whether the above three approaches can be taken hinges on the second one: management and investment. These two conditions are the two weak links in China's high-tech development. Not only do we lack experience but also funds. Given this, we must conscientiously reform management of scientific research and rationally use the limited funding.

12744

Prospects for China's Manned Spacecraft Technology

40080176a Beijing XIANDAIHUA [MODERNIZATION] in Chinese No 6, Jun 88 p 30

[Article by Yu Qingtian [0060 1987 3944]]

[Text] In an announcement made by the Chinese Space Medico-Engineering Institute on 31 March, it was reported that major achievements have recently been made in space medico-engineering research, and a good foundation has been laid in the development of China's manned spacecraft technology. In a keynote speech celebrating the 20th anniversary of the institute, General Nie Rongzhen said: "These achievements have generated great optimism in China's manned space (medico-engineering) development efforts." Currently, this institute is engaged in the research of medico-engineering for space application systems.

Space medico-engineering is a newly developed, state-of-the-art science. Combining the disciplines of space engineering, space medicine, and biology, it applies modern scientific theories to study the interaction between man, spacecraft, and the space environment. It addresses the problem of improving the living and working conditions for astronauts during spaceflight. The results of these studies will be the prerequisite for man's ability to carry out scientific experiments and to establish production and living facilities in space. Therefore, some scientists believe that manned spaceflight is not possible without research in space medico-engineering.

The Chinese Institute of Space Medico-Engineering was established on 1 April 1968. Over the past 20 years, the technical staff of the institute has combined the research techniques of different scientific disciplines such as man-machine environment and systems engineering, medicine and engineering, Chinese and Western medicine, etc. to produce 455 research accomplishments in space medico-engineering. In particular, the research and application of medical engineering to develop a space life support system received first prize for the 1985 national technology advancement award. In recent years, progress is also being made in developing international cooperation and technical exchange.

The little-known research area contains a complete facility of ground simulation equipment used for China's space medico-engineering research. When the reporters were led on a tour of the low-pressure chamber, the

low-pressure variable-temperature chamber and the environmental simulation chamber for the life support system, they all wanted to enter the chambers to experience the living environment of the astronauts. Of particular interest to them were the motorized four-column swing, the hydraulic multi-function rotary chair, and the impact tower. They were also told that this institute has the largest centrifuge in Asia, and some of the equipment are among the few available in the world.

Under the direction of the famous scientist Qian Xuesen, this institute has applied the theory and techniques of system engineering to allow medical scientists and engineers to learn from each other and to work together. Jointly, they carried out many original research projects, and solved a number of difficult problems in developing the spacesuit and the life support system. In the process of conducting the research, a well-trained space medico-engineering team has been established and a strong foundation has been built for developing China's manned spacecraft technology.

One of the important research topics in space medicine is the problem of preventing and protecting against diseases associated with space motion. This institute has applied some unique approaches in studying the problem of protecting against bone loss due to space motion, noise, and radiation; specifically, encouraging results have been obtained using traditional medical theories and techniques (Chinese medical methods, Chinese medicine, and breathing techniques). These techniques have attracted considerable attention from the medical communities abroad; some have initiated proposals for joint study efforts with this institute.

In recent years, the research results from this institute have already been used in many of China's commercial, technical and military applications. For example, the pressure control system used by the Scientific Exploration and Experiment Satellite was developed as part of manned spacecraft research program. The development of the oxygen supply system and the medical sensor device have produced definite social and economic benefits. Also, the research and development of protective earplugs, radio earphones, protective helmets, liquid cooling systems, microwave protective suits, protective equipment for propellant-handling personnel, and anti-microwave medicines will play an important role in ensuring the safety, health, and efficiency of personnel working under special environmental conditions, and in promoting the modernization of China's commercial and military equipment.

3012/6091

Xichang: Center of Spacecraft Technology

40080176b Beijing XIANDAIHUA [MODERNIZATION] in Chinese No 6, Jun 88 p 33

[Article by Yu Qingtian [0060 1987 3944]]

[Text] On 6 March, Beijing was still windy and cold. Our plane took off from the airport toward the southwest; after more than 2 hours' flying time, we reached the key city of southern China, Xichang. Spring had already arrived in Xichang, the city was surrounded by flowers, birds, and green fields. The purpose of our trip was to participate in a satellite launch exercise.

Xichang, the capital of the autonomous state of China's Yi people, is also the home of the famous Xichang Satellite Launch Center. The launch site is located in a long valley in the foothills of Daliang Shan; it is approximately 60 km from Xichang City. As we approached the site, the first thing that came into view was a huge steel structure rising from the ground--the satellite launch tower. We were told that this 70-m structure was built with more than 900 tons of steel. It has 22 steel arms which can rotate horizontally over 180° to embrace the satellite, thus forming 11 movable platforms where technicians can safely perform their work. It is here that the satellite and the rocket are erected and joined together, tested and inspected, adjusted for bearings, and filled with propellant. Then it is ignited and launched into space.

This area was selected as the launch site because of its favorable geographic conditions. In ancient times, Xichang used to be called Moon City because of its year-around clear skies. It is located high above sea level with clear air, cool summers, and mild winters. It has 320 sunny days in a year, with an average temperature of 16°C; hence it provides a unique window for launching satellites. Also, because of its low latitude, one can fully take advantage of the centrifugal force generated by earth rotation; for this reason, it is particularly suitable for launching geosynchronous satellites. In terms of transportation, it can be easily accessed through Xichang airport, which can accommodate large airplanes, and the Chengdu-Kunming Railroad as well as the Sichuan-Yunnan Highway which both pass through here. The launch center also has its own dedicated railroad and highway to reach other regions.

The primary launch vehicle used at the Xichang Launch Center is the Long March-3, which can launch various geosynchronous satellites such as communications satellites, broadcast satellites, and weather satellites. This launch vehicle has three stages; the first two stages are 3.35 m in diameter and the third stage is 2.25 m in diameter. The total length of the vehicle is 43.25 m and the lift-off weight is approximately 202 tons. It has a lift-off thrust of 280 tons which can inject a 1-ton satellite into geosynchronous orbit. The launch center is organized into six major systems: command and control, test and launch, tracking and measurement, communication, weather, and technical services. In addition to the launch site, its modern technical center and command control center have state-of-the-art equipment and well-trained operating and command personnel. The tracking and measurement system, the communication system and the weather system are all well equipped and have a strong technical staff.

Since its completion in 1980, the launch center has successfully launched four satellites into space. The communications satellite launched on 7 March of this year is now stationed at 87° East Longitude above the equator. This satellite will play an important role in improving China's capability in communications and television relay. In recent years, with the implementation of China's open-door policy, its launch vehicles have entered the international aerospace market, and the Xichang Launch Center has also opened its door to the world. One after another, aerospace delegations and foreign experts have arrived here to visit and to discuss joint ventures of launching satellites. Visitors from Europe, Asia, America, and Oceania have come here for tours, inspections, and meetings. Some have signed letters of intent, some have signed contracts, and others have brought back their impression of China's aerospace industry.

3012/6091

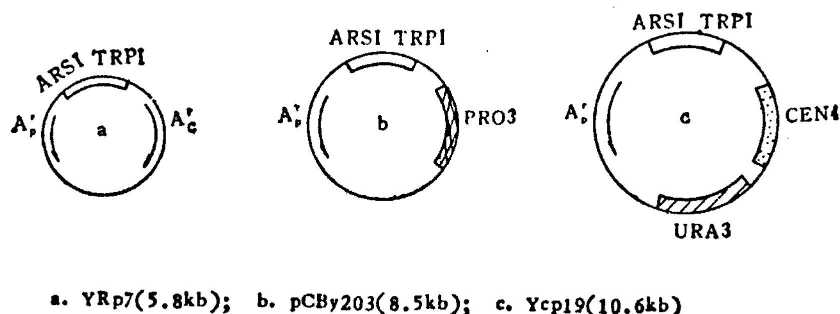
Selective Isolation of Yeast Auxotroph PRO3+

40081083a Beijing KEXUE TONGBAO in Chinese Vol 33 No 6, Mar 88 pp 463-464

[Article by Jiang Weidong [5639 0251 2639] and Kuang Daren [0562 6671 0086] of the Cell Biology Institute of the Chinese Academy of Sciences, Shanghai branch: "Selective Isolation of Yeast Auxotroph in YEPD Rich Medium"; received 24 March 1987]

[Text] The first isolation and characterization of three proline-requiring auxotrophic mutants of Sacchromyces cerevisiae (beer yeast) was reported by Brandriss in 1979[1]. He also observed that the yeast proline auxotrophs failed to grow on the rich yeast extract-peptone-dextrose (YEPD) medium. This failure was not fully understood then, and only after 2 years of study was he able to attribute the cause to ammonium ions in YEPD medium, which repressed the proline transport system of the yeast Sacchromyces cerevisiae[2].

To isolate gene PRO3, we first transformed the proC mutant of E. coli using a yeast genome generated by the plasmid YRp7[3,4]. The modified mutant was then cloned by the complementation method to yield the PRO3 yeast (Figure 1). The plasmid containing gene PRO3 was designated pCBy203[4] (Figure 1b).



a. YRp7(5.8kb); b. pCBy203(8.5kb); c. Ycp19(10.6kb)

Figure 1. Structures of Yeast Plasmids

The beer yeast MB299-7A (α, pro3, lys, Brandriss[1]) was then transformed with pCBy203 in the presence of lithium acetate[5]. On the YEPD medium, the pro⁻ mutant was not able to grow. Yet, the replication of the MB299-7A auxotroph should not be and was not interrupted, because its plasmid contained gene

PRO3. We isolated approximately equal numbers of $PRO3^+$ auxotroph of MB299-7A (∿100/μgDNA) from the selective medium (SD + lys) and from the YEPD medium. This result clearly demonstrated that the YEPD medium can be used directly to culture PRO^+ auxotrophs selectively.

In order to prove that the plasmid pCBy203 can stay stably in the yeast strand MB299-7A and that the mutant can grow in YEPD rich medium, we examined the stability of pCBy203 in MB299-7A cells in the mitosis process (mitotic stability, for short) and the expression efficiencies of gene PRO3 in selective medium and in YEPD medium.

We transformed Eal-103 (α, trp1, leu2, ura3) with the three (TRP1-containing) plasmids, YRp7 (vector), YCp19[6] (containing the centromere, therefore, more stable; Figure 1) and pCBy203. The pCBy203-transformed auxotroph was closely examined and compared with the mutants obtained through the first two plasmids. Yeast transformants were then cultured on a synthetic, selective medium (SD + leu + ura). The mitotic stabilities of the plasmids in the yeast cells were then measured as described in the literature[7]. It was noteworthy that it took the $PRO3^+$ auxotroph of MB299-7A 2 days to propagate (approximately 20 generations) on the SD + lys + pro medium, because its growth rate on this selective medium is much slower than that on the YEPD medium (1 day for 20 generations). The experimental results, given in Table 1, showed that the pCBy203 plasmid was only metastable in yeast Eal-103; in a non-selective medium, pCBy203, like YRp7, was miotically unstable (experiments 4 and 5). We also observed that the growth of pCBy203-modified auxotroph of MB299-7A, in a selective environment fortified with lysine (experiment 1) was comparable to that on the YEPD medium (experiment 2) and the reverse is true. Thus, it was concluded that the selectivity of the YEPD medium helped to retain the inserted plasmid under the experimental conditions. Experiment 1 showed that the pCBy203 plasmid was metastable while experiment 2 clearly demonstrated the selectivity of YEPD medium. Although plasmid pCBy203 was retained 100 percent in the yeast after having been propagating for 20 generations in a non-selective medium in experiment 3, it does not necessarily mean that the pCBy203 had become an integral or a centrometric plasmid of the host yeast; it simply means that the plasmid was retained (experiment 2) after a propagation of 20 generations on the YEPD medium (used as a non-selective medium). Consequently, when pCBy203/MB299-7A was cultured in the SD + lys selective medium, 100 percent of the plasmid pCBy203 was retained. This observation indicated that the YEPD medium is selective only when used as selective medium and not as a non-selective medium.

The Northern blotting procedure showed that PRO3 genes of the pCBy203/MB299-7A auxotrophs, whether cultured on SD + lys medium or YEPD rich medium, could not only transcribe normally to mRNA, but also its translated products--the P5C reductase--was able to maintain its enzymatic activity at the same level. This observation further supports our conclusion that the YEPD medium can be used in the place of SD + lys medium as a selective culture medium.

Table 1. Mitotic Stabilities of Plasmids in Yeast Cells

Experiment	Plasmid/yeast	Chromosome marker	Non-selective media	Selective media	Percent of plasmid remaining after a 20-generation propagation on non-selective media
1	pCBy203/MB299-7A	PRO3	SD + pro + lys	SD + lys	1-2
2	pCBy203/MB299-7A	PRO3	SD + pro + lys	YEPD	1-2
3	pCBy203/MB299-7A	PRO3	YEPD	SD + lys	∿100
4	pCB6203/Ea1-103	TRP1	YEPD	SD + leu + ura	1-2
5	YRp7/Ea1-103	TRP1	YEPD	SD + leu + ura	1-2
6	Ycp19/Ea1-103	TRP1	YEPD	SD + leu + ura	∿98

In order for a foreign gene in a yeast cell to be expressed, a selective medium, supplemented with antibiotics or a synthetic, selective medium is usually required. However, yeast and human cells are eukaryota; therefore, antibiotics, which are effective against the yeast cells are also harmful to human cells. In addition, the synthetic, selective medium is made of nutrients (such as amino acids, nucleic acids and vitamins), which are relatively expensive and not easy to prepare. Furthermore, yeast cells grow much faster on a YEPD medium than on a synthetic, selective medium. The low-cost maltose, commonly used in industrial manufacturing, is a good example of rich medium. In conclusion, the rich culture medium offers many advantages over the synthetic, selective medium. The results of our study have demonstrated that by transforming yeast cells with the plasmid pCBy203 to mutants containing gene PRO3 enables us to grow modified yeast cells on a rich medium, thus, making it possible to achieve selective isolation by direct culturing on a rich medium. We believe this observation is of great significance in practical applications in yeast genetic engineering. For instance, if we can insert the PRO3 gene into a plasmid which can express foreign genes, and in turn, use this modified plasmid to transform MB299-7A or other pro3 mutants, then, we should be able to selectively culture PRO^+ auxotrophs on a rich medium; that is to culture foreign gene genetically engineered products on a rich medium.

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12817/6091

Disease Resistant Tobacco Plant Gene From Viral Satellite RNA

40081083b Beijing KEXUE TONGBAO in Chinese Vol 33 No 6, Mar 88 p 480

[Article by Wu Shixuan [0702 0013 1357], Zhao Shuzhen [6392 3219 3791], Zhang Chunxia [1728 2504 7209], Wang Gejiao [3769 7245 1293], Yang Xicai [7299 1585 2688], Wang Xin [3769 2500], and Tian Po [3944 3134] of the Microbiology Institute of the Chinese Academy of Sciences, Beijing: "Construction of a Cucumber Mosaic Virus Resistant Tobacco Plant Gene Through the Expression of Viral Satellite RNA"]

[Text] This laboratory reported the first success of genetically engineered protection of cucumbers against the cucumber mosaic virus (CMV) by viral satellite RNA in 1983[1,2]. In order to further explore the potential application of viral satellite RNA in plant disease protection and control, we synthesized and cloned the complementary DNA (cDNA) of the satellite RNA-1 of CMV. The nucleotide sequence analysis showed that this cDNA was a single strand, linear molecule. To achieve expression of its sequence in transformed tobacco plants, this cDNA molecule was transferred into an expression vector, ROK-II, based on the binary Ti-plasmid sequence, the terminator fragment of the nopaline synthase (nos) gene of Agrobacterium T-DNA as well as the middle section of the promoter ROK-II of cauliflower mosaic virus 35S RNA. The gene of the reconstructed satellite cDNA was then mobilized into the Ti-plasmid of Agrobacterium tumefaciens, using pRK2013 in a triparental mating. The resultant Agrobacterium strain was then used to infect leaf disks from the G-140 plant, a common extension variety of tobacco plant in China. The disks were cultured in the NBK medium, supplemented with kanamycin, to select segregants containing the linked kanamycin resistance gene. Transformed plants were propagated by transfer of stem cuttings into root induction medium. To determine the biological activity of these transcribed RNA molecules, the transformed plants were infected with a specific strain of CMV, which was a satellite RNA-free isolate of CMV obtained in the field. Non-transformed G-140 tobacco plants were similarly infected as controls. Seven days after inoculation, transformed plants developed mottling in infected leaves, as did control plants. RNA's were isolated from the leaves; and analysis by electrophoresis on polyacrylamide-agarose gels indicated that 9 out of the 10 transformed plants had synthesized large amounts of satellite RNA; and one of the transformed plants, as well as all four of the control plants, failed to produce any satellite RNA. Further observations revealed that mosaic symptoms grew more and more serious

in all infected leaves of non-transformed plants and the one transformed plant which failed to express the satellite RNA. Three weeks after inoculation, young leaves puckered and developed chlorotic lesions; the plants became obviously stunted as well. In contrast, the symptoms gradually improved in the plants which expressed satellite RNA: leaves produced subsequently did not show mosaic symptoms and the plants grew almost as well as non-infected ones. Finally, infectivity tests were conducted to determine the titre of virus in the infected plants. Sap from infected leaves of equal weight, from plants which expressed satellite RNA and from plants which did not, was sampled 19 days after infection and inoculated to leaves of Chenpodium quinoa, a local-lesion host of CMV, by the half-leave correlation method. The sap from transformed plants which expressed satellite RNA produced 42 lesions on 20 half leaves, while the sap from the transformed plant which did not induce the synthesis of satellite RNA produced 446 lesions under similar conditions.

These experiments demonstrated that 9 of the 10 transformed tobacco plants in fact contained the satellite cDNA, which through the induction of CMV, transcribed a large amount of satellite RNA in the plants. As a result of the satellite RNA molecules' ability to inhibit the replication of CMV RNA, the transformed plants have become less susceptible or, in some cases, even totally resistant to CMV infection. Baulcombe et al.,[3,4] from the United Kingdom, also reported achieving plant protection against CMV symptoms by the transformation of tobacco plants (cv. Samsun NN) with cDNA copies of satellite RNA isolated from CMV strain $I_{17}N$. Alien genetic material can be transferred into plant genomes by Ti-plasmid system and the plants' genetic resistance to viruses thus produced, should transcribe and evolve according to Mendel's laws. These effects indicate that it is feasible to obtain virus-resistant crop plants by modifying the natural varieties through genetic engineering.

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12817/6091

Lab Tests For Optimum Way To Produce Alpha Amylase Detailed

40081078a Beijing WEISHENGWUXUE TONGBAO [MICROBIOLOBY] in Chinese Vol 15 No 2, Apr 88 pp 52-54

[Article by Kong Xianliang [1313 7359 5308], Zuo Jing [1563 7234], Sun Zengmei [1327 2582 5019], and Qi Zutong [7871 4371 0681], Microbiology Institute, Chinese Academy of Sciences, Beijing, except for Zuo Jing, who is a fourth year student specializing in applied biochemistry in the Biology Department of Beijing University. (Comrade Jiang Liping [1203 7787 5493] took part in some of the work, and Comrade Zhao Hai [0340 3189 5493] took part in some of the work, and Comrade Zhao Hai [0340 3189] of the Preservatory provided some of the bacteria).: "Research on the Screening of Alpha Amylase Produced as Aspergillus Oryzae and Conditions For Its Production"]

[Text] Abstract: Five strains of bacteria fairly active in the production of alpha amylase were screened from among 51 strains of Aspergillus oryzae, and experiments were performed on one of them, No 5037 strain, to determine conditions required for the production of alpha amylase. The most suitable culturing temperature was between 30 and 33 degrees C for a period of 2-1/2 days. Enzyme activity began to decline 3 days later. A pH of between 3.5 and 6.0 had little effect on enzyme production activity. Addition of a source of carbon such as dextrin and dried melon powder increased production, 5 percent cornstarch being best. Addition of a source of nitrogen such as NH_4NO_3 and urea had an anxo-action on enzyme production; NH_4Cl inhibited enzyme production. Under most suitable enzyme production conditions, enzyme activity averaged 438.8 u/ml.

Key Terms: Aspergillus oryzae; alpha amylase; and enzyme production conditions.

China has a long history of Aspergillus oryzae use. The principal microbes in the distiller's yeast used in ancient times to produce "huangyi and huangzheng" liquor were Aspergillus oryzae. The Taka-diastase made from Aspergillus oryzae that the Japanese Takamine Jokichi prepared in 1898 is extremely widely used in both industry and medicine.[1] During the 1950's, China conducted research on Aspergillus oryzae production of enzymes,[2,3] but subsequently little work was done in this regard. Denmark, the United

States, and Japan use fungus alpha amylase enzyme preparations produced by Aspergillus oryzae, but no such products have been produced in China. Work in this regard must be undertaken in order to keep up with the development of food, pharmaceutical, starch processing, and beer industries. This article reports research on the screening of alpha amylase producing strains of Aspergillus oryzae and the conditions under which the enzyme is produced.

Materials and Methods

(1)

Fifty-one strains of Aspergillus oryzae were provided by the fungus laboratory and the preservatory of the Microbiology Institute. The bacteria were cultured at 30 degrees for 5 days preparatory to use.

(2) Culturing Medium

Slant culturing in a test tube using Czapek slant agar culturing medium.

Solid fermentation culturing medium: One hundred grams of the coarser part of wheat bran was selected. To this was added 120 ml of water (containing 0.5 percent urea). After mixing, it was put in 250 ml flasks.

(3) Preparation of Enzyme Solution

The basic culturing medium was subjected to high pressure steam at a pressure of 1 kg/cm^2 for 30 minutes to kill bacteria. After cooling, it was inoculated and cultured in an incubator at 30 degrees C for 16 hours, being shaken once. After 40 hours, the culture in the flasks was inverted and left to culture for 2-1/2 days, after which it was removed.

To the culture was added 50 ml of distilled water. After pounding to a pulp, another 50 ml of water was added and shaken to mix it in. Then, it was placed in a 37 degrees C incubator to soak for 3 hours. It was then removed and filtered through cotton to provide a crude enzyme solution.

(4) Enzyme Activity Assay Method

Ministry of Light Industry standards were used.[4] The iodine staining reaction method was used to assay alpha amylase activity. One enzyme activity unit consists of the liquefaction of 1 gram of soluble starch by 1 ml of enzyme solution at 60 degrees C, and a pH of 4.8 for 1 hour. This experiment expresses enzyme activity produced per gram of dry yeast.

(5) Reagents

Soluble Starch: Produced by the Linghu Chemical Reagent Plant in Zhejiang Province. Made up on the same day it was to be used.

Results

(1) Screening of Bacteria Strains

Cultured for 3 days at 30 degrees C in the basic culturing medium, and then assayed for activity. Results are shown in Table 1. Enzyme activity was fairly good in five of the 51 strains. Number 5037 was selected for further research in view of its short fermentation time, and the light color of spores.

Table 1. Results of Alpha Amylase Assay of 51 Strains of Aspergillus Oryzae

Enzyme Activity (u/g)	Bacteria Strain Number
More than 200	5
151-200	3
101-150	7
100 and below	36

(2) Conditions For Production of Alpha Amylase

1. Effect of Culturing Time on Enzyme Production Activity

During the culturing process, samples were taken every 12 hours for assay of enzyme activity, the results of which are shown in Table 2. Table 2 shows maximum enzyme production activity after 2-1/2 days of culturing. After 3 days, enzyme activity began to decline.

Table 2. Effect of Culturing Time on Enzyme Production Activity

Time (Days)	Enzyme Activity (u/g)	Time (Days)	Enzyme Activity (u/g)
2.0	191.5	4.0	152.0
2.5	206.1	4.5	135.4
3.0	183.7	5.0	99.1
3.5	168.3		

2. Effect of Temperature on Enzyme Production Activity

Culturing tests were done at different temperatures. Figure 1 shows the suitable temperature range for the bacteria to produce the enzyme in between 28 and 35 degrees C. The most suitable temperature is 30 to 33 degrees C.

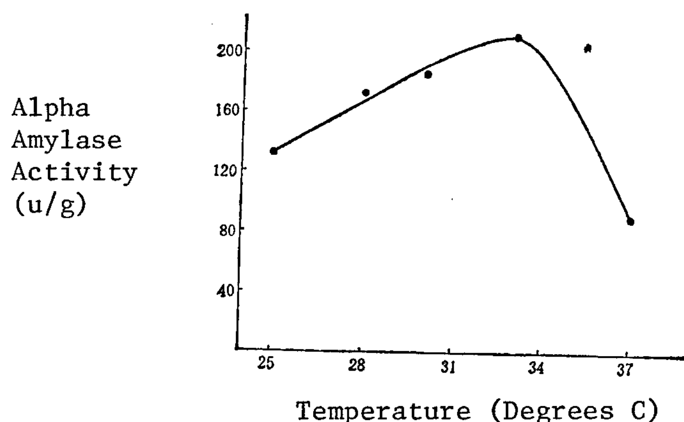

Figure 1. Effect of Different Culturing Temperatures on Enzyme Production Activity

3. Effect of Initial pH on Enzyme Production Activity

Hydrochloric acid and sodium hydroxide were used to adjust the pH of the culturing solution. A pH meter was used for accurate determination of the pH value. After culturing, enzyme activity was tested. Results are shown in Table 3. The proper pH range for the production of enzyme is between 3.5 and 6.0; however, requirements are not strict, and a nautral pH may be used.

Table 3. Effect of Initial pH on Enzyme Production Activity

pH	Enzyme Activity (u/g)	pH	Enzyme Activity (u/g)
3.0	148.6	5.5	154.3
3.5	160.1	6.0	151.7
4.0	169.3	6.5	142.3
4.5	162.6	7.0	140.0
5.0	158.5		

4. Effects of Different Water Content on Enzyme Production Activity

Ten grams of wheat bran were apportioned among 250 ml flasks, and 6, 8, 10, 12, 14, and 16 ml of water containing 0.5 percent urea were added to separate flasks. The bacteria were then killed, and the flasks were inoculated and cultured for 2.5 days after which enzyme activity was assayed (See Table 4). The flasks with a water content ranging between 50.0 and 58.3 percent produced fairly good activity, but enzyme activity was best at 54.5 percent.

Table 4. Effects of Different Water Content on Enzyme Production Activity

Water Added (ml)	Water Content (%)	Enzyme Activity (u/g)	Water Added (ml)	Water Content (%)	Enzyme Activity (u/g)
6	37.5	121.3	12	54.5	191.4
8	44.4	173.7	14	58.3	187.6
10	50.0	183.8	16	61.5	172.8

5. Effects of Different Carbon Sources on Enzyme Production Activity

Different sources of carbon were added to the basic culturing medium. Enzyme production activity following culturing is shown in Table 5. These results show the following: The addition of an appropriate amount of these substances promoted the production of enzyme to varying degrees in the following discending order of presence: dextrin, dried melon powder, glucose, lactose, cornstarch, maltose, soluble starch. Dextrin was best.

Table 5. Effects of Different Carbon Sources on Enzyme Production Activity

Carbon Source	As a Percent of Solids	Enzyme Activity (u/g)	Carbon Source	As a Percent of Solids	Enzyme Activity (u/g)
Soluble starch	5	182.0	Glucose	0.5	220.9
Cornstarch	5	187.8	Maltose	0.5	184.7
Dried melon powder	5	222.1	Lactose	0.5	193.1
Dextrin	5	244.2	Control	0	155.5

6. Effect on Enzyme Production Activity of Increased Amounts of Cornstarch

In order to meet applications to production, different amounts of cornstarch were added as a supplementary source of carbon to the basic culturing medium. After culturing, enzyme activity was assayed. When the amount of cornstarch added was 0.5 g, enzyme activity was highest (See Table 6).

Table 6. Effects on Enzyme Production Activity of the Addition of Different Amounts of Cornstarch

Cornstarch Added (g)	Enzyme Activity (u/g)	Cornstarch Added (g)	Enzyme Activity (u/g)
0	155.5	1.0	187.1
0.1	190.9	1.5	183.6
0.5	205.1	2.0	178.8

7. Effects on Enzyme Production Activity of Various Sources of Nitrogen

To the basic culturing medium were added different nitrogen sources at a 1 percent concentration. Ammonium nitrate, urea, yeast paste, ammonium hyperphosphate, and polypeptone accelerated enzyme formation. Ammonium chloride inhibited enzyme formation. Ammonium nitrate and urea were good nitrogen sources for the bacteria, enzyme activity being greatest when they were added. (Table 7)

Table 7. Effects on Enzyme Production Activity of Various Sources of Nitrogen

Nitrogen Source	Enzyme Activity (u/g)	Nitrogen Source	Enzyme Activity (u/g)
NH_4Cl	67.9	Urea	172.2
NH_4NO_3	174.7	Peptone	136.7
$(NH_4)_2HPO_4$	156.0	Yeast paste	163.1
$(NH_4)_2SO_4$	114.0	Polypeptone	143.7
$NaNO_3$	149.4	Control	140.6

8. Effect on Enzyme Production Activity of Different Urea Concentrations

Ten grams of wheat bran was put into separate 250 ml flasks to make a urea solution of different concentrations through the addition of 12 ml of water. Bacteria were killed and the flasks were inoculated. After 2-1/2 days enzyme activity was assayed. The results, which are shown in Table 8, show the most suitable concentration of urea to be 1 percent.

Table 8. Effect on Enzyme Production Activity of Different Urea Concentrations

Urea Concentration (%)	Enzyme Activity (u/g)	Urea Concentration (%)	Enzyme Activity (u/g)
0	185.9	1.5	195.1
0.5	208.6	2.0	194.2
1	233.3		

9. Effect on Enzyme Production Activity of Different Bean Cake Content

To the basic culturing medium was added different amounts of bean cake as a supplementary source of nitrogen. After culturing, enzyme activity was assayed. When the bean cake content was 1 percent of solids, enzyme activity was highest. (See Table 9)

Table 9. Effect on Enzyme Production Activity of Different Bean Cake Content

Percent of Solids (%)	Enzyme Activity (u/g)	Percent of Solids (%)	Enzyme Activity (u/g)
0.0	129.3	1.0	183.0
0.1	166.1	1.5	176.9
0.5	175.5	2.0	168.5

10. Testing of Optimum Enzyme Production Conditions

To 9.5 g of wheat bran was added 0.5 g of dextrin. This was placed in separate 250 ml flasks to which 12 ml of water with a 1 percent urea content was added, and then 0.1 g of bean cake was added. The mixture was evenly mixed and bacteria were killed. After inoculation, it was cultured at 30 degrees C for 2-1/2 days. Then enzyme activity was assayed. Test results for five batches are shown in Table 10. Enzyme activity averaged 438.8 u/ml.

Table 10. Overall Optimum Conditions For Enzyme Production Activity

Batch Number	Enzyme Activity (u/ml)
1	450.0
2	411.4
3	514.0
4	407.5
5	411.0
Average	438.8

Discussion

Alpha amylase is an enzyme preparation that is produced in large quantities and used widely. China presently has only a single kind of alpha amylase produced from Bacillus subtilis, which hurts use by certain industries. When Aspergillus oryzae strains were first screened for those that produced the most alpha amylase, enzyme activity was not high from liquid fermentation. After changing to the solid wheat bran culturing medium, activity increased more than 10 fold, which was consistent with results reported by Meyrath[5] and Ramesh.[6] Kunda et al[7] believed that starch was the best source of nitrogen for all enzyme producing bacteria. Wheat bran is able to provide starch and high nitrogen content chemical compounds as well as highly concentrated phosphates and various vitamins. Murota et al[8] reported both urea and ammonium sulphate as being rather good sources of nitrogen. In this experiment, the selected No 5037 strain of Aspergillus oryzae was cultured in a solid culturing medium to which was added suitable amounts of carbon and nitrogen sources producing an amylase producing activity averaging 438.8 u/ml. With further testing, this bacterium strain holds promise for being put into production.

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9432/12223

Application of DNA Transformation Method of Alpha Amylase Gene Isolation

40081078b Beijing WEISHENGWUXUE TONGBAO [MICROBIOLOGY] in Chinese Vol 15
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[Article by Zhang Lijuan [1728 7787 1227] and Zhao Baoguo [6392 0202 0948], Applied Microbiology Institute, Heilongjiang Provincial Academy of Sciences, Harbin. (Zhang Lijuan is now with the Chinese Pharmaceuticals and Biolotical Certification Institute in Beijing. Comrade Bei Yinglin [4101 2019 2651] of the Genetics Institute of the Chinese Academy of Sciences assisted in this experiment, for which gratitude is hereby expressed).: "Isolation of Alpha Amylase Gene From Bacillus Subtilis 8a5 by the DNA Transformation Method"]

[Text] Abstract: EcoRi was used for the partial degrading of Bacillus subtilis 8a5 chromosome DNA: then agarose gel electrophoresis was used to isolate and purify each of the degraded segments. The transformation method was used to assay one by one the transformation action of each of the segments. Results of the experiment show that alpha amylase genes may themselves serve as selective markets for use in the isolation of the alpha amylase gene. Hind III degraded 2-DNA served as a molecular weight control. It was determined that it was possible to transform with high efficiency an amylase gene DNA segment approximately 4.3 kb in size. The transformation rate for the alpha amylase gene obtained in this experiment was 1 x 10^4 transformants/μg DNA.

Key Terms: Transformation method; alpha amylase gene

Bacillus subtilis is a strain of bacteria whose 8a5 chromosome produces high yields of amalyse used in production. Alpha amylase is made up of a single peptide chain controlled by a single gene. When a carrier introduces an alpha amylase gene into a receptor cell, the reproduction of the carrier within the cell enables the target gene to achieve highly efficient expression. If a secreting cell is used as a receptor, of if a secreting carrier stimulates the release of enzyme by the cell, a bacteria strain can be built that produces high yields of amylase.

Isolation of a target gene is the basis for gene cloning, and the transformation method is an effective way to go about gene isolation. Yamane et al[1] used the transformation method in agarose gel electrophoresis to locate the zone in which are located the tyrA, trpB, aroI, hisA, leuA and

lys 21 genes of Bacillus subtilis. The current experiment has made some improvements on this original method to fix the location in the gel electrophesis zone of the alpha amylase gene (amyE) in the chromosome of the Bacillus subtilis.

It is generally believed that the amyE gene in the Bacillus subtiliz system cannot serve as a selective marker, but rather that the chain link between the aroI and amyE genes on the chromosome should be used, the aroI gene serving as a selective marker.[2] In the cloning of the amyE gene, the aroI segment containing the amyE gene is isolated first,[3,4,5] after which the amyE gene is cloned. Since alpha amylase is an ectoenzyme produced by Bacillus subtilis, the bacillus cells secrete large amounts of amylase, and hydrolytic starch serves as a source of carbon for continued growth only when starch is present but other readily usable small molecule sources of carbon are lacking. Since amylase negative bacillus strains cannot produce amylase and cannot use amylase, they are unable to grow in a medium that contains only starch as a source of carbon. This experiment benefited from this distinction to use amyE as a selective marker in isolation of the Bacillus subtilis 8a5 alpha amylase gene.

Materials and Methods

(1) Bacteria

Bacillus subtilis 8a5 contained the donor gene. These bacteria are a strain producing large yields of amylase that had been conserved by the Heilongjiang Provincial Applied Microorganism Institute. The receptor bacteria were Bacillus subtilis L-trp$^-$, amyE$^-$, str^r donated by the Genetics Institute of the Chinese Academy of Sciences.

(2) Culturing Medium and Culturing Conditions

1. The donor bacteria were grown in LB broth at 37 degrees C for 14 hours during which the broth was vibrated. The thalli were collected and chromosome DNA was extracted from them.

2. The receptor bacteria received state processing and culturing medium was used for transformation.

BY-liquid culturing medium: 0.5 percent beef extract, 0.5 percent yeast extract, 1 percent peptone, 0.5 percent sodium chloride, and 0.5 percent glucose.

GM-growth culturing medium: 1 x spizizen saline solution, 0.02 percent hydrolytic casein, 0.1 percent yeast extract, 50 μg/ml tryptophan (trp) as an amino acid needed by the receptor bacteria.

TM-transformation culturing medium: 1 x spizizen saline solution, 0.01 percent hydrolytic casein, and 5 μg/ml tryptophan.

3. Starch-agar culturing medium used for transformation SA: 0.02 percent $MgSo_4$, 0.2 percent $(NH_4)_2SO_4$, 1.4 percent $K_2HPO_4.3H_2O$, 0.6 percent $KHPO_4$, 1 percent starch, 50 μg/ml tryptophan, and 2 percent agar. Transformant cultured 3 to 5 days, then assayed.

(3) Method

1. Chromosome DNA extracted using the Dubnau method[6].

2. Electrophoresis isolation of chromosome segment cut by enzyme: To 50 mg of chromosome DNA was added to u of EcoRI for use at 37 degrees C for from 1/2 to 1 hour, and at 70 degrees for 5 minutes when the reaction was halted. For the electrophoresis a 0.5 x 14 x 14 cm parallel plate electrophoresis, and 0.7 percent agarose gel were used. The electrophoresis buffering solution was 0.04 M Tris-HAc, 0.002 M EDTA, at a pH of 8.0. Thirty volts of electricity were used. Bromophenol blue was controlled at 10 cm from the end of the spotted specimen and electrophoresis was halted. The gel was cut into 20 segments 0.5 cm wide, and they were numbered from 1 to 20 beginning from the end where specimens were spotted, separated and put into 1.5 ml centrifuging tubes.

3. Purification of chromosome segments: The tubes filled with gel pieces were placed in liquid nitrogen for 30 seconds to quick freeze them. Then they were removed and allowed to revive naturally at room temperature. They were centrifuged in a desk centriguge at 12,000 rmp, and the precipitate was discarded. Phenol, phenol-chloroform, and isoamyl alcohol were used in that order to make several extractions from the supernate, and double the volume of cold 95 percent ethanol was added. Precipitation continued overnight at -20 degrees C, and centrifuging was done again for 15 minutes after which the precipitate was dissolved in a buffering solution, and its concentration was tested at 1 μg DNA/ml.

4. Receptor bacteria genotype verification: (a) The Bacillus subtilis receptor bacteria were inoculated in an LB plate containing starch and cultured for 24 hours. The isolated single bacteria colony spots were dibbled to a fresh starch LB plate and daubed with an iodine solution 24 hours later, no hydrolysis circles forming. (b) The receptor bacteria were dibbled to an SA plate containing tryptophan and cultured from between 24 and 36 hours, no bacteria colonies forming. (c) The receptor bacteria were inoculated to an LB slant. One generation after another was continuously cultured for several weeks, and they were separately streaked in a starch-agar plate, (SA), and continued to be cultured for from 2 to 5 days. The SA plate showed no growth of bacteria colonies.

5. Receptor state cell transformation: The receptor cells were inoculated into the BY culturing solution at 37 degrees C and vibrated during culturing for from 3 to 5 hours. At the place where the measured wave length measured 620 nm, the OD value was approximately 0.4 Centrifuging was done and the thalli were collected. The supernate was discarded. GM solution was used to wash the thalli once, and the bacteria cells were resuspended in an equal

volume of GM solution to bring the OD value to approximately 0.4. Culturing with vibration was continued at 37 degrees C for 3 to 5 hours, and the OD value (620 nm) was tested at 0.6 to 0.9. At a ration of 1 to 10, the bacteria suspension cultured in the GM solution was diluted in a TM solution. A 20 x 150 (mm) test tube was used for culturing with vibration at 37 degrees C for 90 minutes, and then this was added to the DNA solution and the TM solution as shown in Table 1. This was termed the transformed group. A bacteria control group and a DNA control group were also set up. (Table 1).

Table 1. Transformed Groups of Receptor State Cells

Group	Bacteria Solution (ml)	DN* (ml)	TM Solution (ml)
Transformed Group	0.2	0.1	1.7
Bacteria Control Group	0.2	-	1.8
DNA Control Group	-	0.1	1.9

*DNA concentration of 1 μg/ml.

Each of the above experimental groups were cultured at the same time at 37 degrees C while being vibrated for 30 minutes. Then, they were diluted 10^0 to 10^3 fold with a TM solution. From each diluted specimen, 0.1 ml was spread on a starch-agarose plate. The same spreading method was used without dilution for the receptor bacteria control group and the DNA control group. Culturing was done at 37 degrees C for from 2 to 5 days after which results were observed.

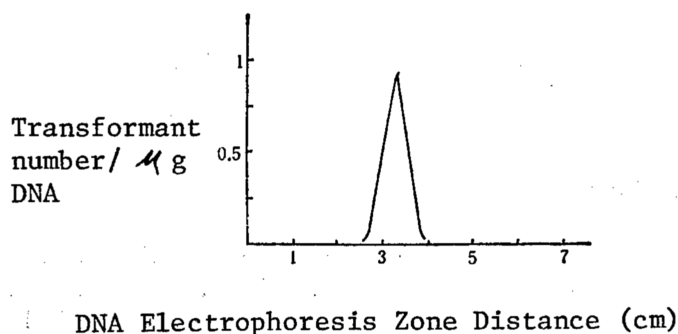
Results and Discussion

The chromosome segments that had been partially degraded by EcoRI and purified by electrophoresis individually transformed into Bacillus subtilis amylase negative receptor bacteria. These receptor bacteria were $recA^+$ system bacteria in a culturing medium in which starch was the sole source of carbon. Unless these bacteria could use the starch as a source of carbon, they would not be able to grow. The amylase positive bacteria strains thrived. After 2 to 5 days of culturing, the bacteria colonies that had grown, i.e., the amylase positive bacteria, were daubed with 0.1 percent iodine solution and all around the colonies transparent hydrolytic circles appeared. In the bacteria control group and the DNA control group, however, no colonies of amylase positive bacteria had formed.

Use of the transformation method to isolate the amylase gene is extremely effective; however, certain points must be noted as follows: Since the culturing solution used in receptor state processing is a complete culturing medium, the amylase negative bacteria can take this opportunity to grow. Results of this experiment show that by controlling the culturing time of the transformant, one can obtain accurate results. By culturing from between 3 to 5 days the plate on which the bacteria solution has been spread, the amylase positive bacteria (the transformants) are able to form rather large colonies (with a diameter of approximately 2 to 3 mm); however,

once the amylase negative bacteria have consumed the minute amount of nutrient, they stop growing, and there is no increase in bacteria colonies even after a long period of culturing. Only the original tiny colonies (with a diameter of less than 1 mm) remain. Therefore, by using the controlled culturing method, it is very easy to separate the amylase positive from the amylase negative colonies. This method obviates the use of iodine solution testing, not only simplifying procedures, but also reducing iodine solution interference with the cells' physiology.

Using the transformant numbers as the vertical axis and each of the gel electrophoresis zone distances (cm) as the horizontal axis to plot a graph, a transformation curve for the amylase gene was derived. (See Figure 2). The transformation rate is 1 x 10^4 transformant/μg DNA



DNA Electrophoresis Zone Distance (cm)

Figure 2. Curve For Isolation of the amyE Gene Using the Transformation Method

Results of the use of EcoRI to degrade the Bacillus subtillis 8a5 Chromosome DNA, 0.7 percent agarose gel electrophoresis, and assay by the transformation method.

After partial degradation of the Bacillus subtilis 8a5 chromosome DNA using EcoRI, agarose gel electrophoresis, and control of the bromophenol blue electrophoresis movement distance specimen end at 10 cm, results of the transformation experiment placed the DNA strand containing the alpha amylase-containing gene within the 2.5 to 4.0 cm area of the electrophoresis zone, and the amylase positive transformant peak was at the 3.0 to 3.5 cm location. Using the Hind III degraded 2-DNA as a molecular control, the size of the DNA segment that was able to transform the amylase gene with high effectiveness was close to 4.3 kb.

This experiment tested the genotype of the receptor bacteria; however, after continuous observation of successive generations, there were no reverse mutations. The experiment showed the receptor bacteria genotype to be stable, and that they were ideal transformed receptor bacteria, thereby showing the selected amylase positive bacteria strain to be the result of DNA transformation.

Molecular cloning of the alpha amylase gene holds important significance for increasing the kaobeishu [0572 6296 2422] of the target gene, thereby increasing the amylase output as well as building a secreting carrier that

can be generally used with Bacillus subtilis. Furthermore, it is extremely useful in the analysis of the evolutionary correlation of alpha amylase genes that are found in microorganisms and in plants and animals.[7,8] Teleological cloning of exogenetic genes requires, first of all the isolation of the target gene, a task which is very difficult unless a gene probe is available. In cloning the amylase gene, many researchers use aroI as a selective market, and they use the chain relationship of the aroI and the amyE gene in the chromosome atlas to isolate the aroI-amyE segment, such as constructing a specific transducing bacteriophage,[3] after which they isolate the amyE gene. Then they link it to the carrier in vitro and clone a corresponding receptor.

This experiment used amyE as a selective market, successfully isolating the Bacillus subtilis amylase gene. The controlled culturing time method was used for easy separation of the amylase positive from the amylase negative colonies and to obviate the step of using an iodine solution to test for amylase positive colonies, thereby avoiding having the iodine solution interfere with the bacteria's physiology.

The transformation method is one effective method for the isolation of genes. In the absence of a gene probe, in particular, this method is simple, workable, and teleologically strong.

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9432/12223

Reliability, Specificity of Vibrio Cholerae Toxin Gene Probe

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[Article by Xu Yongqiang [1776 3057 1730], Su Guofu [5685 0948 1381], and Liu Chuanxuan [0491 0278 2537], Institute of Basic Medical Research, Military Academy of Medical Sciences, Beijing: "Development and Application of a Gene Probe to Detect Vibrio Cholerae Toxin (CT)"]

[Text] Abstract: The cesium chloride-ethidium bromide density gradient ultra centrifuging method was used to isolate and purify recombinant plasmid pCT 332 containing the CT gene, and restrictive nucleic acid endonuclease Xba I + Bgl II was used for enzyme cutting. After agarose gel electrophoresis, the CT gene was recovered from the gel, and then [alpha-^{32}p]dATP was used in the notch translation marking of the gene. The CT gene probe was then hybridized with the Vibrio cholerae and other bacteria strains. The experiment showed the probe to have no autoploidy with E. coli C600, RRI, and with the C 600 containing plasmids pBR 322 or pBR 325. Hybridizing with Shigella dysenteriae, and with ETEC of hog origin, or human origin also showed a negative reaction. When hybridized with Y-1 adrenal gland cells and with Vibrio cholerae that GM-1 ELISA testing had found positive, a positive reaction occurred. Results of the experiment showed the gene probe to be sensitive and reliable, and with good specificity. It can be used not only in the laboratory to screen for recons and CT gene deficient mutants, but may also be used in epidemological surveys.

Key Terms: Vibrio cholerae; cholera toxin gene; gene probe; and bacteria colony orthotopic hybridizing

The recently developed DNA probe technique is used not only in the laboratory to detect recons and to study the correlation among genes, but it is also used in clinical dianosis and in epidemological examinations. Majardino et al[1] used a hepatitis B virus (HBV) DNA gene probe in detection work on hepatitis patients; blood serum and liver specimens, and to study the correlation between hepatitis and liver cancer. Echeverria[2] and Seriwatana[3] et al used LT (heat-labile enterotoxin) and ST (heat-stable enterotoxin) gene probes in epidemological examination of enterotoxin producing E. coli. Rubin et al[4] established gene probes to detect Salmonella typhi. Most recently, Maurell et al[5] cloned into E. coli gene segments that were related to Shigella flexneri intrusion into Hela cells,

and he used them in the selection of recons and in the study of the correlation among different kinds of dysentery. We prepared a CT gene probe from the recombinant plasmid pCT 332 containing a CT gene, and used it in the detection of Vibrio cholerae, E. coli, and ETEC of hog and human origin, obtaining satisfactory results. Currently, we are in process of using gene probes to screen CT gene deficient mutants.

Materials and Methods

(1) Reagents

[alpha-^{32}p]dATP (Amersham), dCTP, dGTP, and TTP (all Boehringer products), DNase 1 (Sigma), E. coli DNA polymerase 1 (BRL), Cow serum albuminin (Servac laboratories), glucose G-50 (Pharmacia), nitrocellulose filtering membranes (Shanghai No 10 Pharmaceutical Plant), calf thymus DNA (Shanghai Dairy Company), ficoll (Pharcacia), polyvinyl pyrollidone (Fluka AG), restrictive nucleic acid endonuclease XbaI and Bbl II (Huamei Company).

(2) Bacteria

Please see Table 1 for the principal bacteria strains used in this experiment. E. coli C 600 (pCT332) was a recombinant plasmid pCT 332 containing a CT gene existing in E. coli C 600. This bacteria strain was donated by Comrade Lu Deru [7120 1795 1172] of the Microbiology Institute of the Chinese Academy of Sciences.

(3) Preparatiaon of CT DNA Segment

The cesium chloride-ethidium bromide density gradient ultra centrifuging method [6] was used to isolate and purify the plasmid pCT 332, and Xba I + Bgl II endonuclease was used for enzyme cutting (in accordance with the manufacturer's recommendations) in the preparation of the agarose gel electrophoresis method[7], the CT gene segment being recovered from the gel.

(4) [alpha-^{32}p]dATP Marked CT Gene

The DNA notch translation method reported by Su Guofu[8] was followed, with [alpha-^{32}p]dATP being used to mark the CT DNA in 100 μl of reaction solution containing 20 μm each of 50-10 μl [alpha-^{32}p]dATP, 50 mm Tris-HCl (pH 7.9), 50 mm $MgCl_2$, 10 mm beta-mercaptoethanol, 5 μg of cow serum albumin, dGTP, dCTP and TTP, as well as 1 μg CT DNA, 100 ng DNase I, which was allowed to react at room temperature for 1 minute after which between 10 and 20 units of E. coli DNA and polymerase I were added and allowed to react at 14 degrees C for 3 hours. Then 60 μl of 0.25 m Na_2EDTA (pH 8.0) was added to halt the reaction. After processing in a Sephadex G-50 column, some was recovered. From each tube was taken 1 μl and counted in an LKB 1215 liquid scintillation counter, the first radiation peak being collected.

Table 1. Bacteria Strains

Bacteria Strain Vibrio cholerae	Enterotoxin Gene	Source
RV79	CT+	Mekalanos
569B	CT+	Biological Products Certification Institute
18001	CT+	Biological Products Certification Institute
18003	CT+	Biological Products Certification Institute
82178	CT+	Biological Products Certification Institute
82179	CT+	Biological Products Certification Institute
16186	CT+	Biological Products Certification Institute
16187	CT+	Biological Products Certification Institute
Wujiang 2	CT+	Biological Products Certification Institute
Bin 43	CT+	Biological Products Certification Institute
E. coli C600 (pCT 332)	CT+	Gennaro
E. coli K12		
C600	LT^-	Microbiology Institute, Chinese Academy of Sciences
RR1	LT^-	Molecular Biology Laboratory, Commonwealth Academy of Sciences, Australia
C600 (pBR 322)	LT^-	Microbiology Institute, Chinese Academy of Sciences
C600 (pBR 325)	LT^-	Provided by Wang Jiaxi [3769 1367 3886], Military Academy of Medical Sciences
44813	LT^-	Biological Products Certification Institute
44814	LT^-	Biological Products Certification Institute
C600 (EUD 299)	LT^-	Provided by Professor Falkaw, U.S.A.
C600 (p 307)	LT+ST+	Provided by Professor Maas, U.S.A.

(5) Colony Orthotopic Hybridizing

First, a checkerboard pattern of certain dimensions was drawn with a pencil on a piece of nitrocellulose filtering membrane. After sterilizing it under an ultraviolet light, it was placed on an LB plate, and a sterilized toothpick was used to dot the bacteria strain to the filtering membrane. Then it was cultured at 37 degrees C overnight. The filtering membrane was removed from the plate and placed for 5 minutes on two layers of Xinhua filtering paper that had been immersed in 0.5 N sodium hydroxide. This was then repeated. The same method was used twice for processing using 1M Tris-HCl (pH 7.6), 7 minutes each time. The filtering membrane should be neutral by this time (a pH test strip may be used to test the pH). Next, it should be processed for 10 minutes in 1.5 N of NaCl-0.5M Tris-Hcl (pH 7.6). After air drying, it was left at 80 degrees C for 2 hours. Next, it was hybridized at 37 degrees C for 4 hours (or overnight), and rehybridized for 24 hours. The filter paper was removed and washed for 2 hours using 2 x SSC-0.5 percent SDS at 56 degrees C. After drying, it was left at 80 degrees C for 2 hours and an autoradiograph made at -60 degrees C.

(6) Y-1 Adrenal Gland Cell Test

The CT toxin was detected using the Y-1 adrenal gland cell method reported by Sack et al.[9]

Results and Discussion

(1) Purity of CT Gene Probe

A recombinant plasmid pCT 332 containing a CT structural gene was made by Gennaro et al.[10] They took the Pst I and EcoR I segment of a Vibrio cholerae chromosome (the segment was 4.3 kb, and there was a cross reaction when hybridized with an ETEC LT gene) and recombined it into a derivative plasmid pAT 153 of plasmid pBR 322 to obtain recombinant plasmid pCT 332. They further verified that the CT structural gene was contained in the Xba I + Bgl II segment of the 4.3 kb segment, the Xba I + Bgl II segment being 1.8 kb.

We used the cesium chloride-ethidium bromide density gradient ultra centrifuging method to purify the recombinant plasmid pCT 332 after which we proceeded as explained above in the materials and methods section using Xba I + Bgl II. Following agarose gel electrophoresis, we recovered the 1.8kb DNA segment from the gel and used the [alpha-^{32}p]dATP DNA notch translation method to produce a CT gene probe.

By way of testing whether the CT gene probe that was produced by using the foregoing method was of sufficient purity for the orthotopic hybridization of bacteria colonies, we hybridized it with Vibrio cholerae RV 79, E. coli C 600, RR 1 and C 600 containing plasmids pBR 322, pBR 325, or pCT 332. Results of these hybridizations are as shown in plate I-1. Plate I-1 [not produced] shows a positive for the hybridization of the CT gene probe with the Vibrio cholerae RV 79, and with the C 600 containing the pCT 332 plasmids, and a negative for the hybridization with the E. coli C 600 and RR 1, and the C 600 containing the plasmids pBR 322 or pBR 325, which was entirely as predicted. These results demonstrate that use of the methods described in this article for the preparation of CT gene probes produces probes of sufficient purity for the orthotopic hybridization of bacteria colonies. They not only contain E. coli chromosome components, but they are also not contaminated with plasmid pAT 153 components. Theoretically, when the Xba I + Bgl II enzyme medium recombinant plasmid pCT 322 is used, there is a possibility of producing a Vibrio cholerae chromosome DNA segment containing some carrier or that is unrelated to toxin production, with a size close to 1.8kb that agarose gel electrophoresis is unable to separate out, thereby damaging the purity of the probe. However, since the recombinant plasmid pCT 332 is itself not large, being only several kb, the possibility that this will happen is very small. Our experiment verified this point.

(2) Reliability and Specificity of Using CT Gene Probes To Detect Vibrio Cholerae

By way of verifying the reliability of this method of preparing CT gene probes for use in the bacteria colony orthotopic hybridization method for the detection of Vibrio cholerae, the probe was crossed with nine strains of known toxins producing Vibrio cholerae. In addition, Y-1 adrenal gland cells as well as GM 1 ELISA were used to determine the toxin producing ability of these nine strains of Vibrio cholerae. The results obtained from these three different methods were compared (See Table 2). Table 2 shows positive for the hybridization of the CT gene probe with the nine strains of CT^{+} Vibrio cholerae. The Table 2 data show results from hybridization of the gene probe to be completely identical with results obtained from the Y-1 adrenal gland cells and the GM 1 ELISA. Subsequent assay of a large number of contagious strains showed the hybridization of all toxin producing Vibrio cholerae strains with the probe to be positive. This shows that reliable results may be obtained from the use of the CT gene probe in the orthotopic hybridizing of bacteria colonies to detect Vibrio cholerae.

Table 2. Results of Three Different Methods of Detecting Nine Strains of CT Positive Vibrio Cholerae

Bacteria Strain	Toxin Gene	Cololy Orthotopic Hybridizing	Y-1 Cell	GM-1 ELISA
Wujiang 2	CT+	+	+	+
Bin 43	CT+	+	+	+
16186	CT+	+	+	+
16187	CT+	+	+	+
18001	CT+	+	+	+
82178	CT+	+	+	+
82179	CT+	+	+	+
569 B	CT+	+	+	+

Since the LT gene and the CT gene are 75 percent homologous[11], in order to test whether there was a cross reaction between the CT gene probe and the LT gene, colony orthotopic hybridizing of the gene probe was done with LT positive E. coli 44813, 44814, EWD 299, and Entp 307 of both human and hog origin. The results showed when hybridizing was done under stringent conditions, and the concentration of formamide in the hybridizing fluid was 50 percent, results from the hybridizing of the CT probe with an LT positive bacteria strain were negative; therefore, the CT gene probe is extremely specific in being able to differentiate LT genes and CT genes. Generally speaking, when the formamide concentration in the hybridizing fluid is lower than 30 percent, CT and LT hybridizing exhibit a weak positive reaction. However, then the formamide concentration fell to 25 percent in our assay, positive results continue to appear.

(3) Use of the CT Gene Probe to Detect Genotypic and Phenotypic Unidentified Vibrio Cholerae

We used the CT gene probe prepared in this experiment to detect 25 strains of genotypic and phenotypic unidentified Vibrio cholerae. (These strains were provided by the Zhejiang Provincial Health and Epidemic Prevention Station and five institutes of the Military Academy of Medical Sciences). Figure 4 [not reproduced] shows strain numbers 5, 7, 9, and 19 as not homologous with the CT gene probe. For the other 21 strains, hybridizing results were positive. In addition, the Y-1 adrenal gland cell and the GM 1 ELISA were used in the detection of these 25 strains. In these latter two methods, results obtained for strain numbers 5, 7, 9 and 19 were also negative, which was entirely consistent with results obtained from the hybridizing experiment. One difference was that in the GM 1 ELISA, strain number 8 also tested negative. Possibly this was because the sensitivity of this method was lower than that of either the Y-1 adrenal gland or the gene probe technique. When the CT gene probe was hybridized with various shigella bacilli (Shigella sornei, S. flexneri, and S. dysenteriae), and with ETEC of both hog and human origin, no autoploidy was found.

To summarize, the experiment showed no autoploidy between the CT gene probe prepared according to the method described in this article and E. coli C600 and RRI, and plasmids PBR 322 or PBR 325. Nor would it hybridize with dysentery bacilli. In a 50 percent formamide system, there was also no cross reaction with ETEC of human or hog origin; there was crossing only with CT positive Vibrio cholerae. When the probe was used to detect genotypic and phenotypic unidentified Vibrio chlerae, the results were consistent with those obtained from testing using Y-1 adrenal glands and GM 1 ELISA, showing the gene probe technique to be not only sensitive, but also specific and reliable. Since the gene probe technique possesses the aforestated advantages, this method is applicable not only to laboratory use in the selection of recons and in study of the correlation among genes, but it may also be used in clinical diagnosis and in epidemological surveys.

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Results Reported For Streptococcus Pneumoniae Sensitivity to Antibiotics

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[Article by Ye Renbang [0673 0086 6721], Du Zongli [2629 1350 0448], Sun Yaping [1327 0068 5493], and Xiao Lei [5135 4320], Chinese Pharmaceuticals and Biologicals Certification Institute, Beijing: "Study of Streptococcus Pneumoniae Drug Sensitivity Testing"]

[Text] Abstract: The paper method was used in testing the sensitivity to 11 antibiotics of 295 strains of Streptococcus pneumoniae isolated in China between 1981 and 1983. Results verified that these strains were most sensitive to erythromycin and cephalosporin II at better than 99 percent, followed by chloromycetin, gentamycin, and penicillin. The streptococcus was least sensitive to neomycin, aureomycin, and kanamycin, its resistance to these antibiotics being greater than 96 percent.

Bacteria strains from different places, of different origins, and from different blood serum types differed in their resistance to penicillin, chloromycetin, and gentamycin. Bacteria strains from Sichuan Province, Shanghai, and Yunnan Province were sensitive to penicillin, and no strains from these places were resistant to it. However, bacteria strains from Anhui province and from Shenyang were more than 63 percent resistant to penicillin. Bacteria strains isolated from the chest and abdominal fluid were fairly resistant to these three antibiotics, and strains isolated from the blood were second most resistant to them. However, strains isolated from fluid in the middle ear were fairly sensitive to them. Strains isolated from the cerebrospinal fluid were more resistant to gentamycin than they were to either penicillin or chloromycetin. There were no marked differences in sensitivity to penicillin of the seven strains most commonly encountered in China, namely type (or group) 1, 2, 5, 6, 14, 19, and 23. Type 5 was fairly resistant to chloromycetin and gentamycin at 82.35 and 94.12 percent respectively, but the group 6 bacteria were fairly sensitive to chloromycetin, the resistance rate being only 20 percent.

Standard antibiotics were donated by Wu Quanfu [0709 5425 0102] of the institute, for which appreciation is hereby expressed.

Key Terms: Streptococcus pneumoniae; drug sensitivity test; drug resistance; sensitivity; and antibiotics

Streptococcus pneumoniae causes infections of the lungs, and is a major pathogen in otitis media purulenta acuta and in bacterial meningitis in infants and children. Serious infection with these bacteria may lead to an appreciable number of deaths.[1-4] The appearance of bacteria strains that are resistant to antibiotics has created problems for clinical treatment of these infections.[4-6] In order to study the sensitivity and the extent of resistance of Streptococcus pneumoniae to frequently used antibiotics, sensitivity testing using 11 different antibiotics was done on 295 strains of Streptococcus pneumoniae isolated from the cerebrospinal, blood, and inner ear fluids of patients from 1981 through 1983. Results are reported below.

Materials and Methods

(1) Bacteria Strains

The test bacteria were 295 strains of Streptococcus pneumoniae that had been isolated from the cerebrospinal fluid, blood, and inner ear fluid of hospital patients in 18 provinces, municipalities, and autonomous regions from 1981-1983. Upon receipt of the strains from various hospitals, they were preserved by the vacuum freeze dry method. Before testing, the strains' growth, morphological, biochemical, and physiological characteristics were checked, and bile solubility tests, Optochin sensitivity tests, and capsule stain tests were run to determine that they were typical Streptococcus pneumoniae. One capsular swelling tests had determined their type, they were used in the drug sensitivity tests.

(2) Drug Sensitivity Test Quality Control

The quality control bacteria that WHO currently prescribes, namely Escherichia coli, ATCC 25922 strain, Staphylococcus aureus (ATCC 25923 strain, and Pseudomonas aeruginosa ATCC 27853 served as controls.

(3) Culturing Media

Five percent fiber fresh sheep blood agar culturing medium (or sheep blood agar culturing medium, for short), and 2 percent rabbit serum broth culturing medium.

(4) Preparation of Drug Sensitivity Test Strips

We first used the 11 kinds of antibiotics and Optochin that see frequent clinical use in China, and we referred to the WHO standard dosages and the Shanghai Municipal Medical Chemical Testing Institute's paper preparation method.[7,8] We used a certain amount of filter paper to make round pieces of paper 6 mm in diameter. After sterilizing them, they were placed in a certain amount of antibiotic and allowed to soak until fully saturated. Then they were dried at 37 degrees C and kept in a 4 degrees C refrigerator for use. Each piece of paper contained one antibiotic in the following amounts: erythromycin, 15 μg; cephalosporin II, 30 μg; penicillin 20 IU, chloromycetin 30 μg; gentamycin 10 μg; streptomycin, 10 μg; kanamycin, 30 μg; tetracycline, 30 μg; terramycin, 30 μg; aeromycin, 20 μg; neomycin, 10 μg; and Optochin 5 μg.

(5) Drug Sensitivity Test

The vacuum freeze dried bacteria were opened and inoculated into a sheep blood agar slant culturing medium. They were cultured at 35 degrees C in a 10 percent carbon dioxide environment for between 18 and 20 hours after which they were reinoculated into a rabbit serum broth culturing medium and cultured at 35 degrees C in a 10 percent carbon dioxide environment for 6 hours. From the 6 hour culture, 0.1 ml was taken and put in a bacteria-free flat dish (90 mm in diameter) to which was added 15 ml of a sheep serum agar culturing medium that had been chilled to approximately 45 degrees C. The flat dish was shaken at once to mix the bacteria fluid with the culturing medium, and then it was placed on a laboratory table. After the agar had solidified, it was placed in a 37 degree C incubator for 30 minutes. Then a small bacteria-free tweezer was used to place one of the antibiotic papers on top of the above mentioned agar plates, and four different antibiotic papers were put into each dish. The dishes were placed in a 37 degree C incubator overnight after which the diameter of the bacterial inhibition circle was measured to determine the results. Two plates were used in each of the antibiotic paper tests of each bacteria strain, the average diameter of the bacterial inhibition circle of both plates being used to figure the results.

Standards For Judging Test Results: In making a determination, reference was made to the standards set by WHO in 1981 for the paper method of testing antibiotic sensitivity.[7] Please see Table 1.

Table 1. WHO Drug Sensitivity Test Standards

Antibiotic	Paper Content (µg)	Bacterial Inhibition Circle Diameter		
		Drug Resistant	Medium Sensitive	Sensitive
Erythromycin	15	$\leq$ 13	14-17	18
Cephalosporin II	30	$\leq$ 14	15-17	18
Penicillin*	10	$\leq$ 20	21-28	29
Chloromycetin	30	$\leq$ 12	13-17	18
Gentamycin	10	$\leq$ 12	13-14	15
Streptomycin	10	$\leq$ 11	12-14	15
Kanamycin	30	$\leq$ 13	14-17	18
Tetracycline	30	$\leq$ 14	15-18	19
Terramycin	30	$\leq$ 12	13-15	16
Aureomycin	20	$\leq$ 12	13-15	16
Neomycin*	30	$\leq$ 12	13-16	17
Optochin	5	$\leq$ 11	12-14	15

*In this test, the penicillin content of the paper was 20 µg, and the neomycin content was 10 g, which differs from the WHO standards.

Results

(1) Drug Sensitivity Testing of 295 Strains of Staphylococcus Pneumoniae To 11 Commonly Used Antibiotics and Optochin (Table 2)

Table 2. Results of Drug Sensitivity Testing of 295 Staphylococcus Pneumoniae Strains

Antibiotic	Paper Content (μg)	Drug Resistant		Medium Sensitive		Sensitive	
		Strain Number	%	Strain Number	%	Strain Number	%
Erythromycin	15	1	0.34	14	4.75	280	94.91
Cephalosporin II	30	1	0.34	5	1.69	289	97.97
Penicillin*	20	92	31.19	125	42.37	78	26.44
Chloromycetin	30	82	27.80	82	27.80	131	44.40
Gentamycin	10	156	52.88	44	14.92	95	32.20
Streptomycin	10	268	90.85	20	6.78	7	2.37
Kanamycin	30	290	98.30	3	1.02	2	0.68
Tetracycline	30	265	89.83	9	3.05	21	7.12
Terramycin	30	208	70.51	45	15.25	42	14.24
Aureomycin	20	285	96.61	8	2.71	2	0.68
Neomycin*	10	291	98.64	4	1.36	0	
Optochin	5	3	1.02	29	9.83	263	89.15

The Table 2 results confirm that the sensitivity of the 295 strains of Staphylococcus pneumoniae to the 11 most commonly used antibiotics varies. They are most sensitive to erythromycin and cephalosporin II. Except for 1 drug resistant strain, all the other 294 strains, or 99.66 percent of the total number, were sensitive to these antibiotics. Sensitivity to Optochin was 98.98 percent followed by chloromycetin, penicillin, and gentamycin at 72.20, 68.81 and 47.12 percent respectively. The strains were least sensitive to kanamycin, neomycin, aeromycin, streptomycin, tetracycline, and terramycin, resistance to these antibiotics being 98.30, 98.64, 96.61, 90.85, 89.83, and 70.51 percent respectively. This is a matter that merits close attention to clinical treatment.

(2) Resistance of Staphylococcus Pneumoniae Isolated From Different Areas to Three Antibiotics in Common Clinical Use (Table 3).

Table 3. Results of Resistance to Three Antibiotics of Staphylococcus Pneumoniae Isolated From Different Areas

Area	Number of Strains	Penicillin		Chloromycetin		Gentamycin	
		Number of Strains	%	Number of Strains	%	Number of Strains	%
Beijing	107	26	24.30	27	25.23	53	49.53
Nanjing	54	4	9.30	11	20.37	25	46.30
Tianjin	27	14	51.85	8	29.63	20	74.07
Wuhan	23	7	30.44	6	26.09	11	47.82
Anhui Province	19	12	63.16	9	47.37	13	68.42
Shenyang	19	12	63.16	8	42.11	10	52.63
Sichuan Province	12	0	0	3	25.00	4	33.33
Shanghai	8	0	0	3	37.50	3	37.50
Yunnan Province	8	0	0	1	12.50	4	50.00

The Table 3 results show that bacteria strains isolated from different areas had different drug resistance to penicillin, chloromycetin, and gentamycin. The strains isolated in Sichuan Province, Shanghai, and Yunnan Province were most sensitive to penicillin, and no strains were resistance to this antibiotic. Only 5, or 9.30 percent, of the 54 strains from Shanghai were resistant to penicillin. A fairly large number of strains from Anhui Province, Shenyang, and Tianjin were penicillin resistant at 63.16 percent (12/19), 63.16 percent (12/19), and 51.85 percent (14/27) respectively. Next were the strains from Wuhan and Beijing at 30.44 percent (7/23), and 24.30 percent (26/107). Few of the strains from Yunnan Province, Nanjing, Sichuan Province, Beijing, Wuhan, and Tianjin were resistant to chloromycetin, only between 12.50 and 29.63 percent; however, a substantial number of strains isolated in the foregoing nine provinces and cities were resistant to gentamycin. In particular, the strains from Tianjin and Anhui Province were most resistant at 74.07 and 68.42 percent.

(3) Resistance to Three Commonly Used Antibiotics of Staphylococcus Pheumoniae Isolated From Different Specimens (Table 4)

Table 4. Results of Resistance to Three Antibiotics of Staphylococcus Pheumoniae of Different Origins

Bacteria Source	Number of Strains	Number of Strains	%	Number of Strains	%	Number of Strains	%
Cerebrospinal Fluid	91	33	36.26	31	34.07	63	69.23
Blood Serum	35	15	42.86	11	31.43	15	42.86
Middle Ear Fluid and Nasal Cavity Secretions	110	28	25.45	23	20.91	45	40.91
Chest Fluid; Abdominal Fluid	20	10	50.00	12	60.00	11	55.00
Other*	37	4	10.81	6	16.22	19	51.35

*Includes eye secretions, pus, accumulated fluid from cerebral dura mater, accumulated fluid from body joints, mastoid secretions, pulmonary alveoli and trachea secretions.

The Table 4 results confirm that Staphylococcus pneumoniae of different origins have a different resistance to penicillin, chloromycetin, and gentamycin. Bacteria strains isolated from cerebrospinal fluid showed considerable resistance to these three antibiotics, ranging from 34.07 to 69.23 percent. The strains isolated from blood serum were second most resistant to the three antibiotics at from 31.43 to 42.86 percent. The strains isolated from middle ear fluid and nasal cavity secretions were slightly resistant to the three antibiotics at only 20.91 to 40.91 percent.

Bacteria strains isolated from other specimens were fairly sensitive to penicillin and chloromycetin, the resistant strains totaling 10.81 and 16.22 percent respectively; 51.35 percent were resistant to gentamycin. Strains isolated from chest cavity fluid and abdominal fluid showed a resistance to the three antibiotics ranging from 50 to 60 percent.

(4) Resistance to the 11 Antibiotics of Staphylococcus Pneumoniae Isolated in Different Years (Table 5)

Table 5. Staphylococcus Pneumoniae Drug Resistance in Different Years

	1982 Number of Strains	Percent	1983 Number of Strains	Percent
Number of Strains	87		202	
Cephalosporin II	0	0	1	0.50
Erythromycin	0	0	1	0.50
Penicillin	38	43.67	54	26.73
Chloromycetin	22	25.29	57	28.22
Gentamycin	51	58.62	100	49.50
Streptomycin	81	93.10	182	90.10
Kanamycin	86	98.85	198	98.02
Tetracycline	77	88.51	183	90.59
Terramycin	59	67.81	144	71.30
Aureomycin	83	95.40	196	97.03
Neomycin	87	100.00	197	97.52

The Table 5 results show a marked difference in the resistance to penicillin and gentamycin of Staphylococcus Pneumoniae isolated in different years. In 1982, resistance to penicillin was 43.67 percent (38/87), but in 1983 it was 26.73 percent (54/202). In 1982, resistance to gentamycin was 58.62 percent, but in 1983, it was 49.50 percent. There were no marked differences in resistance to the other nine antibiotics of the Staphylococcus Pneumoniae isolated in different years.

(5) Comparison of Resistance to Three Antibiotics of Staphylococcus Pneumoniae in Commonly Seen Blood Serum Types

The seven common blood serum types from which Staphylococcus Pneumoniae was isolated in China from 1981 through 1983 were types 1, 2, and 5, group 6, type 14, group 19, and group 23. Please see Table 6 for a comparison of the resistance to three antibiotics of bacteria strains in these seven blood serum types.

Table 6. Results of Resistance to Antibiotics of Bacteria Strains in Common Blood Serum Types

	Penicillin			Chloromycetin			Gentamycin		
	Total Strains	Drug Resistant Strains	%	Total Strains	Drug Resistant Strains	%	Total Strains	Drug Resistant Strains	%
Type 1	26	9	34.62	26	12	46.15	26	15	57.69
Type 2	18	8	44.44	18	8	44.44	18	15	83.33
Type 5	17	8	47.06	17	14	82.35	17	16	94.12
Group 6	35	12	34.29	35	7	20.00	35	13	37.14
Type 14	13	6	46.15	13	6	46.15	13	10	76.92
Group 19	16	8	50.00	16	3	18.75	16	7	43.75
Group 23	18	7	38.89	18	2	11.11	18	10	55.56

The Table 6 results show no marked differences in sensitivity to penicillin of the strains in seven blood types. The strains in Type 5 show appreciable resistance to chloromycetin and gentamycin at 82.35 and 94.12 percent respectively. The Group 6 strains showed little resistance to chloromycetin at 20.00 percent. In Group 14, 76.92 percent of the strains were gentamycin resistant. Group 19 and Group 23 strains were 18.75 and 11.11 percent resistant to chloromycetin. The Type 2 strains were strongly resistant to gentamycin at 83.33 percent.

Discussion

Since the frequently used paper test method for testing drug sensitivity, the agar dilution method, and the broth dilution method, as well as the microdilution method differ, and since the culturing medium and inoculation amounts also vary, results of drug sensitivity tests cannot be compared. For this reason, we recommend use of the WHO recommended standardized testing method.[7].

The 295 strains of Staphylococcus pneumoniae covered by this report were most sensitive to erythromycin and cephalosporin II; they were fairly sensitive to penicillin, chloromycetin, and gentamycin. It should be noted, however, that a fairly large number of strains were resistant to several of the commonly used antibiotics, causing difficulties for clinical treatment.

The results show that strains from different parts of the country, from different sources, and from different blood serum types were resistant to penicillin, chloromycetin, and gentamycin. A substantial number of the strains isolated in Tianjin were resistant to penicillin and gentamycin. Resistance to penicillin, chloromycetin, and gentamycin was strong for strains isolated from chest and abdominal fluids, followed by strains isolated from blood. Strains isolated from cerebrospinal fluid were more resistant to gentamycin than to either penicillin or chloromycetin. Strains isolated from the middle ear were fairly sensitive to penicillin and chloromycetin. Group 6, Group 19, and Group 23 strains were fairly sensitive to chloromycetin, and Type 5 strains were more sensitive to penicillin than to either chloromycetin or gentamycin. In order to boost clinical treatment results and lower the fatality rate, it is of crucial importance that sensitivity testing be done on the sensitivity of isolated Staphylococcus Pneumoniae to antibiotics in order to provide a scientific basis for selecting the antibiotic that is to be used clinically. It is suggested that hospitals having requisite conditions make drug sensitivity testing a rule.

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Identification of Pseudomonas Putrefaciens and Their Pathogenicity

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[Article by Quan Taishu [2938 1132 3219], Li Wei [2621 5633], Li Lemin [2621 867 3046], and Fan Tianrui [5400 1131 6904], Xuzhou Municipal Health and Epidemic Prevention Station: "Isolation and Identification of Pseudomonas Putrefaciens From Acute Diarrheal Patients and Their Pathogenicity"]

[Text] Abstract: This article reports our examination of more than 10 different intestinal tract pathogens found in the stool of 1,792 patients suffering from acute diarrhea during the period 1982 through 1985. These examinations detected 98 strains of Pseudomonas putrefaciens, a 5.47 percent detection rate. In addition to making a biochemical identification of the isolated strains, a pathogenic examination was also done, the results of which showed the following: Pseudomonas putrefaciens was able to make experimental white mice ill and die within a short period of time, and was fairly strongly intrusive into the canthi membranes of guinea pigs. Some strains (6/14) were also able to produce LT enterotoxin. In addition, antibody testing was done on double portions of blood serum from eight patients, which confirmed that there were a fairly high number of antibodies against Pseudomonas putrefaciens in the sera of recovering patients.

Consequently, it is believed that this bacterium is the pathogen causing diarrhea in man.

This article was reviewed by Professor Liu Bingyang [0491 4426 7122], and Professor Cheng Zhiyi [4453 4249 5030] offered valuable views about it. Qiu Xuezhao [5941 1331 2507], a medical doctor and deputy director of the electron microscope laboratory of the Jiangsu Provincial Health and Epidemic Prevention Station provided assistance, for which appreciation is hereby expressed.

Key Terms: Pseudomonas putrefaciens; diarrhea; and pathogenicity

Pseudomonas putrefaciens is a gram negative, rod shaped or slightly curved bacillus that is asporous and acapsular, and that moves by using polar flagella. It is strictly aerobic, and it is a pseudomonas belonging

to the asporous, non-zymotic gram negative group of bacilli. This bacillus was first isolated in 1941 by Derby and Hammer who named it Pseudomonas putrefaciens. Its DNA G + C percentage is 43.5 to 45.5 mol percent.[1] This germ is found fairly widely in the natural world. It can contaminate and rot fish, meat, eggs, milk, and frozen foods, and it can also cause septicemia, tympanitis, tibia infection, and post-traumatic ulcers.[2] Its isolation from blood, urine, pus, traumatic secretions, stool, sputum, and throat swabs has attracted the attention of clinicians and microbiologists alike. For many years, China has produced a substantial number of articles about the study of verdigris pseudomonas; however, no reports have been seen to date in China about research on Pseudomonas putrefaciens. From 1982 through 1985, our station collected 1,792 stool samples from patients suffering from acute diarrhea and used different culturing mediums in the isolation and culturing of more than 10 different intestinal tract pathogens, 98 of those isolated being strains of Pseudomonas putrefaciens. Results of experimental research are reported below.

Materials and Methods

(1) Source of Bacteria Strains

Isolated from the stool of outpatients suffering from acute diarrhea

(2) Isolation and Culturing Medium

A BTB sucrose high salt (4 percent sodium chloride) agar culturing medium was used.

(3) Physiological Identification

The medium, the reagents, and the assay methods were those provided in references 3 and 4. Sodium chloride was added to all culturing media that did not already contain sodium chloride to bring the final concentration to 1 percent.

(4) Testing of Pathogenicity

1. White mice toxicity assay: White mice weighing between 15 and 18 grams were selected, and 0.3 ml (approximately 1 billion/ml) of broth culturing medium containing Pseudomonas putrefaciens was inoculated into their abdominal cavities for 24 hours, three mice being inoculated with each bacterial strain. The same method without the bacteria in the broth was used as a control. Observations were then made of the incidence of illness and fatalities. 2. Enterotoxin LT test: WHO prescribed methods were used in a rabbit intestinal loop ligation assay. Eighteen hours following the surgery, the abdomen was opened and examined, and the average amount of fluid retained per millimeter of intestine was determined. Intrusiveness assay: The Ser'eny method was used in making this assay.

(5) Patient Organism Immunoreaction Assay

Blood serum was taken from patients having diarrhea resulting only from Pseudomonas putrefaciens during the acute stage (4 to 5 days after onset), and during the recovery stage (19 to 26 days after the illness). The antigens detected in each ("O" bacteria fluid diluted to 1 billion/ml) were subjected to quantitative direct agglutination testing, and the titer of the antibodies were measured. The same method was used with the blood serum of healthy people as a control.

(6) Drug Sensitivity Test

The paper diffusion method was used, the paper impregnated with 14 separate drugs having been provided by the Shanghai Medical Laboratory. Both the test method and the judging of results followed the method prescribed by WHO.

Test Results

Pathogens of various kinds were found in 1,164 of the 1,792 stool specimens, including specimens from 93 patients (or 5.18 percent of positive patients) having mixed infections in which a single specimen yielded two or more pathogens. The total pathogen detection rate was 70.14 percent of which 5.47 percent was Pseudomonas putrefaciens. (See Table 1).

Table 1. Pathogens Detected in 1,792 Patients Having Acute Diarrhea

Pathogen	Detected Number of Strains	Positive Detection Rate	Constituent Ratio
Shigella shigae bacillus	479	26.73	38.11
Pseudomonas putrefaciens	98	5.47	7.80
Vibrio algalytica [sic]	92	5.13	7.32
River vibrio	84	4.69	6.68
Jejunem curved bacteria	76	4.24	6.04
Salmonella bacteria	72	4.02	5.73
Hydroerophillis monad	68	3.79	5.41
Leizhihe pilin [7352 1807 6320 3026 6775] monad	56	3.13	4.46
Pathogenic E. coli	54	3.01	4.30
Non-01 Group Vibrio cholerae	51	2.85	4.05
Vibrio parahaemolyticus	42	2.34	3.34
Pseudomonas verdigris	37	2.06	2.94
Colitis yeshijun [5102 3044 5497]	22	1.23	1.75
Pseudovibrio [sic]	7	0.39	0.56
Intrusive E. coli	5	0.28	0.40
Untyped vibrio	14	0.78	1.11
Total	1257	70.14	100.00

(1) Somatic Form

All 98 strains of the Pseudomonas putrefaciens were gram negative, asporous, rod shaped or curved bacilli, some of which had arranged themselves in pairs or short chains. Some strains were examined using the flagella staining method and found to be single rooted terminal hair bacteria. Dark field observation showed them to move about actively, and electron microscope observation revealed an unsheathed polar flagellum and a fimbia at one end of the thallus.

Table 2. Characteristics of Isolated and Cultured Pseudomonas Putrefaciens Colonies (Cultured at 37 Degrees Co for 24 hours

Isolation and Culturing Medium	Colony Characteristics	Salient Points Observed
BTB sucrose high salt agar (containing 4 percent NaCl)	Green, round, glossy, wet, about 2 mm in diameter, opaque, and slightly conves	Do not decompose sucrose; colony center is green
Maikangkai [7796 1660 7030] agar	Light brownish yellow translucent, 1.5-2 mm in diameter, prone to form mucoid lawn and colonies.	Do not decompose lactose; colony growth areas produce yellow alkali
SS agar	Colorless, transparent, round, rather small, 1-1.5 mm diameter	Do not decompose lactose, do not produce H_2S for the most part, colonies difficult to pick up
Sheep blood agar	Grayish brown, round, wet swollen, anhemolytic diameter of 2-3 mm.	Dark green discoloration at bottom of medium
Common agar	Light pink, round, bulging, wet, murky and sometimes sticky, about 2 mm in diameter	Pigment forms readily at about 30 degrees C, but pigment is not diffused.

Table 3. Results of Biochemical Testing of 98 Strains of Pseudomonas Putrefaciens

Particulars		Results +	Results −
Isolating Medium	BTB Sucrose High Salt Agar	98	0
	Maikangkai agar	98	0
	SS agar	92	6
	NAC agar*	0	98

(Table continued on following page)

KIA	Bottom level (acid producing)	0	98
	Slant (acid producing)	0	98
	Gas	0	98
	Hydrogen sulfide	98	0
Undiffused Pigment	Jinshi [6855 3044] agar slant	98	00
	Semi-solid agar surface layer	98	00
Growing Temperature	5 degrees C	39	59
	6 degrees C	94	4
	7 degrees C	98	0
	41 degrees C	94	4
	42 degrees C	91	7
Motility		98	0
Oxidase		98	0
Cell Pigment oxidase		98	0
Catalase		98	0
Growth in potassium cyanide culturing medium		0	98
Nitrate reduction		98	0
Nitrogen generation		0	98
Peptone water containing different concentrations of sodium chloride	0% NaCl growth	2	96
	3% NaCl growth	98	0
	6% NzCl growth	98	0
	7% NaCl growth	87	11
	8% NaCl growth	72	26
	10% NaCl growth	0	98
Saccharide oxidation in O-E culturing media	Glucose	89	9
	Lactose	0	98
	Maltose	0	98
	Mannitol	0	98
	Mannitose	0	98
	Sucrose	0	98
	Arbinose	0	98
	Inositol	0	98
	Salicyl	0	98
	Melitose	0	98
	Cellobiose	0	98
	Tregalose	0	98
	Fructose	32	66
	Ethanol	7	91
	Xylose	3	95
ONPG		0	98
Esculin hydrolysis		6	92
Methyl red		0	98
V-P		0	98
Indigo matrix		0	98
Urease		16	82

(continued on following page)

Gelatinase	98	0
Phenylpropiolic acid deammoniase	0	98
Lysine decarboxylase	0	98
Ornithine decarboxylase	98	0
Arginine double hydrolase	0	98
Simon's [sic] citrate	17	81
Malonate	0	98
Acetate	98	0
Glucosamine	0	98
DNAse	98	0
Lecithinase	98	0
Polysorbate-80 hydrolytic	98	0
Acetamide hydrolytic	0	98
Penicillin sensitive 10 ug	0	98
0/129 susceptibility 10 ug	0	98
0/129 susceptibility 150 ug	0	98
Hemolysis	0	98

*NAC agar: Nalidixic cuprate [sic] hexadecyl trimethylamine bromide agar

(2) Biological Characteristics

1. These bacteria are strictly aerobic, respire to metabolize, and do not have high nutrient requirements. At a pH ranging between 7.0 and 8.8, they grow very well, and most are able to grow at a temperature below 6-42 [sic] degrees C. 2. The bacteria are halophilic, and an overwhelming majority failed to live in salt-free peptone water. 3. When they were cultured in beef broth medium below 37 degrees C for 3 to 6 hours, the broth appeared uniformly murky. After between 18 and 24 hours, a mycoderm appeared on the surface of the broth. If the culturing time was extended, a myxomembranous substance appeared that sank to the bottom of the tube. 4. These bacteria formed colonies having different characteristics in several commonly used isolating cultures (See Table 2). At 30 degrees C on the surface level of the Jinshi agar slant and the common semi-solid agar, they readily produced markedly pink pigmentation. The pigment was not diffuse, however, nor would it dissolve in alcohol and chloroform. Only the lawn itself was pink.

(3) Physiological Test Results

The physiological test results for the isolated bacteria were consistent with those of pseudomonas. This bacterium is extremely weak in its ability to oxidize sugar. (See Table 3).

(4) Results of Pathogenic Test

1. White mice toxicity test: A total of 26 strains were used, and after 6 to 10 hours, the mice began to become ill. Within 10 to 16 hours, death occured, with a small number lasting for between 22 and 26 hours before dying. At first the mice were not very active, and they refused food; their

hair became erect; their backs arched; and they curled into a ball. They discharged mucous stool from their noses, finally dying and becoming stiff. Dissection showed their peritoneum to contain a fairly large amount of brownish, gelatinous fluid, and their intestines were distended with brown fluid. Material was taken from their cardiac blood, peritoneal fluid, and intestines and cultured, a pure culture being obtained that was identical to the test bacteria. Meanwhile, observation of the control group of mice (for 3 days) showed them to be as healthy as always.

2. Rabbit intestinal loop ligation test: Six of the 14 strains of the bacteria filtrate had a value greater than 1.0 and showed positive in the LT assay. In four of the strains, between 1.35 and 1.52 ml/cm of fluid accumulated in the rabbit intestines.

3. Ser'eny assay: After each of a total of 26 strains was inoculated into the canthi membrane of 26 guinea pigs using the classical method, observation on the following day found the canthi membrane of the guinea pigs that had received live bacteria to be murky and congested. The eyes of most of the guinea pigs were badly stricken and oozed a large amount of pus that tightly sealed their eyelids. The infection reaction was similar in a control group that had been infected with Shigella shigae. Examination of the eye secretions produced pure culture growth (of the former strongly intrusive test strains). A pathogenic intestinal E. coli was used in the canthi membranes of the control guinea pigs, but observation 72 hours afterward showed no infectious reaction.

(5) Results of antibody tests

There was a more marked increase in antibodies during the recovery period than during the acute stage of eight patients, the serum of five healthy people tested positive. (See Table 4)

Table 4. Double Serum Antibody Test For Diarrhea Patients

Serum Number	Serum Titer During Acute Stage						Serum Titer During Recovery Period						Control (NS)
	1:15	1:10	1:20	1:40	1:80	1:160	1:15	1:10	1:20	1:40	1:80	1:160	
962	+	-	-	-	-	-	#	#	+++	++	-	-	-
987	++	-	-	-	-	-	+++	+++	++	-	-	-	-
1057	+++	+	-	-	-	-	#	#	+++	+++	+++	++	-
1153	-	-	-	-	-	-	+++	+++	-	-	-	-	-
1315	++	-	-	-	-	-	#	+++	+++	++	-	-	-
1486	-	-	-	-	-	-	+++	++	-	-	-	-	-
1595	+++	++	-	-	-	-	#	#	#	+++	++	+	-
1646	++	-	-	-	-	-	#	#	#	+++	++	+	-

(6) Results of Drug Sensitivity Test

Drug sensitivity test results for 14 antibiotics against 98 strains of Pseudomonas putrefaciens are shown in Table 5.

Table 5. Drug Sensitivity Test Results For 97 Strains of Pseudomonas Putrefaciens

Antibiotic	Drug Sensitivity Test Results		
	Highly Sensitive (Strains	Moderately Sensitive (Strains)	Little or No Sensitivity (Strains)
Gentamycin	98	0	0
Kanamycin	98	0	0
Amikacin	98	0	0
Neomycin	98	0	0
Tobramycin	96	1	1
Polymyxin B	96	2	0
Chloromycetin	62	1	35
Erythromycin	56	9	33
Sulfanalimide plus a synergist	58	2	38
Tetracycline	4	4	90
Streptomycin	2	5	91
Cephapirin	0	0	98
Ampicillin	0	0	98
Penicillin	0	0	98

Discussion

1. The taxonomical position of Pseudomonas putrefaciens has yet to be finally decided. On the basis of its DNA G + C structure, some scholars maintain that it should not be one of the pseudomonas.[5-7] Baumann et al[8] did a taxonomical study of 218 strains of aerobic marine bacteria after which they proposed placing non-zymotic gram negative polar flagella bacteria having a DNA G + C of 43.2 to 48.0 mol percent in a new category, namely alteromonas. They went on to compare the definition of pseudomonas and alteromonas, distinguishing between the two by their G + C content. In an article on the numerical classification of pseudomonas, Lee also stated explicitly that the Pseudomonas putrefaciens that Derby and Hammer had named should really be in a new category termed Alteromonas putrefaciens[9]. However, in Bergey's Manual of Determinative Bacteriology (Eighth Edition), these bacteria are classified as Pseudomonas putrefaciens. Nevertheless, the characteristics of Pseudomonas putrefaciens at the time. Consequently, the taxonomical position of this bacterium awaits further clarification.

2. McMeekin et al[10] isolated Pseudomonas putrefaciens from a rotted chicken leg. The authors also have found it in four smelling salt-preserved eggs (a toxic foodstuff), river water, lake water, and aquatic products. At that time, a mixture of bacteria was believed to cause contamination. In the research that we have conducted since 1982 in detecting intestinal pathogens in the stool of patients in the acute stage of dysentery, and in diarrhea-like patients who have spontaneous fevers, and mucous and blood in their stool, we have frequently found the thriving growth of Pseudomonas putrefaciens. However, we have not isolated dysentery bacilli or other intestinal tract pathogens. This fact holds major significance for research on the diarrhea caused by these bacteria. Among the 98 patients in whom Pseudomonas putrefaciens was detected, except for just a small number (12) who had a mixture of infections, no other pathogens were found, and they were dispersed cases. The 86 patients infected with Pseudomonas putrefaciens were also examined for contagious diseases. A small number (21 out of the 86) had light self-limiting illnesses in which the diarrhea was exhibited in watery and yellow colored thin stool. Most of the patients (65 out of the 86) had mucous and blood in their stool, very much like dysentery. The illness lasted from 2 to 5 days, and in a small number the diarrhea continued for 17 days. It continued for 42 days at the longest. The detection rate in adult patients was 94.90 percent. For infants and children, the rate was 5.10 percent. There were no marked differences between males and females. The infectious season ran from May through October; however, 74.42 percent (64 out of 82) of cases occurred during July, August, and September when temperatures were fairly high. In most cases, the diarrhea was attributable to water sources or contaminated foodstuffs such as poultry, eggs, milk, fish, frog meat, or cold drinks.

3. By way of exploring the pathological significance of this bacteria, (1) we used the above method to examine the stool of 813 people employed in food and beverage industries as part of a physical examination, finding no Pseudomonas putrefaciens. (2) We made a double serum antibody check of patients who were infected with this bacterium alone, verifying that during the recovery period, patients' serum contained fairly high numbers of antibodies against Pseudomonas putrefaciens while the blood of healthy people was without such antibodies, or the titer was low (1:5, and reaching 1:10 in individual cases). (3) Animal experiments revealed that these bacteria could make infected mice sick and cause them to die within a short period of time, and they were strongly invasive in the canthi membrane of guinea pigs. Some strains (six out of 14) were also able to produce LT enterotoxin. Therefore, it may be concluded that the isolated Pseudomonas putrefaciens was the pathogen causing the diarrhea in patients. In addition, the percentage of these bacteria in the make-up of diarrhea pathogens (7.8 percent) merits serious attention.

4. For a long time, insufficient attention has been given to Pseudomonas putrefaciens infections even to the point of there being very little epidemiological data available currently about the pathology of these bacteria. It is very easy to make omissions in pathological examination of diarrhea, making an accurate diagnosis difficult.

The Pseudomonas putrefaciens bacterium is halophilic. Durint the first isolation, propagation of bacteria may be increased by suspending the stool specimen in peptone water containing three percent sodium chloride after which high salt sucrose agar may be used as an isolation culture medium. This method not only produces fine results, but is also suitable for specimens in the external environment. In the identification of these bacteria, a preliminary screening should be done first by using the KIA to determine the presence of Pseudomonas putrefaciens after which biochemical identification may be made. At a KIA high level, these bacteria can produce hydrogen sulfide. Frequently covered, they are unable to ferment, but when oxygen is available, they are able to degrade glucose (albeit weakly), and they are unable to degrade lactose. They are consequently mistaken for salmonella bacteria, degenerative bacilli, or citrobacters. When making tests, it is necessary to perform several characteristic tests in order to distinguish Pseudomonas putrefaciens from these other bacteria. Among these gram negative bacteria, there are two characteristics of crucial importance. One is the type of flagella, i.e., whether polar flagella or peritrichal flagella. The second is whether the breakdown of carbohydrates is through oxidation or fermentation. When the Pseudomonas putrefaciens oxidase experiment is positive, there is respiratory metabolism, and dark field hanging drop specimen examination is done, motility is active. In addition to be able to slow the oxidation of glucose (81/98), other sugars are not used or very little used. These bacteria have DNAse, gelatinase, and ornithine decarboxylase, and some strains contain urease. MR, V-P, indole, and phenylalaline deaminase were all negative. In addition, one of the characteristics of this bacterium is its ability to produce undiffused pink pigment. The foregoing biochemical tests and the easily confused Enterobacteriaceae hold significance for identification.

The 98 strains of Pseudomonas putrefaciens that we isolated are only weakly able to oxidize, or completely unable to oxidase glucose, and we have yet to see a strain that is able to ferment glucose. This point differs markedly from reports in the references[1,2].

5. The 98 strains of Pseudomonas putrefaciens isolated from the diarrhea patients are highly sensitive to gentamycin, kanamycin, amikacin, neomycin, tobramycin, and polymyxin B, which should be the medicines first used in clinical treatment. Sixty percent of the strains were highly sensitive to chloromycetin, erythromyacin, and sulfanalimide with a synergist added. The strains were only slightly sensitive or not at all sensitive to streptomycin, tetracycline, cephapirin, ampicillin, and penicillin. Bacteria strains that with multiple resistance to antibiotics have also appeared.

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9432/12223

Bacteriophage Tested on Various Kinds of Brucella

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[Article by Cui Qinglu [1508 1987 4389] and Guo Xiulan [6753 4423 5695], Inner Mongolia Local Diseases Prevention and Treatment Institute, Hohhot: "Lytic Action of Bacteriophage SA on Various Kinds of Brucella"]

[Text] Abstract: Brucella bacteriophage SA was isolated from a swine brucella culture. When the concentration of the brucella fluid was 10^{-4}, its lytic action was able to generate a mixed lysis of smooth type swine brucella, types 1 and 3, cow brucella, types 1, 3, and 6, and shalinshu [3097 2651 7857; possible gerbil] brucella. The bacteriophage plaque produced was 3 to 4 mm in diameter. When the bacteriophage SA stock solution or a dilution of 10^{-1} or 10^{-2} was used, there was no lysis of goat brucella types 1 and 3. Against goat brucella type 2, rough type cow brucella 45/20, dog brucella, and sheep epididymis brucella, there was either mixed lysis or bacteriophage plaque, the bacteriophage plaque being between 1 and 2 mm in diameter or smaller.

This article was reviewed by Professor Lu Tuliang [7120 0960 5328] and Director Huang Jian [7806 0256], for which appreciation is expressed.

Key Terms: Brucella bacteriophage, lytic action, and bacteriophage plaque

Reports from abroad on the study of brucella bacteriophages date back to 1951; however, as regards the application of bacteriophages to brucella assays only the Soviet Union's isolation of cow brucella bacteriophages from polluted water in 1955, i.e., the Tb (Tbilisi) bacteriophage is internationally recognized. Because the range of lysis is limited, it has not been possible to assay all Brucella. Since the 1960's, M.J. Corbel et al have done a great deal of research on brucella bacteriophages. They have divided into five groups, on the basis of their host range and their lytic action, the more than 40 strains of brucella bacteriophages that have been isolated throughout the world.[1] Inside China, no pertinent overt reports have been seen. In 1985, we isolated a brucella bacteriophage from a swine brucella culture medium, which we named brucella bacteriophage SA. Results of lytic action tests done on it are reported below.

Materials and Methods

(1) Materials

1. Brucella strains used in the test.

(1) Reference strains: Swine brucella bacillus S 1330; cow brucella bacillus, strains A 544, 45/20; goat brucella strains 16 M, 63/9, and Ether; shalinshu brucella strain 5 K 33; dog brucella strain RM 6/66; and sheep epididymis brucella strain 63/290.

(2) Strains proviced by the institute for testing: Twelve different hog brucella strains of types 1 and 3, 15 strains of cow brucella of types 1, 3, and 6, 30 strains of goat brucella of types 1, 2, and 3, one strain of dog brucella (provided by the Guangxi Health and Epidemic Prevention Station), and eight atypical strains of brucella of unidentified kind and type.

2. Brucella Bacteriophages:

(1) Brucella bacteriophage SA. This bacteriophage was isolated in the 1950's from Type 1 brucella bacillus provided by the Beijing Agricultural University. The institute preserved this strain for nearly 30 years. It carried the number S 005.

(2) Reference strains of brucella bacteriophages: Tb, Wb, Bk_2, and Fi (provided by the Institute of Epidemiology and Microbiology of the Chinese Academy of Preventive Medicine and repropagated by our institute).

3. Culture: Improvement of the Albimi brucella broth and agar culture[2], namely by adding tryptose imported from the U.K and 1.5 percent agar plus 10 percent inactivated horse serum to the Albimi culturing medium instead of the formerly added 2 percent M peptone and 2 percent agar.

(2) Method

1. Inoculation: The brucella culture that had been cultured for 48 hours was diluted to make a bacillus suspension of 10^9/ml, which was spread evenly on the improved Albimi agar plate medium and let set for from 30 to 60 minutes.

2. Preparation of the brucella bacteriophage dilute solution: The bacteriophage stock solution was diluted to six concentrations ranging from 10^{-1} to 10^{-6}. For the reference strains of bacteriophages, the conventional assay diluent was used.[3] Each of the diluents were inoculated, a drop at a time, to the supplied test bacilli agar plate medium. After it had dried, it was cultured at 37 degrees C for 48 hours, and the appearance of bacteriophage plaque was recorded.

Assay Results

(1) Bacteriophage SA Stock Solution Lysis Results Against the Test Strains Provided. (Table 1)

Table 1. Bacteriophage Stock Solution Lysis Results Against Various Brucella Bacilli

Reference Bacilli Strains						The Institute's Strains			
Swine	Cow	Goat	Sha-lingshu	Dog	Sheep Epididy-mis	Swine	Cow	Goat	Atypical
S 1330	S 544 R 45/20	S 16M 63/9 Ether	S 5K 33	R RM 6/66	R 63/290	S 12	S 15	S 25 strains R 5 strains	R 6 strains 2 strains
+	+ +	− + −	+	+	+	+	+	− +	− +

Note: + means lysis; - means no lysis. S: smooth type brucella strains; R: Rough type brucella strains.

The Table 1 results show that a fusion lysis or bacteriophage plaque was formed by the bacteriophage SA stock solution against smooth type swine brucella bacilli, cow bacilli, shalinshu bacilli, and rough type cow bacilli 45/20, dog bacilli, sheep epididymis brucella bacilli, goat bacilli type 2, and two strains of atypical brucella bacilli. There was no lytic action against goat brucella bacilli types 1 and 3.

(2) Lytic Activity of Bacteriophage SA at Dilute Concentrations and Bacteriophage Reference Strains at Conventional Concentrations Against the Brucella Bacilli Strains Provided. (Table 2)

Table 2. Lytic Results of Brucella Bacilli Bacteriophages SA, Tb, Wb, Bk_2, and Fi Against Different Brucella Bacilli

			SA									
	Bacilli		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Tb	Wb	Bk_2	Fi
Swine	S1330	(S)	+	+	+	+	PL	PL	-	+	+	+
	S005	(S)	+	+	+	+	PL	PL	-	+	+	+
	Zhong 5	(S)	+	+	+	+	PL	PL	-	+	+	+
Cow	A544	(S)	+	+	+	+	PL	PL	+	+	+	+
	45/20	(R)	+	+	PL	PL	-	-	-	-	-	-
	A8405	(S)	+	+	+	+	PL	PL	-	+	+	+
	A42	(S)	+	+	+	+	PL	PL	+	+	+	+
Goat	M16	(S)	-	-	-	-	-	-	-	-	+	-
	63/9	(S)	+	+	PL	PL	-	-	-	PL	+	-
	Ether	(S)	-	-	-	-	-	-	-	-	+	-
	M819	(S)	-	-	-	-	-	-	-	-	+	-
	M28	(R)	+	PL	-	-	-	-	-	-	-	-
	M5	(R)	+	+	PL	-	-	-	-	-	-	-
	M45	(S)	+	+	+	+	PL	PL	-	+	+	+
Sha-linshu	5K33	(S)	+	+	+	PL	PL	-	PL	+	+	+
Dog	RM6/66	(R)	+	+	+	PL	PL	-	-	-	-	-
Dog	57	(R)	+	+	PL	PL	-	-	-	-	-	-
Sheep	63/290	(R)	PL	-	-	-	-	-	-	-	-	-
Atypi-cal	202	(R)	+	PL	-	-	-	-	-	-	-	-

Note: + means lysis; PL means botyroid bacteriophage plaque or single bacteriophage plaque; - means no lysis; S means smooth type brucella bacillus strain; and R means rough type brucella bacillus strain.

The Table 2 results show that brucella bacteriophage SA diluted at 10^{-1} to 10^{-4} produced lysis in the provided smooth type swine bacilli, and in the cow and the shalinshu bacilli. At a concentration of 10^{-5} and 10^{-6}, either botyroid bacteriophage plaque or single bacteriophage plaque (as shown in Plate I-1-3) formed. The bacteriophage plaque was between 3 and 4 mm in diameter. At a concentration of between 10^{-1} and 10^{-3}, there was no lysis of goat brucella bacilli, types 1 and 3. Against goat brucella bacilli type 2 and rough type cow brucella bacilli 45/20, dog bacilli, sheep epididymis brucella bacilli and the two strains of atypical bacilli, either a lytic reaction occurred, or single bacteriophage plaque formed. The bacteriophage plaque was between 1 and 2 mm in diameter or smaller. Sometimes no bacteriophage plaque appeared. With the exception of the Wb, which produced bacteriophage plaque when used at high concentrations against the goat type 2 brucella bacilli, the four strains of reference bacteriophages' lytic action against the provided bacteriophage strains was similar to the assay results obtained by M.J. Corbel et al, and they produced no lytic action against any of the rough type bacilli.

Discussion

1. The lytic results against the bacilli strains provided for testing using brucella bacteriophage SA and bacteriophages show that except for the SA at high concentrations, which produced lysis against some rough type strains, the other brucella bacteriophages produced similar lytic activity against smooth swine bacilli, cow bacilli, and shalinshu bacilli. The BK_2 produced a lytic reaction against all the goat bacilli, while at high concentrations, both the SA and the Wb produced a lytic reaction against only goat type 2 bacilli.

2. The goat bacillus M 45 against which SA and the reference strains produced a lytic reaction were isolated during the 1950's and 1960's from the blood of human brucella patients. Six strains of this bacillus have been found, which were determined to be goat bacilli at the time. These bacilli strains did not vary despite repeated assays; Tb bacteriophage lysis was negative; and the A factor blood serum agglutination grew in a 2.5×10^{-4} thionine medium. It is similar to the goat type 2 bacillus in this respect; however, it is able to produce large amounts of hydrogen sulfide, which makes it similar to cow type 3 bacillus as well. The bacteriostatic circle that Bacillus pumilus produced on six st ins of the bacilli provided for testing measured 18 to 20 mm in diameter[4], as was the case with the swine type 3 bacillus; however, the swine type 3 bacillus did not produce hydrogen sulfide. the as yet untyped strains were sensitive to the four different bacteriophages.

3. Not only was the SA parent generation bacteriophage strain reproduced, but a mutant strain of the cow bacillus was repeatedly reproduced as well. (This was a cow bacillus A 8405, which grew some single colonies in the bacteriostatic circle produced by a streptomycin tablet. Assay determined the mutant strain to be biochemically different from the parent strain, changing from a strongly toxic strain to a weakly toxic strain. It was unlike the cow bacillus in either kind or type, nor was it like the swine strain). The post-reproduction bacteriophage solution's lysis range was wider than that of the bacteriophage solution from the S 005 reproduced strain. Whether this was because the mutant strain caused changes in the bacteriophage SA that increased its lysis range is a question that awaits thorough study.

4. Inasmuch as there is a limit to the number of bacilli strains and bacteriophage reference strains that the institute can preserve, it was possible only to perform some bacillus lytic action tests and bacteriophage reference strain control tests. As to how to increase the lytic action of bacteriophage SA and determine its lytic range, whether there was a mistake in the original assay of lytic action against the six strains including the goat bacillus M45, or whether it should belong to another kind of brucella bacillus; whether the use of cow bacilli mutant strains in the reproduction of bacteriophage SA can produce changes in the SA, thereby heightening and expanding its lytic range; and control experiments on those mutant strains and the five bacteriophages require further study.

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New Method Outlined For Detection of Plasmid DNA in Corynebacteria

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[Article by Yu Hong [0151 4767], Fang Xu [2455 1645], and Yang Neng [2799 5174], Bioscience and Technology Department, Zhejiang University: "Rapid Method For the Detection of Plasmid DNA in Corynebacteria"]

[Text] Abstract: We compared three different methods for the rapid detection of plasmid DNA in corynebacteria--the strong alkali method, the quick splitting method, and the enzyme method of our own design. Using the strong alkali method, the extracted plasmid band were very light, and only slightly less so using the splitting method, making it impossible to distinguish two plasmids of approximate molecular weight. Using the enzyme method, however, the bands showed up clearly, and it was possible to distinguish very clearly plasmids of approximately the same weight. The differentiation rate was high. We believe the corynebacteria plasmid rapid detection method to be a very good method.

Key Terms: Corynebacteria; plasmid DNA; and plasmid detection.

Corynebacteria are the principal bacteria produced in the fermentation of amino acids and nucleotides. They have a substantial economic value. The building of a nuclear cloning system in corynebacteria holds very great significance.[1] It not only enables us to conduct genetic research on this category of bacteria, but it also provides an extremely effective tool for modifying bacteria. Plasmid DNA detection is a basic task that cannot be overlooked in genertic engineering. The rather special nature of the cell walls of corynebacteria causes many difficulties for plasmid DNA detection. Numerous conventional methods for detecting plasmid DNA leave much to be desired. In our building of a corynebacteria transformation system to meet work needs, after comparing the plasmid detection methods used by Barnes[2] and Kado[3], we proposed an effective method for the rapid detection of plasmid DNA in corynebacteria.

Materials and Methods

1. Bacteria strains and plasmids. The bacterium strain used was Corynebacterium glutamicum, the sources of which and the plasmid content of which was as follows:

Bacteria Strain	Plasmids Carried	Source
1014	pXZ10145(Cm^r)*, pZM 10141	Shanghai Gongwei Institute
1014-1	pZM10141	1014 Yansheng Strain
1014-6T	pXZ10145(Cm^r)	1014 Yansheng Strain
1014-6		1014 Yansheng Strain

*Cm^r: Chloromycetin antibiotic marked

2. Culturing Medium (Percent): Peptone 1, sodium chloride 0.5, beef extrace 1, pH 7.2.

3. Plasmid rapid detection method:

(1) Strong alkali method: To 5 ml of culturing medium in which bacteria had cultured overnight was added to 50 µl and TE buffering solution (40 mm Tris, 2 mm EDTA, and enough acetic to adjust the pH to 7.9) to form a suspension. To this was added 100 µl of bacteriolytic solution (3 percent SDA dissolved in 50 mm Tris, with sodium hydroxide used to adjust the pH to 12.6), and then it was put in a water bath at a fixed temperature for from 10 to 15 minutes and an extraction made using TE saturated phenol solution. The supernate was then checked using electrophoresis.[3]

(2) Quick splitting method: To 5 ml of culturing solution in which bacteria had cultured overnight was added 25 µl of a splitting buffering solution (50 mm sodium hydroxide, 0.5 percent SDS, 5 mm of EDTA, and 0.025 percent bromocresol green) to form a suspension. This was put in a 45 degree C water bath for 10 minutes, then 2.5 µl of 25 percent sucrose was added. It was then centrifuged in a desktop centrifuge at 12,000 rpm and the supernate was checked using electrophoresis.[2]

(3) Enzyme method: To 5 ml of culturing solution in which bacteria had cultured overnight was added 200 µl of TEN buffering solution (Tris 30 mm, EDTA 50 mm, and sodium chloride 50 mm at a pH of 8.0) to form a suspension. To this was added 70 µl of lysozyme (25 mg/ml), which was kept at 35 degrees C while being vibrated for 2 hours. Then 30 µl of 10 percent SDA was added to bring the concentration to 1 percent. This was left at room temperature for 10 minutes to allow full cleavage of the cells. Then TEN saturated phenol was used for extraction, and the supernate was checked using electrophoresis.

(4) Electrophoresis: 0.8 percent agarose gel, boric acid electrophoresis buffering solution (89 mm Tris, 89 mm boric acide, 20 mm Sodium EDTA), 80 volts of electricity, with electrophoresis proceeding for 2.5 hours at room temperature.

Results and Discussion

The strong alkali method is a plasmid detection method founded on E. coli. When this method is applied to corynebacteria, results are less than ideal, the plasmid bands being very light and fuzzy.

Results of the quick splitting method applied to corynebacteria 1014 and Yansheng bacteria strain plasmid DNA are shown in Plate I-2 [not reproduced]. This method is able to detect rapidly the presence of corynebacteria plasmids; however, the plasmid bands are insufficiently distinct, so the differentiation rate is not high. This method does not reveal and two different kinds of plasmids contained in the 1014 bacterium strain. In addition, the success of this method is greatly affected by the bateria concentration, processing time, and temperature.

We founded the enzyme method for rapid detection of plasmid DNA on plasmid DNA extraction. Use of the enzyme method in the rapid detection of glutamic acid corynebacteria strain 1014 and its Yansheng strain enabled clear analysis of the number of plasmids in the bacterium strain and the plasmid DNA molecular weight. As Plate I-3 [not reproduced] shows, two plasmid bands were clearly visible in the 1014 strain. Neither the strong alkali method or the quick splitting method can compare with this. This method is simple and easy to follow, can be readily duplicated, and is suitable for rapid detection of plasmids in large numbers of bacteria strains as, for example, in the straining of bacterial strains containing plasmids, identification of transformants, and detection of inserted segments. This method also yielded rather good rasults in the rapid detection of plasmids in bacilli. This method may also be elaborated. After extraction of the enzyme, the acid phenol method[4] may then be used to get rid of the chromosome DNA after which glacial ethyl alcohol may be used for precipitation. The plasmids thus derived may be used in transformation and enzyme cutting.

The enzyme method that we have sketched above is simpler, and more effective than otherwise reported corynebacteria plasmid DNA quick detection methods[5-7], and may be used for different needs.

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'Magic Light' High-Power Laser Installation Detailed

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[Article by Yu Wenyan [0151 2429 3508] and Lei Shizhan [7191 0013 3277] of the Shanghai Institute of Optics and Fine Mechanics, Chinese Academy of Sciences: "The 'Magic Light' Device"]

[Text] "Magic light" is a high-power laser with a maximum power output of 10^{12} watts. It is also one of a few large lasers in the world. It is primarily used in the basic research of laser-induced nuclear fusion and X-ray lasers, as well as in the study of high-temperature, high-pressure and high-density plasma behaviors. Most of the components, materials and technology used to construct this device are available in China.

1. Fuel is used as a source to generate heat and power. Wood, coal, natural gas and petroleum are common fuels. In the 1960's, people began to use a new kind of fuel--nuclear fuel. It is uranium isotope 235. People are exploring another nuclear fuel--deuterium (denoted as D) and tritium (denoted as T) which are hydrogen isotopes. In the fusion process involving two deuterium nuclei or one deuterium and one tritium nucleus, a large amount of energy is released. This energy will be available for billions of years.

However, in order to allow a nuclear fusion reaction to occur, the two participating nuclei must be close within the nuclear force range (approximately $3x10^{-13}$ cm). The great repulsive Coulombic force between two positively charged nuclei would prevent them from getting close. To make a fusion reaction happen, each nucleus must be given an enormous amount of kinetic energy. Based on calculation, it might be possible for two deuterium nuclei to overcome the repulsive force between them to be fused into a nucleus if they reach a thermal velocity of 1,000-2,000 km/sec. Based on thermodynamics, to make it move at this high speed is equivalent to heating a deuterium atom to 100-200 million degrees. On earth, almost everything evaporates at approximately 3,000 degrees and transfers its energy to other media. Therefore, deuterium fusion reaction cannot happen spontaneously on earth.

How can we heat up nuclear fuel to a hundred million degrees? An atomic blast can produce a high temperature fire ball of a hundred million degrees. As a matter of fact, that is how hydrogen bombs are detonated. However, it is an instantaneous nuclear explosion which cannot be controlled. There is no way to harness it for power. If we want to utilize the energy, we must have controllable nuclear fusion. There are several ways to achieve controllable nuclear fusion and laser nuclear fusion is the most promising approach. A laser is a high intensity, very coherent light source which can be focused by optical elements such as lenses at a fine point. A great deal of energy is concentrated at this point which is capable of instantaneously heating up matter to very high temperature. Based on current capability, it can heat up deuterium nuclei to several thousand degrees.

From the angle of applications of laser technology, laser induced nuclear fusion is a major item. Compared to other applications, it is the most demanding one. For instance, the energy output of the laser system has to reach 1 million joules and the light pulse is only one-billionth to one-tenth of one-billionth of a second. In addition, it also requires a uniform spatial intensity distribution so that it illuminates evenly over the target sphere. The disperse angle of the laser beam must be so small that it is only limited by diffraction. Thus, the laser beam can be focused to a very fine point. The laser system is also required to emit 10 to 100 pulses of light per second. In other words, the laser system must operate repeatedly at high pulses. The difficulty is that these requirements are mutually limiting. For example, with high energy output, the laser pulse would not be very narrow or the wave front would not be very smooth.

2. In order to initiate laser nuclear fusion research, we needed a high-quality high-power laser system. China began to develop high-power lasers in 1963. Between 1980 and 1981, the "magic light" concept was technically proven. At the end of 1981, the assignment of developing "magic light" was officially given to the Shanghai Institute of Optics and Fine Mechanics by the Chinese Academy of Sciences. The institute mobilized nearly one-third of its technical force (over 200 people) to participate in the effort.

"Magic light" was successfully developed in 1986. It was tested for 1 year. A team of experts went in and spent about 8 months conducting experiments to evaluate 22 parameters. In addition, the device was used in target shooting experiments and obtained a great deal of valuable data. More than 1 million neutrons were produced in shooting deuterium filled micro-sphere targets.

The laser system used in the study of laser nuclear fusion not only must have high energy output and short pulse time but also must have a high quality beam with good directivity. The latter two requirements can only be achieved through single mode longitudinal oscillation and lowest order transverse mode oscillation. Nevertheless, a high-energy output large laser usually involves the simultaneous oscillation of multiple longitudinal and transverse modes. Hence, a high-power laser system usually employs the laser oscillator-amplifier mode to operate. A small laser oscillator is carefully designed and adjusted to oscillate in a longitudinal and a transverse mode.

The laser waveform is also modified so that the pulse width and the wave front shape meet the requirements for micro-sphere targets. This beam is sent to the input of a laser amplifier. The laser amplifier in "magic light" can amplify the input energy by a factor of 100,000 to 1,000,000. In this process, other beam characteristics, except for the energy, remain unchanged.

In order to allow the micro-sphere to be illuminated symmetrically, the nuclear fusion device has to be a system which simultaneously produces several laser beams. After an analysis by the designers, it was concluded that the most rational choice for a 10^{12} Watt laser system is two beams because the symmetry and solid angle with two beams are comparable to those with more beams and it is structurally much simpler. Furthermore, it is much easier to control the energy balance and optical path synchronization to make it easy to stabilize in the experiment. The "magic light" employed a two channel laser amplifier system. The energy output at the last stage amplifiers 800 joules and the light pulse width is 1 nanosecond, i.e., the power output is $8x10^{11}$ Watt per channel.

Selecting the working medium for the laser amplifier is a major decision to make. In the early 1960's, the power output of the ruby laser was high relative to other types of lasers. Therefore, the ruby laser was chosen initially. However, neodymium laser soon replaced it. Because ruby is crystalline, it is very difficult to obtain a large ruby rod with good optical characteristics. The amount of laser power the working medium can withstand increases with its diameter. The inability to obtain a large-diameter laser rod is equivalent to limiting the achievable laser power. The matrix of the working medium for a neodymium laser is glass. It is fabricated by the conventional optical glass melting process. It is relatively easier to obtain large and optically uniform materials.

After the diameter of the working medium is determined, the major factor limiting the level of power to pass through the system is the non-linear effect of the medium caused by intense laser light. The electromagnetic field of the light will affect the dielectric constant of the medium to change the index of refraction of the medium. Normally, the light beam has very little effect on the index of refraction and the change can be neglected. However, the situation is quite different with a high-power laser beam. The change in the refraction index would be quite noticeable. When an intense laser beam passes through the medium, the attached index of refraction is higher where the light field is more intense. In general, laser intensity distribution is a function of time and space. The result is that the laser beam would continuously change its direction of propagation in the process due to changes in index of refraction. The light energy is even more concentrated in areas where the light intensity is strong. This is the so-called self-focusing effect. This effect not only destroys the completeness of the wave front but also could seriously damage the working medium to cause rapid deterioration of the optical property of the laser system. The power density threshold for self-focusing is dependent upon the non-linear index of refraction of the medium and can be expressed by the following integral B:

$$B = \frac{8\pi^2}{\lambda c} \times 10^7 \int_0^L \frac{n_2}{n_0} dz, \qquad (1)$$

where λ is the wavelength, c is the speed of light, L is the length of the amplifier, n_0 is the linear index of refraction, and n_2 is the non-linear index of refraction. The larger the integral B is, the more pronounced the self-focusing effect is.

The non-linear index of refraction n_2 of phosphate neodymium glass is 60-70 percent lower than that of silicate neodymium glass. Therefore, if phosphate neodymium glass is used as the medium of the amplifier, then the power it can withstand is approximately 1.6 times of that of silicate neodymium glass of the same diameter. Based on theoretical and experimental results, the gain is approximately 1.6-1.7 times higher with phosphate neodymium glass than with silicate neodymium glass. When the laser beam is amplified by the same extent, the use of phosphate neodymium glass can reduce its length. Based on equation (1), the value of B can be further reduced to enhance the power loading of the laser system. Based on this analysis, "magic light" uses phosphate neodymium glass as the medium (despite the fact that silicate neodymium glass technology was relatively mature and phosphate neodymium glass was still under development when the decision was made).

The first few stages of the amplifier system uses rods and the later stages use plates. In the medium where the pulse width is of sub-nanosecond order, the average power density that the neodymium glass can withstand is less than 3-5 GW/cm^2. This indicates that the diameter of the working medium for a single channel amplifier with an output of 10^{11} Watts must be greater than 70 mm. If the power output is to be further raised, the last stage diameter has to be even larger. In general, a plate should be used in an amplifier greater than 100 mm in diameter. It permits the light to pass through a thin medium over a large cross-section which improves the homogeneity of the light beam. In addition, the thermally induced optical aberration due to distortion is also very small. The disadvantage of using a plate is that all neodymium plates must be placed according to the Brewster angle. The maximum dimension of the plate should at least be twice that of the light beam. When the precision of machining is not sufficient, this type of large angle optical set-up would introduce a great deal of dispersion and other aberrations. Furthermore, the structure of the amplifier is also more complex.

3. Certain measures have to be taken in order to maintain the quality of the laser beam after it is amplified. For example, when the laser beam is transmitted from one amplifier to the next one, the light reflected from the optical element of the latter (or the target surface) would travel in the opposite direction. The gain of this multi-stage traveling wave amplifier is extremely high. Any slight feedback would cause the spontaneous oscillation of the amplifier to hinder the performance of various amplifiers. The quality of the laser beam would deteriorate. In serious cases, components of early stage amplifiers become the target of the high-power laser system and are damaged at times. Therefore, it is necessary to take measures to allow light to pass in one direction. The light beam going in the reverse direction is isolated in every stage. Specifically, an optical isolator is

installed between two amplifiers. Its function is to allow the laser beam to only go forward from one stage to the next until reaching the target. The beam that travels backward is blocked. Usually, electrical and magnetic optical isolators are used. Figure 1 shows the structure of a magnetic optical isolator consisting of a pair of polarizers and a Faraday optical rotary. P_0 is the polarizer and P_1 is the analyzer. The optical rotary operates based on the Faradaic effect, which is composed of a polarizing medium, a solenoid and an LC power supply circuit. The direction of polarization of the light that passes through the optical rotary would rotate. The angle of rotation is proportional to the external magnetic field.

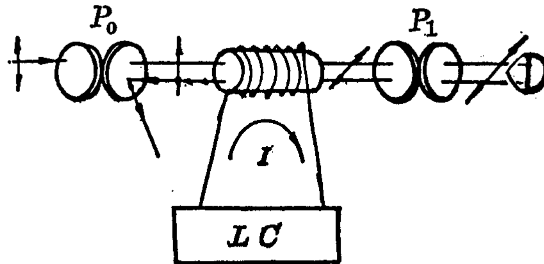


Figure 1. Structure of the Magnetic Optical Isolator

Assuming that the direction of the incident polarized light P_1 coincides with that of the polarizer P_0 and the direction of polarization is rotated 45° by the optical rotary, it happens to coincide with the direction of polarization of the analyzer (see Figure 1). Thus, the light beam can penetrate the analyzer and enter the next stage of amplifier. As for the light beam going in the opposite direction, its polarization is rotated by 45° around the opposite direction when it passes through the Faraday optical rotary. In this case, the direction of polarization is perpendicular to that of the polarizer. Thus, it is reflected by the polarizer and cannot enter the next amplifier, accomplishing the objective of isolating light traveling in the opposite direction.

A high-power laser is composed of hundreds of optical components. Any spot, dust or imperfection in any optical component would cause diffraction. The finite diameter of every optical component acts as a grating which also causes diffraction. These diffraction patterns make the intensity distribution across the cross-section of the optical element non-uniform. This effect is further enhanced after going through various stages of amplification. The laser power density in some local areas in the working medium might get excessively high. In order to prevent self-focusing in the working medium, the laser field distribution ought to be as uniform as possible. A space filter can eliminate spatial disturbance. In 1975, Livermore Laboratories in the United States first satisfactorily employed space filters in a high-power laser system.

A space filter operates based on the principle of Fourier optics. It consists of a pair of conjugate focus lenses and a filter hole on the focal

plane. Figure 2 shows a schematic of the space filter. The incident laser beam is projected onto the input lens and is decomposed into a spatial frequency spectrum by the positive lens and distributed on its focal plane. The small hole blocks the high frequency portion of the spectrum. The highest frequency to pass through the hole (which is called the cutoff frequency) v_f is

$$\nu_f = \frac{\pi D}{\lambda f_R}, \quad (2)$$

where D is the diameter of the filter hole, λ is the laser wavelength, f_R is the effective lens of the lens. The space filter eliminates the high frequency component and also evens out the intensity distribution across the cross-section. A large laser system often employs several space filters. These filters act as an optical system and are also used to transfer images. They treat the light intensity distribution from the amplifier as an "object" and sequentially transfer this image stage by stage to the target surface. Thus, the target surface is uniformly irradiated. After space filters were installed in the "magic light" system, the power that can be focused on the target surface was significantly increased.

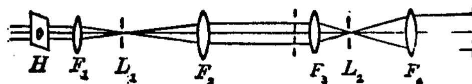


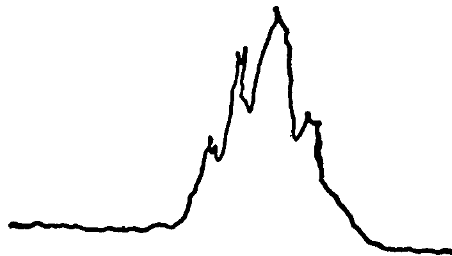
Figure 2. Schematic Diagram of the Space Filter

4. The raw signal of the laser amplifier is supplied by the laser oscillator. What should we use as a working medium for the laser oscillator? The central wavelength of the phosphate neodymium laser amplifier is 1.054 μm. Of course, if phosphate neodymium glass is also used as the working medium for the laser oscillator, then the laser wavelength coincides with the peak wavelength of the amplifier. Thus, we will have the maximum gain. However, it is necessary to perform mode locking Q tuning to obtain a nanosecond laser pulse. Because of its small excitation cross-section and poor thermal properties, it is unfavorable to use phosphate neodymium glass to get a locked single longitudinal Q pulse laser. Nd:YAG crystal is a good laser material. Based on its energy levels, it can produce a radiation at 1.05205 μm which is very close to the central wavelength 1.054 μm of phosphate neodymium. The difficulty is to make the Nd:YAG laser oscillate stably at this wavelength.

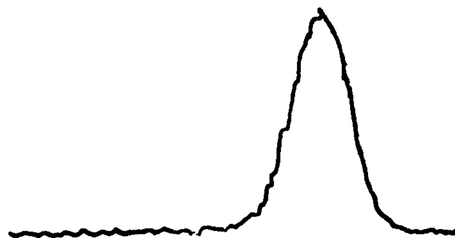
Nd:YLF (neodymium doped yttrium lithium fluoride) is a new laser crystal. Its optical properties are similar to those of Nd:YAG. Moreover, it can stably emit a laser beam at 1.053 μm. This wavelength is even closer to the laser wavelength of phosphate neodymium glass. Hence, the designer of "magic light" decided to employ this new crystal as the working medium for the laser oscillator.

Nd:YLF crystal has two equivalent A axes and a C-axis. When C-axis crystal is used, it does not require any polarizer to get a laser output at 1.053 μm.

As discussed before, the amplitude of the laser pulse from the oscillator must be very stable, the wave front must be very smooth and the signal to noise ratio must be very high (generally greater than 10^7-10^8). The key to getting this kind of high quality laser pulse is that the laser must be in resonance in a single longitudinal mode. To realize this in a solid state laser is technically more difficult because the gain line width is relatively wide for a solid medium. For Nd:YLF crystal, its gain line width is 12.5 cm^{-1}, which is two orders of magnitude higher than that of the He-Ne laser. Therefore, it is easy for several longitudinal modes to resonate at the same time. In addition, the standing wave hole burning effect in a solid laser cavity also makes easy for neighboring longitudinal modes to resonate simultaneously. When several longitudinal modes resonate, the intensity distribution envelop of the laser is no longer smooth. Figure 3 (a) and (b) show the output light pulse from a multi-mode and single mode laser, respectively.



(a) light pulse from a multiple longitudinal mode laser



(b) light pulse from a single longitudinal mode laser

Figure 3

To get a stable output, the frequency of the longitudinal mode chosen must coincide with the peak transmission frequency of the standardizing device and the maximum frequency of the gain curve over an extended period of time. This is the key to attain a stable laser resonating in a single longitudinal mode. It was experimentally found that when a pre-pulse of laser light was used to initiate the resonance different longitudinal modes showed some difference in the time domain. The breakthrough made by the designers of "magic light" was that this characteristic was used to determine the relative position between

the longitudinal mode in the resonance cavity and the transmission curve of the standardizing device. Furthermore, it is used as the reference to control the length of the cavity to allow the principal longitudinal mode sitting at the peak of transmission of the standardizing device at all times to obtain a stable single mode Q tunable laser output. After wave shaving and amplification of this single mode Q tunable laser, we will be able to get huge laser pulses of various width and intensity with smooth envelops.

5. The field where a high-power laser beam is used to conduct a nuclear fusion experiment and to gather various physical parameters is called a target area. A target area usually consists of focusing lenses, focusable light source, various probes to record parameters associated with the laser and the plasma, and a main vacuum system.

In general, the accuracy requirement of the light focusing system is very high in the laser fusion experiment. The aim of the laser at the target with deuterium and tritium must be accurate to within several micrometers. In other words, the laser is focused less than several micrometers from the target surface. The center of the target sphere must not deviate from its ideal position by a few micrometers in a three-dimensional coordinate system. Moreover, it should be simple to change the target and reliable to position it. In order to ensure that the laser energy reaches the target simultaneously for the fusion reaction, the two laser beams must be synchronized. The synchronization requirement of "magic light" is ±10 ps.

These requirements must be ensured by precision machining, optical machining and reliable computer control circuitry. When the beam is stable, the position where the reflected light is most intense is the focus. The accuracy of the focus position is determined by the resolution of the reflected signal. However, if there is some instability in the laser output, such as some energy fluctuation, the focusing accuracy is affected. The target area of "magic light" compares the light intensity to accurately determine the location of the focus.

The second requirement of the focusing system in the target area is to have a very uniform light intensity distribution over the target surface. Normally, even if the near field distribution of the laser beam is uniform, after focusing the near field and quasi-near field distribution in front and behind the focus is not uniform. The "magic light" uses a new focusing method which is called a lens array to attain uniform irradiation over a large range on the focal plane. Figure 4 shows the arrangement of a hexagonal lens array. Figure 5(a) shows the light intensity distribution on the focal plane without the use of a lens array. The modulation percentage is ±45 percent. Figure 5(b) shows the light intensity distribution on the focal plane with a lens array. In this case, the light intensity fluctuation is reduced and the modulation percentage is ±12 percent.

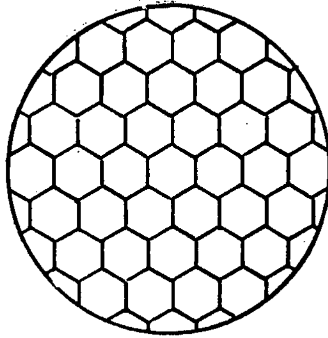
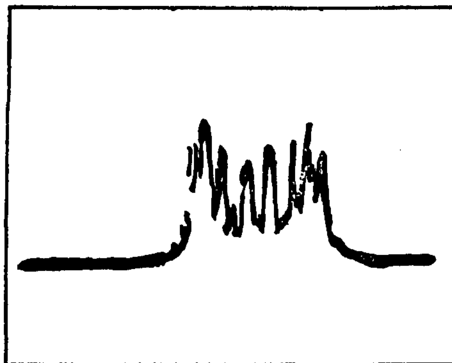
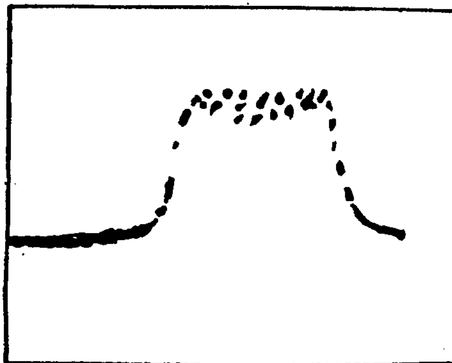


Figure 4. Layout of a Lens Array To Obtain Uniform Illumination Over Large Area on the Focal Plane



(a) light intensity on the focal plane without a lens array



(b) light intensity on the focal plane with a lens array

Figure 5

6. A high-power laser system such as the "magic light" can be used to conduct basic research on new energy resource such as nuclear fusion. In the next several years, it is planned to solve the following problems:

(1) To obtain a practical driver;

(2) To determine the minimum amount required to effectively use deuterium-tritium fuel;

(3) To determine the precise amount of laser energy required to ignite the fusion target;

(4) To study the control of laser plasma and determine its fluid dynamic instability.

Let us not talk about that far about solving the energy problem for mankind forever, even today the γ-ray, X-ray and high energy neutron generated by a small nuclear fusion sphere induced by a high-power laser can more realistically simulate various effects of a nuclear bomb explosion. The accuracy of simulation in the understanding of the effect of X-ray and γ-ray on the electronics in a missile warhead or a communication satellite is several orders of magnitude higher than that of existing methods. In addition, the experiment can be repeated in the laboratory to obtain more reliable data.

We must also point out an interesting finding. In the study of the interaction between high-power laser and matters, laser plasma showed signs of lasting in the X-ray band. It is a revolutionary leap to produce an X-ray laser from the interaction between an intense laser in the optical frequency band and a matter. Because the X-ray laser is considered the most powerful source of radiation in Star Wars, this becomes a topic of interest in the world.

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